

# Abundance and microhabitat association of *Barbourula busuangensis* (Anura: Bombinatoridae) in Busuanga Island, Philippines

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## Abstract

### Abundance and microhabitat association of *Barbourula busuangensis* (Anura: Bombinatoridae) in Busuanga Island, Philippines.

*Barbourula busuangensis*, commonly known as the Philippine Flat-Headed Frog, is a near-threatened species endemic to Palawan, Philippines. Despite its distinct ecological niche and unique characteristics, including a flat head and body to support its fully aquatic lifestyle, limited studies have been conducted to understand its habitat requirements and population status. We conducted visual encounter surveys and hand captures along 30–100 m belt transects in two Barangays, Cheey and New Busuanga. A total of 270 individuals were recorded and microhabitat variables were compared between the two sites. However, the abundance of *B. busuangensis* was significantly higher in Cheey compared to New Busuanga. We found significant differences in six key microhabitat variables (relative humidity, temperature, stream/river depth and width, rocks percentage, water flow and turbidity) between the two locations, suggesting diverse environmental conditions impacting frog populations. Additionally, 14% of the captured frogs (23 individuals) exhibited morphological deformities, though the cause remains undetermined. Our findings underscore the urgent need for further research and targeted conservation efforts to ensure the survival of this species. This study provides essential data on the distribution and habitat preferences of *B. busuangensis*, informing conservation strategies to mitigate population declines and address environmental challenges affecting this unique amphibian.

**Keywords:** Amphibians, Endemic species, Palawan, Stream, Watershed.

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## Resumo

**Abundância e associação de micro-habitats de *Barbourula busuangensis* (Anura: Bombinatoridae) na ilha de Busuanga, Filipinas.** *Barbourula busuangensis*, popularmente conhecida como rã-de-cabeça-chata-das-filipinas, é uma espécie quase ameaçada e endêmica de Palau, Filipinas. Apesar de seu nicho ecológico distinto e características únicas, como a cabeça e o corpo achatados que suportam seu estilo de vida totalmente aquático, são limitados os estudos efetuados para compreender seus requisitos de habitat e status populacional. Realizamos levantamentos visuais e capturas manuais ao longo de transectos de 30–100 m em dois distritos, Cheey e New Busuanga. Um total de 270 indivíduos foi registado, e as variáveis de micro-habitat foram comparadas entre os dois locais. A abundância de *B. busuangensis* foi significativamente maior em Cheey do que em New Busuanga. Encontramos diferenças significativas em seis variáveis-chave do micro-habitat (umidade relativa, temperatura, profundidade e largura do riacho/rio, percentagem de rochas, fluxo e turbidez da água) entre os dois locais, sugerindo condições ambientais diversas com impacto nas populações. Além disso, 14% das rãs capturadas (23 indivíduos) apresentavam deformações morfológicas, embora a causa permaneça indeterminada. Nossas descobertas ressaltam a necessidade urgente de mais pesquisas e esforços de conservação direcionados a garantir a sobrevivência dessa espécie. Este estudo fornece dados essenciais sobre a distribuição e as preferências de habitat de *B. busuangensis*, sugerindo estratégias de conservação para mitigar o declínio da população e abordar os desafios ambientais que afetam esse anfíbio único.

**Palavras-chave:** Anfíbios, Bacia hidrográfica, Espécie endêmica, Palawan, Riacho.

## Introduction

The Philippine archipelago is notably considered as one of the most important centres of amphibian and reptile diversity in Southeast Asia (Brown and Alcalá 1970, Diesmos *et al.* 2002). This accounts for 116 species of amphibians, with 99 (85%) being endemic (Diesmos *et al.* 2015) now known in the country. With its rich archipelago, one of its unique biodiversity islands is the Palawan which is also home to one of the country's evolutionary distinct frog species—the *Barbourula busuangensis* Taylor and Noble, 1924. This is one of the ancient frog genera *Barbourula* that has only two known species worldwide including the Philippine Flat-headed Frog, *B. busuangensis*, and the Bornean Flat-headed Frog, *B. kalimantanensis* Iskandar, 1978. Notably, both are considered threatened by the International Union for Conservation of Nature (IUCN 2019).

*Barbourula busuangensis* was first described in Busuanga Island and was later known to occur in Northern Palawan (Myers 1943, Brown and Alcalá 1983). There remains to be a question on

the validity of the record in the town of Culion (IUCN 2018). It is highly aquatic and requires clean and cool mountain streams (Alcalá and Brown 1987, Alcalá and Brown 1998). It usually hides in crevices or under boulders in the stream bed during the day (Schoppe and Cervancia 2009) and emerges at dusk to forage. Individuals can easily be detected since their eyes reflect the light of a torch or a flashlight. There is few published information available on the ecology and conservation status of the species and most were conducted in mainland Palawan and very little information is available on the Calamian Islands including Busuanga Island from which it gets its name.

The Calamian Islands constitute the easternmost extension of the “Palawan Faunal Region”; a globally important center of species biodiversity and endemism, partially connecting two of the world's major biogeographic regions. The island group comprises a total of about 95 isles and islets, covering a total area of 1,554 km<sup>2</sup>, including the four largest islands of Busuanga, Culion, Coron and Caluit, which are included amongst the 117 Philippine ‘Important Bird

Areas (IBAs)', and 206 'Key Biodiversity Areas (KBA's)' identified in the 2002 Philippines Biodiversity Conservation Priority Setting and Action Plan.

The Municipality of Busuanga has a total freshwater area of 136.87 ha. A total of 12 streams and rivers were surveyed by Afuang and Cielo in 2009, three rivers and streams by Paguntalan *et al.* (2012) and two rivers and two streams by Paguntalan *et al.* (2015) and in the current study one sampled stream, the Chinibayan overlap with the past studies of Afuang and Cielo (2009) and Paguntalan *et al.* (2012). The rivers and streams in Calamian Islands are still unclassified according to DENR-EMB (2019). Given the limited data on *B. busuangensis* in the Calamian Islands and the pressing need to understand its ecology for conservation efforts, this study aims to present ecological information on the species and the conservation status of its habitat in Busuanga Island. Our research is significant for filling the knowledge gaps regarding the species' distribution, population dynamics, and habitat requirements, ultimately contributing to the development of effective conservation strategies in the area.

## Materials and Methods

### Site Description

Busuanga is the largest island in the Calamian group of islands (12°08'42.00" N, 120°05'40.92" E) and is divided into two municipalities: 'Busuanga' in the west and 'Coron' in the east. Its highest peak is Mount Kilien located in the South-eastern section of the Island. The mountain is listed amongst the 206 Key Biodiversity Areas (KBA's) identified in the 2002 Philippines Biodiversity Conservation Priority Setting and Action Plan. It spans at 392.9 km<sup>2</sup> mosaic of habitats including pastureland, agricultural areas, and karst forest (Figure 1). Most of the remaining forests in Busuanga Island were established as a Pastureland Reserve under Proclamation 1387

issued by President Ferdinand Marcos on 13 February 1975.

A total of three rivers and three streams were surveyed in Busuanga Island from 04–10 July 2017. Rivers are defined as relatively large lotic waterbody of >5 m wide, while streams are relatively small lotic waterbodies of < 5 m wide (Davies *et al.* 2008). Chinabayan River (12°13'33.9924" N, 119°56'24.1440" E). Located in Datay Mountain Range in New Busuanga with an elevation of 43 m a.s.l. The river expands up to 20 m wide and up to 126 cm deep. Deep pools with cloudy appearance interspersed with large boulders and rock crevices characterized the area near waterfalls. The canopy coverage on the riverbanks was at 60% and understory coverage was at 50% maximum. The sampling area comprises old-growth (containing large, mature trees, a multi-layered canopy, and diverse understory vegetation) and secondary forest (containing younger, rapidly growing tree species) with a closed canopy and understory filled with shrubs and tree ferns. The Chinabayan River is continuously flowing throughout the year. We established and surveyed a total of seven transects with 100 m length in the area (Figure 2).

The first stream feeding into Chinabayan River (stream 1: 12°13'16.3344" N, 119°56'29.1768" E) has an elevation of 48 m a.s.l. The width and depth of the stream were measured, and it expands of up to 4.5 m wide and 18 cm deep. The water was very clear even the submerged features in stream like rocks and sand particles were visible. We visually measured canopy coverage of the stream, which was at 90% and understory coverage was at 70% maximum. Epiphytes, shrubs, and fern trees were present with few *Rattanus* sp. observed. In this stream, we established surveyed at least two transects with a 100-m length.

The second tributary that also feeds Chinabayan river (stream 2: 12°13'14.4840" N, 119°56'25.6200" E) has an elevation of 74 m a.s.l. and expands up to 3.5 m wide and reaches 38 cm deep. We visually measured canopy

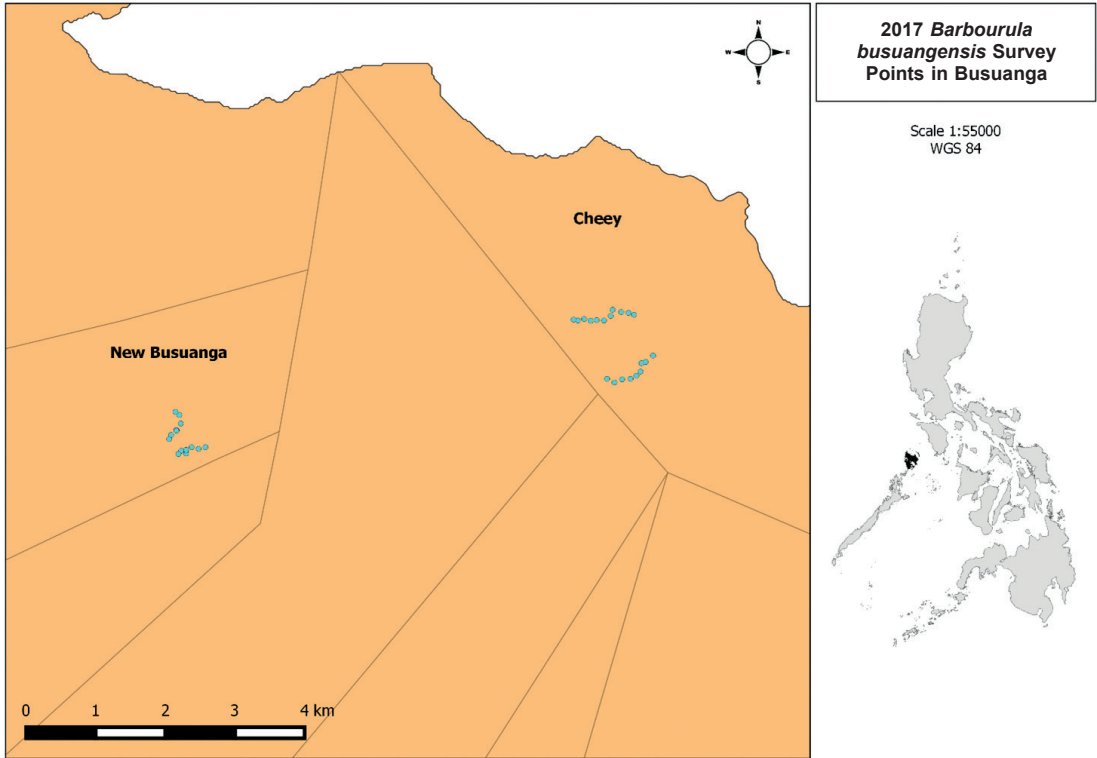


Figure 1. Location of study site (dot) in Busuanga Island.

coverage of the stream along stream banks which was at 90% and understory coverage was at 40%. Epiphytes, shrubs and fern trees were present with few *Rattanus* sp. observed. We established and surveyed at least four transect lines measuring up to 100 m.

The third tributary in Chinabayan river (stream 3: 12°13'25.2480" N, 119°56'24.5760" E) was at an elevation of 40 m a.s.l., width expanding to 4.8 m wide and depth at 20 cm. Like the first and second tributaries, water was very clear where submerged particles were visible. We visually measured canopy coverage of the river which was at 85% and understory coverage was at 60%. Epiphytes, shrubs and bamboo species were present with few *Rattanus* sp. observed. In this stream, we established and surveyed at least two transect lines measuring up to 100 m.

The forest in Cheey serves as the watershed of the barangay and covers less than 100 hectares with an elevation of 74 m a.s.l. The Malapinggan River (12°14'21.4800" N, 119°59'51.8640" E) is the largest river traversing the mountings and has a width of 9.6 m and depth of up to 219.5 cm. with clear water. We visually measured canopy coverage of the river which was at 90% and understory coverage was at 50% maximum. Presence of bamboo, Narra and Ipil—*Intsia bijuga* (Colebr.) Kuntze—dominate the surrounding forests with *Ficus* species scattered within the forests. The understory is mostly dominated by palms, rattan and wildlings of various kinds. We established and surveyed at least eight transect lines measuring up to 100 m (Figure 3A).

Lungon River (12°14'00.1320" N, 120°00' 11.1600" E) is in Barangay Cheey with an



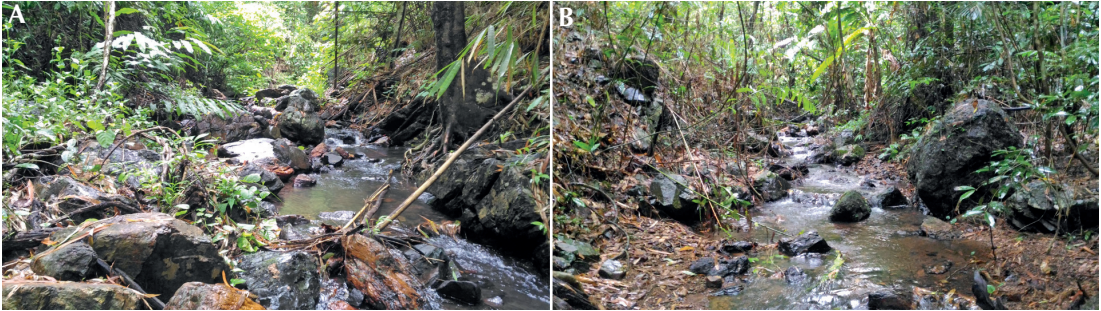


**Figure 2.** Sampling stations including Chinabayan River (A), tributary 1 (B), tributary 2 (C), and tributary 3 (D) in Brgy, New Busuanga.

elevation of 32 m a.s.l. The width and depth of the river were measured, and it expands up to 5.5 m wide and 40.7 cm deep. The water was slightly turbid. Canopy coverage was also at 90% and understory coverage was at 60% maximum. The surrounding area includes a mixed plantation of bananas, coconut trees, jackfruit, citrus, etc. Dipterocarps were also observed interspersed in some areas surrounding the stream and a few species of shrubs dominated the understory. Ferns and mosses were very rare. We established and surveyed at least 10 transect lines measuring up to 100 m (Figure 3B).

#### *Frog Detection Transect*

Frog composition was determined using belt transect method which measured 100 m each and were lined continuously along the streams and rivers of Barangays Cheey and New Busuanga from 04–10 July 2017. Juveniles were identified based on body size and based on Alcala and Brown's (1998) description that colored morph indicates that the frog is juvenile whereas dark morph indicates that the frog is an adult. Females are significantly larger than males in most of the frog species to accommodate mating (i.e.



**Figure 3.** Malapinggan River (A) and Lungon River (B) in Cheey.

amplexus) and females are the ones responsible in laying eggs. Larger females tend to produce more eggs than average-sized females. We surveyed a total of thirty transects with at least one hundred meters in length established on streams. Visual Encounter Survey (VES) and hand capture were conducted. We conducted the surveys from 1800 to 2100 hours where *B. busuangensis* were active.

#### *Stream Microhabitat Assessment Characteristics*

Microhabitat characteristics such as rate of water flow, turbidity, stream width and depth, percentage of rocks, relative humidity (day and night), and the temperatures of water, air and soil (day and night) were determined using 10 × 10 m<sup>2</sup> plots (Table 1). These variables were measured to understand the specific environmental conditions that influence the distribution of and survival of species within a habitat (Dunlop *et al.* 2005, Goldstein *et al.* 2017). A total of 36 plots were sampled following the modified stream visual assessment protocol by Magbanua *et al.* (2008). The description of each microhabitat variable and the methods used for their measurement are provided in Table 1.

#### *Data Analysis*

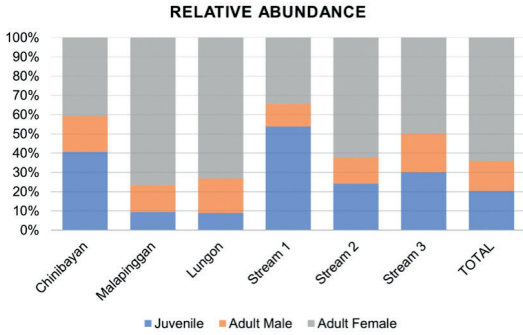
We recorded the biometrics, age and sex of captured individuals in standard data sheets.

Morphometric measurements such as snout–vent length (SVL) and weight were measured. We used Mann-Whitney U test to compare two sample means of each microhabitat variables from the same population. The Mann-Whitney U test is a non-parametric test, making it suitable for comparing two independent groups when the data do not necessarily follow a normal distribution. We used the Mann-Whitney U, given that our microhabitat data did not meet the assumptions of normality and homoscedasticity required for parametric tests.

## **Results**

### *Species Abundance*

A total of 270 individuals of *Barbourula busuangensis* was recorded in rivers and streams in New Busuanga and in Cheey, of which 20.37% were juveniles and 70.63% were adult frogs with the majority represented by females with a relative abundance of 64.07%, while only 15.56% were males (Figure 4). Juveniles were mostly seen and captured in streams in puddles and in slow-moving waters. We saw and captured individuals of *B. busuangensis* in rivers of Malapinggan and Lungon, wherein most of which were adults. We also observed the presence of eggs in some female frogs. Possible nesting sites were not discovered during our study, so the opportunity to understand the mode



**Figure 4.** Comparison of recorded individuals in rivers and streams in Brgy. Cheey and Brgy. New Busuanga.

of reproduction of *B. busuangensis* remains unknown. We identified at least seventy-five gravid females (indicated by the presence of large yellow eggs in their bellies). Females ranged in weight from 18–149.9 g and SVL from 50.7–99.7 mm, while males ranged in weight from 13–87 g and SVL from 47.7–89.5 mm (Table 2).

We observed a few individuals of *B. busuangensis* with morphological abnormalities (e.g., missing hindlimbs, forelimbs and toe phalanges). These deformities were observed on individuals captured in Malapinggan river in Barangay Cheey but not in New Busuanga. Out of the 163 individuals 23 or 14% (15 females and 8 males) had this condition (Figure 6).

### Microhabitat Characteristics Assessment

We used Mann-Whitney U test to compare microhabitat variables between downstream and upstream with the presence of *B. busuangensis*. We observed significant difference in night soil temperature with a difference of 1.55°C ( $p$ -value = 0.04). Mean soil temperatures downstream during the night was 25.9°C while upper stream was 24.3°C. Also, we noted a significant difference of 6.18 cm depth in the upper stream. The widest recorded width was 9.6 m in Malapinggan river while Chinabayan river was the deepest. Apart from this, the percentage of rocks and the water flow velocity were greater in areas downstream. Shallow, cold, and clear waters were mostly observed in the upper stream. Canopy cover and understory cover also thicken going upstream (Table 3).

At least six microhabitat variables such as night relative humidity, air temperature, day soil temperature, day water temperature, night soil temperature, and night water temperature presented significant differences across sites. Also, Chinibayan river in New Busuanga was significantly deeper than that of Cheey’s. In New Busuanga, water in stream tributaries were clearer and had a lesser velocity than the Chinibayan river. However, in Cheey, Malapinggan river was clearer than that of Lungon’s (Table 4).

**Table 1.** Stream and river characteristics, unit and methods used.

Stream characteristics	Method of acquisition
Width (m)	Visual Estimate
Depth (cm)	Steel Tape/Meter Stick
Rocks (%)	Visual Estimate
Water, Air and Soil Temperatures (°C)	Digital Thermometer
Relative Humidity (%)	Psychrometer
Rate of Water Flow/Velocity (scoring)	Visual Estimate
Turbidity (scoring)	Visual Estimate





**Figures 5.** Photos of *Barbourula busuangensis* recorded in Barangays New Busuanga and Cheey. Juveniles (A–B), adult (C).

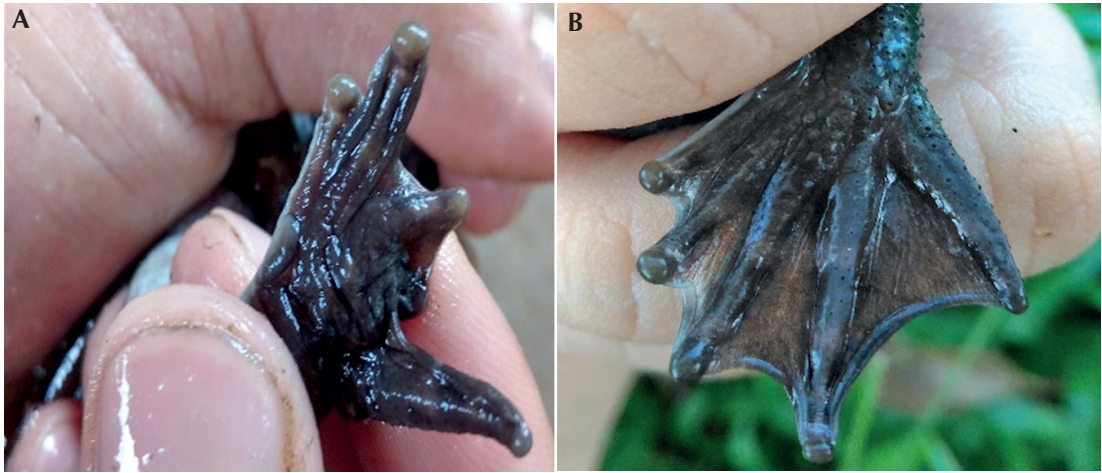
**Table 2.** Comparison of weight and SVL of captured females and male individuals in Brgy. Cheey and Brgy. New Busuanga.

	Weight (g)		Snout–vent length (mm)	
	Range	Mean	Range	Mean
Females (N = 115)	18–149.9	60.98	50.7–99.7	76.50
Males (N = 20)	13–87	35.85	47.7–89.5	86.88

**Table 3.** Results of Mann-Whitney U Test between stream habitat variables of downstream (DS) and upstream (US). \*Significant at 95% confidence interval.

Microhabitat characteristics	DS Mean	US Mean	Difference	p-value
Day Relative Humidity (%)	86.777778	87.94444	-1.1666667	0.21288109
Night Relative Humidity (%)	85.888889	86.66667	-0.7777778	0.59604419
Air Temperature Day (°C)	26.244444	26.04444	0.2000000	0.61009404
Soil Temperature Day (°C)	26.038889	25.92778	0.1111111	0.84830844
Water Temperature Day (°C)	26.166667	25.86667	0.3000000	0.35446707
Air Temperature Night (°C)	25.583333	25.28333	0.3000000	0.48342756
Soil Temperature Night (°C)	25.872222	24.32222	1.5500000	0.03925102*
Water Temperature Night (°C)	25.822222	24.95000	0.8722222	0.12011229
Stream Width (m)	4.770556	4.51000	0.2605556	0.81232551
Stream Depth (cm)	32.533333	38.71111	-6.1777778	0.97475214
Rocks Percentage (%)	45.083333	45.83333	-0.7500000	0.96184032
Rate of Water Flow (scoring)	13.555556	14.22222	-0.6666667	0.64428892
Turbidity (scoring)	15.777778	15.11111	0.6666667	0.54388968





**Figures 6.** Abnormal deformities (absence of 2<sup>nd</sup> toe on the left) vs. normal (right) phalanges of *Barbourula busuangensis*.

**Table 4.** Results of Mann-Whitney U Test between river habitat variables of Cheey and New Busuanga. \*Significant at 95% confidence interval.

Microhabitat characteristics	Cheey mean	New Busuanga mean	Difference	p-value
Day Relative Humidity (%)	86.700	88.187500	-1.487500	0.114
Night Relative Humidity (%)	85.350	87.437500	-2.087500	0.002*
Air Temperature Day (°C)	25.250	27.262500	-2.012500	0.000001*
Soil Temperature Day (°C)	25.305	26.831250	-1.526250	0.0000009*
Water Temperature Day (°C)	25.465	26.706250	-1.241250	0.0000005*
Air Temperature Night (°C)	25.300	25.600000	-0.300000	0.378
Soil Temperature Night (°C)	24.450	25.906250	-1.456250	0.037
Water Temperature Night (°C)	24.925	25.962500	-1.037500	0.006
Stream Width (m)	3.955	5.496875	-1.541875	0.239
Stream Depth (cm)	32.615	39.381250	-6.766250	0.192
Rocks Percentage (%)	44.075	47.187500	-3.112500	0.677
Rate of Water Flow (scoring)	14.100	13.625000	0.475000	0.988
Turbidity (scoring)	15.350	15.562500	-0.212500	0.552

## Discussion

Our observations indicate distinct habitat preferences between adult and juvenile frogs. Adult frogs predominantly inhabit rivers, while juveniles prefer streams or small channels. In

New Busuanga, the prevalence of juveniles in streams is likely due to the presence of three tributaries in the area. Notably, a significant proportion of the captured females were gravid, easily identified by their large yellow eggs. It is also evident that females are generally larger and

heavier than males. This size difference is likely an adaptation to accommodate the reproductive demands of amphibian mating, as larger females can produce and lay more eggs. However, potential nesting sites were not observed during our study, and the mode of reproduction of *B. busuangensis* remains unknown. Additional research and field monitoring of *B. busuangensis* is required to understand their habitat requirement for potential nesting sites, behavioral observation of the species and microhabitat analysis. According to the studies of Wells (2010), Crump (2015), and Glime and Boelema (2017), additional steps include field observations, studying eggs and larvae, conducting histological examinations of gonadal tissues, acoustic monitoring, and genetic investigation to understand mating systems and implementing long-term monitoring to observe seasonal as well as annual variations in reproductive activity (Figure 5).

Considering the two barangays studied, Cheey recorded the highest number of *B. busuangensis*. This variation in population density may be attributed to several factors including habitat quality and availability, water quality, temperature, and anthropogenic factors (Collins and Storfer 2003, Cushman 2006, Stuart et al. 2004, Blaustein et al. 2010). Out of the thirteen microhabitat characteristics tested using Mann-Whitney U test, soil temperature at night was the only variable to show a significant difference between downstream and upstream sites. This indicates that soil temperature at night significantly differs between these two areas, suggesting it may play a crucial role in habitat selection for these frogs. Additionally, six microhabitat variables showed significant differences between Cheey and New Busuanga. Night relative humidity significantly differed between the two sites. Relative humidity is essential for amphibians as they are highly sensitive to desiccation, and high humidity levels are crucial for their skin respiration and hydration (Wells 2010). Daytime air temperature had a strong significant difference between the two

locations. Air temperature affects the metabolic rates and activity levels of amphibians, and cooler daytime temperatures may be preferable for *B. busuangensis*, aligning with their need for a cooler habitat (Infante et al. 2002). Soil temperature also showed significant differences both during the day and at night. Soil temperature can influence the thermal environment of frogs, affecting their behavior, physiology, and microhabitat use. Optimal soil temperatures are crucial for maintaining the body temperature of these ectothermic animals. Water temperature also had significant differences during the day and at night. Water temperature is a critical factor for amphibians, particularly for those like *B. busuangensis* that inhabit streams. Cooler water temperatures are typically associated with higher dissolved oxygen levels, which are beneficial for both the frogs and their prey (Phochayavanich et al. 2010).

Additionally, based on the study of Wells (2010), the abundance of this frog species can also be influenced by various environmental factors, crucial for their survival given the sensitivity of anurans to changes in the environment. According to Infante et al. (2002), *B. busuangensis* inhabit cool and clean mountain streams, making their homes beneath and between rocks. These frogs often hide underwater or in crevices during the day to avoid predators, preferring clean, cool waters and soil/sand surfaces. At night, they are active, hunting for food and resurfacing within the vicinity of rivers and streams with a good environment thus provides a suitable habitat for these frogs. Stream width and depth also play significant roles in the abundance of adult and juvenile frogs. Streams in the studied areas vary greatly in habitat characteristics, influencing the presence of frog species. Research by Porter (2010) and Phochayavanich et al. (2010) indicate that stream-breeding frogs require streams large enough to hold water for extended periods, ensuring tadpole survival. Larger streams provide suitable breeding habitats for a greater variety of frog species due to the extended water presence

and greater diversity of microhabitats and food resources. Our results also suggest that microhabitat characteristics significantly influence the presence and distribution of *B. busuangensis*. The significant differences in soil and water temperatures, as well as relative humidity, highlight the species' preference for specific environmental conditions. These findings underscore the importance of preserving the unique microhabitats that support the survival of this species, particularly in the face of environmental changes and habitat disturbances (Hopkins 2007). Understanding these microhabitat preferences is crucial for developing effective conservation strategies. By maintaining optimal environmental conditions, such as appropriate humidity levels and cooler temperatures, we can support the continued survival of *B. busuangensis* and other sensitive amphibian species in these areas.

In our selected sites, we observed that streams with specific characteristics tended to support different life stages of *B. busuangensis*. For instance, in New Busuanga, the tributaries are located deep inside the forest with nearly 100% canopy cover, slow to moderate water flow, clear turbidity, and narrower, shallower streams compared to the Chinibayan stream. Juveniles, typically less than one inch, were predominantly found in these tributaries and small puddles near the stream, while none were observed in the wider, deeper, and faster-flowing Chinibayan stream. In Cheey, the Malapinggan stream, which has the clearest water based on turbidity rates and slow to moderate flow, supported a higher presence of *B. busuangensis*. Conversely, the Lungon stream, the most disturbed site, recorded a very low presence of *B. busuangensis*, with only 26 individuals seen and captured despite the established transect points.

Prior to the implementation of active enforcement measures in Cheey, the use of pesticides by locals for fishing and catching freshwater crabs and shrimps was widespread (Paguntalan 2015). Additionally, farmers near

the river applied herbicides to their agricultural lands. Research by Bishop and Haas (2009) indicates that the rampant use of chemicals in agriculture significantly affects the survival of both tadpoles and adult frogs. This can lead to morphological abnormalities, such as the presence of extra or missing legs or limbs, a phenomenon also observed in the Philippines (Shuman-Goodier *et al.* 2017, Propper *et al.* 2020). The use of these chemicals has detrimental effects on the frog population. Pesticides and herbicides can contaminate water bodies, leading to toxic environments for aquatic life. For frogs, which have permeable skin and rely on clean water for reproduction and development, exposure to these chemicals can be particularly harmful. Tadpoles are highly susceptible to these toxins, which can interfere with their growth and lead to deformities. These deformities not only affect individual frogs but can also reduce the overall fitness and survival of frog populations.

After engaging with the community, it is evident that the local communities in these areas are aware of the importance of biodiversity, especially due to their firsthand experiences of declining water levels and the drying up of streams. Despite the awareness, the size of these forests is decreasing due to deforestation and conversion to various land uses, driven by human consumption and activities. The ongoing reduction in forest size poses a significant threat to the habitat and survival of *B. busuangensis* and other local species. Understanding the value of the remaining forests, their direct relationship with water quality, and the importance of wildlife in preserving the forest ecosystem is crucial for effective conservation. The forests in Cheey and New Busuanga serve as watersheds, maintaining clean water supplies essential for both human and wildlife communities. Educating local communities about the ecological roles of species like the Philippine Flat-Headed Frog and promoting sustainable practices can help protect these habitats. Engaging communities in conservation efforts and demonstrating the long-term benefits of preserving natural resources are

key steps towards sustainable environmental stewardship. Fragmentation of forests habitat and threats to survival of single-island endemic species remains a major concern. The loss of forests is happening due to insufficient local governance mechanisms towards effective biodiversity conservation and the lack of technical capacity of local government units to manage these unique ecosystems. Added to this, the continuing land conversions, road development, river/stream poisoning, low appreciation on the uniqueness of the biodiversity of Busuanga contributed to the low support for biodiversity conservation. Although Chinibayan river is within the jurisdiction of Busuanga Pasture Reserve at the time and Malapinggan river as source of drinkable water to Barangay. On-going habitat changes brought by human activities pose a threat to *Barbourula busuangensis*'s population and to other species. The most common occurrences observed in these areas are the conversion of its surrounding forests into agricultural lands, streams and rivers as local destination and using of chemicals that poison rivers and streams to capture freshwater fishes and shrimps according to the local guides. With these threats occurring, it is deemed necessary that immediate and appropriate attention and efforts be placed for the establishment of further effective protection for the preservation of *B. busuangensis* and other wildlife species thriving in the same areas.

There are several local organizations advocated for the conservation of wildlife and its habitats. Many activities were conducted to raise biodiversity awareness within schools and local communities. Philippines Biodiversity Conservation Foundation Inc. (PhilBio) and C3 Philippines (Coastal Conservation and Education Foundation, Inc.) which are Non-Government Organizations (NGOs) that play crucial roles in protecting the Philippines' rich biodiversity this is through different aspects of conservation including terrestrial to marine environments, involving local communities. These organizations had assisted in the creation and sustaining two

locally managed conservation areas in the Municipality of Busuanga (Cheey and Bogtong) while one more (New Busuanga) was in the process of declaration through barangay resolution. Barangay Quezon was recently declared as a Local Conservation Area under barangay resolution, while majority of the remaining forests in Busuanga Island falls within the jurisdiction of Busuanga Pasture Reserve Management Office of Forest Management Bureau (FMB) at the time of the survey.

Enforcement activities have been mobilized and strengthened by locals, Busuanga Pasture Reserve (BPR), Local Government Unit (LGU) and Palawan Council for Sustainable Development (PCSDS) through foot patrolling monitoring for the success of protection in Busuanga. Hukbong Pangkalisasan in Barangay Cheey conducted evidence-based monitoring survey and the results of were incorporated in their regular report. Comprehensive Land Use Plan for Busuanga municipality was updated for 2015–2017. It aimed to contribute to the meaningful development of the Municipality of Busuanga, Province of Palawan by providing sound and comprehensive land use plan that will guide the Municipality's actions in the next nine years to ensure and enable the future generations of Busuanga to enjoy the rich and diverse natural resources of the island.

## Recommendations

The Municipality of Busuanga had numerous rivers and streams that are still clean or less polluted and has a continuous waterflow throughout the year. It is precisely for these same reasons that the *Barbourula busuangensis* and other endemic species chose to inhabit these rivers and streams (Infante *et al.* 2002, Bosch *et al.* 2023). River developments should planned carefully to avoid altering the natural river flow or reducing the surface water of rivers and streams. Income generated from these water systems should also allocate budget for watershed protection. Replanting of native trees on the




island should be prioritized within clearings between patches of forests, watersheds and along both sides of rivers and streams banks. The remaining forests and watersheds within the Municipality of Busuanga and other neighboring areas that hold population of the endemic *B. busuangensis* should be prioritized and declared as areas for local conservation. Most of the locals people are not aware of the importance of the endemic wildlife in the area and the importance of protecting the forests (Paguntalan 2015). Community-based biodiversity conservation education activities (ecological camps etc.) must be conducted to raise awareness and foster appreciation in the region.

Initial discussions have already taken place with local governments, communities, and water resources board regarding the use of agro-pollutants such as herbicides and pesticides on agricultural areas near rivers and streams. A follow-up discussion to develop and support river/stream conservation policy is needed to establish a foundation for longer-term sustainability. Additionally, conducting a follow-up survey on remaining priority sites is crucial. Palawan is a vast area with several sites that have not yet been surveyed and areas that require updated information. Given its unique forest habitat, additional surveys should be conducted on clean rivers and streams to update the population status of *Barbourula busuangensis* and to investigate its reproductive mode, habitat requirements, diet, and status of other species of importance in the region.

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### References

- Afuang, L. E. and K. L. S. Cielo. 2009. Biology and conservation of the Philippine Flat-headed Frog (*Barbourula busuangensis*) in the Calamian Islands. Unpubl. Final Report submitted to the Department for Environment, Food & Rural Affairs/Flora and Fauna International Flagship Species Fund Small Grants Programme. 46 pp.
- Alcala, A. and R. Brown. 1998. *Philippine Amphibians: An Illustrated Field Guide*. Makati City. Bookmark Inc. 116 pp.
- Alcala, A. C. and W. C. Brown. 1987. Notes on the microhabitats of the Philippine Discoglossid Frog *Barbourula busuangensis*. *Silliman Journal* 34: 12–17.
- Bishop, D. and C. Haas. 2009. Sustaining America's aquatic biodiversity—Frog biodiversity and conservation. *Virginia Cooperative Extension*: 420–527.
- Blaustein, A. R., S. C. Walls, B. A. Bancroft, J. J. Lawler, C. L. Searle, and S. Gervasi. 2010. Direct and indirect effects of climate change on amphibian populations. *Diversity* 2: 281–313.
- Bosch, J., L. Afuang, M. Miñarro, C. Lansac, R. Márquez, R. Brown, A. V. Lacaste, P. A. Burrowes, and I. De la Riva. 2023. The calls of the poorly known Philippine Flat-headed frog, *Barbourula busuangensis* (Anura: Bombinatoridae). *Salamandra* 59: 297–303.
- Brown, W. C. and A. C. Alcalá. 1970. The zoogeography of the herpetofauna of the Philippine Islands, a fringing archipelago. *Proceedings of the California Academy of Sciences* 38: 105–130.
- Brown, W. C. and A. C. Alcalá. 1983. Modes of reproduction of Philippine amphibians. Pp . 416–428 in A. G. J. Rhodin and K. Miyata (eds.), *Advances in Herpetology*

- and *Evolutionary Biology*. Cambridge. Essays in Honor of Ernest E. Williams, Museum of Comparative Zoology.
- Collins, J. P. and A. Storfer. 2003. Global amphibian declines: Sorting the hypotheses. *Diversity and Distributions* 9: 89–98.
- Crump, M. L. 2015. Anuran reproductive modes: Evolving perspectives. *Journal of Herpetology* 49: 1–16.
- Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation* 128: 231–240.
- Davies, B., J. Biggs, P. Williams, M. Whitfield, P. Nicolet, D. Sear, S. Bray, and S. Maund. 2008. Comparative biodiversity of aquatic habitats in the European agricultural landscape. *Agriculture, Ecosystems & Environment* 125: 1–8.
- Diesmos, A. C., R. M. Brown, A. C. Alcala, R. V. Sison, L. E. Afuang, and G. V. A. Gee. 2002. Philippine Amphibians and Reptiles: an overview of species diversity, biogeography, and conservation. Pp. 26–44 in P. S. Ong, L. E. Afuang, and R. G. Rosell-Ambal (eds.), *Philippine Biodiversity Conservation Priorities: A Second Iteration of the National Biodiversity Strategy and Action Plan*. Quezon City. Department of Environment and Natural Resources Protected Areas and Wildlife Bureau.
- Diesmos, A. C., J. L. Watters, N. A. Huron, D. R. Davis, A. C. Alcala, R. I. Crombie, L. E. Afuang, G. Gee-Das, R. V. Sison, M. B. Sanguila, M. L. Penrod, M. J. Labonte, C. S. Davey, E. A. Leone, M. L. Diesmos, E. Y. Sy, L. J. Welton, R. M. Brown, and C. D. Siler. 2015. Amphibians of the Philippines. Part I: Checklist of the species. *Proceedings of the California Academy of Sciences* 62: 457–539.
- Dunlop, J., G. McGregor, and N. Horrigan. 2005. *Potential impacts of salinity and turbidity in riverine ecosystems*. National Action Plan for Salinity and Water Quality. State of Queensland.
- Glime, J. M. and W. J. Boelema. 2017. Amphibians: Anuran adaptations. Chapt. 14-1 in J. M. Glime (ed.), *Bryophyte Ecology. Volume 14-1-1 2. Bryological Interaction*. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. Available at <http://digitalcommons.mtu.edu/bryophyte-ecology2/>.
- Goldstein, J. A., K. V. S. Hoff, and S. D. Hillyard. 2017. The effect of temperature on development and behaviour of relict Leopard Frog tadpoles. *Conservation Physiology* 5: cow075.
- Hopkins, W. A. 2007. Amphibians as models for studying environmental change. *ILAR Journal* 48: 270–277.
- Infante, C. R., R. M. Brown, A. C. Diesmos, G. Gee, and J. Dimalibot. 2002. The search for the lost treasure of Palawan: the Philippine Flat-headed Frog. *Haring Ibon*: 12–15.
- IUCN SSC Amphibian Specialist Group. 2018. *Barbourula busuangensis*. *The IUCN Red List of Threatened Species*. Version 2018-2. Electronic Database accessible at <https://www.iucnredlist.org>. Captured on 10 August 2019.
- Magbanua, F. S., N. Y. B. Mendoza, A. M. Fontanilla, and P. S. Ong. 2008. *Modified Visual Stream Assessment Protocol: A Field Guide*. UP Biology – EDC Biodiversity Field Guide Series No. 1. Quezon City and Pasig City. Institute of Biology, University of the Philippines Diliman and Energy Development Corporation. 55 pp.
- Myers, G. S. 1943. Rediscovery of the Philippine discoglossid frog, *Barbourula busuangensis*. *Copeia* 1943: 148–150.
- Paguntalan, L. J. 2015. *Biodiversity Conservation Status of Busuanga Island*. Deutsche Gessellschaft für Internationale Zusammenarbeit (GIZ), Busuanga Local Government Unit and Palawan Council for Sustainable Development Staff. 17 pp.
- Paguntalan, L. J., R. K. Gomez, and W. Oliver. 2012. *Threatened Species of the Calamian Islands: developing an integrated regional biodiversity conservation area – Phase II*. National Geographic Society Conservation Trust. 18 pp.
- Phochayavanich, R., H. K. Voris, W. Khonsue, S. Thunhikorn, and K. Thirakhup. 2010. Comparison of stream frog assemblages at three elevations in an evergreen forest, North-Central Thailand. *Zoological Studies* 49: 632–639.
- Porter, A. 2010. Abundance and diversity of Anuran species in Danum Valley, Sabah, Borneo. *Plymouth Student Scientist* 3: 34–50.
- Propper, C. R., L. J. Hardy, B. D. Howard, R. J. B. Flor, and G. R. Singleton. 2020. Role of farmer knowledge in agroecosystem science: rice farming and amphibians in the Philippines. *Human-Wildlife Interactions* 14: 15.
- Schoppe, S. and M. Cervancia. 2009. Herpetological surveys along Pagdanan Range and Dumarán Island, Northern Palawan, Philippines. *Hamadryad* 34: 95–106.
- Shuman-Goodier, M. E., G. R. Singleton, and C. R. Propper. 2017. Competition and pesticide exposure affect development of invasive (*Rhinella marina*) and native (*Fejervarya vittigera*) rice paddy amphibian larvae. *Ecotoxicology* 26: 1293–1304.
- Wells, K. 2010. *The Ecology and Behavior of Amphibians*. Chicago. University of Chicago Press, Chicago. 1400 pp.

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