Food Habits and Distribution of the Lake Taal Sea Snake (*Hydrophis semperi* Garman 1881) and the Sympatric Little File Snake (*Acrochordus granulatus* Schneider 1799) in Lake Taal, Philippines

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Abstract Our knowledge about the food habits of sea snakes and how it is associated with their distribution has seen much development through its description across a number of species available through published literature except for the key threatened species such as *Hydrophis semperi*. This paper aims to describe the food habits of *H. semperi* through gut content and stable isotope analyses. We also compared data with the Little File Snake, *Acrochordus granulatus*, sympatric with *H. semperi*. Recorded captures of *H. semperi* suggest that the sea snake tends to occur in the littoral zones and the shallower portions of the limnetic zone. Gut content analysis of *H. semperi* have shown that gobies and eels are primary prey items. Halfbeaks (Family Hemiramphidae) were recorded as one of the Lake Taal Sea Snake's prey items which is considered as a new prey record for sea snakes. These extracted gut contents are confirmed to be the temporal food preference of *H. semperi* given our detected stable isotope signatures. It appears that *A. granulatus* and *H. semperi* share common prey items suggesting possible diet overlap and resource competition. This study reports the first account of the endemic Lake Taal Sea Snake's distribution and food habits which poses implications towards its conservation as it occurs in a restricted ecosystem that has undergone considerable habitat alteration.

Keywords freshwater, diet, feeding, Hydrophis semperi, Hydrophiidae

1. Introduction

The study of an animal's food habits has allowed expansion of knowledge on various aspects of its biology and ecology (Voris and Voris, 1983). For sea snakes in general, food habits have been reported to some extent in

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scientific literature (Glodek and Voris, 1982; Lobo *et al.*, 2005; Rezaie-Atagholipour *et al.*, 2013; Su *et al.*, 2005; Voris and Voris, 1983; Voris *et al.*, 1978). Fish was found to be the preferred food item of sea snakes with one genus (*Emydocephalus*) being an exception as it only consumes fish eggs (Glodek and Voris, 1982; McCosker, 1975; Voris, 1966; Voris and Moffett, 1981; Voris and Voris, 1983). Despite the number of studies on the food habits of sea snakes, none have documented this in the Lake Taal Sea Snake, *Hydrophis semperi*. From its formal description as a novel species by Garman (1881), the Lake

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^{*}Corresponding author: Vhon Garcia, from the University of Santo Tomas Graduate School, Philippines, with his work on amphibians and reptiles particularly the biology, ecology, systematics, and phylogenetics of the endemic freshwater sea snake, Lake Taal Sea Snake (*Hydrophis semperi*).

Taal endemic sea snake was only mentioned in a limited number of studies which only conveyed information about its locality (e.g. Dunson and Minton, 1978; Dunson, 1975; Minton, 1975) and taxonomic description (e.g. Alcala, 1986; Herre, 1942; Smith, 1926). Despite being rare and endemic in its habitat and being classified as a Threatened ("Vulnerable") species by the International Union for Conservation of Nature (IUCN), many aspects on the biology of *H. semperi* remain unstudied (Gatus, 2010). Thus, we investigated its food habits through the analysis of its gut contents and stable isotope ratios. These analyses provided information about spatial and temporal patterns on the food habits of the poorly studied sea snake that occurs in freshwater. Moreover, we also identified its occurrence and distribution within the lake. The Lake Taal Sea Snake's rarity in its ecosystem, endemicity to its habitat, and high vulnerability to both natural and anthropogenic processes call for the description of its food habits and spatial distribution as these basic aspects pose implications for the sea snake's conservation. We compared the Little File Snake Acrochordus granulatus, a species with a wider geographic distribution occurring from West India through South Asia reaching up to the Solomon Islands, with *H. semperi. Acrochordus granulatus* is commonly found in mangroves and shallow coastal waters as well as rivers and freshwater lakes. In the Philippines, it co-exists with *H. semperi* in Lake Taal (Lillywhite and Ellis, 1994).

2. Materials and Methods

2.1 Sampling Lake Taal is a caldera lake found in the island of Luzon (Figure 1). It is the third largest lake in the Philippines with an area of approximately 268 km². Lake water drains through its sole outlet, Pansipit River to Balayan Bay in the southwest. The Taal Volcano Island sits in the middle of the lake which partially separates its north and south basins (Perez, 2008; Ramos, 2002). Six major areas (1–6, Figure 1) around Lake Taal were sampled for *H. semperi* and *A. granulatus* from June to November 2013. Sampling was done bi-weekly at sites with few to no aquaculture structures present (sites 3–6). Sampling was reduced to once a month at sites with heavy aquaculture use (sites 1 and 2). Gill-nets were

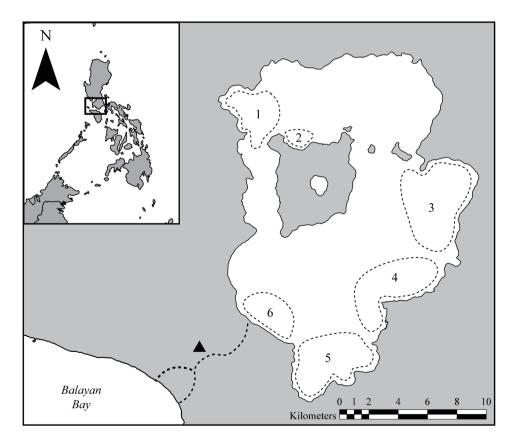


Figure 1 Sampling locations for *H. semperi* and *A. granulatus*. Sampling methods were in accordance with fishing practices in open water areas of the lake (i.e. gill-net fishing). Sampling areas were situated at approximately 300-500 m from shore and were spread across various portions of the lake bounded by dashed lines. Sampling at sites 1 and 2 was reduced due to the presence of aquaculture structures. Immediate area at the outflow of Pansipit River (\triangle) was also surveyed.

deployed for 12 hours overnight and were checked the next day for captures. Sampling size was controlled for *H. semperi* to avoid possible casualties of individuals from gill-net sampling given its rare, endemic, threatened "Vulnerable" species status (Gatus, 2010). We measured (in mm) the snout-vent length (SVL), tail length (TL), neck width, and body weight (in g) of the snakes. Snake sex was determined through hemipenal eversion. All snakes were recorded to summarize encounter rates. Captured live snakes were released back to the lake while dead individuals were dissected for gut content analysis.

2.2 Gut Content Analysis A mid-ventral incision was made to run from the anterior to the posterior portion of the snake to extract as much of the alimentary canal as possible. Regurgitation was employed (when possible) by sliding the stomach and esophageal regions between the thumb and index finger (Carpenter, 1958; Su *et al.*, 2005). Gut contents were preserved in 70% ethanol. All snake specimens were preserved using 10%–15% formalin and were deposited in the National Museum of the Philippines (Accession No. ACD7829–ACD7881). Gut contents, mainly fish, were measured of its length, width, and depth (in mm). Its orientation in the stomach of the snake was noted as well. Food items were identified to the lowest taxonomic level possible and were described of its habits and habitats.

2.3 Food Web Analysis Snake flesh samples (rectangular pieces of 5 cm × 1 cm× 1 cm in size) were extracted from the dorsal side of H. semperi (n = 2) and A. granulatus (n = 3). Snakes with varying snout-vent lengths (SVL) were used to best represent the size range of each species [SVL measurements (in mm) for H. semperi: 448 and 862; for A. granulatus: 340, 630, and 641]. General guidelines for stable isotope (SI) analysis were adapted (Jardine et al., 2003). We isolated fish and snake flesh and dried each for at least 24 h in 60°C. After which, each sample was ground to a fine powder, immersed in chloroform: methanol (2:1) solution for 24 h to remove lipids, and dried again in 60°C for another 24 h. After processing, each sample was pre-weighed (~0.5 mg) and wrapped in tin capsules. These were then combusted using a High Temperature Conversion Elemental Analyzer (ThermoScientific FLASH EA) and analyzed under high vacuum using an Isotope-Ratio Mass Spectrometer (IRMS) (ThermoScientific Delta V Plus) together with known amino acid standards. IRMS data was reported as isotopic notations of carbon (δ^{13} C) and nitrogen (δ^{15} N) and expressed as permil (%) deviation from standards (Vienna Pee Dee belemnite limestone carbonate and atmospheric nitrogen were used for nitrogen and carbon respectively) using the following equation:

 δ^{13} C, δ^{15} N = $[R_{sample}/R_{standard} - 1] \times 1000$ (‰) where $R = {}^{13}$ C/ 12 C or 15 N/ 14 N. These values where used to produce an isotope biplot to elucidate the potential trophic interactions between aquatic snake species and their potential prey items in Lake Taal. For the biplot, we assumed that the trophic enrichment factor would be 3.4‰ for δ^{15} N and 0.8‰ for δ^{13} C (France and Peters, 1997; Fukumori *et al.*, 2008; Shibata *et al.*, 2011; Zanden and Rasmussen, 1999). The analytical precision based on working standards was 0.20 and 0.10 ‰ for δ^{13} C and δ^{15} N respectively (Tayasu *et al.*, 2011).

3. Results

3.1 Sea Snake Distribution Twenty-four individuals of H. semperi and 88 individuals of A. granulatus were captured throughout the sampling duration. Most of the samples were obtained as by-catch from gill-net fishing. Figure 2 shows the encounter rates for the sympatric water snakes at each sampling site of Lake Taal. Most of the samples belonging to H. semperi and A. granulatus were acquired from the littoral to shallow limnetic areas of the lake being almost absent of aquaculture structures. This area of the lake is characterized to have a heavy macrophyte assemblage especially in the littoral zone. The Lake Taal Sea Snake has been also encountered in the shallow limnetic zone and was observed to have lifted its head above water; perhaps to breathe. On other parts of the lake, as in the northern and western sampling areas, only snakes belonging to A. granulatus were captured. Throughout most of Lake Taal, our data suggest that H. semperi and A. granulatus are more of syntopic species than just sympatric. The snakes occur side-by-side and at the same habitat zones of the lake rather than just the same geographical range. Although much of the lake is yet to be explored, this may explain the observable diet overlap and resource competition between the two snakes upon the examination of its spatial and temporal food habits.

3.2 Food items Of the 10 individuals of *H. semperi* examined for gut contents, three had food in their stomachs. The contents were identified as *Psammogobius biocellatus* (Valenciennes, 1837) (Family Gobiidae), *Zenarchopterus buffonis* (Valenciennes, 1847) (Family Hemiramphidae), and *Anguilla marmorata* Quoy & Gaimard, 1824 (Family Anguillidae). Forty-three individuals of the sympatric *A. granulatus* were also examined and were found to contain gut contents

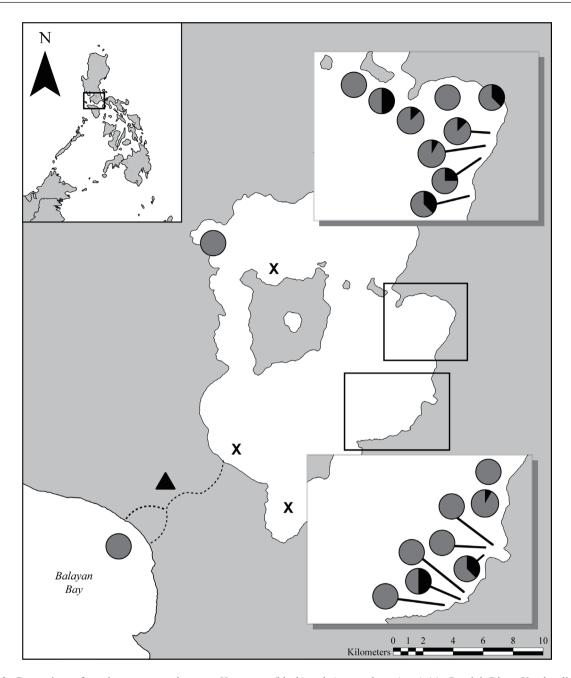


Figure 2 Comparison of species encounters between *H. semperi* (black) and *A. granulatus* (gray) (▲=Pansipit River, X = localities with no captures). Most samples were retrieved from the eastern and south-eastern portions of the lake where little to no aquaculture structures are present. Note the absence of *H. semperi* at lake regions with habitat alteration (aquaculture structures); although there is restriction in sampling opportunities on this part of the lake. Information from Balayan Bay was acquired from a visit done during the latter part of the sampling period. Immediate bay area near the outflow of Pansipit River was also surveyed. The lack of record of *H. semperi* in the bay supports the endemicity of *H. semperi* to Lake Taal and its inability to inhabit a marine habitat unlike other members of the Family Hydrophiinae.

identified as fish belonging to the Family Gobiidae. These items were classified as *P. biocellatus* (Valenciennes, 1837), *Acentrogobius suluensis* (Herre, 1927), and *Glossogobius giuris* (Hamilton, 1822) (Table 1). For both species, all prey items were ingested head-first.

3.3 Stable Isotope Analysis Our stable isotope analysis

has detected the trophic niche of the Lake Taal Sea Snake (i.e. piscivorous) and further strengthened our findings from the extraction of food items from the snake's gut. Stable isotope analysis for *H. semperi* and *A. granulatus* flesh samples generated mean $\delta^{13}C$ and $\delta^{15}N$ values. Table 2 shows the differences between the snake's carbon and nitrogen isotope values from their identified prey items

further confirming predator-prey association. Moreover, relative trophic positions of both snakes show close proximity with each other suggesting that both play similar roles as top predators in Lake Taal (Figure 3).

4. Discussion

4.1 Water snake distribution in Lake Taal The distribution of H. semperi in Lake Taal shows association with the sea snake's prey habits and habitats. As in previous studies, habits and habitats of the prey items of sea snakes have been valuable source of indirect data which helped in the formulation of inferences about its locations and distribution (Voris and Voris, 1983). Sites with high encounters of H. semperi are described to have heavy macrophyte assemblage. These macrophytes make good "sitting" grounds and refuges for sedentary or burrowing fishes which are the common prey items of H. semperi. This may account for the relatively high H. semperi encounters at these sites since the sea snake is able to employ its hunting strategy of investigating nooks and holes for any prey item. In comparison, sites that yielded low *H. semperi* captures and encounters were aquaculture sites in the lake. The effect of aquaculture on H. semperi distribution at these areas relate well to the findings of Papa *et al.* in 2011 where they reported a decline in water quality at locations near the vicinity of aquaculture structures. Moreover, increased microbial activity especially at the benthic portion of the lake was observed and was attributed to uneaten fish feed as well as feces from reared fish (Hallare *et al.*, 2009). This consequence of mismanaged aquaculture practices reduced the quality of the habitats of sedentary fishes and of the sea snake as well. Such disturbance resulting to habitat degradation has been noted as a potent cause of dispersal, population decline, and even species loss in aquatic snakes (Dodd Jr., 1987; Lukoschek *et al.*, 2013).

4.2 Food items and observed prey patterns The Lake Taal Sea Snake is an exception to its family (Hydrophiinae) whose members are classified as true sea snakes in that it inhabits Lake Taal which is a freshwater lake (Papa *et al.*, 2011; Rezaie-Atagholipour *et al.*, 2013). Despite the sea snake occurring in a freshwater habitat, its prey items are consistent with the general food habits of sea snakes found in seawater. Here, food items came from a limited sample size but represented previous food item observations (Glodek and Voris, 1982; Heatwole *et al.*, 1978; Voris and Voris, 1983) and interestingly added a new record in the food habit of sea snakes in general.

Eels and gobies being common prey items of sea

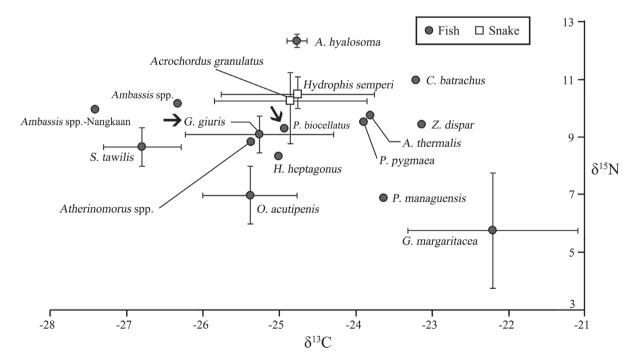


Figure 3 Stable isotope plot for *H. semperi* and *A. granulatus* with other Lake Taal biota (\bullet = Fish; \square = Snake). Arrows indicate fish species that were extracted from the guts of the snakes. Although some of the fish species were not found in the guts of these water snakes (e.g. *Atherinomorus* spp., *Hippichthys heptagonus*, *Oligolepis acutipenis*), stable isotope signatures reflect that these prey appear to be potential food habits of the snakes. It is worthy to note that these fish species share a common morphology. Moreover, the positions of the two water snakes suggest that they are the top predators in the lake ecosystem.

Table 1 Prey items of *H. semperi* and *A. granulatus*. Take note of the similarity in the diet in terms of fish family as well as prey item habit. This suggests that both snakes could have similar hunting modes (investigation of nooks and crannies) which further support previous findings. The sympatric water snakes also share Gobiid species as a preferred food item. With the natural restriction due to their habitat (i.e. lake), a possible diet overlap and resource competition could be present.

| Snake Species | Gut content | | |
|------------------------|------------------------------------|---------------|---------------|
| | Prey Item | Family | Habit |
| Hydrophis semperi | Psammogobius biocellatus (n = 1) | Gobiidae | Benthopelagic |
| | Zenarchopterus buffonis $(n = 1)$ | Hemiramphidae | Littoral |
| | Anguilla marmorata $(n = 1)$ | Anguillidae | Demersal |
| Acrochordus granulatus | Psammogobius biocellatus $(n = 9)$ | Gobiidae | Benthopelagic |
| | Acentrogobius suluensis $(n = 4)$ | Gobiidae | Demersal |
| | Glossogobius giuris $(n = 1)$ | Gobiidae | Benthopelagic |

Table 2 Mean stable isotope values of *H. semperi* and *A. granulatus* together with other Lake Taal biota. Take note of the values between the snakes and the fish items. An assumption for a stepwise enrichment of trophic level can be made for an increase of 3.4 and 0.8 for δ ¹³C and δ ¹⁵N respectively. This trend can also be approximated for prey items that were not extracted from the gut of the snakes.

| Species | δ ¹³ C | δ ¹⁵ N |
|---------------------------------------|-------------------|-------------------|
| Snake | | |
| Hydrophis semperi | -24.76 ± 0.49 | 10.46 ± 0.61 |
| Acrochordus granulatus | -24.85 ± 1.46 | 10.23 ± 1.01 |
| Fish | | |
| Glossogobius giurisª | -25.26 ± 0.97 | 9.07 ± 0.64 |
| Psammogobius biocellatus ^a | -24.93 | 9.28 |
| Atherinomorus spp.b | -25.37 | 8.83 |
| Hippichthys heptagonus ^b | -25.01 | 8.33 |
| Oligolepis acutipenis ^b | -25.38 ± 0.62 | 6.97 ± 1.00 |

^a Fish actually taken as food items by the snakes (extracted from gut)

snakes due to their morphology, scale type, and habitat is a result consistent with our study (Voris and Voris, 1983). The elongated morphology of these fishes facilitates ingestion upon capture. Also eels and gobies have naked scales, a characteristic of fishes that occur in the benthic zone, which allow easy penetration of fangs for energyefficient prey capture. Their habitat as well matches the microcephalic characteristic of H. semperi, as in other species of the genus Hydrophis. Sea snakes of this genus have exceptionally small head regions relative to the size of their posterior girths which allows them to investigate nooks and crevices for "sitting" gobies or find eels that occur as burrowers when taking refuge (McCosker, 1975). Should a prey be captured, their strategy of prey ingestion is also advantageous to their characteristic small heads. Sea snakes, as in H. semperi, ingest its food headfirst to avoid injury from fins and spines that might pierce the small Hyrophiid head.

In this study, aside from commonly known sea snake prey of eels and gobies, we report a species from the Family Hemiramphidae (Halfbeaks) as a prey item of *H. semperi*. This family is not included in the study of Voris and Voris (1983) which summarized published data on sea

snake diets. Halfbeaks are characterized to have similar morphology as gobies and eels as they possess elongated body forms. Despite being an unusual record, this further supports previous studies that reported sea snakes, even though generally known to be specialist predators, prey on fishes with similar morphologies across different fish families (Rezaie-Atagholipour *et al.*, 2013; Su *et al.*, 2005).

It is important to note the addition of fishes from the Family Hemiramphidae as prey items of sea snakes and *H. semperi* in particular. Fishes from this family are known to be planktivorous and utilize the pelagic zones of lakes (Barletta and Blaber, 2007). With that habit, sea snakes that characteristically rise to the surface of the water in order to respire may encounter such fish and consider them as prey due to the similarity in morphology with its other food items.

Diet overlap has been observed to be minimal between sympatric sea snake species (Su *et al.*, 2005). In Lake Taal, *H. semperi* is sympatric with *A. granulatus* and is suggested to be syntopic given previous findings. In this study, the stomach contents of *A. granulatus* only included Gobiid species which are also prey items for *H.*

^b Potential food items as interpreted from the SI plot

semperi showing that both species share a similar fish family as prey. This is further supported by stable isotope analysis confirming that these prey items captured the long-term food habits of H. semperi and A. granulatus. Furthermore, this similarity in prey between H. semperi and A. granulatus in Lake Taal suggests that there is a possible diet overlap between the two water snakes that occurs temporally as well. This result is parallel with the findings on Lapemis hardwickii Gray 1834 (Hydrophiidae) and A. granulatus at Sungai Buloh and Parit Botak in Malaysia. Sharing four species as their prey items, maximum diet overlap was observed between the L. hardwickii and A. granulatus (Voris and Glodek, 1980; Voris and Voris, 1983). However, H. semperi could be addressing this possible overlap in diet by considering other fish prey with the same morphology, as in other sea snakes; a pattern not observed in A. granulatus (Glodek and Voris, 1982; Voris and Glodek, 1980). Nonetheless, the limitation in available prey brought by a lake ecosystem aggravated by habitat alteration may intensify the presence of diet overlap between the two syntopic species. However, a more detailed investigation is needed to confirm the level of diet overlap between the two species.

4.3 Baseline information on *H. semperi* The results highlighted in this study fills in the lack of published information on the snakes in Lake Taal particularly on the endemic *H. semperi*. As other more complex aspects on the biology of *H. semperi* remain to be explored, the baseline information presented here can provide suggestions towards efforts in conservation of the *H. semperi* and its habitat. This study underlines the presence of an important sea snake species in Lake Taal and may initiate management actions towards its protection. The relevance of this study can be further emphasized considering the sea snake's habitat that experiences intensified habitat alteration; a known cause of snake population decline in nature (Dodd Jr., 1987).

This study presents the first account on the food habits and distribution of *H. semperi* which are typical of sea snakes in having specialized feeding on fish from few families having the same morphology. Similar food items as well were shown to be shared between *H. semperi* and *A. granulatus*. Moreover, spatial and temporal food habit data are consistent and confirm predatorprey relationships between the snakes and fish species as shown by the snakes' gut contents and stable isotope signatures respectively. A possible diet overlap could be present between the species and the nature of their habitat could play largely towards this condition, although much

investigation regarding this is needed. Also, the reported limited distribution of *H. semperi* in the lake can be associated with habitat alteration due to aquaculture-induced eutrophication. Despite our limited sampling across the lake area, this study gives baseline information regarding the endemic Lake Taal Sea Snake which is virtually unstudied in scientific literature.

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