ISSN Print : 1656-4707
ISSN Online: 2467-5903

The Palawan Scientist

Volume 13(2) December 2021

A Research Journal of the Western Philippines University
Aborlan, Palawan
www.wpu.edu.ph

www.palawanscientist.org

The Palawan Scientist, a recipient of a 3-year Commission on Higher Education - Journal Incubation Grant for the year 2017-2019, is an internationally circulated annual peer reviewed multi-disciplinary journal published by the Western Philippines University, Palawan, Philippines. It accepts original research articles, reviews, notes and short communications in agriculture, fisheries and aquatic sciences, environment, education, engineering, mathematics, sociology and related disciplines.

Articles published in the Palawan Scientist are indexed in the Emerging Sources Citation Index (Clarivate Analytics), ASEAN Citation Index (ACI), Google Scholar, and Philippine E-Journals. It is under evaluation by ASFA

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Cover Photo
Philippine Scrubfowl *Megapodius cumingii* (left) a large ground dwelling bird in the Megapodiidae family that can be found in northeastern Borneo, Sulawesi and Philippines; Mantanani Scops Owl *Otus mantananensis* (center) is a small owl, belonging to family Strigidae, that lives on small offshore islands in the western Philippines and northern Borneo; and Grey Imperial Pigeon *Ducula pickeringii* (right) that belongs in the family Columbidae is a small island specialist with very small range of less than ten locations including Sulu Archipelago, Miangas and Talaud Island. Photos courtesy of P Godfrey Jakosalem.

The Palawan Scientist, Vol. 13(2)

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https://doi.org/10.69721/TPS.J.2021.13.2.x

EDITORIAL

Dear Readers,

Each new issue of **The Palawan Scientist** represents an impressive amount of work from both local and international scientists. More importantly however, with each new issue of the journal, we improve our understanding of topics from various fields, ranging from the natural sciences to sociology. This knowledge can be applied to build a better, fairer and more sustainable future both for ourselves and our next generations.

This December 2021, we proudly present the Volume 13(2) of the journal. Working in the aquaculture and fisheries sector, I am delighted to see that it includes several papers related to fish and the aquatic environment.

As we all know, our planet is in a dire state due to overconsumption and a rapidly growing human population. This is especially true for the aquatic environment. Unknown to most however, is that water covers a whopping 71% of our planet, with our oceans and seas containing over 96% of the world's water. Due to the depth of our oceans, the livable volume that they represent is 900 times larger than the livable habitat of all our combined terrestrial environments. Yet, we comparatively know very little of the aquatic environment: over 80% of our oceans remain unmapped and only 5% of the seafloor has been properly topographically imaged, leaving 65% of our planet's surface largely unexplored.

As Dr. Gene Carl Feldman, an oceanographer at NASA's Goddard Space Flight Center, recently said in an interview with conservation nonprofit Oceana: "In some ways, it's a lot easier to send people into space than it is to send people to the bottom of the ocean." Although the number of land species we have discovered is about 25 times higher than the number of species we have found in oceans and seas, the greatest diversity of phyla is present in the marine environment- which is estimated to be 300 times larger compared to the diversity in land habitats. One of the key species groups found in salt water is algae. These lifeforms form the basis of the food chain and produce 50% to 70% of all the oxygen on Earth. Because life on Earth would not be possible without our seas and oceans, we should take great care of them.

Most life in the sea is concentrated in the coastal zone. Equally, many humans also live in this zone, with roughly 40% of all people living within 100 kilometers of the coast. In addition, over three billion people rely on the sea for their livelihood. As the human population grows, economic activity intensifies and pressure on coastal ecosystems increase. This has already caused the depletion of an estimated 90% of big fish populations, with 50% or so of the planet's coral reefs lost or severely damaged.

To reverse this trend, we need to increase our understanding, awareness and appreciation of the aquatic environment. This starts with solid science. We cannot protect what we don't understand and I strongly believe that conservation should start from the bottom-up, by creating a stronger appreciation for our natural environment. We can do this by sharing our discoveries with the world.

The Palawan Scientist has the opportunity to encourage scientific research and share newly-gained scientific insights. The current and future generation of scientists will play a key role in this. As such, we encourage researchers to submit papers that are multidisciplinary in nature, result from collaborative research, and will contribute to the collection and conservation of knowledge.

Feel free to reach out for assistance. We are here to help you.

United, we walk towards a sustainable future!

Mabuhay,

Jonah van Beijnen
Editor – The Palawan Scientist
Founder - VB Consultancy
Co-founder - Fins and Leaves (formally Centre for Sustainability)

CONTENTS

Mangrove leaf litter production in the Iwahig River estuary ecosystem of Puerto Princesa Bay, Palawan, the Philippines

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https://doi.org/10.69721/TPS.J.2021.13.2.01

ABSTRACT

This study aims to quantify mangrove leaf litter's contribution to the Iwahig River estuary ecosystem's primary productivity in Puerto Princesa Bay, Palawan, Philippines. There are several studies of this nature in the Indo-west Pacific and Malesian regions, but none, so far, in the island province of Palawan. A sampling protocol using the net traps was employed, and the dry leaf production in gram dry-weight per sq. m per day (g DW $m^{-2} d^{-1}$) was computed. The amount of calculated mangrove leaf litter was at 2.34 ± 0.42 g DW $m^{-2} d^{-1}$ of which 49.6% was from the species *Lumnitzera littorea* (Jack) Voigt. The contribution of five other species, *Rhizophora mucronata* (Lamk.), *Rhizophora apiculata* Bl., *Xylocarpus granatum* König, *Bruguiera sexangula* (Lour.) Poir., and *Xylocarpus moluccensis* (Lamk.) Roem came in varying quantities. The seasonal variability was evident, but this did not differ significantly between the rainy (1.48 ± 0.3 g DW $m^{-2} d^{-1}$) and the dry (2.12 ± 1.0 g DW $m^{-2} d^{-1}$) seasons with a *P*-value of 0.432 ($\alpha = 0.99$). None of the four environmental parameters (temperature, rainfall, wind speed and day lengths) correlated well with the average monthly leaf litter production. Nonetheless, the computed value for this is high and can be associated with the Iwahig River estuary ecosystem's high biodiversity. A year-round assessment, with the inclusion of relevant variables such as tides, nutrients, species density, and diameter-at-breast-height (DBH), should be done. Understanding the inter-annual variability in mangrove leaf litter production and its contribution to the Iwahig River estuary ecosystem in Palawan, the Philippines, are imperative.

Keywords: dry weight, litterfall, productivity, species, variability

The Palawan Scientist, 13(2): 1-12

INTRODUCTION

The mangrove forest of the Iwahig River estuary in Puerto Princesa Bay, Palawan, south-western Philippines, is a bio-diverse ecosystem with 19 mangrove species scattered at nearly 280 ha of forest cover (Dangan-Galon et al. 2016). It harbors 91 mangrove-associated terrestrial vertebrates, consisting of 20 herpetofauna, 63 birds, and eight mammals (Dangan-Galon et al. 2015). It is also home to nearly 15 bivalves and 50 gastropod species from 25 families and 45 genera (Dolorosa and Dangan-Galon 2014).

The high biodiversity of this forest can be associated with its productivity. Mangroves are among the most productive ecosystems on earth with an average productivity of 2,500 mg C m^{-2} d^{-1} (Bunt 1995). Nutrient input from land and sea and the internal recycling of organic matter determined the mangrove's productivity (Holquin et al. 2001). Between the two processes, the latter is a more efficient way of meeting the high mangrove demand for nutrients to sustain the forests' productivity (Alongi et al. 1989; Ovalle et al. 1990; Bunt 1995; Jennerjahn and Ittekkot 2002).

The internal recycling of organic matter in mangrove forest begins with the leaching of soluble organic and inorganic compounds from vegetative and reproductive plant parts due to senescence, mechanical factors, stress, death, weathering of the whole plant, or a combination of these factors in a given time (Kozslowski 1973). Colonization of microorganisms that initiate fragmentation of plant materials will then follow (Hossain and Hoque 2008). Organic matter production, in this case, can reach up to 12 t ha^{-1} y^{-1} (Amarasinghe and Balasubramaniam 1992), and in general, 50-85% of such production is from littered leaves (Saenger and Snedaker1993; Navarrete and Rivera 2002; Imbragen and Dittman 2008; Bernini and Rezende 2010).

Mangrove litter production varies widely with species, forest type, stand age, geographical locations, tidal inundation, and environmental parameters such as temperature, rainfall, and wind (Twilley 1995; Twilley and Day 1999; Bernini and Rezende 2010). Litter production tends to be higher in old, dense, mixed stands and riverine forests at lower latitudes (e.g. tropical regions) during the dry season because of increased soil, water salinity, and evapotranspiration rate (Hossain and Hoque 2008). This correlation trend, although evident, is not uniform and varies across regions.

In the Philippines, quantification of mangrove leaf litter production is limited to the works of De Leon et al. (1992), Calumpong and Cadiz (2012), and Rafael and Calumpong (2018) in the central Philippines. In most parts of the country, including the Iwahig River estuary mangrove forest of Palawan in the south-western Philippines, a similar study has never been conducted. Given the recent findings on its rich biodiversity, it is equally interesting to determine the forest contribution to the primary productivity of the Iwahig

River estuary and to account for variability in mangrove leaf litter production as influenced by environmental factors and seasons.

METHODS

Study Site

Mangrove forests adjacent to the Iwahig River estuary located off the mid-eastern portion of Palawan Island, between 9.7359°N and 118.6969°E with a river stretch of 9.1 km was the site of this study (Figure 1). Three permanent stations (Station 1: 9.4408°N and 118.4150°E; Station 2: 9.4403°N and 118.4101°E; Station 3: 9.4434°N and 118.4926°E) were established along this riverbank, representing the upper, mid, and lower portion of the river estuary. These stations harbored a mixed mangrove species with relatively excellent forest cover.

Sampling Procedure

The littered leaves were collected from mangrove forests along the riverbank for nine months, June-October 2013, December 2013, January-February 2014, and April 2014. By installing the net traps, measuring 1 x 1 m tied on mangrove trunks or branches at 0.5-1.5 m high from the sediment to prevent losses by flooding (De Leon 1992; Sukarjo 2010; Calumpong and Cadiz 2012) and with three replications per site, a weekly collection of trapped leaves had materialized (Figure 2). The collected leaves were then sorted per species in the laboratory, air-dried for 48 h, weighed using the top loading balance, wrapped in aluminum foil, and oven-dried at 60°C until reaching a constant dry weight.

The dry leaf litter production, expressed in g DW m^{-2} d^{-1}, was obtained by dividing the mean weekly dry weight of collected leaves from the net traps by seven days. The 2013-2014 environmental data indicated that the dry season in Palawan, Philippines, extends from December to May while the rainy season, June to November. The mean temperature was at 28°C whereas, the mean precipitation or rainfall, 151 mm, with the highest (274.3 mm) recorded in June 2013. These data were from the Philippine Atmospheric, Geophysical, Astronomical Services Administration, Department of Science and Technology (PAGASA, DOST), Puerto Princesa station.

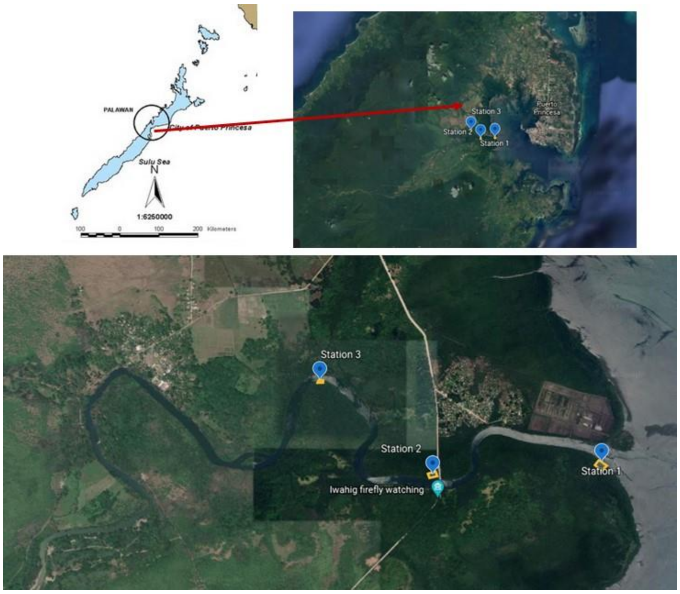

Figure 1. The map of Palawan (top-left) indicating the location of Puerto Princesa City and an aerial view of Puerto Princesa Bay (top-right), where the Iwahig River estuary is situated. The zoomed-in aerial view of the study site shows the three collection stations (bottom).

Figure 2. The leaf litter net traps established in the mangrove forests of the Iwahig River estuary in Puerto Princesa Bay, Palawan, the Philippines.

Statistical Analyses

This study used nonparametric tests such as the Wilcoxon Signed-Rank and Kendall Rank correlation tests of the RStudio 4.0.3 statistical software (R Core Team 2020). These nonparametric tests applied only for data sets that did not satisfy bivariate normality assumptions.

Particularly, Wilcoxon Sign-Rank test was used to determine the mean differences in mangrove leaf litter production between the rainy and the dry seasons. On the other hand, the Kendall Rank correlation test determined the relationship between the average monthly mangrove leaf litter production (as a dependent variable) and a particular physicochemical parameter such as the air temperature, rainfall, wind speed, and day-length (as the independent variables).

RESULTS

Mangrove Leaf Litter Production

Quantified mangrove leaf litter production in the Iwahig River estuary, Puerto Princesa Bay, Palawan, was at 2.34 ± 0.42 g DW m^{-2} d^{-1}. Out of the 19 mangrove species present in the area, only six had a high contribution to mangrove litter production. These included *Lumnitzera littorea* (Jack) Voigt, *Rhizophora mucronata* (Lamk.), *Rhizophora apiculata* Bl., *Xylocarpus granatum* König, *Bruguiera sexangula* (Lour.) Poir., and *Xylocarpus moluccensis* (Lamk.) Roem. Of these species, the *L. littorea* had the highest production, 0.87 ± 0.54 g DW m^{-2} d^{-1}, constituting 49% of total leaf litter weighed for the six species. The *R. mucronata*, *R. apiculata* and *X. granatum* followed with productivity values of 0.43 ± 0.38 (25%), 0.22 ± 0.12 (12%), and 0.164 ± 0.13 (9%) g DW m^{-2} d^{-1}, respectively. The remaining 5% was for *B. sexangula* with litter production of 0.043 g DW m^{-2} d^{-1} and *X. moluccensis* with 0.037±0.03 g DW m^{-2} d^{-1} (Figure 3).

Seasonal Variability

Seasonal variability in mangrove leaf litter production at the river estuary was evident. The monthly yield of these species ranged from 0.72 to 2.87 g DW m^{-2} d^{-1}. The month of January 2014 had the highest, followed by 2.83 g DW m^{-2} d^{-1} in the preceding month of December 2013, while February 2014 had the lowest production value (Figure 4). The result of the Wilcoxon Signed-Rank test had indicated no significant difference in mean monthly leaf litter production between the rainy (1.48 ± 0.3 g DW m^{-2} d^{-1}) and the dry (2.12 ± 1.0 g DW m^{-2} d^{-1}) seasons with a *P*-value of 0.432 (α = 0.99).

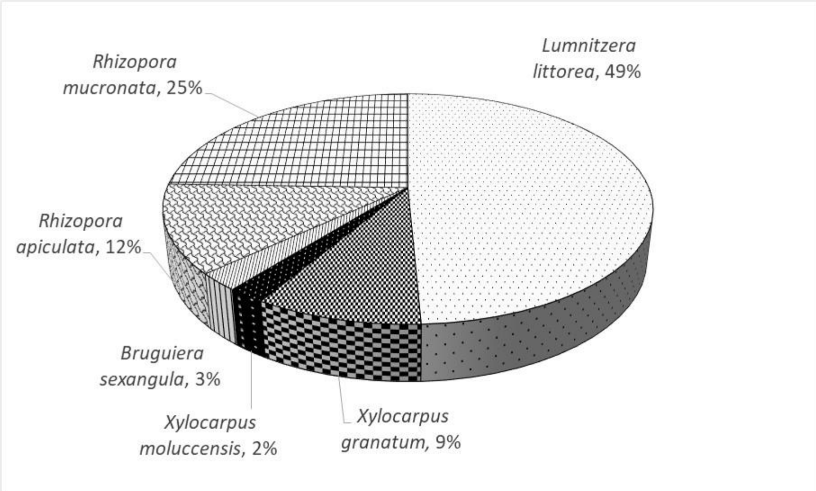

Figure 3. The leaf litter production (g DW m^{-2} d^{-1}) and percent leaf litter contribution of the six predominating mangrove species in the Iwahig River estuary, Puerto Princesa Bay, Palawan, the Philippines.

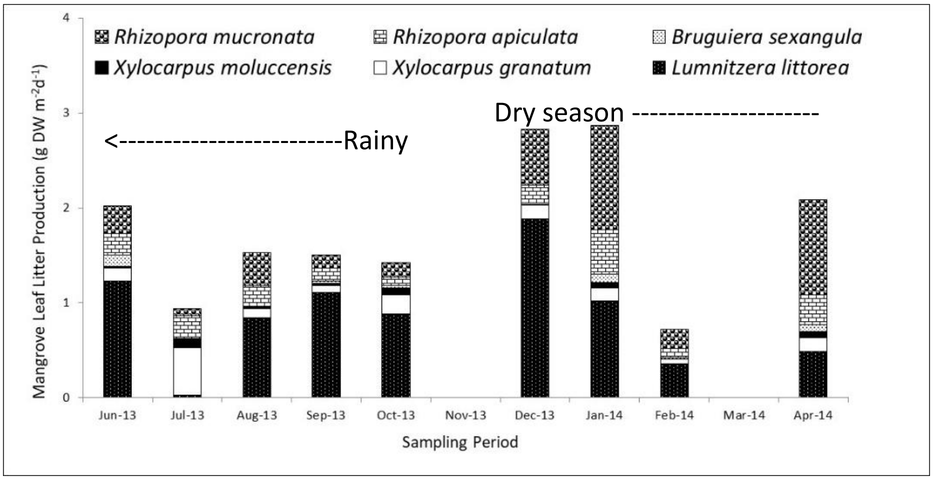

Figure 4. The seasonal variability in mangrove leaf litter production in the Iwahig River estuary, Puerto Princes Bay, Palawan, the Philippines.

Leaf Litter Production and Environmental Factors

None of the four measured physicochemical parameters, the temperature, rainfall, wind speed, and day-length, correlated well with the mean monthly mangrove leaf litter production (Figure 5). However, the highest leaf litterfall was recorded in December 2013 and January 2014 when the temperature and day length was relatively high, and the wind speed and rainfall were low (Figures 6 and 7).

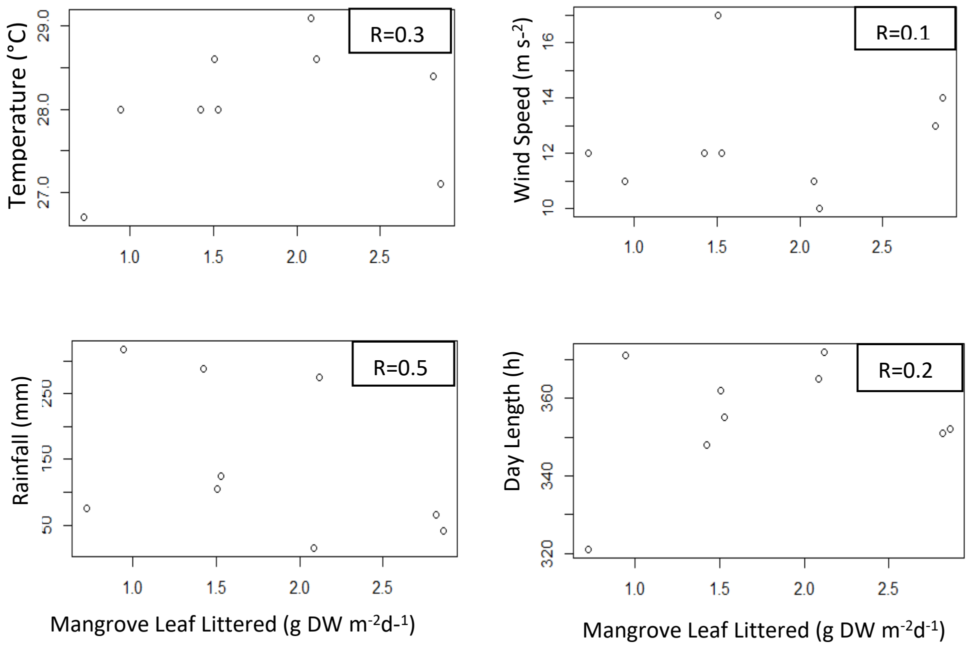

Figure 5. The scatter graphs between the mean monthly mangrove leaf litter production and the temperature, rainfall, wind speed, and day-length values from June 2013 to April 2014 in the Iwahig River estuary, Puerto Princesa Bay, Palawan, the Philippines.

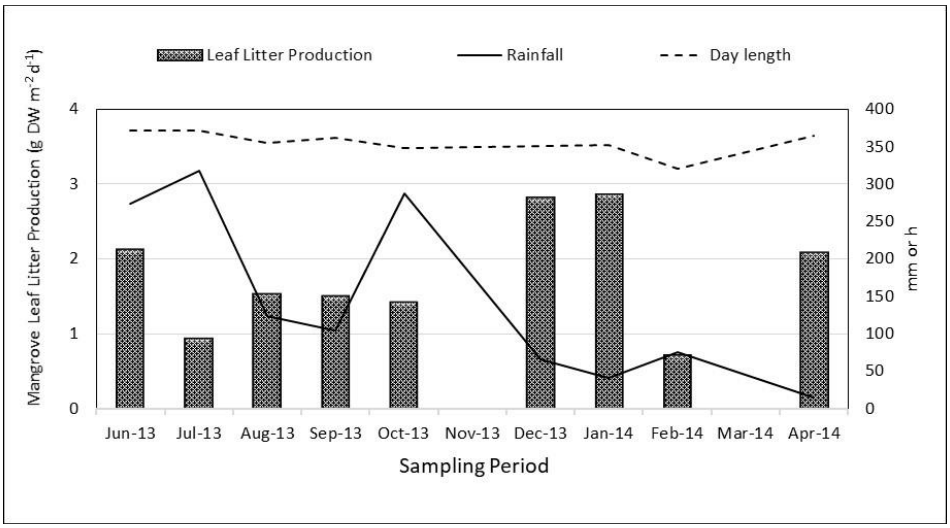

Figure 6. The profile of rainfall, and day length, from June 2013 to April 2014 in the Iwahig River estuary, Puerto Princesa Bay, Palawan, the Philippines.

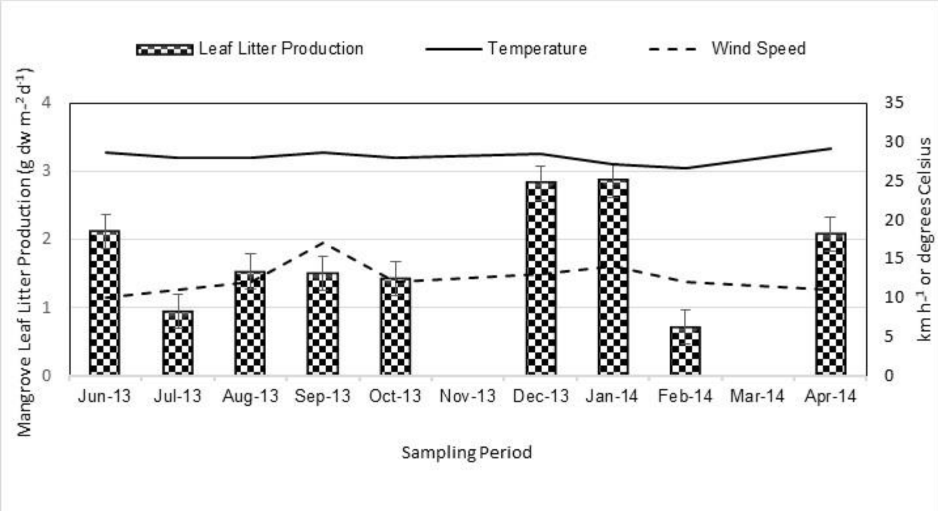

Figure 7. The profile of temperature and wind speed, from June 2013 to April 2014 in the Iwahig River estuary, Puerto Princesa Bay, Palawan, the Philippines.

DISCUSSION

Mangrove Leaf Litter Production

Mangrove leaf litter production in the Iwahig River estuary, Puerto Princesa, Palawan differs among species. The most dominant, *L. littorea* has the highest contribution, almost 50% of the total value from the five other prominent species, *R. mucronata, X. granatum*, *R. apiculata*, *B. sexangula*, and *X. moluccensis*. The entire litter production, 2.34 ± 0.42 g DW m^{-2} d^{-1}, contributed by these species is relatively low compared to natural mangrove forests in the central Philippines (Bais Bay, Negros Oriental; Bohol; and Cebu) with an average litter production of 6.04 ± 1.9; 7.06 ± 3.9; 7.43 ± 4.33; g DW m^{-2} d^{-1}, respectively (Rafael and Calumpong 2018). It is important to note that litter production from the central Philippines included mangrove-littered leaves and twigs, flowers, and fruits. On per species basis, leaf litter production of *R. apiculata*, 0.43 ± 0.38 g DW m^{-2} d^{-1} and *R. mucronata*, 0.22 ± 0.12 g DW m^{-2} d^{-1} in the study site were relatively high compared to data obtained by De Leon et al. (1992) from Bais Bay, Negros Oriental, with only 0.17 and 0.29 g DW m^{-2} d^{-1}, respectively. Species could influence the variations in mangrove litter production, sampling stations, stand density, and DBH (Rafael and Calumpong 2018), phenology (Nazim et al. 2013), and other physiological characteristics (Twilley et al. 1997). Nevertheless, the amount of mangrove leaf litter production in the Iwahig River estuary was almost five times higher than that of Tiris mangrove forest in West Java, Indonesia

(Sukardjo 2010). It is, therefore, a high value compared to the mangrove forest in the Indo-West Pacific and Malesian regions. This high leaf litter production contributes mainly to the overall productivity of the mangrove forest and river estuary system. When comprehensively studied, this can help explain the presence of diverse organisms in the area. Several studies had documented the positive relationships between productivity and biodiversity in forest ecosystems (Waide et al. 1999; Costanza et al. 2007; Liang et al. 2016; Brun et al. 2019).

Seasonal Variability

There was no seasonal trend observed on mangrove leaf litter production in the Iwahig River estuary ecosystem. An immense mangrove leaf shedding associated with seed maturation (Nazim et al. 2013), which generally transpired during the dry season in Asian countries, like Vietnam (Clough et al. 2000) and South Australia (Imgraben and Dittman 2008) and the rainy season in Brazil (Bernini and Rezende 2010) were not evident in the study site.

Leaf Litter Production and Environmental Factors

None of the tested Physicochemical parameters (temperature, rainfall, wind speed, and day-length) correlated well with the mean monthly mangrove leaf litter production. This finding conformed to the study of Bernini and Rezende (2010) in Brazil, which is also a tropical country where fluctuations of environmental parameters are minimal. Even in the warm-temperate region of Mgazana, South Africa, mangrove leaf litter production showed no seasonal trends (Emmerson and McGwynne 1992). A similar study in the central Philippines by Rafael and Calumpong (2018) showed no significant correlation between the average monthly leaf litter and rainfall. The factors that showed a positive correlation with mangrove leaf litter included solar radiation, pH, nutrients, tides, and salinity (Twilley 1995; Twilley and Day 1999). Accordingly, the low salinity of interstitial water favors nutrient enrichment (Bernini and Rezende 2010), enhancing mangrove productivity. Unfortunately, this study could not elucidate such an effect of salinity. Lumping of data sets obtained from the sampling stations was inevitable due to varying net-traps retrieved every collection period.

Therefore, conducting a year-round assessment of mangrove litter production, including the effects of several other variables such as the tides, nutrients, species density, and DBH, is essential. Considering that environmental factors may differ from year-to-year, a series of annual litterfall cycles could be done to understand further the inter-annual variability on mangrove leaf litter production in Iwahig River estuary, Palawan, the Philippines.

ACKNOWLEDGMENTS

This study was a component of the research project “Marine Biodiversity along the Bohol and Sulu Seas” with funding support from the Commission on Higher Education (CHED). We are thankful to all PSU-MSL staff, especially Darren Mag-Apan and Arvin N. Roco I, for their assistance during the sampling period and to the PSU administration for the full support in this endeavor. The comments and suggestions of the two anonymous reviewers helped improve this paper.

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ARTICLE INFO

Received: 31 March 2020
Revised: 10 March 2021
Accepted: 17 March 2021
Available online: 15 April 2021

Role of authors: FDG – conceptualized the study, acquired funding, gathered and analyzed the data, wrote the paper; RGD and ROS – gathered the data and co-wrote the paper.

Biological performance of African catfish *Clarias gariepinus* (Burchell, 1822) fingerlings fed with raw chicken entrails

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https://doi.org/10.69721/TPS.J.2021.13.2.02

ABSTRACT

The African catfish *Clarias gariepinus* (Burchell, 1822) grows fast, feeds on a large variety of agriculture by-products and can be raised in high densities resulting in high net yields. It is therefore considered as a good candidate for aquaculture. Generally, the increasing aquaculture activities worldwide led to a more expensive feed costs, thus looking for alternative and cheaper feeds coupled with optimum feeding rate is a focus in aquaculture research field. This study therefore aimed to determine the most efficient feeding rate and the potential of raw chicken entrails as feeds for *C. gariepinus* in terms of fish weight increment (WI), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR). *Clarias gariepinus* were fed with chopped raw chicken entrails at three different feeding rates (3%, 4% and 5%) based on the average weight for 60 days. The fish weight continually increased regardless of the feeding rate, but generally, those that were fed at 4% and 5% of the body weight showed significantly higher WG (g), SGR (% day^{-1}) and better FCR. Regardless of the feeding rate, all fish attained 100% SR. The feeding rate of 4% is recommended due to its similar WI with those fed at 5% but showed better FCR at the end of the culture.

Keywords: chicken wastes, freshwater fish, growth parameters

INTRODUCTION

The costs of commercial aquatic feeds are currently increasing due to the growing numbers of aquaculture activities. It is therefore crucial to efficiently use the feeds during the aquaculture operation not only for the growth of the culture but also for economic reasons since improper food supplication directly affects the production cost (Mihelakakis et al. 2007). Thus, it is essential to optimize the feeding rate in the culture of all commodities. This optimization becomes one of the critical areas in the aquaculture research field for the reduction of the excessive feeds (Dong-Fang et al. 2003).

Each species with its respective developmental stage has varying feeding rates that is also influenced by the culture system, fish size, and the nutrients in the diet (Cho et al. 2003; Mihelakakis et al. 2007). Overfeeding could result to waste of food and the interruption of the water quality (Ng et al. 2000). With appropriate feeding rates, fish farmers could reduce the production cost, maximize the culture growth and manage the water quality for a successful farming operation (Aderolu et al. 2010; Marimuthu et al. 2011).

The African catfish *Clarias gariepinus* (Burchell, 1822) is a large eel-like fish usually of dark gray or black coloration on the back, with white belly and is locally known as “hito” and “pantat” belonging to family Clariidae (FAO 2010). It is hardy with an accessory air-breathing organ (labyrinth organ) and it is found to be suitable for both small-scale and commercial aquaculture since it does not require extreme efforts and costs and has faster growth rate (Goos and Richter 1996; FAO 2010). Generally, this fish is an omnivore feeding on insects, plankton, snails and plant matter in the natural water bodies (Uys 1989).

Clarias gariepinus commands high price when sold in the market and its production greatly increases (FAO 2010). The demand for catfish in the Philippines continued to increase with production volume ranging between 3,729.29 metric tons in 2016 and 5,420.77 metric tons in 2020 (PSA 2021). It also contributed an annual value of PHP 5.77 million in 2020 (PSA 2021).

Clarias gariepinus are usually reared in ponds or different types of tanks at different stocking densities (FAO 2010). For the extensive culture of this fish, the larvae are fed with cow brain and egg yolk after 4-6 days prior to stocking in fenced nursery ponds. The post-larvae are then fed with single ingredient or compounded feeds. Fingerlings are graded and harvested after 24-48 days and are transferred to the production pond or being sold by the farmers. Different systems are currently being used in growing the catfish including the traditional flooded ponds, pits or ditches, earthen ponds, tanks, raceways and even in cages.

Large numbers of indigenous raw materials from poultry by-product meal, blood meal, oilcakes, cereal by-products, vegetables and leaf meals are used in developing feeds for the culture of various fish species (Akand et al. 1991; Bhadra et al. 1997; Manuel et al. 2020). Chicken entrails are poultry by-product with potential use as feed to African catfish. Chicken is one of the most commonly consumed meat worldwide, given that it is considered acceptable in most religions and cultures. The world’s poultry meat per capita consumption is reported to increase more than threefold and is projected to reach up to 45.3 kg capita^{-1} by 2030 (FAO 2003). In Western Visayas, Philippines, the number of dressed chickens in poultry plants reached up to 44.8 million metric ton (PSA 2019). This also equates to the amount of the chicken by-products every day from the slaughterhouses. Edible chicken by-products include heart, liver, spleen and kidney. These constitutes significant ratio of live chicken weight ranges from 5-6% (Ockerman and Basu 2004). Aside from the kidney and intestines which are being grilled and eaten by humans, other components of the chicken entrails could be used as feeds. Dried chicken intestines contain protein (59.58%), lipid (17.78%), ash (6.79%) and nitrogen free extract (15.85%) (Nahar et al. 2000). Other internal parts of the chicken like liver, cecum, crop, heart, duodenum and lungs contain significant amounts of protein, vitamins, fatty acids, amino acids and minerals (Seong et al. 2015).

This study therefore aimed to determine the most efficient feeding rate and the potential of raw chicken entrails as feeds for *C. gariepinus*. Specifically, it aimed to identify the optimum raw chicken entrails feeding rate to *C. gariepinus* in terms of weight increment (WI), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR).

METHODS

This study was conducted in a private earthen fish pond in Brgy. Lanot, Roxas City Capiz Philippines. The 6 x 4 x 1.5 m freshwater-filled outdoor ponds were installed with nine adjacent 1 x 1 x 2 m hapa nets. The edges of the nets were fixed at 0.5 m away from the sides of the pond and the bottoms were lifted to 0.5 m from the pond substrate. These hapa nets served as compartments for particular treatment replicates.

Feed Preparations

For the feed preparation, raw chicken entrails were taken from VECS Poultry Processing at Brgy. Mianay, Ivisan Capiz Philippines. These were washed, chopped into 2.00 ± 1.00 mm and stored in a freezer (-4 ± 4°C) until use. Three treatments at 3% (T1), 4% (T2) and 5% (T3) wet weight (w/w) feeding rates were prepared based on the weight of *C. gariepinus*.

Rearing and Monitoring of *C. gariepinus*

Ninety pieces African catfish fingerlings with an average (± SEM) total length and weight of 5.14 ± 0.16 cm and 50.67 ± 1.51 g respectively were obtained from the local private hatchery in Brgy. Lanot, Roxas City, Capiz. Conditioning was done in a pond for 45 days by feeding the fish with commercial fry mashed feeds containing 38% crude protein. After conditioning, *C. gariepinus* fingerlings were stocked in hapa nets within an earthen pond at a density of 10 fish 0.6 m^{-3} assigned through a complete randomized design (CRD) each with three replicates. The stocks were fed with the prepared and thawed for 1-2 h chicken entrails twice a day, every 0800 h and 1700 h based on the assigned feeding treatments (3%, 4% and 5% of the body weight) for 60 days. Samplings were done every after 15-day period. These were done by lifting each hapa net from the water and scooping the pooled fish with a plastic bucket. From the pooled stock in a bucket, each fish was transferred in a plastic container for individual weighing using a stainless meshed scoop.

Statistical Analysis

Average ± standard error of mean (SEM) of the WG, SGR, SR and FCR were computed using the software Statistical Package for Social Sciences (SPSS) version 20. The same software was used to compare the treatments. The growth and feed efficiency data were tested for homogeneity of variance using Levene's tests and then subjected to one-way analysis of variance (ANOVA) at 0.05 level of significance to determine the differences between the three treatments. Furthermore, post-hoc Tukey's tests were carried out to confirm where the differences between the groups occurred.

RESULTS

Weight Increment

The WI were steadily increasing with a slight reduction on the 45^{th} day, followed by a sharp trend towards the 60^{th} day. At day 15, those fed at 3% rate had lower WI at 0.97 ± 0.13 g compared to those that were fed at 4% and 5% with 1.90 ± 0.00 g and 1.57 ± 0.07 g, respectively. At day 30, however, those fed at 3% and 5% rate showed significantly higher WI than those fed at 4% rate. At day 45, all treatments showed significant differences in WI having those fed at 5% to be highest, followed by those fed at 4% and the lowest increment was observed to those fed at 3% rate. At the end of the culture period, 4% and 5% appeared to have the highest WI at 35.40 ± 0.45 g and 36.00 ± 0.29 g, respectively. The lowest WI was found in *C. gariepinus* fed at 3% rate at 30.40 ± 0.12 g (Figure 1).

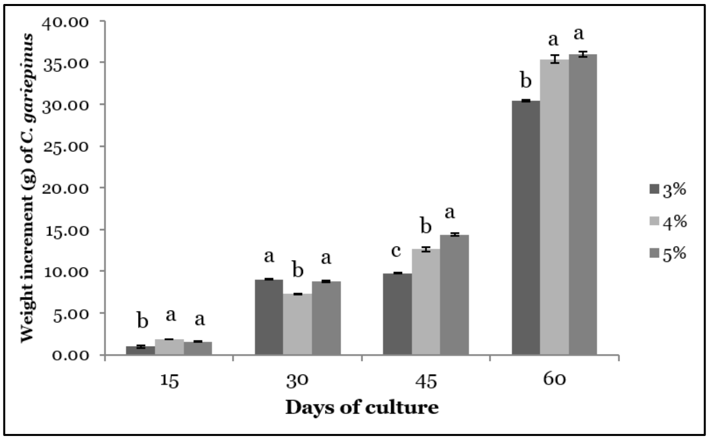

Figure 1. Weight increment of *Clarias gariepinus* fed raw chicken entrails at 3%, 4% and 5% feeding rate. Data presented as mean ± SEM ($P < 0.05$).

Specific Growth Rates

The SGR of *C. gariepinus* showed a consistent increase within the culture period. Highest SGR was found in fish with 5% feeding rate at day 45 and 60 of the culture reaching up to 3.21 ± 0.01% day^{-1} and 3.96 ± 0.01% day^{-1}, respectively. This is followed by those at 4% feeding rate with SGR of 3.10 ± 0.01% day^{-1} and 3.90 ± 0.01% day^{-1} at days 45 and 60 respectively. Those at 3% feeding rates appeared to have the lowest SGR at day 45 and 60 with 3.05 ± 0.00% day^{-1} and 3.81 ± 0.00% day^{-1}, respectively.

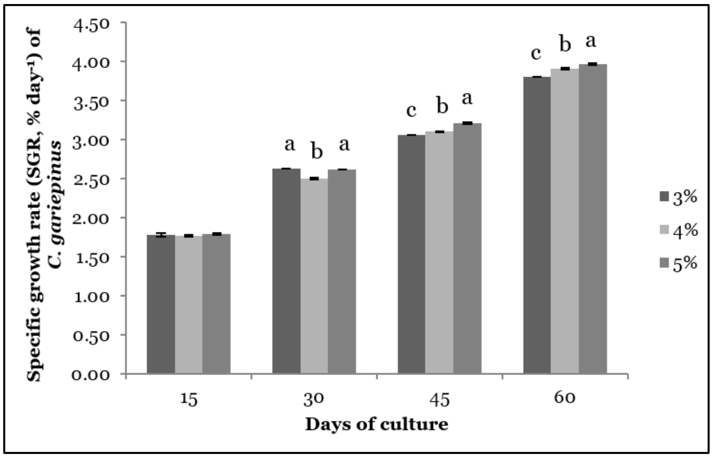

Figure 2. Specific growth rate (SGR, % day^{-1}) of *Clarias gariepinus* fed raw chicken entrails at 3%, 4% and 5% feeding rate. Data presented as mean ± SEM ($P < 0.05$).

Feed Conversion Ratio

The FCR was relatively higher on the first 15 days, requiring 1.54 to 3.23 kg of feeds to convert a kilogram of fish. However, the FCR improved in the succeeding sampling events, only requiring 0.78 to 1.17 kg of feed to produce a kilogram of fish. At the end of the culture period, T2 (4%) had significantly better FCR compared to the other treatments (Table 1).

No mortality was observed throughout the culture period resulting to 100% survival rates in all treatments.

Table 1. Average (± SEM) feed conversion ratio (FCR) of *Clarias gariepinus* fed with raw chicken entrails at 3%, 4% and 5% feeding rates. Values with the same superscripts in every feeding rate at the same culture period are not significantly different ($P < 0.05$).

Treatment (Feeding Rate %)	Days of Culture			
	15	30	45	60
T1 (3)	3.23 ± 0.47^{a}	0.78 ± 0.01^{b}	1.17 ± 0.00^{a}	0.83 ± 0.00^{a}
T2 (4)	1.54 ± 0.01^{b}	0.85 ± 0.01^{a}	0.94 ± 0.01^{b}	0.79 ± 0.00^{b}
T3 (5)	1.93 ± 0.08^{b}	0.79 ± 0.01^{b}	0.93 ± 0.01^{b}	0.82 ± 0.00^{a}

DISCUSSION

Weight Increment

The WI of the fish continued to increase from the start up to the end of the culture period (Figure 1). This was probably attributed by the sufficient protein content, vitamins and minerals in the fed chicken entrails (Nahar et al. 2000; Seong et al. 2015). Those at 3% FR, however, showed lower WI after 15 days and from day 45 up to the end of the culture. Furthermore, an unusual result at day 30 appeared showing that the WI of the fish fed at 4% feeding rate was lower compared to those fed at 3% and 5%. This incident is similar to the results of the study conducted by Tiamiyu et al. (2018) showing that *C. gariepinus* fed at 10% feeding rate obtained lower mean WI than those fed at 5% and 15% feeding rates. This was probably attributed to the fish behavioral, physiological and structural responses to utilize the energy reserves to compensate their metabolic requirements during starvation or feed restriction (Navarro and Gutierrez 1995.) At day 30, the fish fed at 4% feeding rate probably experienced decrease on the overall condition factor which, perhaps exhibited the changes in lipid content, blood metabolites, muscle and organ mass that have possibly resulted to a decreased growth. However, when feeding resumed until day 45, the fish probably exhibited faster growth rate known as compensatory or catch-up growth up to the end of the culture period (Ali et al. 2003). Also, 4% and 5% feeding rates have higher growth increment at the end of the culture period reaching up to 35.40 ± 0.45 g and 36.00 ± 0.29 g respectively. This confirmed that the fish weight has generally linear increase with the increasing feeding rate and the chicken entrails significantly affected the growth performance of the fish in general (Marimuthu et al. 2011). Given the duration of this study, the result of the WI is comparable to that of Nahar et al. (2000) where *C. gariepinus* attained 69.6 ± 0.17 g WI after 120 days of culture. This is also similar to those conducted with juvenile bagrid catfish (*Mystus nemurus*), Europen sea bass (*Dicentrarchus labrax*), Channel catfish (*Ictalurus punctatus*) and Pacu (*Piacartus mesopotamimus*) wherein higher WI were exhibited when given more amounts of feeds than those fed with lesser amounts (Borghetti and Canzi 1993; Ng et al. 2000; Eroldogan et al. 2004; Robinson and Li 2007). The result of the study conducted by Nahar et al. (2000) on *C. gariepinus* fed with chopped raw chicken intestine showed increased mean WI for the first two months only and there was a gradual decline thereafter. This study on the other hand, showed continuous increase in mean WI from the start up to the 60-day culture. However, this study showed lower mean WI (36.00 ± 0.29 g) compared to the study of Nahar et al. (2000) that reached a maximum of 53 g.

Specific Growth Rate

The SGR recorded in this study directly reflected the results of the WI. Those at 4% and 5% feeding rate showed higher SGR at days 45 and 60, while those at 3% feeding rate had lower SGR (Figure 2). The result of this study on 5% feeding rate (3.90 ± 0.01% day^{-1}) is relatively similar to those conducted by Marimuthu et al. (2011) at 3.39 ± 0.17% day^{-1} but higher compared to the study of Tiamiyu et al. (2018) with an SGR of 2.28 ± 0.19% day^{-1} with both studies also at 5% feeding rates. It further showed that the higher the amounts of feeds given to the fish, the better the SGR. This is further supported by the result of the study conducted by Sun et al. (2006) showing that cobia (*Rachycentron canadum*) fed 7% of the body weight had higher SGR than those fed 3% body weight per day.

Feed Conversion Ratio

The ability of the *C. gariepinus* to convert the feeds into flesh in this study was measured through its FCR. The observed improvement in the FCR after 15 days of culture (Table 1) could have been due to various factors, but one major reason could be the adjustment of the diet from commercial fry mashed during the conditioning to chicken entrails. Generally, it appeared that *C. gariepinus* fed at 4% and 5% feeding rates had better FCR ranging from 0.79 ± 0.00 to 1.93 ± 0.08 compared to those fed at 3% of the body weight, suggesting that this species could efficiently convert the raw chicken entrails into fish flesh.

Usually, the FCRs of the cultured fishes were above 1.0, but some lower values were obtained from this study. According to USAID (2011), it is possible to have an FCR lower than 1.0 since the fish contains water in its flesh while the feeds used were dry and contain minimal amount of water. When the fish, therefore, converted the dry feeds into moist flesh using a highly efficient diet, it could produce heavier moist flesh than the weight of the dry feeds used. Another possible reason for this was the high density of natural food present in the culture system that could have been consumed by the fish. A study conducted by Hender et al. (2021) on *Lates calcarifer* (Bloch, 1790) fed diets containing Black soldier fly also resulted to FCRs between 0.73 ± 0.02 and 0.78 ± 0.02. Another study conducted by Nhu et al. (2015) also resulted to Snakeskin gourami FCRs between 0.4 and 0.6 when fed with pig manure or its digestate with carbohydrate-rich pelleted feeds.

The results of the FCRs in this study were comparable to those conducted by Marimuthu et al. (2011) on *C. gariepinus* fed commercial feeds (38% protein) at 8% feeding rate which resulted to an FCR of 1.00 ± 0.09. Tiamiyu et al. (2018) reported a lower FCR of 2.69 ± 0.16 for *C. gariepinus* fed with commercial diets at 5% FR. In addition, Anderson and Fast (1991) described that if the feed ration was greater than the optimum feed level, this could result to the increased food waste and FCR. In this study, the maximum feeding rate used was 5% but the result of the FCR of *C. gariepinus* fed at this rate is comparable to those fed at 4% body weight and in general did not result to higher FCR. These further showed that raw chicken entrails were efficient and suitable diet since only few amounts were required to produce a unit WI (Nahar et al. 2000). However, the total amount of feeds given to the fish was also not expected to be totally converted into flesh since these organisms were not completely efficient feed converters (Tiamiyu et al. 2018). This is because some of the energy from the feed is used by the fish first for metabolic activities including digestion, respiration, nerve impulses, salt balance, swimming and other life activities (Craig and Helfrich 2009). In this study, however, only few amounts were necessary for the production of fish flesh. This study therefore, recommends the use of raw chicken entrails for *C. garieipinus* at 4% feeding rate since it has similar WI with those fed at 5% of the body weight but showed lower FCR at the end of the culture. This could limit the rations of feed while still achieving acceptable long-term growth trajectories leading to a minimized feeding cost.

Feeding *C. gariepinus* with raw chicken entrails did not only result to increased growth rate and lower FCR but also showed 100% SR. This result was higher compared to the studies of Nahar et al. (2000) and Tiamiyu et al. (2018) which achieved a SR of 95.0 ± 5.00 % and 83-96% SR respectively. This is an indication that raw chicken entrails fed to the fish did not contain harmful elements that could cause culture mortality. Furthermore, though the water parameters were not monitored in this study, 100% SR was an indication that the cultures were not overfed that could alter the optimum condition which might also lead to mortality. Nonetheless, it is still recommended to monitor the water parameters such as temperature, pH, and ammonia levels of the culture system to confirm the effects of the chicken entrails to the water quality. It is also recommended to include proximate analyses for this raw feed.

Lastly, it is important to note that the related studies mentioned were conducted in concrete or earthen ponds but none in hapa nets. Using this new protocol, however, resulted to comparable biological performance of the African catfish in general. Also, using the hapa nets in this study provided ease on culture management in terms of feed distribution and monitoring and catfish sampling and harvesting. For the feed distribution, using hapa nets have eliminated the probability of the feeds to have a direct contact with the soil or concrete substrates which could increase its contamination with the organic matter. The volume of uneaten feeds could easily be monitored by lifting the net. For the sampling and harvesting, the stocks could be pooled, scooped or transferred easily to a container or weighing scale by also lifting the hapa nets.

ACKNOWLEDGMENTS

The author is grateful to Mr. John Alex B. Arguelles, Mr. Clefford B. Valderas, former Assistant Professor Victor A. Billanes, Dr. Diony G. Cahilig, Dr. Joselito C. Sitjar, Dr. Fatima P. Sustento and Dr. Vivian A. Alejaga for the inputs in this study. Gratitude is also extended to the reviewers and editors for their assistance in improving this manuscript.

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ARTICLE INFO

Received: 30 March 2020
Revised: 27 January 2021
Accepted: 25 June 2021
Available online: 29 July 2021

Opercular girth, maximum girth and total length relationships for eight fish species from the Saros Bay (northern Aegean Sea, Turkey)

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https://doi.org/10.69721/TPS.J.2021.13.2.03

ABSTRACT

This study was carried out to uncover the relationship between opercular girth (G_{ope}), maximum girth (G_{max}) and total length (TL) for eight fish species [(*Sardina pilchardus* (Walbaum, 1792), *Sardinella aurita* (Valenciennes, 1847), *Serranus cabrilla* (Linnaeus, 1758), *Serranus scriba* (Linnaeus, 1758), *Symphodus mediterraneus* (Linnaeus, 1758), *Symphodus tinca* (Linnaeus, 1758), *Trachurus mediterraneus* (Steindachner, 1868), and *Trachurus trachurus* (Linnaeus, 1758)] from four families (Carangidae, Clupeidae, Labridae, and Serranidae) sampled between August 2016 and December 2017 in Saros Bay (Northern Aegean Sea, Turkey). G_{ope} and G_{max} were found to increase linearly with the total length for all the species. All r^2 values were statistically significant ($r^2 > 0.83$, $P < 0.001$). The values about length-girth relationships (LGRs) of *S. mediterraneus* were estimated for the first time worldwide. This information will contribute to the development of ecosystem-based fisheries management.

Keywords: biometric relationship, fisheries management, fish morphlogy

INTRODUCTION

Fisheries management often requires the use of biometric parameters in order to transform data collected in the field into appropriate indices (Ecoutin and Albaret 2003). Biometric parameters are of utmost importance not only to fill up the gap of our present-day academic knowledge but also to increasing the technological efficiencies of the fishery entrepreneurs for evolving judicious pisciculture management (Swain and Foote 1999). Fish morphology is inseparably related to the study of the mode of life. The analysis of size and shape variations becomes fundamental to highlights variability in living organisms (Turan et al. 2004). In this connection, the morphometric measurements have been used to identify fish stocks and remain the simplest and most direct way among methods of species identification (Turan et al.

2004). In addition, the studies on differences and variability in morphometric relationships of fish stocks are significant in phylogenetics as they provide information for subsequent studies onthe genetic improvement of stocks (Umaru et al. 2015). The morphometric relationships between length and girth could be quite useful (Mendes et al. 2006). Length-girth relationships (LGRs) are an important component for (a) biological (e.g. condition and swimming capability) (Wootton 1998); (b) ecological (e.g. predator-prey relationships, trophic level estimation) (Stergiou and Karpouzi 2003); and (c) fisheries assessments (e.g. quantifying the catching efficiency of fishing gear) (Kyritsi et al. 2018). Moreover, the species-specific LGRs allow the computation of girth from length measurements, the latter of which is easier and less expensive to be obtained (Moutopoulos et al. 2017).

The published data on LGRs for fish species are scarce (Mendes et al. 2006) and studies on LGRs of fish species in the Aegean Sea have only been carried out in southern (Stergiou and Karpouzi 2003; Aydın and Düzgüneş 2007) and central (Beğburs et al. 2020) regions, up to the present. In this context, the present study provided preliminary information on LGRs of eight fish species for the Northern Aegean Sea. The following species were studied: *Sardina pilchardus* (Walbaum, 1792), *Sardinella aurita* (Valenciennes, 1847), *Serranus cabrilla* (Linnaeus, 1758), *Serranus scriba* (Linnaeus, 1758), *Symphodus mediterraneus* (Linnaeus, 1758), *Symphodus tinca* (Linnaeus, 1758), *Trachurus mediterraneus* (Steindachner, 1868), and *Trachurus trachurus* (Linnaeus, 1758). At present, there are no available data on LGRs estimates for *S. mediterraneus* worldwide. Hence, the reported results will be useful for developing ecosystem-based fisheries management.

METHODS

The northern Aegean areas are characterized by an extended continental shelf, smooth muddy/sandy bottoms and higher nutrient concentrations (Maravelias and Papaconstantinou 2006). The area is known for having higher phytoplankton and zooplankton abundance compared with the southern Aegean Sea (Theocharis et al. 1999). The northern Aegean coasts of Turkey are divided into sub-regions as the Saros Bay, the Gallipoli Peninsula, the Gökçeada and Bozcaada Islands and the Edremit Bay (Cengiz and Paruğ 2020) (Figure 1). The length of Saros Bay is about 61 km and the width at the opening to the Aegean Sea is about 36 km (Eronat and Sayın 2014). As the bay had been closed to bottom trawl fishing since 2000 (Cengiz et al. 2014) and no industrial activity was prevalent in the area (Sarı and Çağatay 2001), it can be considered as a pristine environment (Cengiz et al. 2015). Therefore, Saros Bay and its coastal area were declared as Special Environmental Protection Area (SEPA) due to its landscape, geomorphological, ecological, floristic biogenetic and touristic properties (Güçlüsoy 2015).

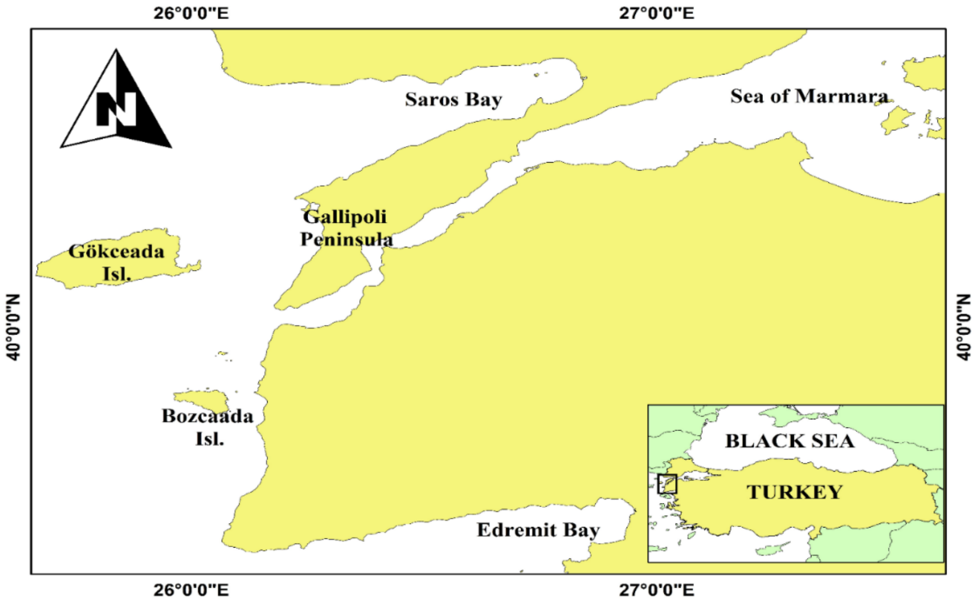

Figure 1. Saros Bay and the northern Aegean coasts of Turkey.

Samples were obtained, monthly, between August 2016 and December 2017 in stratified random sampling, from catches of commercial fisheries around Saros Bay. Most of these were stationary set and drive in fishing methods with gillnets which have 20, 22, 23, 25 mm mesh sizes, net height of 105 meshes and 210d/3 twine thickness. The total length and the body girth measurements were taken to the nearest centimeter using a tape measure: (1) behind the gill-cover (G_{ope}) and (2) in front of the first dorsal fin (G_{max}). Relationships between fish total length (TL) and opercular or maximum girth (y) were estimated by linear regression analysis: y = *a*+*b*TL, where the intersect (a) and slope (b) were found by least-squares estimation (Neter et al. 1988). The correlation coefficient (r^2) was used to evaluate the strength of this linear relationship.

RESULTS

Overall, 786 individuals were sampled, belonging to the eight fish species (*Sardina pilchardus, Sardinella aurita, Serranus cabrilla, Serranus scriba, Symphodus mediterraneus, Symphodus tinca, Trachurus mediterraneus,* and *Trachurus trachurus*) from four families (Carangidae, Clupeidae, Labridae, and Serranidae). The relationships of G_{ope} and G_{max} with TL for the eight fish species are summarised in Table 1 and Table 2, respectively. For all species, both opercular and maximum girth were generally linearly related to body length. For the relationship between opercular girth and the total length, the slope (*b*) is lowest for *Trachurus mediterraneus* and highest for *Symphodus tinca* representing a greater increase in girth with the length for the latter species. The correlation coefficient (r^2) shows a range of 0.85-0.95, with the lowest value obtained for *Sardina pilchardus* and the highest for *Sardinella aurita* and *Serranus cabrilla*. For the relationship between maximum girth and total length, the slope (*b*), is lowest for *Trachurus trachurus* and highest for *Serranus scriba*, representing a greater increase in girth with the length for the latter species. The correlation coefficient (r^2) indicates a range of 0.83-0.95 with the lowest value obtained for *Sardina pilchardus* and the highest value for *Serranus cabrilla*. All linear regressions were statistically significant (P < 0.001). These relationships pointed out that opercular and maximum girth increased faster with the length for all species.

DISCUSSION

The length-girth relationships for the eight fish species have been estimated for the first time in the Northern Aegean Sea. In addition, no LGRs estimates existed for *S. mediterraneus* worldwide with the exception of the present study. Both the opercular girth-total length and maximum girth-total length relationships reported herein were found to be linear. Due to the size-selective characteristics of the fishing gear used (e.g. Mendes et al. 2006; Santos et al. 2006; Jawad et al. 2009), the samples were based on a restrictive range of lengths. Small-sized individuals and immature stages of these species were absent from the samples. Thus, the use of these relationships should be strictly limited to the size ranges used in the estimation of the linear regression parameters. Table 3 and Table 4 reveal the findings of the opercular girth-total length and maximum girth-total length relationships of these fish species between the present study and other studies, respectively. The differences in LGRs may be attributed to biological (e.g. sex, food availability) and/or abiotic (e.g. water temperature) factors (Wootton 1998) together with different variations in the size range. Concordantly, the spawning/reproduction frequency and gonad activity/development could give rise to cause seasonal variations in the LGRs (Santos et al. 2006; Kyritsi et al. 2018). The possible reasons for differences in the results involved between other studies and the present study may be related to one or more factors given above. Fish body girth could be estimated by way of three procedures: (1) body perimeter around the eye (G_{eye}), (2) behind the gill-cover (G_{ope}) and (3) in front of the

Table 1. Relationship between opercular girth and total length for eight fish species from Northern Aegean Sea (Turkey). *N*: sample size, min: minimum, max: maximum, *TL*: total length (cm), G_{ope}: opercular girth (cm), r^2 = the coefficient of determination, *SE* = standard error, *a* = intersect, *b* = slope.

Family/Species	N	Mean TL ± SE (min-max)	Mean G_{ope} ± SE (min-max)	Lenght - Opercular Girth equation	r^2	SE of *a*	SE of *b*
Carangidae							
Trachurus mediterraneus	296	15.1 ± 0.14 (13.0 – 27.1)	6.6 ± 0.07 (5.4 – 12.6)	G_{ope} = -0.3053 + 0.462TL	0.93	0.2040	0.0302
Trachurus trachurus	64	14.5 ± 0.17 (13.0 – 19.0)	6.8 ± 0.09 (5.8 – 8.6)	G_{ope} = -0.0372 + 0.4674TL	0.88	0.6041	0.0890
Clupeidae							
Sardina pilchardus	146	13.0 ± 0.08 (11.0 - 16,1)	4.8 ± 0.05 (3.5 – 6.5)	G_{ope} = -2.2056 + 0.539TL	0.85	0.2676	0.0556
Sardinella aurita	126	20.2 ± 0.52 (16.4 – 24.6)	7.9 + 0.29 (6.1 – 10.4)	G_{ope} = -3.1133 + 0.5455TL	0.95	0.6610	0.0824
Labridae							
Symphodus mediterraneus	31	12.1 ± 0.26 (9.9 – 13.8)	7.0 ± 0.16 (5.8 – 8.3)	G_{ope} = 0.0928 + 0.5696TL	0.85	1.0191	0.1454
Symphodus tinca	32	13.3 ± 0.58 (10.9 – 17.1)	8.1 ± 0.48 (5.8 – 10.6)	G_{ope} = -2.4288 + 0.787TL	0.94	0.8113	0.0988
Serranidae							
Serranus cabrilla	49	14.6 ± 0.51 (11.9 – 23.5)	7.8 ± 0.32 (6.2 – 13.5)	G_{ope} = -1.245 + 0.6177TL	0.95	0.5296	0.0665
Serranus scriba	42	15.7 ± 0.42 (12.1 – 22.6)	9.2 ± 0.27 (7.1 – 14.1)	G_{ope} = -0.6448 + 0.625TL	0.92	0.7305	0.0784

Table 2. Relationship between maximum girth and total length for eight fish species from Northern Aegean Sea (Turkey). *N*: sample size, min: minimum, max: maximum, *TL*: total length (cm), G_{max}: maximum girth (cm), r^2 = the coefficient of determination, *SE* = standard error, *a* = intersect, *b* = slope.

Family/Species	N	Mean TL ± SE (min-max)	Mean G_{max} ± SE (min-max)	Lenght - Maximum Girth equation	r^2	SE of *a*	SE of *b*
Carangidae							
Trachurus mediterraneus	296	15.1 ± 0.14 (13.0 – 27.1)	7.3 ± 0.08 (5.7 – 13.5)	G_{max} = -0.701 + 0.5312TL	0.92	0.2110	0.0284
Trachurus trachurus	64	14.5 ± 0.17 (13.0 – 19.0)	7.3 ± 0.09 (6.4 – 9.2)	G_{max} = 0.0008 + 0.5025TL	0.90	0.5537	0.0755
Clupeidae							
Sardina pilchardus	146	13.0 ± 0.08 (11.0 – 16.1)	5.9 ± 0.05 (4.7 – 7.6)	G_{max} = -1.2212 + 0.5492TL	0.83	0.3722	0.0628
Sardinella aurita	126	20.2 ± 0.52 (16.4 – 24.6)	9.3 ± 0.38 (7.1 – 12.9)	G_{max} = -4.8748 + 0.7036TL	0.90	0.8118	0.0853
Labridae							
Symphodus mediterraneus	31	12.1 ± 0.26 (9.9 – 13.8)	8.4 ± 0.16 (7.6 – 9.6)	G_{max} = 1.3316 + 0.5849TL	0.86	1.1558	0.1371
Symphodus tinca	32	13.3 ± 0.58 (10.9 – 17.1)	9.6 ± 0.38 (7.7 – 11.9)	G_{max} = 1.3559 + 0.6185TL	0.89	1.5457	0.1596
Serranidae							
Serranus cabrilla	49	14.6 ± 0.51 (11.9 – 23.5)	8.7 ± 0.38 (7.0 – 16.3)	G_{max} = -1.8132 + 0.7222TL	0.95	0.5460	0.0609
Serranus scriba	42	15.7 ± 0.42 (12.1 – 22.6)	10.4 ± 0.33 (8.0 – 16.8)	G_{max} = -1.6634 + 0.764TL	0.92	0.6740	0.0640

first dorsal fin (G_{max}). In this study, there was a linear relationship between the increasing opercular girth (G_{ope}), maximum girth (G_{max}) and total length (TL), which is in agreement with the results of Stergiou and Karpouzi (2003), Mendes et al. (2006), Jawad et al. (2009, 2015), Daliri et al. (2012), and Moutopoulos et al. (2017).

In general, fish retention by fishing gear is primarily related to girth rather than to length (Jawad et al. 2009). The different girth types calculate the probability of different ways of capture by fishing gear, assessed by G_{eye} when fish are tangled (i.e. held in the gear by teeth, maxillaries, or other projections), by G_{ope} when fish are gilled (i.e. being prevented from backing out of the gear by a mesh caught behind the gill-cover), and by G_{max} when fish are wedged (i.e. being held tightly by a mesh around the body) (Reis and Pawson 1999). Concerning this, the maximum girth is generally used to describe cod-end mesh retention, whereas in gillnets, opercular and maximum girths are the two parameters related to the methods of the capture of gilled and wedged fish, respectively (McCombie and Berst 1969). Baranov (1948) has made the first study that the fish morphology strongly influences the retention by fishing gear. The probability of a fish being retained by a given mesh is primarily determined by the relationship between the body shape and the mesh opening. Thereby, the girth has been considered as a significant parameter in understanding the gear selection process (Reis and Pawson 1999). In addition to this information, the data on girth have been used to define selection patterns during indirect selectivity experiments with gillnets (Kurkilahti et al. 2002). Similarly, length-girth relationships have been one of the critical parameters in cod-end selectivity studies to understand the selection pattern of species that differ in behavioural and morphological characteristics (Tokai and Omoto 1994). As stated above, the gillnet selectivity could be calculated by using the girth than length. This approach has been uncovered so as to predict selectivity curves of gillnet by Sechin (1969) and Kawamura (1972). Both selectivity models estimates the probabilities of fish retention as a function of morphometric features of the body between the operculum (G_{ope}) and the maximum girth of the fish (G_{max}).

In conclusion, the given length-girth values in the present study and previous ones could be used in the experimental design for selectivity surveys, particularly for gillnets where decisions on the suitable mesh size ranges need to be taken. In addition, these values are of great significance, owing to the fact that they determine fish growth patterns, which in turn are necessary for the development of ecosystem-based fisheries management. Because, the ecosystem-based fisheries management is a holistic method of managing fisheries and entire marine resources by keeping in view all ecosystem of the species being managed. Herewith, these data will help fisheries management authorities worldwide.

Table 3. Comparison of opercular girth-total length relationships of eight fish species between the present study and other studies. *N*: sample size, min: minimum, max: maximum, *TL*: total length (cm), *FL*: fork length (cm), G_{ope}: opercular girth (cm), r^2 = the coefficient of determination, *SE* = standard error, *a* = intersect, *b* =slope, * *FL*: fork length. [1] first *TL*-G_{ope} relationship reference for Northern Aegean Sea. [2] first *TL*-G_{ope} relationship reference for the species worldwide.

Fish species	References	Location	N	TL_{min} - TL_{max}	Length - Opercular Girth equation	r^2	SE of *a*	SE of *b*
Serranus cabrilla[1]	Mendes et al. (2006)	from Póvoa do Varzim to Santo Andre (western Portugues coast)	49	16.3 – 25.5	G_{ope} = -2.126 + 0.653TL	0.89	-	0.0320
	Santos et al. (2006)	Algarve coast (southern Portugal)	68	18.4 – 24.8	G_{ope} = -1.3469 + 0.5664TL	0.95	0.3411	0.0164
	Aydın & Düzgüneş (2007)	Bodrum Peninsula (southern Aegean Sea, Turkey)	263	13.4 – 19.8	G_{ope} = 1.2393 + 0.3982TL	0.83	-	-
	This study	Saros Bay (northern Agean Sea, Turkey)	49	11.9 – 23.5	G_{ope} = -1.245 + 0.6177TL	0.95	0.5296	0.0665
Serranus scriba[1]	Stergiou & Karpouzi (2003)	Cyclades (southern Aegean Sea, Greece)	105	13.0 – 24.1	G_{ope} = -0.945 + 0.608TL	0.71	-	0.0380
	This study	Saros Bay (northern Agean Sea, Turkey)	42	12.1 – 22.6	G_{ope} = -0.6448 + 0.625TL	0.92	0.7305	0.0784
Symphodus mediterraneus[2]	This study	Saros Bay (northern Agean Sea, Turkey)	31	9.9 – 13.8	G_{ope} = 0.0928 + 0.5696TL	0.85	1.0191	0.1454
Symphodus tinca[1]	Stergiou & Karpouzi (2003)	Cyclades (southern Aegean Sea, Greece)	231	13.2 – 25.0	G_{ope} = -2.557 + 0.708TL	0.83	-	0.0210
	This study	Saros Bay (northern Agean Sea, Turkey)	32	10.9 – 17.1	G_{ope} = -2.4288 + 0.787TL	0.94	0.8113	0.0988
Sardina pilchardus[1]	Mendes et al. (2006)	from Póvoa do Varzim to Santo Andre (western Portugues coast)	60	17.2 – 23.7	G_{ope} = -2.351 + 0.505TL	0.82	-	0.0310
	Beğburs et al. (2020)	Izmir Bay (central Agean Sea, Turkey)	81	10.5 – 17.0	G_{ope} = -0.495 + 0.411TL	0.83	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	146	11.0 – 16.1	G_{ope} = -2.2056 + 0.539TL	0.85	0.2676	0.0556
Sardinella aurita[1]	Aydın & Düzgüneş (2007)*	Bodrum Peninsula (southern Aegean Sea, Turkey)	253	15.4 – 24.0	G_{ope} = -1.5595 + 0.508FL	0.90	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	126	16.4 – 24.6	G_{ope} = -3.1133 + 0.5455TL	0.95	0.6610	0.0824
Trachurus mediterraneus[1]	Stergiou & Karpouzi (2003)	Cyclades (southern Aegean Sea, Greece)	234	18.3 – 33.7	G_{ope} = -1.743 + 0.487TL	0.73	-	0.0190
	Beğburs et al. (2020)	Izmir Bay (central Agean Sea, Turkey)	75	27.0 – 33.5	G_{ope} = -4.462 + 0.622TL	0.97	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	296	13.0 – 27.1	G_{ope} = -0.3053 + 0.462TL	0.93	0.2040	0.0302
Trachurus trachurus[1]	Mendes et al. (2006)	from Póvoa do Varzim to Santo Andre (western Portugues coast)	233	15.8 – 39.8	G_{ope} = 1.321 + 0.400TL	0.93	-	0.0070
	Santos et al. (2006)	Algarve coast (southern Portugal)	596	12.9 – 44.2	G_{ope} = 0.1611 + 0.4322TL	0.97	0.0727	0.0029
	Beğburs et al. (2020)	Izmir Bay (central Agean Sea, Turkey)	134	13.5 – 30.8	G_{ope} = -0.784 + 0.475TL	0.97	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	64	13.0 – 19.0	G_{ope} = -0,0372 + 0,4674TL	0.88	0.6041	0.0890

Table 4. Comparison of maximum girth-total length relationships of eight fish species between the present study and other studies. *N*: sample size, min: minimum, max: maximum, *TL*: total length (cm), *FL*: fork length (cm), G_{max}: maximum girth (cm), r^2 = the coefficient of determination, *SE* = standard error, *a* = intersect, *b* = slope, * *FL*: fork length. [1] first *TL*-G_{max} relationship reference for Northern Aegean Sea. [2] first *TL*-G_{max} relationship reference for the species worldwide.

Fish species	References	Location	N	TL_{min} - TL_{max}	Length – Maximum Girth equation	r^2	SE of *a*	SE of *b*
Serranus cabrilla[1]	Mendes et al. (2006)	from Póvoa do Varzim to Santo Andre (western Portugues coast)	46	16.3 – 25.6	G_{max} = -0.902 + 0,627TL	0.89	-	0.0320
	Santos et al. (2006)	Algarve coast (southern Portugal)	68	18.4 – 24.8	G_{max} = -3.0089 + 0.7467TL	0.94	0.4688	0.0225
	Aydın & Düzgüneş (2007)	Bodrum Peninsula (southern Aegean Sea, Turkey)	263	13.4 – 19.8	G_{max} = 0.2834 + 0.5473TL	0.84	-	-
	This study	Saros Bay (northern Agean Sea, Turkey)	49	11.9 – 23.5	G_{max} = -1.8132 + 0.7222TL	0.95	0.5460	0.0609
Serranus scriba[1]	Stergiou & Karpouzi (2003)	Cyclades (southern Aegean Sea, Greece)	74	14.3 – 23.9	log(G_{max}) = −0.298 + 1.075 log(TL)	0,78	-	0,0680
	This study	Saros Bay (northern Agean Sea, Turkey)	42	12.1 – 22.6	G_{max} = -1.6634 + 0.764TL	0.92	0.6740	0.0640
Symphodus mediterraneus[2]	This study	Saros Bay (northern Agean Sea, Turkey)	31	9.9 – 13.8	G_{max} = 1.3316 + 0.5849TL	0.86	1.1558	0.1371
Symphodus tinca[1]	Stergiou & Karpouzi (2003)	Cyclades (southern Aegean Sea, Greece)	169	13.2 – 25.0	G_{max} = -2.486 + 0.744TL	0.83	-	0.0270
	This study	Saros Bay (northern Agean Sea, Turkey)	32	10.9 – 17.1	G_{max} = 1.3559 + 0.6185TL	0,89	1,5457	0,1596
Sardina pilchardus[1]	Mendes et al. (2006)	from Póvoa do Varzim to Santo Andre (western Portugues coast)	60	17.2 – 23.7	G_{max} = -1.508 + 0.531TL	0.81	-	0.0330
	Beğburs et al. (2020)	Izmir Bay (central Agean Sea, Turkey)	81	10.5 – 17.0	G_{max} = -1.688 + 0.557TL	0.72	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	146	11.0 – 16.1	G_{max} = -1.2212 + 0.5492TL	0.83	0.3722	0.0628
Sardinella aurita[1]	Aydın & Düzgüneş (2007)*	Bodrum Peninsula (southern Aegean Sea, Turkey)	253	15.4 – 24.0	G_{max} = -5.973 + 0.822FL	0.94	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	126	16.4 – 24.6	G_{max} = -4.8748 + 0.7036TL	0.90	0.8118	0.0853
Trachurus mediterraneus[1]	Stergiou & Karpouzi (2003)	Cyclades (southern Aegean Sea, Greece)	192	18.3 – 33.7	G_{max} = -3.265 + 0.580TL	0.69	-	0.0280
	Beğburs et al. (2020)	Izmir Bay (central Agean Sea, Turkey)	75	27.0 – 33.5	G_{max} = -7.793 + 0.763TL	0.96	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	296	13.0 – 27.1	G_{max} = -0.701 + 0.5312TL	0.92	0.2110	0.0284
Trachurus trachurus[1]	Mendes et al. (2006)	from Póvoa do Varzim to Santo Andre (western Portugues coast)	230	15.8 – 39.8	G_{max} = 1.916 + 0.402TL	0.92	-	0.0070
	Santos et al. (2006)	Algarve coast (southern Portugal)	598	12.9 – 44.2	G_{max} = 0.0441 + 0.4972TL	0.97	0.0889	0.0036
	Beğburs et al. (2020)	Izmir Bay (central Agean Sea, Turkey)	134	13.5 – 30.8	Gmax = -0.797 + 0.497TL	0.97	-	-
	This study	Saros Bay (northern Aegean Sea, Turkey)	64	13.0 – 19.0	G_{max} = 0.0008 + 0.5025TL	0.90	0.5537	0.0755

ACKNOWLEDGMENTS

The author would like to thank the commercial fishermen and the two anonymous reviewers for their assistance.

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ARTICLE INFO

Received: 19 April 2021
Revised: 29 July 2021
Accepted: 04 August 2021
Available online: 24 August 2021

Measuring the historical conservation status of dragonet fishes in Tosa Bay, Southwestern Japan: ecological and genetic approach

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https://doi.org/10.69721/TPS.J.2021.13.2.04

ABSTRACT

Dragonets are one of the dominant species in Tosa Bay, Southwestern Japan. However, until now, there is no baseline information on the conservation status of its species and populations. This study gathered genetic and ecological data and information to analyze and measure the historical conservation status of dragonets in the Bay. Quantitative values were converted into qualitative ranges to measures the conservation status of dragonet species. Eight dragonet species/populations were found to be in stable condition in the early 1990s, namely: *Callionymus planus* Ochiai, 1955; *Callionymus lunatus* Temminck and Schlegel, 1845; *Callionymus curvicornis* Valenciennes, 1837; *Callionymus japonicus* Houttuyn, 1782; Callionymus *enneactis* Bleeker, 1879; *Synchiropus altivelis* (Temminck and Schlegel, 1845); *Repomucenus virgis* (Jordan and Fowler, 1903); and *Repomucenus huguenini* Bleeker, 1858. Others were globally endangered and rare (*Callionymus draconis* Nakabo, 1977), locally highly vulnerable (*Callionymus valenciennei* Temminck and Schlegel, 1845; *Callionymus beniteguri* (Jordan and Snyder, 1900), locally vulnerable (*Callionymus formosanus* Fricke, 1981; *Bathycallionymus kaianus* (Günther, 1981)), and globally highly vulnerable (*Callionymus sokonumeri* Kamohara, 1936). The information gained in this study provided baseline knowledge on the historical species risk status of dragonets in Tosa Bay, which can be used as a basis for future studies. It also provided some scientifically-based options for managing biodiversity in a defined spatial management unit, which is applicable to e.g., marine protected areas, parks, bays, islands, lakes, etc.

Keywords: biodiversity indicators, risk assessment, spatial management unit

The Palawan Scientist, 13(2): 37-52

INTRODUCTION

Every species on earth has its special value as conferred by its evolutionary history, unique ecological roles (Primack 1995), and beauty. Loss of genetic diversity reduces future evolutionary options, and high genetic diversity variation within populations is positively related to fitness (Meffe and Carroll 1994). The human beings are dependent on other species to exist, as species depend on other species; thus, what is bad for biological diversity is bad for humans. However, in-spite of their value, vast numbers of species have declined rapidly, to some point of untimely extinction at a rate that far exceeds the rate of species replacement (Primack 1995). Cheung et al. (2005) stress that effective conservation of threatened species requires timely identification of vulnerable species, and careful measurement of environmental trends and progress will provide a foundation for effective policymaking (Garcia 1996). As such, there is a need to identify which species are at risk of extinction (Dulvy et al. 2004) and which populations are threatened to form a basis to decide suitable management options. Furthermore, the United Nations Environmental Programme (UNEP) Key Marine and Coastal Biodiversity uses data sets to monitor biodiversity changes through time or identify areas of high biodiversity value (Martin et al. 2014). Shin et al. (2005) agree that several indicators may be needed to track the state of several components and attributes of biodiversity.

To this effect, the Food and Agriculture Organization of the United Nations has established the international framework for the protection and sustainable development of the marine and coastal environment and its resources by laying out the rights and obligations of states (FAO 1992). This provision obliges the coastal states to undertake measures to maintain biodiversity, including a marine biodiversity survey and an inventory of endangered species and critical marine habitats. Consequently, Yankova et al. (2014) and Pešić et al. (2021) prepared a list of rare and endangered fish species of the Adriatic Sea and the checklist of the marine fishes in the Black Sea, as well as their current conservation status, respectively.

Hence, it is necessary to develop biodiversity indicators defined as variables, pointers, or indices of a phenomenon (Garcia et al. 2000) which are widely used for environmental reporting, research, and management support (Spellerberg 2005). Twenty-five percent of the ecologists choose indicators that assess a combination of local abundance, ecological significance, or conservation status (Siddig et al. 2016). For better accuracy, indicators compose of different disciplines are needed to cover more population components and traits within a defined management area. Garcia (1996) stresses that the scope of indicators should encompass both the sustainable resources, commodities, services and important societal factors derived from the system. In addition, Jennings (2005) considered an ecosystem (or more realistically a spatial management unit) with components (e.g. population or species) and attributes (e.g. diversity, abundance, trophic structure). On the other hand, the Indicators for Sustainable Development of Fisheries (ISDF) emphasizes species and genetic diversity loss as biodiversity criteria (Garcia 1996). Meanwhile, the Traffic Light (TL) approach integrates multiple quantitative and qualitative criteria to assign management responses to fisheries management (Caddy 1999, 2015).

While genetic approaches became popular with the conservation of rare species (e.g. Ashbaugh et al. 1994; Primack 1995), Gaston and Lawton (1990) and Huston (1990) use correlations between population ecology and rare species to determine conservation priorities. Burgman et al. (1993) use such qualitative categories as abundance, spatial distribution, geographic range, frequency of occurrence indicators to assess species or population status, while Kirpichnikov (1992) correlated the genetic variability to population size. It is thus important to work on the elements of biodiversity in a locality, involving the combination of species diversity, genetic diversity, ecological traits, microhabitat, and phylogeny.

Callionymids are one of the dominant species in Tosa Bay, Japan. Despite the numerous biodiversity studies conducted on different species in the area, there is still no information on the conservation status of the species. Therefore, this study aims to determine the historical conservation status of identified populations and species of dragonets in Tosa Bay and Uranouchi Inlet, Japan, using the combination of two parameters (ecological and genetics) and infer on the historical conservation status of the species (rare, stable, or vulnerable). This information could serve as a historical baseline on the conservation status of various species of dragonets in the Bay.

METHODS

The information on the depth distribution of 12 dragonets species collected in Tosa Bay and Uranouchi Inlet, Japan, was from the work of the authors (Gonzales and Taniguchi (1997b), while genetic information of dragonets in Tosa Bay was based on the allozyme study of Gonzales et al. (1997a). The correlation coefficient (2-tailed test: Spearman's rho) of genetic and ecological indicators was used to determine the relationships among the indicators of the conservation status of dragonet species and populations.

Measurement of Historical Conservation Status using the Population Ecology and Genetic Variation

The varying degrees of the conservation status of different populations and species of dragonets in Tosa Bay and Uranouchi Inlet were measured based on the quantitative categories, both from their population ecology and genetic variations where quantitative values were converted into qualitative ranges. Population ecology (four qualitative categories) and genetic variation (two qualitative categories) were used as indicators to assess the risks of population and species (Table 1). An arbitrary score (points) ranging from zero to two were designated for each of the qualitative category ranges to estimate the degree of risk of each population (Table 1). The minimum score for each species in all categories were six points (Table 2) and a maximum of 12 to be considered stable.

Table 1. Arbitrary scores used in different indicators in determining the conservation risk of dragonets population and species in Tosa Bay and Uranouchi Inlet, Japan. Polymorphism (P) value average and lower margin were based on Kirpichnikov (1992). *other populations of the species can be found in the far seas as in the waters of Indonesia and Australia, and the Indian ocean; **found in Japan or waters adjacent to it; e.g. the South China Sea, East China Sea, and in waters off the Korean Peninsula; +reported only in Tosa Bay or the Pacific Coast of Southern Japan.

Arbitrary Score	Population Biology				Genetic Variation	
	Abundance (ind mo^{-1})	Depth Distribution (no. of depth zone)	Geographical Range	Frequency (frequency of occurrence in the trawl catches)	Polymorphism (P)	Heterozygosity (H)
2	abundant (> 30)	widespread (> 5)	widespread*	ubiquitous (> 60%)	high (P > 20%)	high (H> 5%)
1	average (15-30)	average (3-5)	domestic**	average (40-60%)	average (P between 8-20%)	average (H between 2-5%)
0	rare (< 15)	restricted (< 3)	endemic+	scarce (< 40%)	low (P < 8%)	low (P < 2%)

The species *Callionymus Draconis* Nakabo, 1977, *Callionymus valenciennei,* and *Foetorepus masudai* Nakabo, 1987 were not included in the point system due to a lack of data in some categories. This study is limited to dragonet species found only in Tosa Bay and the adjacent Uranouchi Inlet (southwestern Japan – as a spatial management unit). Other dragonet species found in other areas of Japan, like the *Callionymus ornatipinnis* Regan, 1905 in northern Japan (Awata et al. 2010), were not included.

Table 2. The score points used to assess the conservation status of dragonets population and species in Tosa Bay and Uranouchi Inlet, Japan. *Based from Burgman et al. (1993).

Score points	Conservation status	Interpretation
≥ 50% (6)	Stable	Population not at risk
< 50% (5)	At risk	Unstable, may become vulnerable if no management intervention is in place
25–42% (3-5)	Vulnerable	*Population prone to be endangered about 75–100 years if factors tending to push the population into decline continue to operate.
≤ 25% (3)	Highly vulnerable	*Population prone to be endangered at about 50-75 years if factors tending to push the population into decline continue to operate.

Determination of Genetic Variation and Species Identification

The genetic heterozygosity and polymorphism used in this study was from Gonzales et al. (1997a). Fish samples were collected in Tosa Bay, Uranouchi Inlet, and nearby fish market (Figure 1). Fish sample tissues used for the electrophoretic analysis were skeletal muscle, liver, and eye. Horizontal starch-gel electrophoresis and staining procedures followed Sumantadinata and Taniguchi (1982), with slight modifications. The buffer systems used were citric acid-aminopropyl morpholine (C-APM) at pH 6.0 and Tris-citrate (T-C) at pH 8.0.

The gene nomenclature for protein-coding followed Shaklee et al. (1990). Allelic frequencies were calculated following Allendorf and Ferguson (1990). Nei (1972) genetic distance ($D=\log_e I$) was calculated for each species pair, using all loci at which genotypes were scored for both species. Test of conformance to expected Hardy-Weinberg proportions was carried out by conventional chi-square tests with Yate's correction (Pasteur et al. 1988).

All scientific names of dragonets followed the FishBase (Froese and Pauly 2021) and were validated using the work of Fricke et al. (2020) and WoRMS database (WoRMS Editorial Board 2021).

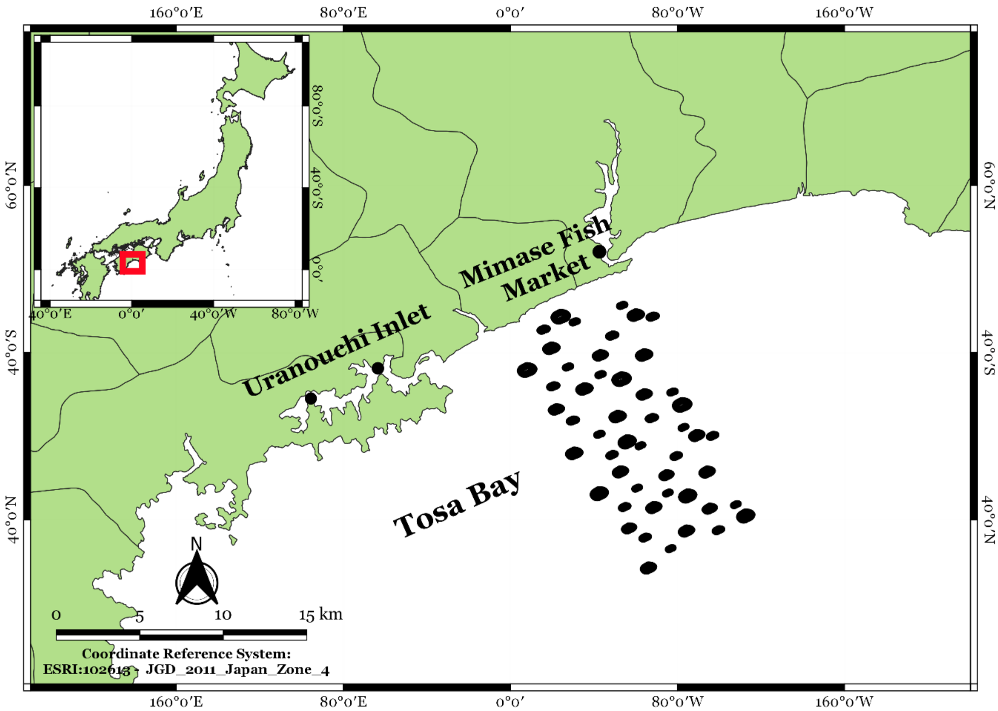

Figure 1. Map of Tosa Bay and Uranouchi Inlet, Kochi, Japan showing the sampling areas in black patches/circles.

RESULTS

Historical Conservation Status

Eight dragonet populations/species are found to be in stable conditions namely: *Callionymus planus* Ochiai, 1955, *Callionymus lunatus* Temminck and Schlegel, 1845, *Callionymus curvicornis* Valenciennes, 1837, *Callionymus. japonicas*, *Callionymus enneactis* Bleeker, 1879, *Synchiropus altivelis* (Temminck and Schlegel, 1845), *Repomucenus virgis* (Jordan and Fowler, 1903), and *Repomucenus huguenini* Bleeker, 1858. Others are globally endangered, locally vulnerable, locally highly vulnerable, and globally highly vulnerable (Table 3).

Genetic Variation and Species Identification

In terms of genetic diversity, *R. virgis* (18.0%) shows the highest percentage of polymorphism (P), while the lowest is *Callionymus beniteguri* Jordan and Snyder, 1900 (0.0%) (Figure 1). For heterozygosity (H), *C. planus* is the most diverse (15.6%), and *C. beniteguri* (0.0%) is the lowest. For the

Table 3. Qualitative categories for the conservation status based on the arbitrary scores of the reported dragonet populations in Tosa Bay. P-polymorphism; H-heterozygosity. *recently not collected in Tosa Bay; **no data.

Species	Genetic variation		Abundance	Depth distribution	Geographic range	Frequency	Conservation status (total=12)
	P	H					
Bathycallionymus kaianus	ave. (1)	ave. (1)	rare (0)	restricted (0)	widespread (2)	scarce (0)	locally vulnerable (4)
Callionymus beniteguri	low (0)	low (0)	rare (0)	restricted (0)	domestic (1)	scarce (0)	locally highly vulnerable (1)
Callionymus curvicornis	ave. (1)	ave. (1)	ave. (1)	widespread (2)	domestic (1)	ubiquitous (2)	stable (8)
*Callionymus draconis**	**	**	**	**	endemic (0)	**	globally endangered (0)
Callionymus enneactis	ave. (1)	ave. (1)	ave. (1)	restricted (0)	widespread (2)	ubiquitous (2)	stable (7)
Callionymus formosanus	ave. (1)	ave. (1)	rare (0)	restricted (0)	domestic (1)	scarce (0)	locally vulnerable (3)
Callionymus japonicas	ave. (1)	ave. (1)	abundant (2)	ave.(1)	widespread (2)	ubiquitous (2)	stable (9)
Callionymus lunatus	high (2)	high (2)	abundant (2)	widespread (2)	domestic (1)	ubiquitous (2)	stable (11)
Callionymus planus	ave. (1)	high (2)	ave. (1)	restricted (0)	endemic (0)	ubiquitous (2)	stable (6)
Callionymus sokonumeri	ave. (1)	low (0)	rare (0)	restricted (0)	endemic (0)	scarce (0)	globally highly vulnerable (1)
*Callionymus valenciennei**	**	**	**	**	domestic (1)	**	locally highly vulnerable (1)
Foetorepus masudai	**	**	rare (0)	**	endemic (0)	scarce (0)	Insufficiently known (0)
Repomucenus huguenini	ave. (1)	high (2)	abundant (2)	widespread (2)	domestic (1)	ubiquitous (2)	stable (10)
Repomucenus virgis	high. (2)	high (2)	abundant (2)	widespread (2)	domestic (1)	ubiquitous (2)	stable (11)
Synchiropus altivelis	ave. (1)	ave. (1)	abundant (2)	widespread (2)	domestic (1)	ave. (1)	stable (8)

frequency, *R. huguenini* and *Callionymus japonicus* Houttuyn, 1782 (15.9%) are the most frequent species caught, while *C. beniteguri* (0.9%) and *Callionymus sokonumeri* Kamohara, 1936 (0.0%) are the least frequent (Figure 1). In numerical abundance (NA), *R. huguenini* (68.6%) is the most abundant, followed by *C. japonicus* (9.2%), and *C. lunatus* (7.8%) (Figure 1).

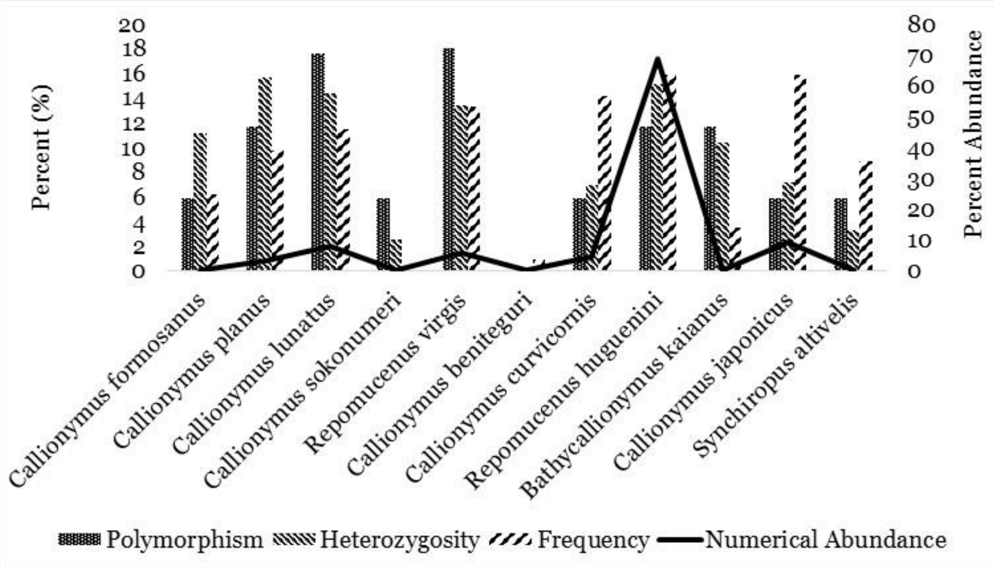

Figure 2. Genetic variation (proportion of Polymorphic (P) loci and mean Heterozygosity (H)), percent monthly Frequency (F) appearance, and with Numerical Abundance (NA) of 11 dragonets during the 18-month trawling in Tosa Bay (1993-1995).

The combination of an ecological and genetic set of indicators showed a significant result in measuring the conservation status of the identified population/species of dragonets in Tosa Bay and Uranouchi Inlet. Among the indicators used, Polymorphism (P) and Heterozygosity (H); Frequency (F) and Numerical Abundance (NA) were highly correlated. Heterozygosity (H) and Numerical Abundance (NA); Depth Distribution (DD) and Numerical Abundance (NA) were correlated (Table 4).

Table 4. The correlation coefficient of genetic (Polymorphism-P, Heterozygosity-H) and ecological (Numerical Abundance-NA, Frequency-F, Depth Distribution-DD, Geographic Range-GR) indicators, using a 2-tailed test (Spearman's rho). **highly significant, *significant.

Parameters	P	H	NA	F	DD	GR
P Correlation Coefficient	1.000	0.799**	0.501	0.367	0.383	0.000
Sig. (2-tailed)		0.003	0.117	0.267	0.245	1.000
H Correlation Coefficient	0.799**	1.000	0.655*	0.506	0.211	-0.095
Sig. (2-tailed)	0.003		0.029	0.113	0.533	0.780
NA Correlation Coefficient	0.501	0.655*	1.000	0.952**	0.663*	0.286
Sig. (2-tailed)	0.117	0.029		0.000	0.026	0.394
F Correlation Coefficient	0.367	0.506	0.952**	1.000	0.710*	0.311
Sig. (2-tailed)	0.267	0.113	0.000		0.014	0.353
DD Correlation Coefficient	0.383	0.211	0.663*	0.710*	1.000	0.158
Sig. (2-tailed)	0.245	0.533	0.026	0.014		0.642
GR Correlation Coefficient	0.000	-0.095	0.286	0.311	0.158	1.000
Sig. (2-tailed)	1.000	0.780	0.394	0.353	0.642	

DISCUSSION

Historical Conservation Status

There are varying degrees of conservation risks to the populations of *Callionymus valenciennei* Temminck and Schlegel, 1845, *C. beniteguri, C. draconis, C. sokonumeri, Callionymus Formosanus* Fricke, 1981, and *Bathycallionymus kaianus* (Günther, 1981). However, it is possible that because dragonets are not popular fish food and have low commercial value, people locating their samples may fail to record or report their existence in certain locations. Therefore, current and further exhaustive fieldwork must locate the undiscovered and unreported dragonet species.

Two previously reported species in Tosa Bay are not found in our samples – *C. valenciennei* and *C. draconis* (Table 3). The non-appearance of *C. valenciennei* and *C. draconis* in our samples indicates the possibility that their populations may not anymore exist in Tosa Bay, making it very tempting to declare them as locally extinct or presumed extinct species or populations. However, extinct species are those for which there is no doubt that the last member of the species has died; and presumed extinct species are those that have not been located in the wild for the last 50 years (Burgman et al. 1993). Thus, these conditions do not apply to the present status of *C. valenciennei* and *C. draconis*. On the other hand, an endangered species is defined as those facing a high risk of extinction within one or two decades, and vulnerable species are those not currently endangered but are at risk over longer periods (usually 50 to 100 years) if factors tending to push the species into decline continue to operate (Burgman et al. 1993). According to Fricke (pers. comm.), *C. draconis* is a very rare species, therefore difficult to collect and rarely reported, while *C. valenciennei* is more common, even commercially used in Japan, which is consistent with our results in Table 3 – *C. draconis* is globally endangered, only found in the Pacific Coast of southern Japan, while *C. valenciennei* is locally highly vulnerable in Tosa Bay. When something is rare, it is not necessarily threatened with imminent extinction, just as species that are likely to become extinct soon are not necessarily rare, restricted, or specialized (Burgman et al. 1993). Rare, restricted, and specialized species are not presently vulnerable and may be present in stable populations, but some characteristics of their population sizes or distributions make them conceivably at risk in the long term (Burgman et al. 1993). Hence, although the population of *C. draconis* in Tosa Bay is very rare (Nakabo 1977), it could be in stable condition.

Genetic Variation and Species Identification

The genetic variability values of *F. masudai* could not be obtained, because only one sample was collected, and our survey did not cover its entire depth distributional range, 120-400 m in Tosa Bay. Thus, we termed its conservation status as 'insufficiently known" defined by Burgman et al. (1993) as those species with insufficient information on which to base a judgment concerning either their abundance and distribution or the degree of threat they face. *Callionymus beniteguri* is locally highly vulnerable. The values for genetic variation, numerical abundance, and frequency of occurrence in Tosa Bay (Table 3 and Figure 1), show that *C. curvicornis* and *R. huguenini* are dominant and widely distributed species in the area. The rare *C. beniteguri* occurs sympatrically with its four widely distributed congeners like *R. huguenini*, *C. lunatus*, *C. planus*, and *C. curvicornis* in the shallow waters of Tosa Bay, which have similar dietary resource requirements (Gonzales et al. 1996a), co-occurring spawning seasons (Eda et al. 1994; Gonzales and Taniguchi 1997b) and similar spawning behavior (Gonzales et al. 1996b). Furthermore, some dragonets have territorial behavior, and fighting among males occur during spawning (Takita and Okamoto 1979; Gonzales et al. 1996b). These similarities in the basic biological and ecological requirements (e.g. in reproductive resources, in prey organisms) of the four species indicate close competition among them, which most likely have resulted in the decrease in reproductive fitness of *C. beniteguri* in Tosa Bay.

The survival rate of 40-days juveniles of *C. curvicornis* and *C. valenciennei* is 7-8 times greater than *C. beniteguri*. Although *C. beniteguri* has a larger length-sized newly hatched pro-larvae, it has a relatively smaller forty-day-old juvenile than *C. curvicornis* and *C. valenciennei* (Eda et al. 1994), which show relatively low survival and growth rates in the early life-stages of *C. beniteguri*. This loss in the early life fitness of *C. beniteguri* in Tosa Bay may be an effect of genetic drift or inbreeding in its low-density population, as observed by Meffe (1990) in other fishes. The situation might have been further aggravated when the individuals in the already small population presumably inbred, likely expressing deleterious recessive alleles in the population (Meffe 1990), subsequently resulting in the loss in early-life fitness of the population. Anthropogenic effects may also be a probable factor, as dragonets were often observed caught as by-catch species during fishing operations.

Additionally, the genetic dendrogram of Gonzales et al. (1997a) shows that *C. beniteguri* is a fairly newly-evolved species. The more recently derived rather than the oldest members of a community that show evidence of interspecific interactions that could lead to competition-mediated extinction (Brooks et al. 1992). Hence, the natural phenomena on the speciation and extinction cycle could not be a driver for the rarity of *C. beniteguri*. In summary, the cause of the decline of the population of *C. beniteguri* in Tosa Bay could be its competition for reproductive and food resources against the widely distributed *R. huguenini*, *C. lunatus*, and *C. curvicornis*, and partly by human actions.

The geographic distribution of *C. sokonumeri* shows that its extinction in Tosa Bay may mean extinction in its whole geographic range. Hence immediate, more detailed investigation on the causes of its rarity and subsequent management is highly recommended. *Callionymus sokonumeri* is endemic in the Pacific coast of southern Japan, and its present habitat shows that it could be a resident species in that area, presumably occurring within the dispersal area of dragonets (90-120 m) Pacific coast of southern Japan as supported by Gonzales and Taniguchi (1997b). Those species that have been part of any given biota for the longest periods maybe are most in need of protection against exploitation and removal (Brooks et al. 1992). Thus, the management of *C. sokonumeri* must include the conservation of its area of endemism (Pacific coast of southern Japan), though there is much to know about their microhabitat. *Callionymus planus* and *C. draconis* are also known to be endemic in the same area. While, *C. formosanus*, *C. sokonumeri*, and *B. kaianus* have no genetic information, and its available data is only ecological –depth and geographic distribution. This constrains the making of inferences on the rarity of the above species, though the rarity of *C. formosanus* is highlighted by Gonzales and Okamura (1995).

Scientists and researchers use different strategies to provide scientific approaches to protect biodiversity. Some workers utilize ecology-based indicators for biodiversity assessment (Jennings 2005, Siddig et al. 2016; Wendling et al. 2018), while genetic indicators approaches are popular to others (e.g. Meffe 1990; Ashbaugh et al. 1994; and Primack 1995). However, to monitor biodiversity loss at the global, regional, and local levels, a wealth of indicators was created over the last two decades, but genetic diversity indicators are regrettably absent from a comprehensive bio-monitoring scheme (Graudal et al. 2014). Hence, the use of both ecological and genetic indicators may not be common and must be promoted to biodiversity workers.

In support to the above argument, this study shows that combining ecological and genetic indicators has successfully determined the status of different populations and species of closely associated fishes in a management unit and provides information on their conservation status and priorities. This study provides historical baseline information on the two-decade conservation status of dragonets in the Bay; hence, it is important to conduct follow-up studies to compare the before and after changes and trends of the fish species/population status in the Bay.

Using two or more parameters increases the coverage of an assessment, thereby measuring more array of characters and traits, and cross-checking possible bias that may be brought about when using only indicators from one parameter–ecological or genetic. In *C. planus*, for example, vulnerable status was revealed when only ecological indicators were used, but when combined with genetic variation indicators, the population turn out to be in stable condition (Table 3). The temporal aspect of ecological and genetic evolutions is an interesting topic to further the inferences when combining biodiversity indicators from different parameters.

The conservation assessment (Table 3) showed that varying degrees of conservation status could be measured using quantitative categories converted into qualitative ranges. This is a viable tool in population risk assessment that also provides some scientifically-based options in management policies for the preservation of biodiversity in a defined spatial management unit–like southwestern Japan.

This conservation status measurement approach is suitable to any local government or entity managing a spatial management unit, e.g., marine protected areas, key biodiversity areas, parks, sanctuaries, bays, islands, lakes, and any area with high biodiversity value. This approach may not yet be perfect and have much space for improvement, but it does provide a substantial point for sound decision-making based on hard science.

Since the conservation status of the populations in this study was measured based on the 1990s survey, it could serve as a historical baseline for future similar or follow-up studies in Tosa Bay or may be used to assess other species and populations. The Pacific coast of Southern Japan should also be further investigated for possibly more endemic species. The subsequent management policies for the species must include not only the preservation of populations, but also the protection of habitats, especially endemism area. On the other hand, social and governance aspects of conservation shall be closely considered in the final development of the species conservation plan and a specific conservation program shall be developed for *C. planus* and *F. masudai*.

ACKNOWLEDGMENTS

This study is funded by the Monbushio Fellowship Program of the Ministry of Education and Culture of the Government of Japan. We thank the evaluators for improving the early versions of this paper. Thanks are due to my laboratory mates, and the captain and crew of Toyohata Maru for the field assistance. We thank Drs. Ronald Fricke and Tetsuji Nakabo for providing valuable references and information. We are grateful to Dr. Osamu Okamura and Dr. Masao Ono for the suggestions and use of their laboratories. And to Dr. Shingo Seki for his advice and assistance in the electrophoresis experiments. Thanks are due to Niño Jess Mar F. Mecha, Sharon Rose Anunciado and Clarilyn Lariza for their assistance to this paper.

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ARTICLE INFO:

Received: 15 May 2020
Revised: 15 July 2021
Accepted: 07 August 2021
Available online: 24 August 2021

Role of authors: BJG – conceptualized the study, collected, processed and analyzed the data, and wrote the paper; NT – supervised the first author, co-conceptualized the study, provided insights to the discussion, sourced out funds, facilitated field works, and reviewed the manuscript.

Social actor representation in mining discourse in the Philippines: A critical discourse analysis

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https://doi.org/10.69721/TPS.J.2021.13.2.05

ABSTRACT

This paper addresses the need to investigate media's representation of mining issues in the Philippines, an unexplored linguistic field within Critical Discourse Analysis (CDA). It focuses on referential strategies used in naming the social actors (SAs) involved in mining issues from 2012 to 2017 by three national broadsheets. A sample of 224 news reports was analyzed at the sentence level, and was validated by software and two inter-coders. The study found that SAs were commonly named by their unique identities mainly through nomination and categorization strategies. The valuing of the "elites" in the mining discourse is also traceable in the prominent use of nomination strategies, particularly formalization and honorification. While these strategies reflect the conventional linguistic features of the news genre which reinforces specificity, formality, and brevity, it is evident that they were seldom used to refer to the mine workers, disenfranchising them in the process. The tendency of the media to influence public perception through its construction of societal issues demands readers to be skeptical of what they read and to be aware of the machination which underlies the process of discourse production.

Keywords: categorization, environmental discourse, nomination, referential strategies

INTRODUCTION

The Philippines is one of the mineral-rich countries in the world (Manicad 2011), with an estimated US$840 billion worth of untapped mineral wealth (Angub 2013). The statistics of the Mines and Geoscience Bureau indicates that in 2012 the mining sector had created 252,000 jobs and contributed PhP22 billion to the country's treasury from taxes, fees, and

The Palawan Scientist, 13(2): 53-67

royalties (Angub 2013). On one hand, the substantial contribution of the mining industry to the national revenue and its generation of employment opportunities for Filipino people may have carved its reputable space in the Philippine society. On the other hand, the detrimental impact of mining to the country has also been heavily criticized as it causes massive potential destruction to environment and wildlife ecology such as acid mine drainage and contaminant leaching, soil erosion, and tailing impoundments among others (Sam 1999; ELAW 2010).

These conflicting interests are reflected in the mining discourses stoked or dimmed by media, which influence the readers' construal of meanings in the mining texts, social actors' roles in the mining industry, and the urderlying contexts of the mining reality. Fairclough (1989) posits that in constructing a reality, media exercises power that lies on its sole producing rights in determining what/who can be included and excluded in the text, how events are represented, and how the readers are positioned in the texts. Demarest et al. (2020) share the same argument in saying that media do not merely convey information to the public but they also participate in constructing, maintaining, and transmitting certain narratives and discourse.

Moreover, Flores (2009) observes that print media in the Philippines employed manipulative prototypes in reporting on a certain alleged political rigging, realized through discursive and linguistic devices. Though not directly related to media's influence in public discourse, Holzcheiter (2016) sympathizes with the notion of representation as a form of power. She claims that representation, to some extent, realizes the possibility and authority to support, defend and advocate some interests without the concerned party's direct involvement and control of the situation.

Therefore, this study described the national and local media's allocation of referential strategies in discursively constructing the identities of social actors (SAs) involved in mining issues. The discourse and linguistic patterns employed by relevant SAs as recontextualized in the national and local media from 2012 to 2017 as well as the recurring discourse themes in the national and local domains constitute the "Philippine mining discourse," an unexplored field for linguistic investigation. This paper adopted the perspectives of Critical Discourse Analysis (CDA), an approach in the humanities and social sciences that systematically examines the structure and functions of text and talk in their linguistic and discoursal context, specifically van Leeuwen's (2008) Social Actors Network (SAN) framework, a model in CDA that accounts for the referential strategies or the linguistic devices used in naming and labelling of SAs in text.

METHODS

Data Collection Technique

Data for this study were collected by manually searching the online archives of the top three national broadsheets using the keyword “mining” within the time frame June 2012 to June 2017. The three news portals were chosen as data sources because their printed editions are the leading national broadsheets in the country in terms of readership and circulation (Inquirer.Net 2015). From the 746 news reports downloaded, 224 articles were taken as samples by employing a lottery technique.

In addition, 112 weekly editions of a local newspaper yielded 27 mining news reports, which comprised the local news data. However, the samples were not a complete representation of the five-year publication due to archival limitations.

Analytical Framework

The study employed the six categories of referential strategies adapted from van Leeuwen’s (2008) framework. First was nomination, representing SAs by referring to their unique identity, typically realized by proper nouns through formalization, use of surname, with or without honorifics; semiformalization, the use of given name and surname; or informalization, referring to SAs using given name. Nominations might be titulated, either in the form of honorification, the addition of standard titles, ranks, and other honorific titles, or in the form of affiliations, the addition of personal or kinship relation terms. Pseudo-titulation, the use of pseudo titles or “controversial” labels before the SA’s name, was also part of this category.

Next was categorization, used when SAs were represented in terms of identities and functions they shared with others through functionalization and identification. Functionalization occurred when SAs were named through their occupation, or role (e.g. teacher). Identification was realized when SAs were defined in terms of their societal memberships, more or less permanently. Identification had three distinct categories: social classification (SC) where SAs were named by their societal or organizational categorization (e.g. Muslim); relational identification (RI) when SAs were represented by their personal, kinship, or work relations; physical identification (PI) when they were labeled in terms of physical characteristics which uniquely identify them in a given context based on age or gender. A sub-type of PI was appraisement, it gave SAs positive or negative attributions through a set of nouns and idioms denoting such descriptions. The third referential strategy was indetermination, occurred when SAs were represented using indefinite pronouns used in the nominal function. Assimilation was another referential strategy, which occurred when SAs were being referred to as groups, realized either through aggregation or the quantification of SAs, or collectivization as a collective unit. Abstraction happened when SAs were labeled through the quality assigned to them (e.g. poor, skilled). Finally, objectivation, occurred when SAs were named by referring to a place (spatialization) or a thing (instrumentalization) closely associated with either the person or the action in which they were represented as being engaged, or through their utterance (utterance autonomization), or in reference to a part of their body (somatization).

The framework was modified to account for other strategies that could not be accommodated by the existing list. The modified taxonomy includes functionalized nomination, referring to SAs using institutional titles which were only used for a select few (e.g. the Senator); affiliation, adding personal or kinship relations or SAs' functions and/or organizational affiliation; overdetermination, representing SAs as participants of more than one social practice; accompaniment, referring to SAs together with other participant/s; and hypocoristic nomination, calling SAs through their nicknames. Moreover, abstraction and objectivization though presented as individual categories were subsumed under impersonalization strategies in the presentation of findings, keeping van Leeuwen's system.

For the local news, all strategies in each category with a less than five frequency were combined and labeled as "others."

Coding and Analysis

The data consisting 12,276 sentences were coded manually at the sentence level employing Halliday and Matthiessen's (2014) matrix of participants, termed in this study as SAs. After the manual identification of SAs, the data were subjected to a computer-assisted analysis using UAM Corpus Tool, a software for linguistic annotation of texts. This step was necessary to validate whether all items that function as participants in the data were properly identified. The computer-generated list was cleaned manually to weed out double-tagged or mistakenly-coded tokens before the final coding using van Leeuwen's (2008) framework ensued.

To further establish the validity of the findings, two university professors with a doctorate in Applied Linguistics inter-coded 30% (67 news articles) of the data, yielding a 99.96% agreement rate with the researcher's analysis.

RESULTS

Referential Strategies in the National Media

Nomination. Among the ten nomination strategies, formalization (37.81%) and honorification (35.16%) were widely used while informalization (0.96%) and pseudo-titulation (0.69%) were seldom employed by the text producers (Table 1).

Table 1. Nomination strategies in the national news reports (n = 2,187)

Strategy	Frequency	Percentage (%)
Formalization	827	37.81
Honorification	769	35.16
Affiliation	159	7.27
Over-determination	136	6.22
Accompaniment	100	4.57
Functionalized Nomination	98	4.48
Semi-formalization	37	1.69
Hypocoristic Nomination	25	1.14
Informalization	21	0.96
Pseudo-titulation	15	0.69
Total	2,187	100.00

Categorization. Functionalization (50.06%) was predominant while appraisement (9.02%) and PI (2.43%) were barely employed (Table 2). Findings further revealed that categorization strategies were commonly used to refer to "ordinary" SAs (e.g. miners, activists).

Table 2. Categorization strategies in the national news reports (n = 865).

Strategy	Frequency	Percentage (%)
Functionalization	433	50.06
Relational Identification (RI)	204	23.58
Social Classification (SC)	129	14.91
Appraisement	78	9.02
Physical Identification (PI)	21	2.43
Total	865	100.00

Indetermination. Only 55 cases of indetermination manifested in the data. They all appeared as part of quoted statements. Some instances of indetermination were realized by indefinite pronouns: anybody, some, and few.

Assimilation. There was a disproportionate frequency of collectivization (88.63%) and aggregation (11.37%) in the data (Table 3).

Table 3. Assimilation strategies in the national news reports (n = 2,243).

Strategy	Frequency	Percentage (%)
Collectivization	1,988	88.63
Aggregation	255	11.37
Total	2, 243	100.00

Impersonalization. Utterance autonomization (42.91%) topped in this category of referential strategies, followed by abstraction (25.37%) and spatialization (15.17%). The least employed strategies were instrumentalization (13.06%) and somatization (3.48%) (Table 4).

Table 4. Impersonalization strategies in the national news reports (n = 804).

Strategy	Frequency	Percentage (%)
Utterance Autonomization	345	42.91
Abstraction	204	25.37
Spatialization	122	15.17
Instrumentalization	105	13.06
Somatization	28	3.48
Total	804	100.00

Referential Strategies in Local News Reports

The prevalence of nomination strategies (41.94%) was evident in the local news reports, followed by assimilation (31.18%) and categorization (16.85%) strategies. Meanwhile, the impersonalization strategies (10.04%) were nearly absent (Table 5).

Table 5. Referential strategies in the local news reports (n = 279).

Strategy	Frequency	Percentage (%)
Nomination	**117**	**41.94**
Formalization	63	22.58
Honorification	45	16.13
Others	9	3.23
Assimilation	**87**	**31.18**
Collectivization	61	21.86
Aggregation	15	5.38
Accompaniment	11	3.94
Categorization	**47**	**16.85**
Functionalization	22	7.89
Classification	11	3.94
Relational Identification	9	3.23

Appraisement	5	1.79
Impersonalization	**28**	**10.04**
Spatialization	7	2.51
Instrumentalization	7	2.51
Utterance Autonomation	6	2.15
Others	8	2.87
Total	**279**	**100.00**

DISCUSSION

Referential Strategies in the National News Reports

Nomination. The widespread of formalization among the nomination strategies can be attributed to the conventions of news genre, which enables the identification of specific actors in the news in the most possible concise manner, one of the marks of journalese writing (Bhatia 1993). Notably, most of the surnames that appeared in the data belong to government officials, mining executives, among other prominent personalities, and only a few for "ordinary" individuals.

Informalization was the second least employed nomination strategy. The informal and personal tone construed by the "first-name-basis" might not be a journalistic feature but could be the linguistic motif of other texts, i.e. short stories, narratives. Some instances of informalization were used to refer to the wife of a miner who died in a mining accident. She was introduced using semi-formalization in the lead but was constantly represented by her first name throughout the article. With informalization, the journalist sustained the emotional appeal phrased in the headline "It's hard to wait, I want to help dig" (Burgos 2013). This quality of the news exemplifies color, one of the news values (Bednarek 2006). It suggests that news stories sometimes highlight the emotionally relevant aspect of the event. Moreover, appeals to emotion may be considered as deliberate manipulations to provoke an emotional reaction and to bypass rational thought (Fairclough 1989; van Dijk 2009).

Functionalized nomination occurred considerably in the data. The term was coined in this study in response to van Leeuwen's (2008) report that this way of representing social actors blurs the line between functionalization and nomination because the generic functional titles which are supposed to be shared with other members in the social category are exclusively used by only one or a very few social actors such as "the Vice-President."

Pseudo-titulation was the least employed nomination strategy, signifying its inappropriateness in the news genre as it seems to project informal rather than formal tones. This finding strengthens van Leeuwen's

(2008) observation that pseudo-titles are much commonly used in children's stories. Hypocoristic nomination, using SAs' nicknames as in "Digong" was the seldom employed. This phenomenon was not included in van Leeuwen's (2008) taxonomy; hence, the term was coined to describe its occurrence in the data. The use of this strategy conveys personal tone, familiarity, and affection, which is uncommon for this text genre. Its occurrence in the data reflects a certain characteristic of Filipino culture.

Categorization. Functionalization, labelling of SAs in terms of their societal functions, was predominant, realized by: legislators, miners, environmentalists, and barangay officials, among others. Relational identification, naming of SAs in terms of their personal, kinship, or work relations came second in terms of frequency. Some of its manifestations were: appointee, relatives, and co-worker. Social classification, naming of SAs in reference to social categories, which may include provenance, class, wealth, race, ethnicity, religion, sexual orientation, among others (van Leeuwen 2008), manifested with a relatively low number.

Appraisement, dichotomizing SAs as good or bad, loved or hated, among others, was infrequent in the data. This strategy exemplified Fairclough's (1989) claims that there are ideologically-charged words or those that have inherent negative meanings, instances of which in the data include: bandits, perpetrators, and rebels. Physical identification, the naming of SAs denoting their physical features such as "his pregnant wife," "the gunmen," and "rescuers using hand-held tools," was barely employed. According to van Leeuwen (2008), referring to SAs through physical attributes may have connotations that could obliquely classify or functionalize them.

Evidently, ordinary members of the society are the ones mostly named in this manner, which may indicate the play of power and social struggle. Fairclough (1989) argued that social struggle is a process whereby groupings of different interests (e.g. of men and women, young and old) take place. Although some instances may be obvious, others may be implicit.

Indetermination. As presented earlier, only 55 cases of indetermination appeared in the data. The finding is quite expected because journalistic writing, especially news reporting, aims to provide clear and specific information to the public—and that includes naming of all the actors in the news story.

Assimilation. Government agencies (e.g. DENR, the government) and mining companies were among the most-cited collectivized social actors in the data. The prevalence of "the government" is predictable because of its mandate to govern the country's natural wealth and to promote equitable economic opportunities for Filipinos. Such obligation is enshrined in Section

2, Article XII of the 1987 Constitution (Refworld 2021), justifying its frequency in the data. Instances of assimilation are usually reflected in news reports highlighting the authority of "the government" or "the DENR" in imposing suspension on mining companies that committed environmental infractions (Gamil and Domingo 2016).

Aggregation or the quantification of SAs could be a potent intensification strategy, a pressuring tactic to move the concerned SAs into action. Aggregation seems more effective if the contested situation poses a grave threat to a significant number of people or things, such in "over 7,000 people" who are dependent on mining would suffer mass starvation if the DENR would impose the proposed mine closure (Panganiban 2017). The cited statistics compels the authorities to hasten the process of solving the problem. While aggregation might not be a linguistic feature of the mining news reports, it might be common in other news sub-genres, i.e. business column section which usually covers economic topics and presents financial statistics.

Meanwhile, the media's representation of the mining issues in the country tends to highlight the unified actions of individuals or groups, justifying the much higher frequency of collectivization over the aggregation strategy in the data.

Impersonalization. Among the impersonalization strategies, utterance autonomization (UA), referring to SAs by means of their utterance was mostly employed. Some instances of UA were realized by "temporary environmental protection order (TEPO)" and "President Aquino's Executive Order 79." For instance, a statement in a news report stating that the TEPO halted the operation in a forest reserved (Aning 2014) seems to distance the doer (the Supreme Court) from the action by employing UA. All manifestations of UA in the text satisfied the felicity condition required of the utterances. In pragmatics, the speaker's utterances necessitate certain conditions to make the expressions valid and felicitous. Austin's (1992) theory explains that words are not just mere expressions, but something has to be done out of them under the appropriate conditions. Thus, UA requires a set of conditions for the utterance to be felicitous, that is, the speaker must be in authority over the addressee so that he or she could execute a valid declaration, such in the case of the Supreme Court for the TEPO and the President for the executive order.

Abstraction was mostly realized by the adjective "poor", as exemplified in, "President[e] Duterte should live up to his commitment to defend our country from those who destroy her, to help poor people rise from misery, the true enemies of his ideals aren't the poor addicts...." (Aurelio 2017). In the labeling of self and others, Bourdieu (1991) explained that the perspective of the world, the point of view of individuals, and their position become the basis of how they name themselves and others, which are often self-interested. Moreover, the quoted statement is laden with emotionally-loaded words such as her, the poor people, the poor addicts, appealing to the readers' sentiment. The two metaphorical representations, nature as "her" and environmental violators as the "true enemies," are effective predication discourse strategies to convince President Duterte to focus on environmental concerns rather than on the "poor addicts" if indeed he is bent on protecting "her", the country, and help the poor people in the process. It must be noted that the poor people that are being attributed to in the extract are those whose livelihoods are dependent on the environment (e.g. farmers) and the poor addicts are literally those who waged lives in the government's campaign against illegal drugs.

Spatialization, referring to SAs by reference to a place with which they are associated, was the third most employed strategy in this category. Some instances are: "Manila has warned it" and "the pit took my husband," in which Manila and "the pit" exemplified the strategy.

However, instrumentalization, personifying the objects to perform human actions, was the second least employed strategy. It was illustrated in a news report about the rejection of former DENR secretary's appointment in which President Duterte quipped that "money" influenced the decision (Corales 2017). Instrumentalization can then shift the reader's attention from the actor to the object performing the action, a potent tool for downplaying the actor—a case of obfuscating the message and the accountability of the actor. In this particular case, the media might be construed to have intentionally or unintentionally reverberated Duterte's exclusion of the actor or actors who were responsible for the decision and who should be punished for the "money" used in the process, an act which could be considered a form of corruption.

Somatization, the least employed strategy, was highlighted in a news report quoting the presidential spokesperson saying that President Duterte can "head" the DENR but he would rather not advise him to do because his "hands" were already full with his other state functions (Geducos 2017). Somatization was also a configuration of the part-whole relationship as with synecdoche in which reference to the whole is made by reference to a salient part (Taylor 1995). This concept denotes that failure of the part means failure of the whole. As with instrumentalization, somatization shifts the attention of the readers away from the actors by simply referring to parts of his/her body, a mitigating strategy.

Referential Strategies in the Local News Reports

The prevalence of formalization and honorification strategies in the local news reflects the mainstream media's representation of SAs, which is consistent with the conventions of the genre. Although the widespread of formalization was quite expected, the extensive use of honorification strategies seemed unpredictable. This finding likewise echoes the national media's representation, seemingly favoring the discourse positions of the "elite" members of the society. The ubiquity of honorification and the infrequency of affiliation strategies were quite surprising as they signify under-representation of "ordinary" citizens in the local news. Unlike in the mainstream media where key officials and government agencies received more attention than those from the mining sector, the local news seemed to foreground the discourse position of a mining company. Meanwhile, the strategies with almost nil frequencies were affiliation, functionalized nomination, overdetermination, and pseudo-titulation; semi-formalization and informalization were totally absent. As have been mentioned, the thin distribution of referential strategies in the local news could be attributed to a quite limited data, which could also be a limitation of this study.

Generally, media's representations of SAs appeared to be tied to their social roles and ascribed status. Findings revealed that prominent personalities in the mining industry were individually specified, while ordinary citizens were collectively characterized or generalized (e.g. the protesters, the villagers). The same observation reverberated to the local media. The only difference, perhaps, was that the nominated SAs locally were mostly representatives of non-governmental organizations (NGOs) and the private sectors. This finding reinforces van Leeuwen's (1996) observation that "the social roles that agents are given and their grammatical roles in discourse" tend to have a correlation. Interestingly, the data, specifically in the local news, showcased the detrimental impact of mining on the local people as well as the active and salient role of NGOs in lobbying for the protection of the environment and in advancing the cause of the marginalized sectors in the mining industry of the country.

The ubiquity of titulation strategies, particularly honorification, encodes the value given to titles or qualifications of social actors. Bourdieu (1991) explained that the social status or educational qualification, which is valid on all markets and which, as an official definition of one's official identity, "saves its bearers from the symbolic struggle of all against all, by establishing the authorized perspective, the one recognized by all, and thus universal from which social agents are viewed".

The professional title is a sort of legal rule of social perception, guaranteed as a right particularly in the Philippine culture which puts premium on educational attainment (Heathfield and Fusco 2016). It is a symbolic capital in an institutionalized, legal form (Bourdieu 1991). Professional titles have value in themselves and function like a great name, one which procures all sorts of symbolic profit (that one cannot directly acquire with money). Further, the text producers' usage or non-usage of a particular linguistic device is always motivated by their communicative goals, and these are encoded in the representation of themselves and of the other personalities in the discourse they produce. Arguably, linguistic decisions are always entwined on the speakers or writers' communicative intentions (Halliday and Matthiessen 2014).

Furthermore, the text producers' linguistic choices seem to be anchored on the conventions of the text genre imposed by specialists in the discourse community. Bhatia (1993) explained that various genres display "constraints on allowable contributions in terms of their intent, positioning, form, and personal value." That is, though the writers have freedom in producing a text, they still must conform to the acceptable standard imposed by the discourse community. This does not mean, however, that the text producers' positioning, ideological orientations, and communicative intentions say nothing about their linguistic choices.

In sum, social actors represented in mining news reports primarily consist of the key players in the mining industry such as the Philippine government, public officials (e.g. the President of the Republic, DENR officials), mining investors, mining companies, mine workers, and other stakeholders (e.g. environmentalists, church leaders). Seemingly, the more socially prominent a social actor is in the industry and beyond, the more he or she is provided spatio-temporal space in the news report. In contrast, the marginalized such as the mine workers or the small-scale investors tend to be highlighted only when unfortunate incidents occurred such as involvement in mining mishaps or illegal activities. In this respect, the society, as concretized by the media, has its system in stratifying its members and groups depending on their assumed or ascribed status. This social stratification may/can only be done by "those who have more" access to public resources (e.g. information), a play of power difference.

The findings also revealed the power of media in amplifying or silencing a certain type of discourse as seen in the reported statements by which the original speakers made use of impersonalization strategies that tend to downplay the doers of the actions or exclude them entirely, lessening the accountability of the actors in matters that are of public interests like involvement in graft or corruption or simple bribery. In this case, media practitioners should become more aware of this power to shape public opinions that eventually affect societal values such as normalizing falsehood and corruption. It is hoped that with this recognition, they will affirm their role not only as gatekeepers of truth but also as societal engineers that help in the reconstruction of society for humanity's welfare.

Finally, since the media can shape discourses (un)intentionally, the public should be aware that not everything they read or heard are meant to be accepted. They should learn to be more critical in accepting or rejecting certain realities and perspectives by verifying various sources of information.

When necessary, the national and local media should perform a complementary role in reporting societal events that are of greater interest to the public. They should also reaffirm their role in providing all actors, dominant or marginalized, space where they can be heard and represented for a fair and coherent construction of social realities.

ACKNOWLEDGMENTS

The author would like to express her sincerest gratitude and appreciation to the generosity of the Commission on Higher Education for the grant received for this research project. Likewise, gratitude is directed to the two inter-coders for their assistance in validating the analysis, and to the two anonymous reviewers for the valuable suggestions that greatly improved this paper.

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ARTICLE INFO:

Received: 23 February 2020
Revised: 16 March 2021 July 2021
Accepted: 30 July 2021
Available online: 24 August 2021

Phytochemical Screening, Macronutrient Content, Antimicrobial and Cytotoxic Properties of Selected Edible Plants consumed by the Palaw'an tribe in Bataraza, Palawan, Philippines

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https://doi.org/10.69721/TPS.J.2021.13.2.06

ABSTRACT

This study investigated three edible plants, namely, *Ardisia iwahigensis* Elmer, *Baccaurea gracilis* Merr., and *Manihot glaziovii* Müll.Arg consumed by the Palaw'an tribe in Bataraza, Palawan. Specifically, the phytochemical components, macronutrient contents, antimicrobial properties, and toxicity of the crude ethanol extracts of the fresh and/or air-dried leaves were determined. Qualitative phytochemical screening of fresh leaves and chemical profiling of air-dried leaves both revealed the presence of alkaloids, flavonoids, sterols, and tannins. Macronutrient analysis indicated that *B. gracilis* contained the highest crude protein (13.4% weight by weight) and crude fiber (6.65% w/w), while *M. glaziovii* contained the highest crude fat (0.807% w/w). Disc Diffusion Assay demonstrated significant ($P < 0.05$) antibacterial property against gram-positive (*Staphylococcus aureus* Ogston, 1880 and *Bacillus subtilis* Ehrenberg, 1835) and gram-negative (*Escherichia coli* Escherich, 1885 and *Pseudomonas aeruginosa* Schroeter, 1872) bacteria. The mean zones of inhibitions for *A. iwahigensis* against gram-positive (21.65–22.58 mm) and gram-negative (19.59–22.27 mm) bacteria were comparable with the positive controls (oxacillin 19.25–19.32 mm; Amikacin 16.52–27.32 mm). However, the three plants did not exhibit antifungal properties. Brine Shrimp Lethality Assay showed that *A. iwahigensis* was the most toxic with 100% mortality at 1000 ppm (LC_{50} = 4.270 ppm) after 24h exposure followed by *M. glaziovii* (97% mortality at 1000 ppm with LC_{50} = 7.918 ppm). The three edible plants are good sources of various phytochemicals that may have essential biological activities. This indicates that they can be used, not only as food ingredient, but also for therapeutic purposes and as potential sources of bioactive compounds with antibacterial and cytotoxic activities.

The Palawan Scientist, 13(2): 68-86

Keywords: chromatography, crude fat, crude fiber, crude protein, diffusion assay, lethality assay

INTRODUCTION

Natural products have a remarkable role in drug discovery and development due to their chemical novelties and diversity (Calixto 2019). From 1981 to 2019, there were 1,881 FDA-approved drugs derived from natural products (Newman and Cragg 2020). Although most people use conventional drugs and therapies, there are still some communities in remote rural areas, particularly in the Philippines, that rely on medicinal plants in treating common illnesses due to limited access to formal health care system (Dela Cruz and Ramos 2006).

More than 1,500 medicinal plants from over 12,000 plant species found in the Philippines are used by traditional healers (Dela Cruz and Ramos 2006) coming from 109 indigenous ethnic groups (Cariño 2012). However, there are limited ethnopharmacological studies on these ethnic groups, particularly in the province of Palawan which is home to several Indigenous peoples including the Palaw'an tribe (Cariño 2012). Palawan is also home to about 1,700-3,500 flowering plant species of which 15-20% are endemic to the province (Sopsop and Buot 2009) but only a small portion of these plants has been investigated in detail (Garcellano et al. 2019a, b).

In addition to their medicinal uses, plants are also utilized as food or food ingredients. There is an increasing research interest in the health benefits and possible clinical applications of food plants since the combination of nutrition and drug therapy may provide optimum defense against diseases. Investigations are being conducted on the potential of food plants as novel remedies to various diseases because these contain pharmacologically-active compounds (Ramalingum and Mahomoodally 2014). There is also a need to conduct toxicity tests, particularly in wild edible plants, since these contain antinutritional and toxic components in addition to their nutrient content (Guil et al. 1997).

Phytochemical screening of plants used by Indigenous people is considered an effective approach in discovering bioactive components with potential therapeutic applications. In this study, the authors investigated the edible plants used by the Palaw'an tribe. This group, in earlier times, were hunters and lived in upland areas near the forest where they get their food and other daily needs (Ethnic Group Philippines 2017). This study examined and documented the bioactive profile (phytochemical components, macronutrient contents, antimicrobial properties and toxicity) of three plants (*Ardisia iwahigensis* Elmer, *Baccaurea gracilis* Merr., and *Manihot glaziovii* Müll.Arg) used as food ingredient by the Palaw'an tribe in Bataraza, Palawan.

METHODS

Plant Materials

The plant samples for this study, *Ardisia iwahigensis* (fam. Primulaceae), *Baccaurea gracilis* (fam. Phyllanthaceae), and *Manihot glaziovii* (fam. Euphorbiaceae) (Figure 1) are known to the Palaw'an tribe as "Tambilikan", "Duro-manok", and "Sapikol", respectively. *Ardisia iwahigensis* is endemic to Palawan while both *B. gracilis* and *M. glaziovii* have a more widespread distribution (Pelser et al. 2011). The fresh mature leaves of *A. iwahigensis* and young leaves of *B. gracilis* and *M. glaziovii* are used as food ingredients by the Palaw'an tribe in Sitio Budis-Budis, Bataraza, Palawan. In this study, about three kilograms (3 kg) of fresh leaves from each plant were collected in Sitio Budis-Budis.

Figure 1. The three edible plants consumed by the Palaw'an tribe in Sitio Budis-Budis, Brgy. Tarusan, Bataraza, Palawan. A,B: *Ardisia iwahigensis* (Tambilikan); C,D: *Baccaurea gracilis* (Duro-manok); E,F: *Manihot glaziovii* (Sapikol).

Phytochemical Screening, Macronutrient Content Determination, and Disc Diffusion Assay of Fresh Leaves of the Three Edible Plants

Three hundred grams of fresh leaves of each plant were submitted to Department of Science in Technology – Mindoro Marinduque Romblon Palawan Regional Standards and Testing laboratory (DOST-MIMAROPA RSTL) in Puerto Princesa City. The samples were subjected to ethanol extraction and the crude extracts were then sent to the Department of Science and Technology – Industrial Technology Development Institute–Standards and Testing Division (DOST-ITDI-STD) in Bicutan, Taguig City, Philippines for phytochemical screening, macronutrient content determination, and disc diffusion assay (Figure 2).

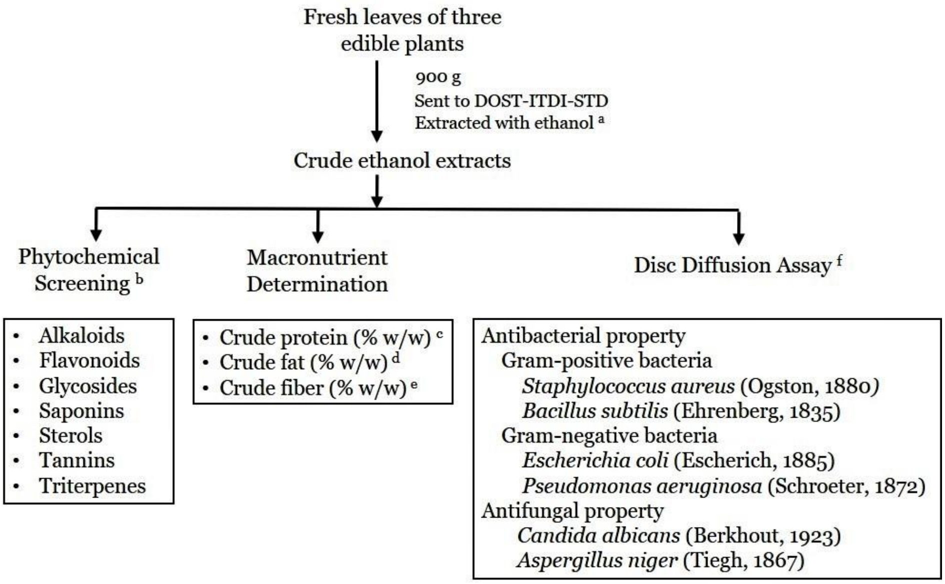

Figure 2. Schematic diagram for the tests conducted on the fresh leaves of *Ardisia iwahigensis*, *Baccaurea gracilis*, and *Manihot glaziovii* at DOST-ITDI-STD. Reference methods: [a] BTD Manual Qualitative; [b] Evans 2002; [c] Block digestion-Kjeldahl (*A. iwahigensis* and *M. glaziovii*) and Combustion (*B. gracilis*) methods; [d] Direct ether extraction; e962.09 AOAC official method; [f] USP30-NF25 2007.

At DOST-MIMAROPA RSTL, 200 g of finely cut fresh plant materials were soaked in 300 ml of 95% ethyl alcohol for 24 h and then filtered using filter paper. The flask and plant material were rinsed with fresh alcohol and then combined with the first filtrate. The residue was discarded. The filtrate was concentrated over a water bath at 40-60°C to about 20 ml and then stored in a desiccator.

At DOST-ITDI-STD, the ethanol leaf extract of the three edible plants were screened qualitatively to determine the presence of phytochemicals while various methods were utilized to determine the macronutrients present (Figure 2).

Disc Diffusion Assay was also conducted to assess the antibacterial and antifungal properties (Figure 2) in terms of reactivity based on the mean zones of inhibition (MZI) of bacterial growth (Table 1). The data were subjected to one-way analysis of variance (ANOVA) and Kruskal-Wallis Test (using SPSS and MegaStat software) to determine the level of significant difference ($P < 0.05$) among the three edible plants against each bacterial strain.

Table 1. Reactivity grades based on mean zone of inhibition (MZI) (USP30-NF25 2007).

Grade	Reactivity	MZI
0	none	No detectable zone around or under specimen
1	slight	Malformed or degenerated cells under the specimen
2	mild	Zone limited under the specimen
3	moderate	Zone extends 5-10 mm beyond specimen
4	severe	Zone extends greater than 10 mm beyond specimen

Chemical Profiling and Brine Shrimp Lethality Assay (BSLA) of Air-dried Leaves of the Three Edible Plants

Plant extraction. Two kilograms each of fresh leaves were air-dried for a month then ground to a fine powder (20 g) and soaked in a 95% ethanol solution (250 ml) for 72 h. The mixture was filtered and simple distillation was conducted to recover the solvent and obtain the crude extracts which were then subjected to chemical profiling and Brine shrimp lethality assay (BSLA) (Figure 3).

Chemical profiling. The crude ethanol extracts were separated on silica gel thin layer aluminum plates. Solvent systems of different polarities were prepared to best separate the components of the ethanol extract of air-dried leaves. Extracts were spotted manually using capillary tubes on pre-coated plates and developed in a glass chamber using different solvent systems namely, petroleum ether:methanol, petroleum ether:ethanol, petroleum ether:acetone, and petroleum ether:chloroform. The developed chromatograms were observed under ultraviolet light (long wavelength) and visible light, then dipped in various visualizing agents such as Dragendorff, Bornträger, potassium ferricyanide-ferric chloride, and acetic anhydride-sulfuric acid. Suitable color of the spots and zones were noted as an observable result for a positive test. The retention factor (Rf) value of the spots was calculated by dividing the distance traveled by the solute (mm) over the distance traveled by the solvent (mm).

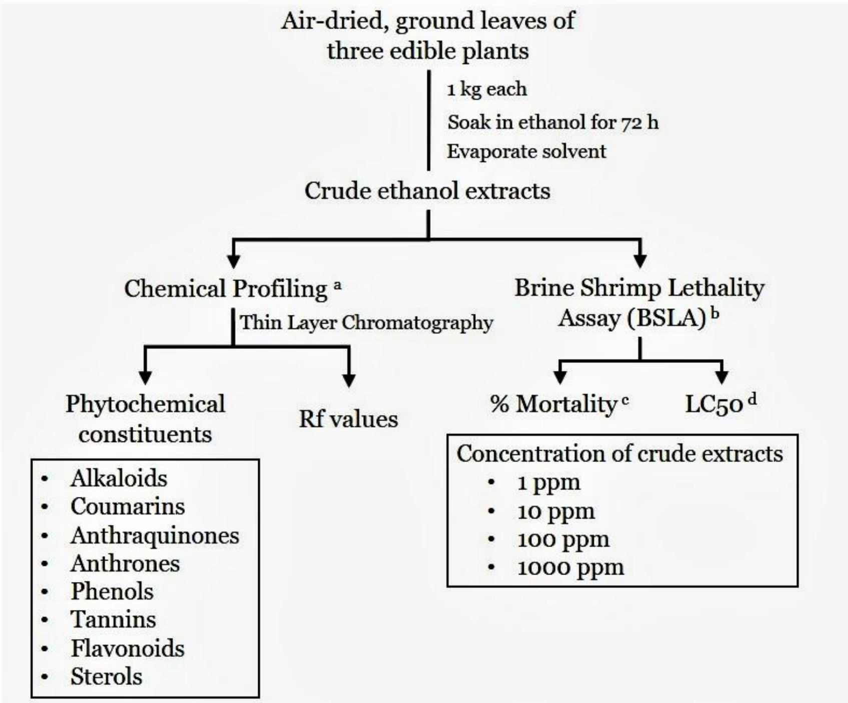

Figure 3. Schematic diagram for the tests conducted on air-dried leaves of *Ardisia iwahigensis, Baccaurea gracilis*, and *Manihot glaziovii*. Reference methods: [a] Guevara et al. 2005; [b] Sarah et al. 2017; [c] Meyer et al. 1982; [d] Mekapogu 2016.

Brine shrimp lethality assay (BSLA). This assay has been widely used to screen the toxicity of plant extracts and as a bioassay guide for cytotoxic and antitumor agents (Sarah et al. 2017). A small tank was filled with filtered and sterilized seawater, fully aerated, and divided into two compartments interconnected with holes: darkened and illuminated area. Brine shrimp eggs (*Artemia salina* L.) were placed on the darkened compartment. The hatched brine shrimp nauplii then migrated into the illuminated compartment after 48 h of incubation to reach their mature state (Figure 4A). The illumination is important to simulate the optimum temperature (30°C) of their natural habitat.

Twenty (20) mg each of crude extracts was dissolved in 3 ml of 95% ethanol to prepare 10,000 parts per million (ppm) stock solution. This was then used to prepare four concentrations - 1000 ppm, 100 ppm, 10 ppm, 1 ppm - through serial dilution (Figure 3). Triplicates were prepared for each concentration and placed in separate Eppendorf tubes (Figure 4B-D). The volume of each sample was adjusted to 5 ml by adding artificial seawater. Negative controls were used containing artificial seawater and 0.5 ml of 95% ethanol. Ten brine shrimp nauplii were collected with a pipette, added to each vial, and left uncovered under the lamp. The number of brine shrimp survivors was observed, counted, and recorded after 24 h.

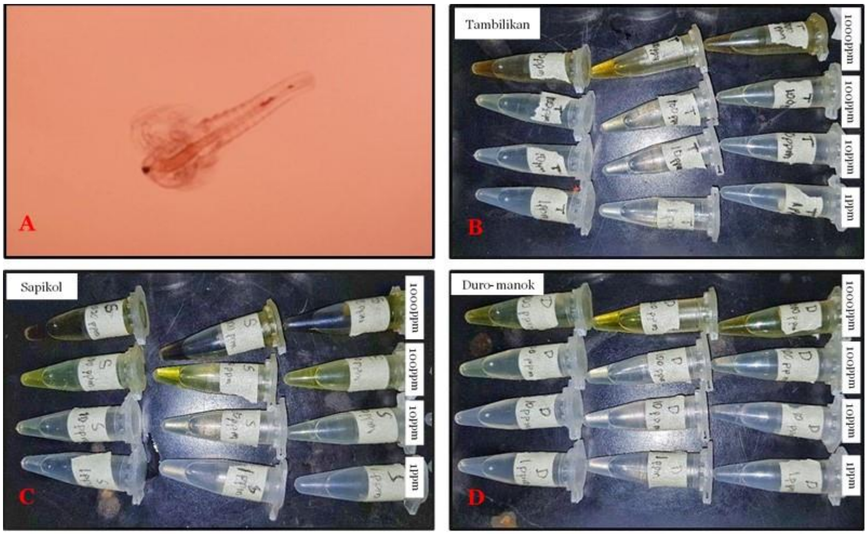

Figure 4. A: Brine shrimp under the dissecting microscope. Eppendorf tubes for Brine Shrimp Lethality Assay containing the edible plants' crude extracts in four concentrations (1 ppm, 10 ppm, 100 ppm, 1000 ppm). B: Tambilikan (*Ardisia iwahigensis*); C: Sapikol (*Baccaurea gracilis*); D: Duro-manok (*Manihot glaziovii*).

Toxicity Analysis and Testing Criteria

A calculator developed by Mekapogu (2016) based on Finney's (1952) probit analysis method was used to compute the Lethal Concentration on 50% of the population (LC_{50}). The relative toxicity of the extracts to the living organisms were then determined (Table 2). The percentage mortality was calculated by dividing the number of dead nauplii by the total number, and then multiplying by 100.

Table 2. Stephan's (1977) toxicity assessment of plant extracts.

LC_{50} (ppm or μgml^{-1}) concentration	Toxicity profile
<100 ppm	potent (active)
<1000 ppm	toxic
>1000 ppm	non-toxic

RESULTS

Phytochemical Screening

The fresh leaves of the three edible plants contained six to seven kinds of phytochemicals. Flavonoids and tannins were abundant in the fresh leaves *M. glaziovii* while *B. gracilis* was rich in saponins (Table 3).

Table 3. Phytochemical constituents present in the fresh leaves of three edible plants. Note: (+) Traces, (++) Moderate, (+++) Abundant, and (-) Absence

Constituents	*Ardisia iwahigensis*	*Baccaurea gracilis*	*Manihot glaziovii*
alkaloids	(+)	(+)	(+)
flavonoids	(+)	(+)	(+++)
glycosides	(+)	(+)	(+)
saponins	(-)	(+++)	(+)
sterols	(+)	(++)	(++)
tannins	(++)	(+)	(+++)
triterpenes	(+)	(-)	(+)

Macronutrient Content

All macronutrients tested were present in fresh leaves of the three plants with concentrations ranging from 0.125 to 7.34%. *Baccaurea gracilis* had the highest crude protein and crude fiber contents while *M. glaziovii* showed the highest crude fat content (Table 4).

Table 4. Macronutrient content in fresh leaves of the three edible plants in % weight by weight.

Macronutrient (% weight by weight)	*Ardisia iwahigensis (%)*	*Baccaurea gracilis (%)*	*Manihot glaziovii* (%)
crude protein	2.75	13.40	7.34
crude fat	0.13	0.37	0.81
crude fiber	2.79	6.65	3.27

Antibacterial Property

Among the three plant species, *A. iwagensis* had the highest MZI for all bacterial strains tested and were significantly different when compared to the positive controls. Both *B. gracilis* and *M. gracilis* had similar MZI for the bacterial strains tested (Table 5).

Antifungal Property

The three edible plants did not show antifungal property against *C. albicans* and *A. niger* (Table 6).

Chemical Profile

The air-dried leaves of the three plants showed eight phytochemicals with Rf values ranging from 0.02 to 0.96 (Table 7). Four to five phytochemicals were observed in petroleum ether:methanol solvent systems while only one phytochemical was detected in both petroleum ether:acetone and petroleum ether:chloroform (7:3) solvent systems

Level of Toxicity in Terms of LC_{50} and Percent Mortality

The LC_{50} for *A. iwahigensis* was the highest at 4.270 ppm. At this concentration, the extract caused the death of half of the nauplii population. Maximum mortality (100%) was observed in *A. iwahigensis* at 1000 ppm whereas *B. gracilis* and *M. glaziovii*, in any concentration, did not give 100% mortality. Moreover, in the negative controls used, all the nauplii survived after 24 h indicating that the mortalities were entirely caused by the crude extracts of the three edible plants (Table 8).

DISCUSSION

Phytochemical Screening and Chemical Profiling

Qualitative phytochemical screening and chemical profiling of the fresh and air-dried leaves of *A. iwahigensis, B. gracilis*, and *M. glaziovii* showed the presence of phytochemicals like alkaloids, flavonoids, sterols, and tannins. Phytochemicals determine the biological activities of plants and its significance in traditional medicine, and their presence can be associated with the potential of plant as a source of phytomedicines (Lawal et al. 2019).

Table 5. Antibacterial activity of the three edible plants against ***Staphylococcus aureus*** (SA), ***Bacillus subtilis*** (BS), ***Escherichia coli (EC)***, ***Pseudomonas aeruginosa*** (PA); MZI, mean zone of inhibition; *Oxacillin; **Amikacin. Note: When ***P*** < 0.05 – statistically significant difference (Kruskal-Wallis Test).

Bacterial strain	*Ardisia iwahigensis*		*Baccaurea gracillis*		*Manihot glaziovii*		Positive controls		***P*-value**
	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity	
SA	21.65	4	10.00	2	10.00	2	*19.25	*4	0.0138
BS	22.58	4	13.55	2	10.00	2	*19.32	*4	0.0145
EC	19.59	3	10.00	2	10.00	2	**16.52	**4	0.0138
PA	22.27	4	10.00	2	10.00	2	**27.32	**4	0.0135

Table 6. Antifungal activity of the three edible plants against ***Candida albicans*** and ***Aspergillus niger*** . MZI, mean zone of inhibition; Reactivity rating: 3 – Moderate.

Fungal strain	*Ardisia iwahigensis*		*Baccaurea gracilis*		*Manihot glaziovii*		Positive control Oxacillin (1 µg)	
	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity	MZI (mm)	Reactivity
Candida albicans	0.00	0	0.00	0	0.00	0	16.31	3
Aspergillus niger	0.00	0	0.00	0	0.00	0	19.91	3

Table 7. Chemical profile of air-dried leaves of the three edible plants. Note: PE, petroleum ether; MeOH, methanol; EtOH, ethanol; Result: (+) present; (-) absent; Rf, retention factor.

Solvent System	Reagent	Constituents	*Aridisia iwahigensis*		*Baccaurea gracilis*		*Manihot glaziovii*	
			Result	Rf values	Result	Rf values	Result	Rf values
PE:MeOH 9:1	Dragendorff	Alkaloids	(+)	0.38	(+)	0.56	(+)	0.40
	Bornträger	Coumarins	(+)	0.67	(+)	0.6	(+)	0.10
		Anthraquinones	(+)	0.57	(+)	0.46	(+)	0.54
		Anthrones	(+)	0.07	(+)	0.54	(+)	0.02
PE:MeOH 7:3	Dragendorff	Alkaloids	(+)	0.52	(+)	0.96	(+)	0.60
	Bornträger	Coumarins	(+)	0.50	(+)	0.02	(+)	0.30
		Anthraquinones	(+)	0.60	(+)	0.56	(+)	0.60
		Anthrones	(+)	0.40	(+)	0.02	(+)	0.40
PE:EtOH 7:3	Potassium ferricyanide-ferric chloride	Phenols Tannins Flavonoids	(+)	0.19	(+)	0.07	(+)	0.17
PE: Acetone 3:2	Acetic anhydride-sulfuric acid	Sterols	(+)	0.04	(+)	0.04	(+)	0.08
PE: $CHCl_3$ 9:1	Potassium ferricyanide-ferric chloride	Phenols Tannins Flavonoids	(+)	0.64	(+)	0.52	(+)	0.46
PE: $CHCl_3$ 7:3	Dragendorff	Alkaloids	(+)	0.10	(+)	0.02	(+)	0.02

Table 8. Results of brine shrimp lethality assay for the three edible plants.

Plant sample	Concentration (ppm)	Number of surviving nauplii after 24 h			Total number of survivors	Total number of dead nauplii	% Mortality	LC_{50} (ppm)
		T1	T2	T3				
Ardisia iwahigensis	1	7	6	6	19	11	37	4.270
	10	4	4	3	11	19	63	
	100	3	4	3	10	20	67	
	1000	0	0	0	0	30	100	
Baccaurea gracilis	1	8	8	9	25	5	17	48.606
	10	6	6	6	18	12	40	
	100	6	5	6	17	13	43	
	1000	1	3	1	5	25	83	
Manihot glaziovii	1	7	6	7	20	10	33	7.918
	10	5	4	4	13	17	57	
	100	6	4	3	13	17	57	
	1000	0	0	1	1	29	97	
Negative Control								
Artificial Sea Water		10	10	10	30	0	0	
0.5 mL 95% Ethanol		10	10	10	30	0	0	

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Ardisia plants are used as traditional medicine in the treatment of various ailments including cancer, heart diseases, liver poisoning, diarrhea, dysmenorrhea, gout, and mental disorder (Pournami and Pratap Chandran 2021) and have been reported to exhibit antituberculosis, antioxidant, cytotoxic, and thrombolytic activities (Khatun et al. 2013). These medicinal uses and activities may be attributed to the presence of alkaloids, steroids, flavonoids, phenols, tannins, and triterpenes reported in *Ardisia* species (Khatun et al. 2013; Amin et al. 2015). These constituents were also observed in *A. iwahigensis* used in this study.

Baccaurea species are reported to display various pharmacological activities such as analgesic, anticancer, antidiarrheal, antimicrobial, and antioxidant activities which may be ascribed to the phytochemicals abundant in this genus like alkaloids, flavonoids, saponins, sterols, and tannins (Charu et al. 2021). These phytochemicals were also present in *B. gracilis* used in this study.

An earlier study on *M. glaziovii* reported that the leaves of this plant is rich in alkaloids, saponins, and tannins (Nduche et al. 2018). These phytochemicals were also observed in the present study, in addition to flavonoids, glycosides, saponins, sterols, and anthraquinones which in turn were reported in its congener, *Manihot esculenta* (Crantz) (Ebuehi et al. 2005).

Moreover, chemical profiling showed that the phytochemicals present in the three plants have wide range of polarities as indicated by their Rf values. A high Rf value indicates that the component is less polar, while a lower Rf value means the component is more polar (Bele and Khale 2011). Sterols are very polar, anthraquinones are semi-polar, while the other phytochemicals present ranges from very polar to non-polar.

Macronutrient Content

This study provides the first report on the macronutrient content of the three edible plants. The leaves of *Ardisia* species, *Ardisia solanacea* (Poir.) Roxb., was reported for its nutrient content which includes protein (31.25%), fat (0.00067%) and fiber (6.6%) and was recommended as food additive for livestock (Pratap Chandran 2015). This species has higher protein and fiber contents than *A. iwahigensis*. Conversely, there was no study found on the nutrient content of the leaves of *Baccaurea* species but the fruit of one congener, *Baccaurea sapida* (Roxb.) Müll.Arg., was found to contain protein (5.43%), fat (1.24%) and fiber (3.60%) (Pandey et al. 2018). This species has lower protein and fiber contents than *B. gracilis*. The most studied *Manihot* species in terms of nutrient content was *M. esculenta* (cassava), a staple food in many countries. Its leaves contain much higher protein (28.02%), fat (5.63%), and fiber (21.41%) (Idris et al. 2020) than *M. glaziovii*.

In addition to their macronutrient nutrient, the phytochemicals present in these plants can provide both pharmacological and health benefits when consumed as food (Demir and Akpinar 2020). For instance, the phytochemicals present in *M. esculenta* are attributed to the use of this plant in the treatment of various ailments like allergies, bone problems, celiac disease and diabetes (Zekarias et al. 2019). *Baccaurea angulata* (Merr.) was also reported as a potential functional food and exhibits effective anti-inflammatory, antioxidant, and cholesterol-lowering activities (Erwin et al. 2018).

Antibacterial and Antifungal Properties

This study provides the first report on antimicrobial test conducted on the three edible plants. *Ardisia iwahigensis* exhibited significant antibacterial property with higher MZI than the standards used, more so than the other two plants, against *S. aureus, B. subtilis* and *E. coli*. This result is of great value particularly in the case of *S. aureus*, which is known for its resistance to several antibiotics and for its ability to cause septicemia by secreting various types of enterotoxins (Al-abd et al. 2017). This antibacterial activity shown by *A. iwahigensis* infers that it could be explored as source of bioactive compounds that can be utilized against bacterial resistance. Another congener, *Ardisia elliptica* Thunb., also showed significant antibacterial activity against *S. aureus* and *E. coli*, among others. The antibacterial property could be attributed to the flavonoids and phenolics present in the leaf extracts (Al-abd et al. 2017).

The three edible plants did not exhibit antifungal property. This observed inactivity may be due to ochratoxin, a mycotoxin produced by *Aspergillus* spp. and other fungi which confers resistance to antifungal drugs. In addition, *C. albicans* is known to exhibit resistance against most antimicrobial drugs (Adeonipekun et al. 2014). However, one species of *Ardisia*, *A. solanacea***,** was reported to be effective against *C. albicans* and *A. niger* (Amin et al. 2015). One species of *Manihot*, *Manihot multifida* (L.) Crantz**,** was also reported effective against *C. albicans* (Fabri et al. 2014). However, no report was found on the leaves *Baccaurea* species being effective against the two fungal strains used.

Toxicity

A previous study on the leaves and twigs of *A. iwahigensis* reported that it exhibited ≥50% immobilization of brine shrimp at 100 ppm and displayed an IC_{50} ≤ 20 ppm against various cancer cell lines (Horgen et al. 2001). This toxicity of *A. iwahigensis* against brine shrimp is in agreement with the results obtained from the present study. The leaves of another *Ardisia* species, *Ardisia humilis* Vahl., also displayed toxicity (LC_{50} at 2.26 ppm) against brine shrimp (Khatun 2013). These toxicity results support the traditional use of *Ardisia* plants in the treatment of cancer (Horgen et al. 2001).

As per the findings of this study, the leaves of *A. iwahigensis, B. gracilis, and M. glaziovii* are strong sources of different phytochemicals that may have essential biological activities, in addition to their use as food or food ingredient. Moreover, *A. iwahigensis* exhibited significant inhibitory property against gram-positive (*S. aureus* and *B. subtilis*) and gram-negative (*E. coli* and *P. aeruginosa*) bacteria comparable with commercial antibiotics and it also displayed high mortality against brine shrimp nauplii which may imply that it contains bioactive components that may be utilized as antibacterial and cytotoxic agents.

It is recommended that future studies be conducted to isolate the components responsible for the observed antibacterial and toxic properties in *A. iwahigensis*. Further toxicity tests should also be conducted on the three plants to ascertain their safety when taken orally, either as food or as medicine.

ACKNOWLEDGMENTS

We would like to acknowledge the Philippine Council for Health Research and Development, under the Department of Science and Technology, for the financial assistance and support through the Undergraduate Thesis Grant in Natural Products; Assistant Professor Elizabeth P. Geronilla, the curator at the Palawan State University Herbarium, for the identification and authentication of the three plants used in this study; and the two anonymous reviewers, for their comments and suggestions which greatly improved this paper.

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ARTICLE INFO:

Received: 14 Julyl 2020
Revised: 26 July 2021
Accepted: 17 August 2021
Available online: 16 November 2021

Role of authors: NELA, EJPP, MJUN, MRFPN – conceptualization, data collection, conduct of the study and manuscript writing; RCG – conceptualation, fund sourcing, supervision and manuscript writing.

Essential elements in *Etlingera elatior* (Jack) R. M. Sm. and *Etlingera philippinensis* (Ridl.) R. M. Sm.

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https://doi.org/10.69721/TPS.J.2021.13.2.07

ABSTRACT

Despite the advancements contributed to botanical research, scientific attention on many Zingiberaceae plants in spite of their numerous health-promoting applications is still few. Existing reports mostly focus on the common species of Zingiberaceae specifically on the rhizomes, with less emphasis on Philippine endemic gingers such as *Etlingera philippinensis* (Ridl.) R. M. Sm. In this study, the concentration of essential elements (Fe, Cu, Zn, Ni) in *Etlingera elatior* (Jack) R. M. Sm. and *E. philippinensis* leaves and rhizomes were determined using atomic absorption spectroscopy. Among the essential elements, Cu was found highest in *E. elatior* leaves and *E. philippinensis* rhizomes. However, the identified levels of Fe, Cu, Zn, and Ni for these two zingiberaceaes indicate that all are below the permissible limit set by World Health Organization (WHO) for plants. Thus, this study ventures in the exploration of the baseline information on the essential element content of the less studied Philippine endemic *E. philippinensis*.

Keywords: leaves, metal content, rhizomes, Zingiberaceae

INTRODUCTION

Zingiberaceae plants, known as gingers, have been popular for medicinal and culinary uses since time immemorial. Zingiberaceae family with 53 genera and over 1,200 species is known as the largest family of the order Zingiberales (Mahdavi et al. 2017). *Etlingera* is a genus belonging to the Zingiberaceae family, which is native to the Indo-Pacific region. This genus consists of more than 100 species that grow from sea level to the altitude of 2,500 m (Vairappan et al. 2012).

The plant torch ginger or scientifically known as *Etlingera elatior* (Jack) R. M. Sm., abundantly grows in Southeast Asia (Krajarng et al. 2017). The inflorescences of *E. elatior*, characterized to have a unique flavor and aroma, are traditionally used for medicinal and culinary purposes such as in traditional dishes like "Ulam" and "Asam laksa" in Malaysia. It has been further reported that the daily intake of raw inflorescence could reduce diabetes and hypertension. Furthermore, when these are taken with bitter leaves *Vernonia amygdalina* Del (Asteraceae), it is said to relieve flatulence in postpartum women (Wijekoon et al. 2011).

Etlingera elatior contains a high amount of total phenolics, flavonoids, and vitamin C contents (Rachkeeree et al. 2018; Sungthong et al. 2018). Both leaves and rhizome of *E. elatior* exhibit antibacterial, antioxidant, antiproliferative, and apoptotic activities (Juwita et al. 2018). The inflorescence of *E. elatior* contains potassium, calcium, magnesium, phosphorus, and sulfur (Wijekoon et al. 2011). *Etlingera elatior* possesses essential oils which could be potentially used as a new source of natural antioxidant and antibacterial in the pharmaceutical and food industries (Abdelwahab et al. 2010). *Etlingera elatior* is found to contain appreciable levels of total phenolic and total flavonoid contents. GC-MS studies resulted in the identification of 73 compounds in the plant. Accordingly, the most abundant components of this plant include β-pinene (24.92%) and 1-dodecene (24.31%).

A certain species of ginger which is endemic in the Philippines is *Etlingera philippinensis*. (Ridl.) R. M. Sm. with basionyms such as *Hornstedtia philippinensis* Ridl., *Amomum philippinense* (Ridl.) Merr., *Achasma philippinensis* (Ridl.) B.L. Burtt and R.M. Sm. (Newman et al. 2004). *Etlingera philippinensis* reaches a height of 2–2.5 m tall. Its rhizomes are long and creep along the soil, and have cone-shaped inflorescence (with 7-12 flowers) which either grow distantly from the main rhizome or are partially buried in the soil (Mendez et al. 2017). This plant has a dominant reddish color in its entire inflorescence and the flowers do not bloom altogether at the same time (Mendez et al. 2017). High 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were also observed in the water extracts of *E. philippinensis*, and the plant's extract contained alkaloids, flavonoids, saponins, tannins and steroids (Barbosa et al. 2016). Its leaves contain chlorogenic acid (Barbosa et al. 2017). Furthermore, this species showed high antioxidant activity and total phenolic content (Mabini and Barbosa 2018).

Having identified the aforementioned elements found in the *E. philippinensis* component is not that surprising since medicinal and some spice plants normally contain essential and non-essential metals (Wagesho and Chandravanshi 2015). However, having deeper understanding on the essential element contents of plants could aid in the development of mineral biofortification. Thus, this study aims to determine the levels of essential metals (copper, Cu; iron, Fe; zinc, Zn; and nickel Ni) found in the leaves and rhizomes of *E. elatior* and *E. philippinensis* and in the soil source where the plants were grown.

METHODS

Plant and Soil Materials Collection

Leaves and rhizomes of *E. elatior* (Figure 1A, B) were collected from Purok 16 of Musuan, Maramag, Bukidnon (7°46’49”N and 125°03’29”E) while leaves and rhizomes of *E. philippinensis* (Figure 1C, D) were collected from Gutapol, Kibawe, Bukidnon (7°29’15”N and 125°03’38”E).

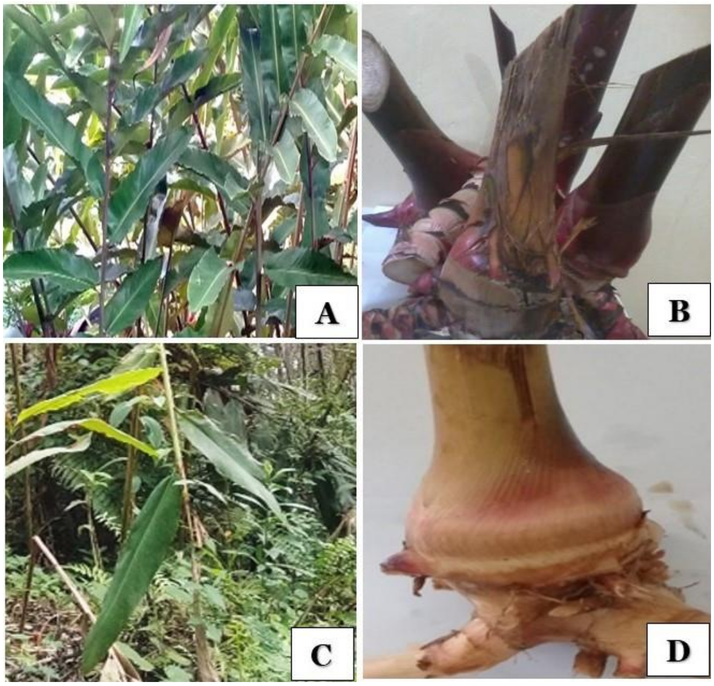

Figure 1. *Etlingera elatior* (Jack) R. M. Sm. (A) leaves and (B) rhizomes and *Etlingera philippinensis* (Ridl.) R. M. Sm. (C) leaves and (D) rhizomes.

Soil samples were collected with slight modification based on the method described by NRCS (2007). Six representative soil samples, with equal amounts of about a standard shovel (roughly ~1 kg), were collected in a zig-zag manner from the source field of the plant samples. Each sampling depth was 60.96 cm deep. Each of the collected representative soil samples was placed in a polyethylene bag, mixed, and air dried. The dried soil samples were then thoroughly mixed. Finally, the 1 kg dried soil sample was then sent for metal analysis.

The collected plant samples were also placed in separate clean plastic bags and were transported to Central Mindanao University (CMU), University Town, Musuan, Bukidnon.

Sample Preparation

The collected leaf samples were thoroughly washed with tap water, then rinsed with distilled water, and were air-dried for three to four weeks under the shade at ambient temperature. In the same manner, the rhizomes were thoroughly washed with tap water followed by distilled water, and then peeled, grated, and lastly, air-dried. Finally, the air-dried samples were separately powdered using a food processor and were securely stored in correctly labelled ziploc bags.

Meanwhile, foreign debris from the collected soil samples were removed. This was followed by the soil being pulverized and then were air-dried for three weeks by spreading thinly on a clean surface. Once dried, the soil samples were sifted in a 2 mm sieve and were stored in properly labelled ziploc bags.

Digestion/Ashing and Metal Analyses of Samples

Soil samples for analysis of Fe, Cu and Zn. Soil samples (12.50 g) were placed into a 50 ml centrifuge tube, added with 25 ml Diethylene triamine pentaacetic acid (DTPA), and then were shaken for 1 h. The mixtures were filtered and read in an atomic absorption spectrophotometer (AAS-Agilent 280 Fast Sequential) (Biddle 1997).

Soil samples for analysis of Ni. One gram sample for digestion was placed in a 250 ml flask. The sample was then heated to 95°C with 10 ml of 50% HNO_3 without boiling, cooled, and was refluxed with repeated additions of 65% HNO_3 until no brown fumes were given off. The solution evaporated until the volume was reduced to 5 ml, cooled, and was gradually added with 10 ml of 30% H_2O_2. The mixture was refluxed with 10 ml of 37% HCl at 95°C for 15 min. The digestate obtained was filtered through a 0.45 µm membrane paper. The filtrate diluted to 100 ml with deionized water and was stored at 4°C for analyses (USEPA 1996). The same procedure was conducted to the other soil samples.

Plant samples for analysis of Fe, Cu and Zn. One gram of sample was placed in a crucible and heated in a furnace at 500°C for 5 h, cooled, was added with 5 ml of 6N HCl and left to stand for 1 h. The mixture was filtered and diluted to 50 ml of distilled water. The same procedure was conducted to the other plant samples.

Metal analyses for the plant samples were done by using 1 ml aliquot of plant digest solutions into 50 ml centrifuge tubes (Nalgene type) using 1 ml volumetric pipette, adding deionized water to dilute to a final volume of 50 ml and reading the mixture in an AAS (Agilent 280 FS) (Biddle 1997).

Plant samples for analysis of Ni. One gram of sample was placed in a beaker and treated with a 10 ml of concentrated HNO_3. The mixture was then heated for 45 min at temperature of 90°C. The temperature was then gradually increased to 150°C and the sample was boiled for a minimum of 8 h to obtain a clear solution. Following this step, 5 ml of concentrated HNO_3 was added into the sample at least three times and plant digestion was continued until the volume was reduced to about 1 ml. Afterwards, distilled water was used to wash down the inner walls of the tube to keep it clean and the tube was swirled throughout the digestion process to prevent loss of sample. The sample placed inside the digestion tube was allowed to cool down, then was additionally treated with 5 ml of 1% HNO_3, and then was filtered through using Whatman filter paper No. 42 and Millipore filter paper (<0.45 µm). The solution was then transferred quantitatively to a 100 ml volumetric flask by adding distilled water (Zarcinas et al. 1987; Zheljazkov and Nielson 1996). Finally, the metal analyses were done by using Agilent 4200 Microwave Plasma Atomic Emission Spectrophotometer (MP-AES). The same steps were followed for each of the plant samples.

Statistical Analysis

An Analysis of variance (ANOVA) using SPSS version 24 was done to determine the differences in the metal content of the plants' leaves and rhizome and in the soil where the plants grew. Also, a post hoc analysis was done using Tukey's Test.

RESULTS

Variable amounts of essential trace metals (Fe, Zn, Cu and Ni) were observed among the plant and soil samples. For *E. elatior* that the concentration values in plant parts and soil ranged from 0.297 to 20.849 mg kg^{-1} for Fe, 1.687 to 8.317 mg kg^{-1} for Zn, below detection limit to 9.682 mg kg^{-1} for Cu, and below detection limit to 4.607 mg kg^{-1} for Ni (Table 1).

Zinc level was highest in *E. elatior* soil (8.317 mg kg^{-1}) followed by *E. elatior* rhizome (6.493 mg kg^{-1}) and *E. elatior* leaves (1.687 mg kg^{-1}). Cu content in *E. elatior* was significantly higher in leaves (9.862 mg kg^{-1}) than in soil (7.193 mg kg^{-1}). Copper content in *E. elatior* rhizomes was below in instrument detection limit. The rhizomes (4.607 mg kg^{-1}) of *E. elatior* contained higher level of Ni than the leaves (1.726 mg kg^{-1}).

Table 1. Metal concentrations in the leaves and rhizomes of *Etlingera elatior* and the soil where the plant was grown as determined using atomic absorption spectrophotometer. The superscript indicators of the same letters within a column implies that the sample results are not significantly different from each other; BDL indicates below detection limit.

Sample	Concentration, mg kg^{-1}			
	Fe	Zn	Cu	Ni
E. elatior leaves	1.001 ^{b}	1.687 ^{c}	9.682 ^{a}	1.726 ^{b}
E. elatior rhizome	0.297 ^{c}	6.493 ^{b}	BDL	4.607 ^{a}
E. elatior Soil	20.849 ^{a}	8.317 ^{a}	7.193 ^{b}	BDL

Analysis of variance (ANOVA) and subsequent Tukey's test revealed that *E. elatior* leaves contain significantly higher Fe content (1.001 mg kg^{-1}) than its rhizomes (0.297 mg kg^{-1}). Fe content in soil (20.849 mg kg^{-1}) was significantly higher than that in the *E. elatior* leaves and rhizomes.

The essential elements concentration values in *E. philippinensis* parts and soil ranged from 1.136 to 17.421 mg kg^{-1} for Fe, 0.727 to 6.067 mg kg^{-1} for Zn, 1.217 to 9.659 mg kg^{-1} for Cu, and below detection limit to 4.758 mg kg^{-1} for Ni (Table 2).

Table 2. Metal centrations found in the leaves, the rhizomes of *Etlingera philipinensis,* and the soil where the plant was grown as determined using an atomic absorption spectrophotometer. Superscript indicators of the same letters within a column implies that the results are not significantly different with each other. Those indicated with BDL means concentrations are below detection limit.

Sample	Concentration, mg kg^{-1}			
	Fe	Zn	Cu	Ni
E. philippinensis leaves	1.136 ^{b}	0.727 ^{b}	2.614 ^{b}	4.686 ^{a}
E. philippinensis rhizome	1.326 ^{b}	6.067 ^{a}	9.659 ^{a}	4.758 ^{a}
E. philippinensis Soil	17.421 ^{a}	0.840 ^{b}	1.217 ^{c}	BDL

In *E. philippinensis*, Zn level results show that it is significantly higher in rhizomes (6.067 mg kg^{-1}) than in the leaves (0.727 mg kg^{-1}) and that of the soil (0.840 mg kg^{-1}) (Tables 2). Moreover, Cu content is significantly highest in the rhizomes (9.659 mg kg^{-1}) followed by those in the leaves (2.614 mg kg^{-1}) and lastly in the soil (1.217 mg kg^{-1}). Meanwhile, the leaves and rhizomes of *E. philippinensis* contain similar levels of Ni.

The ANOVA results revealed that *E. philippinensis'* Fe content is statistically comparable with the rhizomes (1.326 mg kg^{-1}) and the leaves (1.136 mg kg^{-1}) but is significantly lower than in the soil (17.421 mg kg^{-1}). Fe content has a lower value in both leaves and rhizome samples of *E. philippinensis*. However, there is high Fe presence in the soil where the *E. philippinensis* samples were grown.

DISCUSSION

Rhizomes of selected ginger species such as *Alpinia officinarum*, *Alpinia galangal*, *Alpinia zerumbet*, *Alpinias calcarata*, and *Kaempferia galangal* are reported to contain quite low Cu content (0.48–2.16 ppm), moderate Zn content (1.66-14.5 ppm), Ni content (0.130–21.02 ppm), and high Fe content (17.23–85.50 ppm) (Indrayan et al. 2009). On the other hand, the Cu for the *E. elatior* and *E. phillippinensis* were found to be relatively higher than the reported range, but the Zn and Fe contents were relatively low within the range of the other ginger species. Factors influencing the concentrations of these metals include the plant species, the microclimate conditions, environmental pollution, and other factors affecting the plant growth (Mishra et al. 2006; Broadly et al. 2007).

For this study, the Fe levels in plant samples were all below the permissible limit of 20 mg kg^{-1} set by the World Health Organization (WHO) (Shah et al. 2013). Different results, however, were observed by Rachkeeree et al. (2018) wherein Fe was found below the detection limit in the *E. elatior* flowers. Juwita et al. (2018) reported that the inflorescence of *E. elatior* contained high levels of major minerals like potassium, calcium, magnesium and phosphorus. Scientifically speaking, Fe serves to carry oxygen from the lungs to the body tissues, in operational immune system maintenance, and to support metabolic energy production (Gupta et al. 2014). It plays an important role in physiological and biochemical processes. For instance, it is involved in various enzymatic activities such as the electron transport chain in cytochromes, in the synthesis of chlorophyll, and maintenance of chloroplast structure and function (Rout and Sahoo 2015). Iron toxicity from foods are rare since the body regulates iron absorption by only absorbing less iron, if the iron stored in the body are adequate. However, ingestion of elemental iron (e.g. supplement) with more than 60 mg kg^{-1} can results in severe toxicity such as gastrointestinal mucosa which can cause nausea, vomiting, abdominal pain, and diarrhea leading to a severe morbidity and mortality (Yuen and Becker 2021).

Meanwhile, the results also show that the rhizome of *E. elatior* is a good accumulator of Zn, suggesting that it is a good candidate for phytoextraction or phytoremediation in heavily contaminated soils. Hence, it is recommended that further investigations must be conducted to determine the ability of *E. elatior* and *E. philippinensis* to accumulate heavy metals.

Based on the results, Zn levels were all below the World Health Organizations permissible limit of Zn in plants which is 50 mg kg^{-1} (Shah et al. 2013). Zn is an immune response stimulant and membrane stabilizer, and its deficiency leads to impaired growth and malnutrition (Indrayan et al. 2009). Zinc (Zn) is a cofactor involved in many catalytic activities and structural proteins in plants. It has key structural functions in the protein domains, known as "Zn finger" domain, which interact with other molecules. The Zn finger proteins mediate DNA binding of transcription factors and protein–protein interactions (Cabot et al. 2019). Also, Zn is essential for many enzymes which are needed for nitrogen metabolism, and energy transfer (Haffez et al. 2013). Intake of naturally occurring zinc in food has no evidence of adverse effects. However, excessive intake of supplemental zinc (50-150 mg day^{-1}) is associated with the suppression of immune response, decrease in high-density lipoprotein (HDL) cholesterol, reduced copper status, and gastrointestinal distress (Trombu et al. 2001)

The Cu content in the plants obtained from this study indicates that they were below the permissible limit of Cu in plants (10 mg kg^{-1}) set by WHO (Hasan et al. 2012). Copper is a component of cytochrome oxidase, lysyl oxidase, and ceruloplasmin (Mills 1981). Copper, is an essential cofactor of numerous proteins. It is known to participate in photosynthetic electron transport, cell wall metabolism, mitochondrial respiration, hormone signaling and oxidative stress responses. Furthermore, Cu plays a significant role in many enzymatic activities such as Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, and polyphenol oxidase (Yruela 2005; Printz et al. 2016). However, consuming plants containing high amount of Cu could result to great concern to health due its toxicity to humans and animals (Kabata-Pendias and Mukherjee 2007). A high intake of Cu has been related to liver damage (Korfali et al. 2013).

On another instance, the Ni levels for this study indicates that the content were all below the permissible limit (10 mg kg^{-1}) of Ni in plants set by WHO (Nazir et al. 2015). The Ni level in soil where the plant samples were collected from was found to be below detection limit. Nickel, an essential element, acts as an activator of many enzymes such as ureases which hydrolyzes urea in plant tissue (Fabiano et al. 2015). It helps some plants to protect themselves against pathogens and herbivorous insects. At higher concentrations, however, this element is toxic to plants and other living organisms (Harasim and Filipek 2015). Human exposure to high amount of nickel is known to cause a variety of human health problems including allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer (Chen et al. 2017).

The high level of Ni in the rhizome and leaves despite its non-detection in the soil could be due to the ability of the plants to accumulate this element. Most of the Ni, however, is transported to seeds during the senescence of leaves as reported for *Mimulus guttatus* (Tilstone and Mac Nair 1997) and for soybean (Cataldo et al. 1978; Sengar et al. 2008). This may explain the results of this current study where Ni was detected in the leaves and rhizomes of *E. elatior* and *E. philippinensis* despite their non-detection in the soil.

Generally speaking, anthropogenic activities including the burning of fossil fuels for power generation, mining, smelting, emissions from vehicles, disposal of household, municipal and industrial waste, steel manufacturing, agricultural fertilizers, pesticides and cement industry accelerates the release of heavy metals (Ni, Zn, Cu, Co, and Mo) into the environment. Subsequently, there will be plant uptake through roots from the soil and by leaf adsorption from air (Mishra et al. 2017).

Certainly, at a lower level, the heavy metal micronutrients are essential elements for growth and development in both animals and plants (Tchounwou et al. 2012). Exposure of plants to high levels of heavy metal micronutrients usually results to several phytotoxic symptoms, such as leaf chlorosis, growth retardation, nutrient imbalance or low nutrient uptake, decreased stomatal conductance, inhibition of chlorophyll synthesis, reactive oxygen species (ROS) production that causes membrane, DNA and protein damage, and distortion of photosynthetic machineries (Arif et al. 2016; de Macedo et al. 2016). In humans, prolonged ingestion or exposure to a high concentration of heavy metals result to the production of free radicals that attack the DNA which cause cellular damage and mutation leading to an array of diseases (Jaishankar et al. 2014). Nevertheless, based on the results of this study the levels of Cu, Ni, Zn, and Fe in both rhizomes and leaves of *E. elatior* and *E. philippinensis* are below the permissible limit in plants as set by WHO, which means that their presence is not harmful for consumption but instead a good source of heavy metal micronutrients.

ACKNOWLEDGMENTS

The researchers are grateful to the CMU administration for the financial support and the University Research Office for facilitating the conduct of this research, and for the anonymous reviewers for patiently correcting this paper.

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ARTICLE INFO

Received: 10 January 2020
Revised: 04 January 2021
Accepted: 30 September 2021
Available online: 16 November 2021

Role of authors: GBB - Conceptualized the study, supervised the sampling and laboratory assay, interpreted the data, wrote the manuscript; CVA – conducted the sample collection and preparation, facilitated the laboratory analyses, and wrote the manuscript; AGG – Supervised the sampling and laboratory analyses, guided the data analysis, and revised and improved the manuscript.

Bird surveys in Turtle Islands Wildlife Sanctuary, Philippines

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https://doi.org/10.69721/TPS.J.2021.13.2.08

ABSTRACT

Bird observations were conducted in all six islands within the Turtle Islands Wildlife Sanctuary, Tawi-Tawi, Philippines in May 2017, March 2018 and August 2019. Purposive sampling technique using digital cameras, binoculars and spotting scopes were used to document the bird species and to assess their population. A total of 44 species were observed including the IUCN Vulnerable Grey Imperial Pigeon *Ducula pickeringii* (Cassin, 1854), the near-threatened Mantanani Scops Owl *Otus mantananensis* (Sharpe, 1892) and the Philippine Megapode *Megapodius cumingii* (Dillwyn, 1853). Baguan Island recorded the highest number of species (38) followed by Taganak Island with 25 species, Boan Island with 21 species, Great Bakkungan and Lihiman Islands has 18 species each, while the island with the least number of species observed was Langaan (14). Among the islands, only Baguan retained mature beach forest. Locals in Boan Island were observed keeping native birds as pets. The presence of these restricted-range bird species as well as the updated baseline data, provide significant contribution in defining priority islands for avian conservation. Additional surveys are recommended in the area particularly in the relatively undisturbed Baguan Island during migratory season.

Keywords: Mantanani scops owl, Sulu, Tawi-Tawi

INTRODUCTION

The Philippines consists of 7,641 islands (Maritime Industry Authority 2021) is located at the western most part of the Pacific Ocean and is identified as one of the world's biologically rich countries when it comes to diversity of ecosystems, species and genetic diversity. Avian diversity in the country is among the highest in the world where more than 7% of the land area was declared as Important Bird Areas (IBAs) and secondary regions (Stattersfield et al. 1998; Collar et al. 1999). Important Bird Areas are sites that are significant for the conservation of bird populations in a worldwide scale due to the presence of threatened, endemic and restricted-range species (Haribon Foundation 2014). Among the outstanding IBAs is the Sulu Archipelago (Alban 2005).

A number of threatened birds are known to occur only in Sulu archipelago and nowhere else in the world (Ong et al. 2002; BirdLife International 2019). Despite the high concentration of endemic and restricted-range species, very few ornithological expeditions have been conducted on the smaller islands including the Turtle Islands (Dickinson et al. 1991; Kennedy et al. 2000; Mallari et al. 2001) and earlier collections were concentrated on Tawi-Tawi, Jolo, Sitangkai and Simunol (Guillemard 1885; Mearns 1909; Dickinson et al. 1991; Kennedy et al. 2000; Peterson et al. 2000).

The Turtle Islands is located at the southwestern edge of Sulu Sea right at the tip of the international treaty limits separating the Philippines and Malaysia. It ranked as the 11^{th} major marine nesting grounds in the world for the endangered Green Sea Turtle *Chelonia mydas* (Linnaeus, 1758). This small group of islands is highly valued and recognized for its critical marine turtle habitat. Hence, the entire municipality covering six islands, namely Langaan, Lihiman, Baguan, Great Bakkungan, Boan, and Taganak, was declared as a protected area known as Turtle Islands Wildlife Sanctuary (TIWS) pursuant to Republic Act 7586 as amended by RA 11038. It has a total area of 242,967 ha (242.97 km^2), including its surrounding waters (PAMB 2018).

Knowledge on the birds of TIWS is important in helping us understand the relationships of the birds in TIWS to Borneo and Philippines and whether the endemic and restricted-range species of the Sulu archipelago are also found in TIWS. The bird observations of Ivan Sarenas in Baguan and Taganak Islands (Yu et al. 2016) added 28 new records and was the only available recent information on the avifaunal community of TIWS. This study presents the status of the forest habitats on each island, bird species composition, abundance, and diversity in the islands of Baguan, Taganak, Boan, Lihiman, Langaan and Great Bakunggan. These are essential in identifying priority islands for bird conservation.

METHODS

Site Description

Langaan (6°12'17.306"N, 118°8'59.02"E) - The island measures 7 ha with a relatively flat, sandy landscape. Its vegetation is dominated by coconut *Cocos nucifera* (L.) and with some beach forest trees covering a quarter of the island (3 ha). This was visited on 22-23 May 2017 and 24 August 2019.

Lihiman (6°13'56.114"N, 118°4'7.53"E) - A 29 ha island with an active mud volcano on the northern section. The mud volcano forms a 20 m crater that drains directly to the sea. A plantation of "agoho" *Casuarina equisetifolia* (L.) (estimated at 4 ha) surrounds the volcano and are the only known plants growing around the mud volcano. On the lower sections of the steep hill are native species of trees e.g. "talisay" *Terminalia catappa* (L.) and "kapok" *Ceiba pentandra* (L.). Patches of mangroves are observed on the uninhabited coastal areas. This was visited on 22-23 May 2017 and 24 August 2019.

Baguan (6°6'13.171"N, 118°26'50.411"E) - This 29.1 ha island is designated as a strict protection zone. It is the only uninhabited island within TIWS. It is mostly flat in the southern section with a hilly northern portion that reached an elevation of 40 m above sea level and leads to a steep drop with large volcanic boulders on the northern sandy shoreline. The "Balinghasai" *Buchanania arborescens* (Blume), coconut and other beach forest trees dominated the terrestrial flora. This was visited on 21 May 2017, 21 March 2018, and 22-23 August 2019.

Great Bakkungan (6°11'14.045"N, 118°7'10.514"E) - This 51 ha hilly island is dominated by coconuts *C. nucifera*, mangoes *Mangifera indica* (L.) and bananas *Musa* sp. interspersed with grassland that serve as grazing areas for goats. An active mud volcano in the northern end is surrounded with grasslands and occasional "bignay" *Antidesma* spp. trees. The highest altitude reaches 58 m elevation. This was visited on 22-23 May 2017 and 24 August 2019.

Boan (6°17'6.912"N, 118°4'41.43"E) - A 76 ha island has an elevation of 59 m above sea level. The island features a cluster of coastal communities and a hilly landscape. Few native trees are observed on the island. A patch of residual forest on the northern side was reported in the 1990s, but only *Ficus* spp., coconuts, bananas and brushland are observed during the survey. A patch of mangroves composed mostly of *Rhizophora* spp. is observed in the southern coastal area. We visited on 22-23 May 2017 and 24 August 2019.

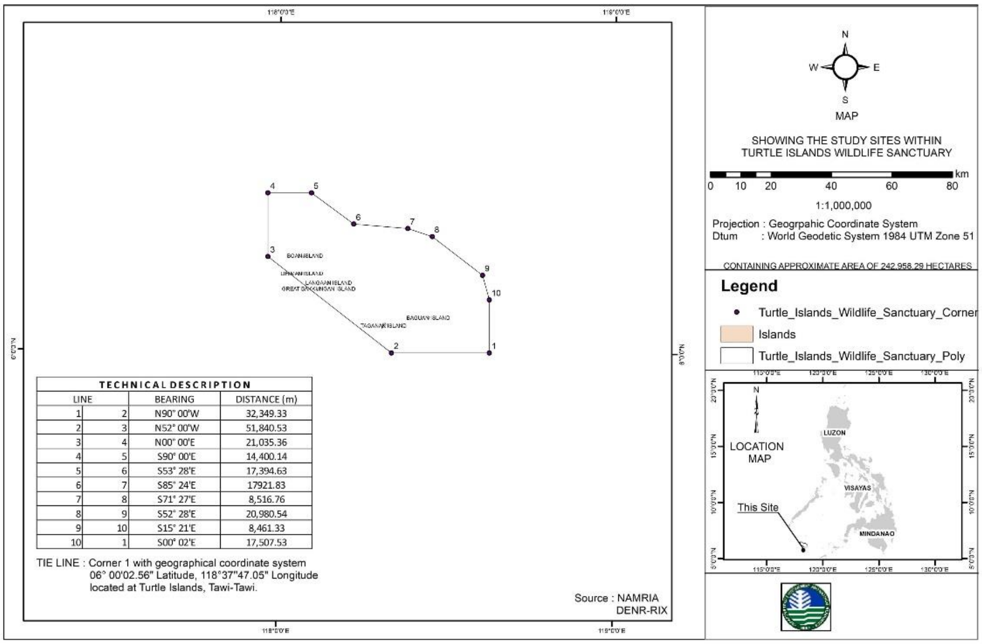

Figure1. Map of Turtle Islands Wildlife Sanctuary, Philippines.

Taganak (6°4’44.375’’N, 118°18’53.611’’E) - The island has an estimated land area of 116 ha and elevation at 148 m above sea level. It is mostly flat on the southern side with a low plateau-like feature at the central section. Mainly devoid of forest except for the native vegetation on the rocky section in the north. The rest of the island is covered with “cogon” *Imperata cylindrica* (L.) with mango and banana plantation as well as other seasonal agricultural plants. We visited last 19-20 May 2017, 21 March 2018, and 25 August 2019. The exact location of the study sites visited during the survey is shown in Figure 1.

Status of Forest Habitats

To identify priority islands for bird conservation, basic description of the general type of habitat was conducted by a) recording the most numerous plants identified at least at the family level, b) presence of fruiting and flowering plants, c) average height of canopy and understory plants and d) anthropogenic disturbances. A drone was also used to take images and videos to determine the different land uses and in estimating remaining natural vegetation. Photos of flowering and fruiting trees were also taken for taxonomic identification using the works of Pelser et al. (2011) and Primavera (2009).

Birds Species Composition and Abundance

A purposive sampling method and photo documentation were carried out in the six islands. Birds seen and heard calling were recorded using 8 x 42, 10 x 42 roof prism binoculars and 80 x 45 degree spotting scopes, and photographed using digital cameras with 600 mm telephoto lenses. In the case of Baguan Island, four count stations were used by observers to search and record the species and numbers of birds for at least eight minutes (Bibby et al. 2000; Lee and Marsden 2008). Taxonomy and nomenclature followed the Handbook of Birds of the World and the BirdLife International Illustrated Checklist of the Birds of the World (del Hoyo et al. 2014; del Hoyo and Collar 2016). Kennedy et al. (2000), Lee et al. (2018) and Jakosalem et al. (2019) were used in identifying birds while Allen (2020) was used as reference for the residency and conservation status. A total of 27 observation hours were spent in the whole area as follows; 16 hours - Baguan; 8 hours - Taganak; 2.5 hours - Lihiman; 4.5 hours - Langaan; 5.5 hours – Boan; 2 hours - Great Bakkungan.

Birds Species Diversity

The Shannon – Weiner index of diversity was determined between islands. This index takes both abundance and richness into account using the formula: H = - SUM [pi*ln(pi) where*, Sum* is the summation; pi is the proportion of each species in a sample.

RESULTS

Status of Forest Habitats

Very little natural vegetation exists on the islands except in Baguan and Langaan. In Baguan, 89% of the island was covered with beach forest (Table 1). The "balinghasai" *Buchanania* spp. was the most common species encountered followed by figs *Ficus* spp., "culasi" *Lumnitzera littorea* (Jack) Voigt, "buta-buta" *Excoecaria agallocha* (L.), "tabigi" *Xylocarpus granatum* (Koen.), "piagao" *Xylocarpus moluccensis* (Lam.) M. Roem and "tungog" *Ceriops* spp. There were still traces of secondary growth beach forests in Taganak and Langaan Island. In Great Bakkungan and Boan, *Antidesma* spp. grows in the grassland areas. The *Buchanania* spp., *Antidesma* spp. and *Ficus* spp. appear to be the major source of fruits for fruit-eating wildlife e.g., doves *Ptilinopus* sp., *Ducula* sp. and many others.

Table 1. Estimated forest cover on the islands of Turtle Islands Wildlife Sanctuary.

Name of Island	Size (ha)	Estimated forest (ha)	Description of habitats	Encountered Plant Species
Baguan	29	26	Secondary beach forest	*Buchanania* spp., *Ficus* spp., *Xylocarpus* spp., *Exoecaria* sp.
Taganak	116	8	Second growth with grassland	*Xylocarpus* spp., *Exoecaria* sp., *Cocos nucifera* (L.), *Mangifera indica*(L.), *Musa* sp.
Lihiman	29	5	*Casuarina* tree plantation	*Casuarina equisetifolia* (L.), *Cocos nucifera, Terminalia catappa* (L.)
Langaan	7	2	Beach forest	*Excoecaria agallocha* (L.), *Cocos nucifera*
Boan	76	24	Brushland and mangroves	*Cocos nucifera, Ficus* spp., *Macaranga* sp., *Terminalia catappa*
Great Bakkungan	51	20	Brushland and grassland	*Cocos nucifera, Antidesma* spp., *Mangifera indica*, *Terminalia catappa*

Birds Species Composition and Abundance

Of the six islands visited, Baguan Island (38) recorded the highest number of species followed by Taganak (25), Boan (21), Lihiman (19), Great Bakkungan (18), and lastly was Langaan with 14 species (Table 2).

Table 2. Counts and relative abundance of birds (in parenthesis) recorded during current surveys on the six islands of TIWS. Note that 1 – Baguan; 2 – Boan; 3– Langaan; 4 – Lihiman; 5 – Great Bakkungan and 6 – Taganak. An asterisk placed before the scientific name denotes migratory birds.

Scientific Name	1	2	3	4	5	6
* *Fregata ariel* (Gmelin, 1789)	4 (3)					
* *Fregata minor* (Gray, 1845)	1 (1)					
Halieaeetus leucogaster (Gmelin, 1788)	1 (1)	1 (1)	1 (3)	1 (1)	1 (2)	1 (2)
Haliastur indus (Boddaert, 1783)	1 (1)	2 (2)	1 (3)	3 (2)	1 (2)	2 (3)
Butorides striata (Linnaeus, 1758)		1 (1)		1 (1)	1 (2)	
Amaurornis phoenicurus (Pennant, 1769)		1 (1)				
Hypotaenidia torquatus (Linnaeus, 1766)		1 (1)				
Egretta sacra (Gmelin, 1789)	1 (1)	1 (1)	1 (3)	1 (1)		1 (2)
Bubulcus ibis (Linnaeus, 1758)	1 (1)					

Scientific Name	1	2	3	4	5	6
**Tringa brevipes* (Veillot,1810)	1 (1)					1 (2)
**Charadrius leschenaultia* (Lesson, 1826)	1 (1)					1 (2)
Sterna sumatrana (Raffles, 1822)	16 (13)	4 (4)	2 (6)	5 (3)	2 (4)	1 (2)
**Chlidonias hybrida* (Pallas, 1811)	8 (7)				5 (11)	5 (8)
Onychoprion anaethetus (Scopoli, 1786)	1(1)					
Thalasseus bergii (Lichtenstein, 1823)				64 (44)		
**Phalarophus lobatus (Linnaeus, 1758)*			2 (6)			
Megapodius cumingii (Dillwyn, 1853)	9 (8)					3 (5)
Treron vernans (Linnaeus, 1771)	4 (3)	3 (2)			7 (15)	2 (3)
Treron axillaris (Bonaparte, 1855)	1(1)					
Ptilinopus melanospila (Salvadori, 1875)		3 (2)				6 (10)
*Ducula pickeringii (*Cassin, 1854)	8 (7)					
Ducula bicolor (Scopoli, 1786)	5 (4)					
*Columba vitiensis (*Quoy & Gailmard, 1830)	1(1)					
Chalcophaps indica (Linnaeus, 1758)	1(1)	2 (2)				1 (2)
Spilopelia chinensis (Scopoli, 1786)	3 (3)	3 (4)	1 (3)	26 (18)	4 (9)	1 (2)
* *Cuculus saturatus* (Blyth, 1843)	1 (1)					
*Eudynamys scolopaceus (*Linnaeus, 1758)	1 (1)					
Otus mantananensis (Sharpe, 1892)	1 (1)					2 (3)
Pelargopsis capensis (Linnaeus, 1766)	2 (2)					1 (2)
Todiramphus chloris (Boddaert, 1783)	10 (8)	8 (10)	8 (24)	3 (2)	3 (6)	4 (7)
Aerodramus cf amelis (Oberholser, 1906)	1 (1)					
Gerygone sulphurea (Wallace, 1864)	2 (2)	1 (1)	1 (3)	4 (3)	1 (2)	1 (2)
**Hirundo rustica* (Linnaeus, 1758)	3 (3)	2 (2)	6 (18)	1 (1)	6 (13)	3 (5)
Pycnonotus goaivier (Scopoli, 1786)	1 (1)	2 (2)		2 (1)	1 (2)	5 (8)
Rhipidura nigritorquis (Vigors, 1831)	2 (2)	1 (1)	1 (3)	1 (1)		2 (3)
Aplonis panayensis (Scopoli, 1783)	3 (3)	23 (28)	1 (3)	1 (1)	2 (4)	1 (2)
Lalage nigra (Forster, 1781)	2 (2)			1 (1)	1 (2)	1 (2)
Artamus leucorhynchus (Linnaeus, 1771)	1 (1)	1 (1)		1 (1)	2 (4)	1 (2)
Oriolus chinensis (Linnaeus, 1766)	1 (1)			1 (1)		
**Motacilla cinerea* (Tunstall, 1771)	1 (1)		1 (3)			
Anthreptes malacensis (Scopoli, 1786)	5 (4)	5 (6)	3 (9)	2 (1)	2 (4)	1 (2)

Scientific Name	1	2	3	4	5	6
Cinnyris jugularis (Linnaeus, 1766)	1 (1)				2 (4)	
Lonchura atricapilla (Veillot, 1807)	7 (6)	2 (2)		2 (1)	2 (4)	3 (5)
Passer montanus (Linnaeus, 1758)	6 (5)	18 (22)	3 (9)	27 (18)	4 (9)	9 (15)
Total individuals	119	85	32	147	47	59
Total species	38	21	14	19	18	25
Total breeding residents	30	19	11	18	19	22
Total migratory birds	8	1	3	1	1	3

A total of eight species were found in all islands: White-bellied Sea eagle *Halieaeetus leucogaster* (Gmelin, 1788), Brahminy Kite *Haliastur indus* (Boddaert, 1783) Spotted Dove *Spilopelia chinensis* (Scopoli, 1786), Collared Kingfisher *Todiramphus chloris* (Boddaert, 1783), Yellow-vented Bulbul *Pycnonotus goaivier* (Scopoli, 1786), Asian Glossy Starling *Aplonis panayensis* (Scopoli, 1783), Brown-throated Sunbird *Anthreptes malacensis* (Scopoli, 1786) and Eurasian Tree Sparrow *Passer montanus* (Linnaeus, 1758). The Asian Glossy Starling was the most frequently encountered bird followed by Eurasian Tree Sparrow. Seven of the bird species were represented with only one individual (Table 2).

A total of nine species were newly recorded in TIWS e.g., Barred Rail *Hypotaenidia torquatus (*Linnaeus, 1766*)*, Whiskered Tern *Chlidonias hybridus* (Pallas, 1811), Black-naped Fruit Dove *Ptilinopus melanospila* (Salvadori, 1875), Pied Imperial Pigeon *Ducula bicolor* (Scopoli, 1786), Metallic Pigeon *Columba vitiensis* (Quoy & Gailmard, 1830), Grey-capped Emerald Dove *Chalcophaps indica* (Linnaeus, 1758), Red-necked Phalarope *Phalarophus lobatus* (Linnaeus, 1758), Black-naped Oriole *Oriolus chinensis* (Linnaeus, 1766) and Great Frigatebird *Fregata minor* (Gmelin, 1766).

Birds Species Diversity

Baguan recorded the highest index of species diversity (H' = 3.2) compared with the other islands (Figure 2). This is followed by Taganak (H' = 2.9) and Great Bakkungan (H' = 2.7). The smallest island (Lihiman) recorded the lowest species diversity index (H' = 1.8).

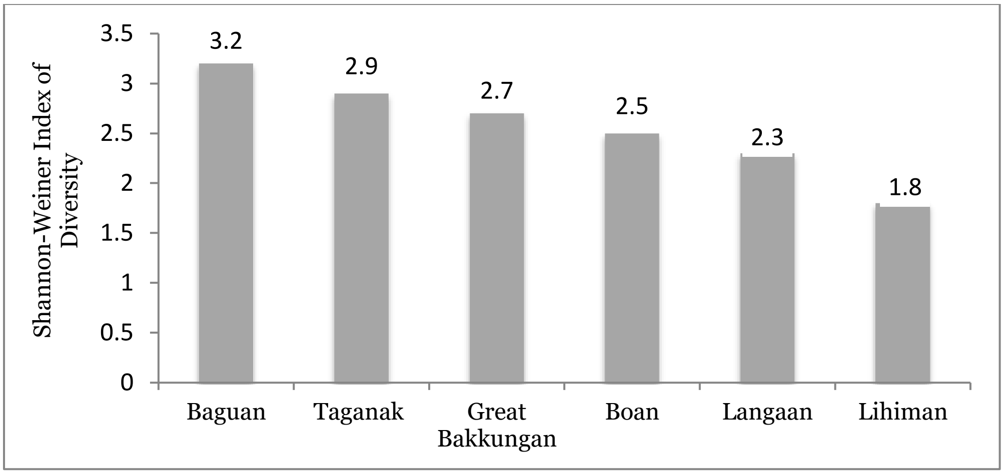

Figure 2. Comparison of species diversity indices (Shannon-Weiner) of all six islands in Turtle Islands Wildlife Sanctuary.

DISCUSSION

Status of the forest habitat

The size of the islands did not appear to influence bird diversity but islands with forest cover recorded more species. Among the six islands, Baguan still retain a substantial beach forest cover. There were small patches of beach forest on Langaan, on steep rock cliffs in Great Bakkungan and surrounding the mud volcanoes in Lihiman. Beach forest on these isolated off-shore islands serve as important habitats for specialized small-island birds e.g. Mantanani Scops Owl and Grey Imperial Pigeon. The increasing scarcity of suitable islands threatens the survival of these species.

The islands of Baguan and Taganak are important habitats for the Vulnerable *D. pickeringii*, the near-threatened *Megapodius cumingii pusillus* (Dillwyn, 1853) and *O.m. mantananensis* as well as terrestrial migratory birds *Lanius tigrinus* (Drapiez, 1828) and *Larvivora cyane* (Pallas, 1776). The islands also recorded species that were affiliated with Bornean avifauna *Aerodramus cf amelis* (Oberholser, 1906) and the *P. moluccensis*. We anticipate that with more surveys (including nocturnal birds), the bird species list is likely to increase particularly during the migratory season.

Based on the current data, priorities should focus on Baguan and Taganak islands for bird monitoring. We also suggest that bio-monitoring should include monitoring of *M.c. pusillus* nests and *O.m. mantananensis*. They are known to prefer to breed in small islands (Lambert 1993; Kennedy et al. 2000; Sloan 2017). A Biodiversity Assessment and Monitoring System (BAMS) in TIWS should include be conducted within the migratory season but should also include all six islands as sites of this study.

Birds Species Composition and Abundance

In 1997, there were only 30 birds recorded on TIWS (PAMB 2018). It took another 18 years before the list of birds were updated to 58 species (Yu et al. 2016). It should be noted that more bird observations were spent on Baguan and Taganak than the rest of the TIWS islands. The surveys were also conducted off migratory season. These factors introduced bias in our results and we caution readers to take this in consideration when interpreting the information provided.

There were 13 new localities recorded from this survey bringing the total number of birds on TIWS to 71 species (Table 3). At least 21 migratory species recorded in 2015 (Yu et al. 2016) that were not observed during the survey (Table 3). Migrants comprise 37% (26 species) of the total birds in TIWS. Waterbirds on the other hand share 24% (17 species) of the total birds while 13% (10 species) were doves. There were also three species that were not observed during the visit e.g. Oriental Dollarbird *Eurystomus orientalis* (Linnaeus, 1766), Black-headed Munia *Lonchura atricapilla* (Linnaeus, 1766) and Northern Boobok *Ninox japonica* (Temnick & Schlegel, 1844).

Table 3. Checklist of birds in Turtle Islands Wildlife Sanctuary. Note: * means migratory, ^ means new record on TIWS, X means recorded in TIWS and ? means needs further validation. The 1997 records were taken from TIWS (2018) while the 2015 records were lifted from Yu et al. (2016).

Species Name	1997	2015	2017 - 2019
*Lesser Frigatebird *Fregata ariel*	X	X	X
*^Great Frigatebird *Fregata minor*			X
White-bellied Sea Eagle *Halieaeetus leucogaster*	X	X	X
Brahminy Kite *Haliastur indus*	X	X	X
Peregrine Falcon *Falco peregrinus*		X	
Striated Heron *Butorides striata*		X	X
^White-breasted Waterhen *Amaurornis phoenicurus*			X
^Barred Rail *Hypotaendea torquatus*			X
Red-legged Crake *Rallina fasciata*		X	
Pacific Reef Egret *Egretta sacra*	X	X	X
Eastern Cattle Egret *Bubulcus ibis*		X	X
Little Egret *Egretta garzetta*		X	
Intermediate Egret *Egretta intermedia*		X	
*Grey-tailed Tattler *Tringa brevipes*		X	X
*Common Sandpiper *Actitis hypoleucos*	X	X	
*^Greater Sand Plover *Charadrius leschenaultii*			X

Species Name	1997	2015	2017 - 2019
*^Red-necked Phalarope *Phalarophus lobatus*			X
Black-naped Tern *Sterna sumatrana*	X		X
*^Whiskered Tern *Chlidonias hybrida*			X
Bridled Tern *Onychoprion anaethetus*	X		X
Greater Crested Tern *Thalasseus bergii*		X	X
*Gull-billed Tern *Gelochelidon nilotica*		X	
Philippine Megapode *Megapodius cumingii*	X	X	X
Pink-necked Green Pigeon *Treron vernans*	X	X	X
^Philippine Green Pigeon*Treron axillaris*			X
Black-naped Fruit Dove *Ptilinopus melanospila*			X
Grey Imperial Pigeon *Ducula pickeringii*	X	X	X
Green Imperial Pigeon *Ducula aenea*		X	
^Pied Imperial Pigeon *Ducula bicolor*			X
^Metallic Pigeon *Columba vitiensis*			X
^Grey-capped Emerald Dove *Chalcophaps indica*			X
Spotted Dove *Spilopelia chinensis*	X	X	X
Philippine Collared Dove *Streptopelia dusumieri*	X		
*Oriental Cuckoo *Cuculus optatus*		X	X
*Himalayan Cuckoo *Cuculus saturatus*	X	X	
Asian Koel *Eudynamys scolopaceus*	X	X	X
*Chestnut-winged Cuckoo *Clamator coromandus*		X	
Hair-crested Drongo *Dicrurus hottentotus*		X	
Mantanani Scops Owl *Otus mantananensis*		X	X
*Northern Boobook *Ninox japonica*		X	
Collared Kingfisher *Todiramphus chloris*	X	X	X
*Common Kingfisher *Alcedo atthis*		X	
^Stork-billed Kingfisher *Pelargopsis capensis*			X
Sunda Pygmy Woodpecker *Picoides moluccensis*		X	
Ameline Swiftlet *Aerodramus cf amelis*		X	X
Oriental Dollarbird *Eurystomus orientalis*		X	
Golden-bellied Gerygone *Gerygone sulphurea*	X		X
*Barn Swallow *Hirundo rustica*	X	X	X
*House Swallow *Hirundo javanica*	X	X	
Yellow-vented Bulbul *Pycnonotus goaivier*	X		X
*Arctic Warbler *Phylloscopus borealis*	X	X	
Philippine Pied Fantail *Rhipidura nigritorquis*	X		X
*Grey-streaked Flycatcher *Muscicapa grisiesticta*		X	
*Asian Brown Flycatcher *Muscicapa dauurica*		X	
*Dark-sided Flycatcher *Muscicapa sibirica*		X	
*Siberian Blue Robin *Larvivora cyane*		X	
*Blue Rock Thrush *Monticola solitarius*		X	
Mangrove Blue Flycatcher *Cyornis rufigastra*	X		
Glossy Starling *Aplonis panayensis*	X	X	X
*Chestnut-cheeked Starling *Sturnus philippensis*	X	X	
Pied Triller *Lalage nigra*	X	X	X

Species Name	1997	2015	2017 - 2019
White-breasted Woodswallow *Artamus leucorhynchus*	X		X
^Black-naped Oriole *Oriolus chinensis*			X
*Grey wagtail *Motacilla cinerea*		X	X
*Eastern Yellow Wagtail *Motacilla tschutsichensis*		X	
*Brown shrike *Lanius cristatus*	X	X	
*Tiger Shrike *Lanius tigrinus*		X	
Brown-throated Sunbird *Anthreptes malacensis*		X	X
Olive-backed Sunbird *Cinnyris jugularis*	X		X
Black-headed Munia *Lonchura atricapilla*	X	X	X
Eurasian Tree Sparrow *Passer montanus*	X	X	X
Total number of species	30	49	44
Total number of breeding residents	22	17	34
Total number of migratory birds	8	32	10

The majority of the birds encountered were expected to occur in a wide range of habitats (Kennedy et al. 2000). The *H. torquatus*, *T. chloris* and *Cinnyris jugularis* are considered generalists and survive even in highly modified habitats (Steadman and Freifeld 1998; Kennedy et al. 2000; Sekercioglu 2006; Jakosalem et al. 2019). Rails are also known for their ability to disperse and survive even on remote small islands (Kennedy et al. 2000). We did not encounter birds endemic to the Sulu and Tawi-Tawi archipelago.

The islands support at least 12 frugivorous birds, 10 of which were pigeons. The Vulnerable *D. pickeringgi* was only observed on Baguan. It was frequently encountered feeding on the ripe fruits of *Buchanania* sp. in groups of five to 10 individuals and sometimes flocks with *D. bicolor* and *C. vitiensis*. They appeared to take advantage on the optimal density of *Buchanania* spp., *Antidesma* spp., figs and other fruit trees in the area. We did not observe the species in the other islands but we suspected that the birds were moving across the islands to opportunistically search for fruit. IUCN (2021) reports on the continuing decline of the population and estimated the number of mature individuals from 1,500 to 7,000. Baguan Island is an important site for this Vulnerable small-island specialist.

The Near-threatened *O. m. mantananensis* was distributed on small islets off Borneo and the Philippines (Allen 2020; Kennedy et al. 2000). There have been no previous confirmed records of Mantanani Scops Owl in Turtle Islands (Kennedy et al. 2000). At least two individuals were briefly seen and heard calling on Baguan Island last August 2019. There were at least four subspecies that occur in the country but we could not determine if it was subspecies *sibutuensis* or *mantananensis* occurring in Turtle Islands. Local reports indicated the presence of the bird on Taganak Island but we were not able to validate the record due to the limited time spent on the island.

We observed six adult individuals of the Near-threatened *M.c pusillus* on Baguan on 22-23 August 2019. One individual was seen digging a one-meter deep burrow in one section of a 2 m wide mound (23 August 2019). Three individuals were encountered on separate occasions on Taganak Island (19-20 May 2017). We presumed the bird also occur on the other islands but we only observed breeding mounds on Baguan.

These were also noteworthy records in TIWS. A female Sunda Pygmy Woodpecker *Picoides moluccensis* (Gmelin, 1788) was photographed searching for insects on the main branch of a mango tree on Lihiman Island (Yu et al. 2016). This was the first record of the Sunda Pygmy Woodpecker in the Philippines. The Tiger Shrike *L. tigrinus* and a female Siberian Blue Robin *L. cyane* (Pallas, 1776) were photographed in Baguan while a Chestnut-winged Cuckoo *Clamator coromandus* (Linnaeus, 1776) was observed in Taganak Island (Yu et al. 2016). All three species have been recorded in the Philippines (Allen 2020; Kennedy et al. 2000). The Ameline Swiftlet *A. cf amelis* was first reported on Baguan Island in 2015 (Yu et al. 2016). We have observed one medium-sized individual with a generally light brown coloration and slightly forked tail flying above a cleared section of the beach forest on Baguan Island on 23 August 2019.

The high proportion of migrants (37%) in TIWS illustrate the importance of the islands as stop over points for migrating birds particularly those that are rarely recorded in the country e.g. *L. cyane* and *L. tigrinus*. According to Clarke et al. (2016), the *L. cyane* is a common migrant in the East Asian Australasian flyway and is a winter migrant in the Philippines.

Birds Species Diversity

The high diversity of bird species in Baguan and Taganak islands can be attributed to several factors e.g. presence of a beach forest, island size, disturbance as well as field effort. Bird species composition and abundance are dependent on the overall habitat requirements such as availability of food resources, breeding grounds and protection (Gonzalez et al. 2010; Alviola and Mohagan 2017; Paguntalan et al. 2021). The presence of native trees on beach forest influenced the bird species composition and diversity on Puerco Island (Paguntalan et al. 2021) and may have similar patterns with Baguan Island. The conduct of purposive search in areas where there is still natural vegetation, secondary growth, beach forest, as well as coastal mudflats will likely add new records on each island.

The presence of *D. pickeringii*, *O. m. mantananensis* and *M.c pusillus* raises the importance of the TIWS as refuge to threatened birds found on small islands. These birds are vulnerable to hunting for local pet trade. We observed six households on Boan Island that kept birds as pets. The *H. leucogaster* (adult and juvenile), Pink-necked Green Pigeon *Treron vernans* (Linnaeus, 1771) (2 individuals), *H. torquatus* (2 individuals) and White-breasted Waterhen *Amaurornis phoenicurus* (Pennant, 1769) (2 individuals) are placed in home-made bamboo cages or shackled with ropes as pets. The conservation management of the TIWS under the Bangsamoro Autonomous Region of Muslim Mindanao (BARMM) should consider conserving forests habitats for restricted-range and forest dependent small island specialists.

ACKNOWLEDGMENTS

We thank the Department of Environment and Natural Resources Region IX, Provincial Environment and Natural Resources (PENRO) Zamboanga Sibugay, Biodiversity Management Bureau, Philippines Biodiversity Conservation Foundation, Armed Forces of the Philippines through the Philippine Coast Guard, BRP Bagacay, Philippine Navy BRP Ramon Alcaraz and BRP Cebu and the Protected Areas Management Board of Turtle Islands Wildlife Sanctuary for supporting this project. We also thank the two anonymous reviewers and the following: R. Hutchinson, D. Allen, CMP. Rodriguez, F Mirasol, R Gadot, CS Julsadjiri, MJ Bairulla, F Samimi, JR Nadugo, A Valida Jr., R Lorenzana, Z Peña, K Tenajeros, LB ALejo, IA Sarenas, CMT Yu, F Yusop, A Niever as, S Marah, P Quintinita, F Atentar, E Alpuerto, A Punzalan, S Oliveros, K Oweng, J Abidin and AS Sahial.

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ARTICLE INFO

Received: 27 April 2020
Revised: 16 September 2021
Accepted: 09 December 2021
Available online: 16 December 2021

Role of Authors: LJP – conceptualization, data gathering, analysis and editing of the manuscript; JFDC – conceptualization, and manuscript writing; DAO – data gathering and documentation; PGCJ – data gathering and documentation; MDL - facilitated acquisition of permits, produced maps and conducted literature review; GF – data gathering, compiled field data and facilitated field sampling.

Notes on the presence of dwarf spinner dolphins *Stenella longirostris roseiventris* (Wagner, 1846) in Puerto Bay, Puerto Princesa City, Palawan

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https://doi.org/10.69721/TPS.J.2021.13.2.09

ABSTRACT

This research note aimed to document the presence of dwarf spinner dolphins *Stenella longirostris roseiventris* (Wagner, 1846) in Puerto Princesa Bay to update the cetacean species inventory of the bay and the little-known distribution of the subspecies in the country. During a dolphin watching expedition, the subspecies was spotted for the first time in Puerto Princesa Bay, Puerto Princesa City, Palawan just east of Turtle Bay on 29 May 2021. The presence of Gray's spinner dolphins *S. longirostris longirostris* (Gray, 1828) in the same vicinity highlighted the difference in size and shape of individuals of the two groups. The subspecies was confirmed using biometrics on a picture taken of a mother and its calf. The dorsal fin height and the pectoral fin ratios to the snout-to-dorsal fin length fit that of the dwarf spinner.

Keywords: biometrics, cetacean species inventory, subspecies distribution

Puerto Princesa Bay in Puerto Princesa City, Palawan is a known habitat to a variety of large marine wildlife which include elasmobranchs, marine turtles, and marine mammals. Apart from the dugong *Dugong dugon* (Müller, 1776) Palmer, 1895, a total 10 species of cetaceans have been previously recorded in the bay, namely: (1) Bryde's whale *Balaenoptera edeni* Anderson, 1879 (Dolar et al. 2012a); (2) pygmy killer whale *Feresa attenuata* Gray, 1874 (Aquino 2009; Aquino et al. 2012a); (3) short-finned pilot whale *Globicephala macrorhynchus* Gray, 1846 (Aquino 2009; Aquino et al. 2012b); (4) Risso's dolphin *Grampus griseus* G. Cuvier, 1812 (Torres 2008; Aquino 2009; Aquino et al. 2012c); (5) Fraser's dolphin *Lagenodelphis hosei* Fraser, 1956 (Torres 2008; Aquino 2009; Dolar et al. 2012b); (6) melon-headed whale

Peponocephala electra Gray, 1846 (Sabater et al. 2012); (7) Pantropical spotted dolphin *Stenella attenuata* Gray, 1846 (Torres 2008; Aquino 2009; Dolar et al. 2012c); (8) Gray's spinner dolphin *S. longirostris longirostris* (Gray, 1828) (Torres 2008; Aquino 2009; Dolar et al. 2012d); (9) Sperm whale *Physeter macrocephalus* Linnaeus, 1758 (Aquino 2009; Dolar et al. 2012e); and (10) Cuvier's beaked whale *Ziphius cavirostris* Cuvier, 1823 (Torres 2008; Aquino 2009; Aquino et al. 2012d).

With the high frequency of encounters with cetaceans, a unique dolphin watching enterprise as developed in the Bay before the onset of the COVID19 pandemic in 2019. Unlike most dolphin watching operations elsewhere, the local enterprise utilized fishers operating in the area as spotters. This, more often than not, assured cetacean encounters for the tourists they catered to. When the pandemic negated tourist travel to the city, the fishers continued to fish but the dolphin watching tours were grounded to a halt along with the updates they often collected on the cetacean populations in the Bay. After almost two years with no new information on the cetaceans in the Bay, the authors decided to go out and check on the animals. Thus, these notes were written to document and primarily provide update on the cetaceans encountered in the Bay.

In the morning of 29 May 2021, the team went out looking for cetaceans in Puerto Princesa Bay. Unlike the previous dolphin watching operations in the Bay, the observers had no prior access to the fishermen who could have directed them to the current location of the dolphins. After about three hours of random searching, however, a small group of spinner dolphins, about 16-24 individuals, were finally spotted off the coast of Puerto Princesa City, Palawan on the southern part of Puerto Bay approximately 9°39'47.6"N 118°46'00.7"E (Figure 1). The team initially saw three fishing vessels going back and forth and decided to check them out for the possibility of cetaceans feeding near them. Upon their approach, two dolphins jumped out of the water north of the vessels. Even before closer interaction, the observers determined the species to be spinner dolphins based on the acrobatic activity some individuals were engaged in.

During the interaction, several clusters of individuals were observed to be scattered in the general vicinity, most of which were exhibiting feeding/milling behavior. Two calves were noted swimming close to their respective mothers. One individual exhibited a large, deep wound on its back near the tail which may have been a cookie cutter shark bite but it did not appear to hinder its movements despite its size and depth (Figure 2). At some point of the interaction, several individuals approached the observers' speedboat to bow ride. These individuals had streamlined bodies and a very white underside (Figure 3).

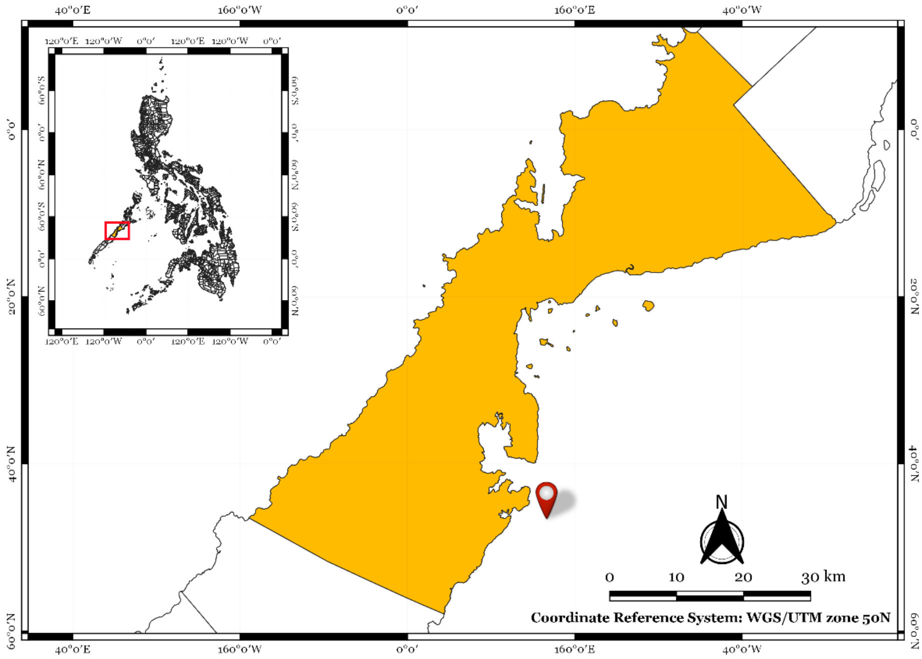

Figure 1. Map showing the general location of the dwarf spinner encounter in Puerto Bay, Puerto Princesa City, Palawan.

Figure 2. One dolphin had a rather deep and large wound on its back but it did not appear to affect its swimming.

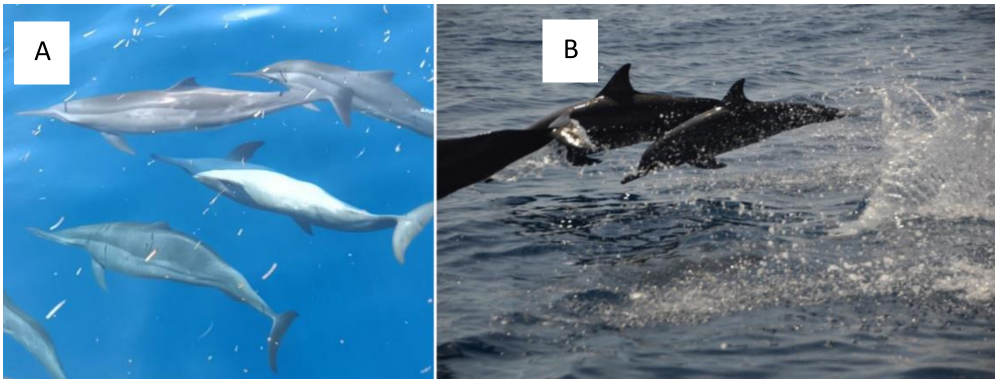

Figure 3. Most of the bow riding spinners had pale/white bellies (A) and streamlined bodies (B).

The team took note of a group of individuals constantly porpoising (i.e. alternately jumping fully out of the water and plunging back it while swimming) around the boat. These individuals were somewhat shorter than the rest but more rotund (thicker around the girth) with relatively smaller heads (Figure 4). These individuals were the first to break away from their interaction with the observers. When they had distanced themselves from the boat, they again started porpoising and the observers were presented with more opportunities to take pictures of individuals fully out of the water.

Figure 4. Dwarf spinners (both photos) are thicker around the girth and had smaller heads compared to the Gray's spinner dolphins (not in the photos above).

The difference in shape and size from the rest prompted the observers to suspect that these may be dwarf spinner dolphins loosely mixed in with a group of Gray's spinner dolphins. A review of the pictures taken by the group yielded pictures of a mother and calf completely out of the water and perpendicular to the observers, making biometrics possible. The dorsal fin height and pectoral fin length ratios to the snout-to-dorsal-fin length of the mother fit that of dwarf spinner dolphins. The calf's measurements were recognized since it was far from fully grown. Nevertheless, the calf's close presence confirmed that the individual measured was its mother and, therefore, an adult female despite its small size (Figure 5).

Figure 5. Mother and calf dwarf spinners *Stenella longirostris roseiventris*.

Dwarf spinner dolphins were initially identified from 10 dead specimens yielded by a local shrimp fishery in the Gulf of Thailand (Perrin et al. 1989). These were similar in size as the spinner dolphins that had been accidentally caught in the Arafura and Timor Seas off northern Australia. The subspecies has been re-described to have a shorter body (about 158 cm long), smaller skull, and lesser teeth and vertebrae counts. It also had proportionately larger pectoral and dorsal fins than the Gray's spinner dolphin. Since all specimens at the time were dead, no description of its external size, shape, and coloration could be given. The study also concluded that the subspecies was distributed in the shallow waters of Southeast Asia including the Gulf of Thailand, Timor and Arafura Seas, and similar waters of Malaysia, Indonesia, and northern Australia but not, at the time, in the Philippines (Perrin et al. 1999).

However, during a boat survey conducted in the waters surrounding Balabac Islands, Palawan in 2006 by a team led by Dr. Louella Dolar, a pod of dwarf spinner dolphins was encountered which yielded photographs of adequate quality to clearly illustrate its external appearance for the first time (Perrin et al. 2007). The dorsal fin height and the pectoral fin length ratio to the dorsal-fin/beak-tip length measured 21% and 28%, respectively, which fit the previously described proportions of the dwarf spinner dolphin (Perrin et al. 1999; Perrin et al. 2007).

Since then, the subspecies' presence has been documented in Tañon Strait in the Visayas (Aquino and Aca 2019), the Bohol Sea (Sabater pers. comm.), and now in Puerto Princesa Bay, Palawan (Figure 6). It was noted that the Balabac and Tañon Strait encounters involved pure dwarf spinner pods while that in the Bohol Sea (Sabater pers. comm.) and Puerto Princesa Bay were loosely mixed with Gray's spinners. The sightings in the Philippines seemed to indicate that the distribution of the dwarf spinner dolphin may be wider in Southeast Asia than previously known. There is also still much to be understood about the subspecies, especially its behavior. To do so, there should be no doubt that the spinners encountered are dwarf and not Gray's spinners. Understandably, it is difficult to confirm the species identification since an adequate photo is needed to do biometrics on. Unfortunately, the rose-colored belly cannot be used for identification since it is a result of a physiologic reaction and other spinners may exhibit such coloration at some point as well (Perrin et al. 1999). The team was lucky that it was mixed with some Gray's spinner dolphins and thus had a basis for comparison. However, this may not always be the case at the Balabac and Tañon Strait. Thus, good photographs are necessary to confirm identification and learn more about the subspecies.

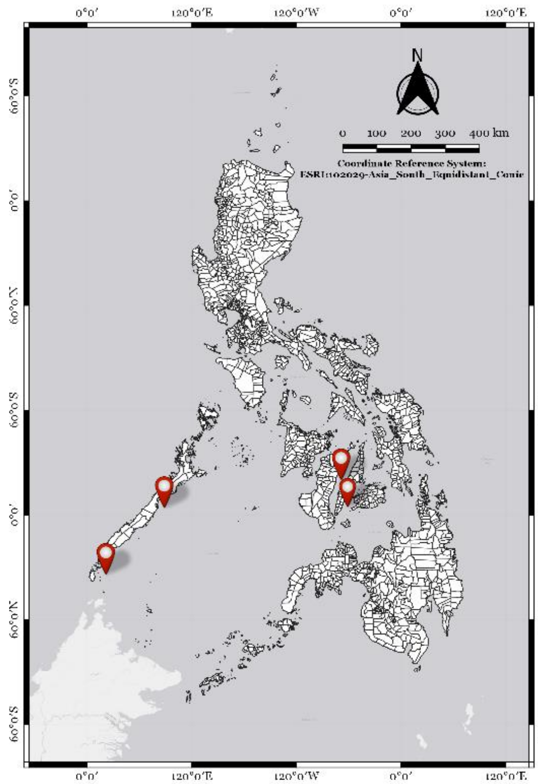

Figure 6. Map showing the location of confirmed encounters of dwarf spinner dolphins in the Philippines.

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ARTICLE INFO

Received: 21 June 2021
Revised: 29 October 2021
Accepted: 04 December 2021
Available online: 16 December 2021

Role of Authors: MTRA –spotter, photographer and writer; RMC, GMC, MADC and CHC – spotter and photographer.

Guide for Authors

The Palawan Scientist, a recipient of the CHED's Journal Incubation Grant, is an annual peer-reviewed multi-disciplinary journal published by the Western Philippines University, with its main campus in Aborlan, Palawan, Philippines. It accepts original research papers, reviews, notes and short communications in agriculture, fisheries and aquatic sciences, environment, education, engineering, mathematics, sociology and other discipline.

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

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

CITES (Convention on International Trade of Endangered Species). 2014. The CITES Appendices. Convention on International Trade in Endangered Species of Wild Flora and Fauna. www.cites.org. Accessed on 05 January 2014.

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

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

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

12. Citing a Report

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

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Volume 13(2), December 2021

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