**นิพนธ์ต้นฉบับ**

# ความแปรผันของขนาดและปริมาตรไข่ในสะเทินน้ำสะเทินบกกลุ่ม Anura 7 ชนิด จากประเทศไทย

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**บทคัดย่อ**

**ความเป็นมาและวัตถุประสงค์**: สัตว์สะเทินน้ำสะเทินบกเป็นองค์ประกอบสำคัญของระบบนิเวศป่าเขตร้อน โดยทำหน้าที่เป็นดัชนีชี้วัดทางชีวภาพของคุณภาพสิ่งแวดล้อม มีบทบาทในการถ่ายทอดพลังงานและหมุนเวียนสารอาหารระหว่างระบบนิเวศทางน้ำและบนบก ตลอดจนช่วยควบคุมประชากรสัตว์ไม่มีกระดูกสันหลังตามธรรมชาติ การทำความเข้าใจกลไกทางชีววิทยาที่เกี่ยวข้องกับการสืบพันธุ์ของสัตว์สะเทินน้ำสะเทินบกจึงมีความสำคัญต่อการพัฒนาแบบจำลองประชากร การประเมินความอยู่รอดของประชากร และการกำหนดแนวทางการอนุรักษ์อย่างมีประสิทธิภาพ ผลผลิตทางการสืบพันธุ์ของกบเพศเมียสามารถประเมินได้จากลักษณะสำคัญ ได้แก่ ขนาดครอก (Clutch size) ซึ่งหมายถึงจำนวนไข่ที่วางในแต่ละครั้ง และปริมาตรรวมของไข่ในครอก ซึ่งสะท้อนถึงการลงทุนด้านการสืบพันธุ์ของเพศเมีย ลักษณะเหล่านี้สัมพันธ์กับการจัดสรรพลังงานและกลยุทธ์การสืบพันธุ์ของแต่ละชนิด โดยทั่วไป ขนาดร่างกายของเพศเมีย วัดจากความยาวจากปลายปากถึงช่องเปิดทวาร (Snout – vent length; SVL) อาจสัมพันธ์กับจำนวนและขนาดของไข่ เนื่องจากข้อจำกัดด้านพื้นที่ภายในช่องท้องและพลังงานที่ใช้สร้างไข่ อย่างไรก็ตาม ความสัมพันธ์ดังกล่าวอาจแตกต่างกันระหว่างชนิดและสภาพแวดล้อมที่อาศัยอยู่ ตามทฤษฎีชีวประวัติ การสืบพันธุ์อาจเกี่ยวข้องกับการแลกเปลี่ยนระหว่างจำนวนไข่กับขนาดไข่ กล่าวคือ การผลิตไข่จำนวนมากอาจจำเป็นต้องแลกกับการลงทุนด้านทรัพยากรต่อไข่แต่ละฟองที่ลดลง ขณะที่การผลิตไข่ขนาดใหญ่อาจช่วยเพิ่มทรัพยากรสำรองสำหรับการพัฒนาของตัวอ่อน อย่างไรก็ตาม รูปแบบการลงทุนดังกล่าวอาจแตกต่างกันตามลักษณะทางนิเวศวิทยาและกลยุทธ์การสืบพันธุ์ของแต่ละชนิด ดังนั้น การศึกษาจำนวนไข่และมิติของขนาดไข่จึงเป็นพื้นฐานสำคัญในการทำความเข้าใจความแปรผันของลักษณะการสืบพันธุ์ในสัตว์สะเทินน้ำสะเทินบก แม้ว่าประเทศไทยจะตั้งอยู่ในภูมิภาคอินโดจีน ซึ่งมีความหลากหลายทางชีวภาพสูงและเป็นแหล่งอาศัยของสัตว์สะเทินน้ำสะเทินบกอันดับ Anura จำนวนมาก แต่ข้อมูลเชิงปริมาณเกี่ยวกับขนาดครอก มิติและปริมาตรของไข่ ตลอดจนความแปรผันของลักษณะเหล่านี้ระหว่างชนิด ยังมีอยู่อย่างจำกัด โดยเฉพาะข้อมูลที่ได้จากการตรวจวัดตัวอย่างไข่โดยตรง การศึกษาลักษณะดังกล่าวจึงช่วยเพิ่มข้อมูลพื้นฐานด้านชีววิทยาการสืบพันธุ์และเป็นประโยชน์ต่อการศึกษาเปรียบเทียบลักษณะทางสัณฐานวิทยาของไข่ระหว่างชนิด ตลอดจนสนับสนุนการศึกษาด้านนิเวศวิทยาและการอนุรักษ์สัตว์สะเทินน้ำสะเทินบกในอนาคต การศึกษานี้มีวัตถุประสงค์เพื่อ (1) ตรวจวัดและบันทึกจำนวนไข่ต่อครอก ความยาวและความกว้างของไข่ และปริมาตร

ของไข่แต่ละฟองในสัตว์สะเทินน้ำสะเทินบกอันดับ Anura จำนวน 7 ชนิด และ (2) วิเคราะห์ความแปรผันของมิติไข่ระหว่างแกนการวัดทั้งสองด้าน เพื่อทราบลักษณะทางสัณฐานวิทยาและรูปทรงของไข่แต่ละชนิด **วิธีการ**: ศึกษาตัวอย่างไข่ของสัตว์สะเทินน้ำสะเทินบกจำนวน 7 ชนิด ซึ่งเก็บรักษาไว้ในห้องเก็บตัวอย่างสัตว์มีกระดูกสันหลัง สาขาวิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น และพิพิธภัณฑ์ธรรมชาติวิทยา องค์การพิพิธภัณฑ์วิทยาศาสตร์แห่งชาติ จังหวัดปทุมธานี โดยตัวอย่างดังกล่าวเก็บรวบรวมจากพื้นที่ 4 ภูมิภาคของประเทศไทย ได้แก่ ภาคเหนือ (เชียงใหม่) ภาคตะวันออกเฉียงเหนือ (บึงกาฬ ขอนแก่น และศรีสะเกษ) ภาคตะวันออก (จันทบุรี) และภาคกลาง (นครนายก) ตัวอย่างไข่ได้รับการเก็บรักษาในสารละลายฟอร์มาลินความเข้มข้น 10% ร่วมกับแอลกอฮอล์ความเข้มข้น 70% จากนั้นนับจำนวนไข่ทั้งหมดในแต่ละครอก และวัดขนาดไข่ 2 มิติ ได้แก่ ความยาว (แกน $X$ ตามแนวร่อง) และความกว้าง (แกน $Y$ แนวตั้งฉากกับร่อง) โดยใช้ไมโครมิเตอร์ชนิดวัดในกล้องจุลทรรศน์สเตอริโอ คำนวณปริมาตรไข่รายฟอง (มม$^3$) จากสูตร $V = 4/3 * \pi * r^3$ โดยใช้รัศมี (r) จากค่าเฉลี่ยของมิติทั้งสองด้าน คำนวณสถิติเชิงพรรณนา (ค่าเฉลี่ย ± ส่วนเบี่ยงเบนมาตรฐาน และช่วงข้อมูล) สำหรับขนาดครอก มิติขนาดไข่ และปริมาตรไข่ **ผลการศึกษา**: ขนาดครอกของไข่มีความแปรผันระหว่างชนิดสูง โดยแตกต่างกันมากกว่าสองขนาด ตั้งแต่ 42 ฟอง ในกบบอกหนาม ($n$ = 1) ถึง 6,269 ฟอง ในอึ่งเผ้า ($n$ = 1) ในกลุ่มชนิดที่มีตัวอย่างหลายครอก ($n$ = 3) พบว่าเขียดบัวมีขนาดครอกเฉลี่ย 498.00 ± 36.39 ฟอง (456 – 520) ขณะที่อึ่งข้างดำมีความแปรผันภายในชนิดสูงกว่า โดยมีค่าเฉลี่ย 395.33 ± 116.76 ฟอง (292 – 522) สำหรับตัวอย่างที่มีข้อมูลเพียงหนึ่งครอก ($n$=1) พบขนาดครอกเท่ากับ 431 ฟองในอึ่งกรายตาขาว 1,171 ฟองในเขียดอ่อง และ 122 ฟองในปาดบ้าน โดยพบความแตกต่างอย่างชัดเจนทั้งในด้านขนาดครอกและขนาดของไข่ที่ประเมินได้จากปริมาตรไข่เฉลี่ย โดยชนิดที่ปรับตัวเข้ากับลำธารมีขนาดและปริมาตรไข่รายฟองสูงที่สุด นำโดยกบบอกหนาม (16.54 ± 3.59 มม$^3$/ฟอง; $X$: 3.15 ± 0.25 มม, $Y$: 3.19 ± 0.25 มม) และอึ่งกรายตาขาว (14.40 ± 2.21 มม$^3$/ฟอง; $X$: 3.01 ± 0.15 มม, $Y$: 3.08 ± 0.14 มม) ในทางตรงกันข้ามอึ่งเผ้า ซึ่งเป็นชนิดขุดรูที่ผสมพันธุ์แบบรวดเร็วและผลิตไข่จำนวนมากที่มีปริมาตรปานกลาง (3.60 ± 1.45 มม$^3$/ฟอง; $X$: 1.87 ± 0.24 มม, $Y$: 1.92 ± 0.36 มม) ปริมาตรไข่ระดับปานกลางพบในปาดบ้าน (2.24 ± 0.42 มม$^3$/ฟอง) เขียดบัว (2.14 ± 0.48 มม$^3$/ฟอง) และเขียดอ่อง (1.64 ± 0.26 มม$^3$/ฟอง) ขณะที่อึ่งข้างดำมีไข่ขนาดเล็กที่สุด (0.57 ± 0.26 มม$^3$/ฟอง; $X$: 1.01 ± 0.15 มม, $Y$: 1.11 ± 0.14 มม) ปริมาตรรวมของไข่ต่อครอกมีความแตกต่างกันระหว่างชนิด โดย อึ่งเผ้ามีปริมาตรสูงที่สุด (22,539.11 mm$^3$) รองลงมาคืออึ่งกรายตาขาว (6,204.62 mm$^3$) และ เขียดอ่อง (1,914.90 mm$^3$) ส่วน เขียดบัว กบบอกหนาม ปาดบ้าน และอึ่งข้างดำมีปริมาตรรวมของคลัตช์เท่ากับ 1,067.47, 694.84, 273.52 และ 224.89 mm$^3$ ตามลำดับ **สรุป**: ลักษณะการสืบพันธุ์ของสัตว์สะเทินน้ำสะเทินบกอันดับ Anura ในประเทศไทยสะท้อนให้เห็นถึงความแปรผันของกลยุทธ์การสืบพันธุ์ ซึ่งอาจสัมพันธ์กับลักษณะทางนิเวศวิทยาของถิ่นวางไข่และขนาดร่างกายของเพศเมีย โดยชนิดที่วางไข่ในลำธารมีการลงทุนด้านการสืบพันธุ์ในรูปของไข่ที่มีขนาดใหญ่ เพื่อเพิ่มทรัพยากรสำรองสำหรับการพัฒนาของตัวอ่อน ขณะที่ชนิดที่วางไข่ในแหล่งน้ำชั่วคราวมีแนวโน้มผลิตไข่จำนวนมากเพื่อเพิ่มโอกาสในการสืบพันธุ์ให้สำเร็จภายใต้ข้อจำกัดด้านระยะเวลาที่มีน้ำ อย่างไรก็ตาม

รูปแบบดังกล่าวควรได้รับการตรวจสอบด้วยข้อมูลเชิงปริมาณเพิ่มเติม เนื่องจากความสัมพันธ์ระหว่างจำนวนไข่ ขนาดไข่ และสภาพแวดล้อมอาจแตกต่างกันระหว่างชนิด ทั้งนี้ ข้อมูลการศึกษาครั้งนี้สามารถใช้เป็นข้อมูลพื้นฐานสำหรับการศึกษาเปรียบเทียบลักษณะการสืบพันธุ์และการทำความเข้าใจความสัมพันธ์ระหว่างลักษณะไข่กับนิเวศวิทยาของแต่ละชนิดต่อไป

**คำสำคัญ**: นิเวศวิทยาสัตว์สะเทินน้ำสะเทินบก กลยุทธการสืบพันธุ์ ความยาวจากปลายปากถึงก้น ความดกไข่ ลักษณะชีวประวัติ ป่าเขตร้อน

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**ORIGINAL ARTICLE**

# Clutch Size and Volume Variations in Seven Anuran Species from Thailand

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## ABSTRACT

**Background and Objectives**: Amphibians represent indispensable components of tropical forest ecosystems, functioning as sensitive bioindicators of environmental health, energetic linkages facilitating nutrient cycling between aquatic and terrestrial habitats, and vital natural regulators of invertebrate populations. A comprehensive understanding of the biological mechanisms governing amphibian reproduction is fundamental for developing accurate demographic matrix models, assessing population viability, and formulating effective conservation strategies. Maternal reproductive output–quantified primarily by clutch size (the total number of eggs produced during a single oviposition event) and cumulative clutch volume–serves as a primary index of female fecundity, energetic allocation, and evolutionary fitness. In female anurans, reproductive capacity is frequently constrained by maternal snout – vent length (SVL), wherein physical body dimensions dictate abdominal volumetric limits and available metabolic reserves. Classical life-history theory predicts fundamental trade-offs between maternal energy investment, numerical egg output, and individual egg size across diverse microhabitats. Species spawning in lotic environments often prioritize larger egg sizes to provide higher yolk reserves for hatchling development, whereas those in ephemeral habitats maximize numerical egg output to ensure survival against rapid desiccation. Although Thailand is located within the Indochinese biodiversity hotspot and harbors a species-rich anuran fauna, empirical baseline data on clutch size variations, volumetric properties, and their mathematical relationships with maternal body size remain remarkably scarce. Resolving these natural history data gaps is essential for establishing robust baseline parameters that inform global amphibian conservation frameworks and ecological modeling. To address these empirical baseline gaps, this study aimed to: (1) quantify clutch sizes, linear egg dimensions along two perpendicular axes (length and width), and individual egg volumes across seven native Thai anuran species representing diverse taxonomic families and ecological niches; and (2) evaluate dimensional variations between the two measured axes to determine egg morphology and precise shape characteristics.

**Methodology**: Egg specimens representing seven species were examined from the vertebrate specimen collection of the Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen Province, Thailand and the Thailand Natural History Museum, National Science Museum, Pathum Thani Province, Thailand. The specimens had been collected from four regions of Thailand: northern Thailand (Chiang Mai), northeastern Thailand (Bueng Kan, Khon Kaen, and Si Sa Ket), eastern Thailand (Chanthaburi), and central Thailand (Nakhon Nayok). The sampling localities and number of female specimens examined were as follows: Chiang Mai Province, *Leptobrachium huashen* (*n* = 1); Bueng Kan Province, *Polypedates megacephalus* (*n* = 1); Khon Kaen University, Khon Kaen Province, *Microhyla heymonsi* (*n* = 3) and *Hylarana erythraea* (*n* = 3); Si Sa Ket Province, *Glyphoglossus molossus* (*n* = 1); Chanthaburi Province, *Quasipaa fasciculispina* (*n* = 1); and Nakhon Nayok Province, *H. nigrovittata* (*n* = 1). The specimens were preserved in a solution containing 10% formalin and 70% alcohol. The total number of eggs in each clutch was counted, and egg length (*X*-dimension) and width (*Y*-dimension) were measured using an ocular micrometer attached to a stereomicroscope. Individual egg volumes ($mm^3$) were calculated using the spherical volume formula $V = 4/3 * \pi * r^3$, where radius (r) was calculated as the average of the two measured dimensions divided by two. Descriptive statistics (mean ± standard deviation and range) were calculated for clutch size, linear egg dimensions, and egg volumes.

**Main Results**: Clutch sizes showed extreme interspecific variation spanning over two orders of magnitude, ranging from 42 eggs in *Q. fasciculispina* (*n* = 1) to 6,269 eggs in *G. molossus* (*n* = 1). Among species evaluated with multiple clutches (*n* = 3), *H. erythraea* yielded a mean clutch size of 498.00 ± 36.39 eggs (456 – 520), whereas *M. heymonsi* demonstrated higher intraspecific variation with a mean of 395.33 ± 116.76 eggs (292 – 522). For single observations (*n* = 1), clutch sizes were 431 eggs in *L. huashen*, 1,171 eggs in *H. nigrovittata*, and 122 eggs in *P. megacephalus*. Clear physical trade-offs were observed in egg size and calculated volumes: stream-adapted species produced the largest individual egg dimensions and volumes, dominated by *Q. fasciculispina* (16.54 ± 3.59 $mm^3$/egg; *X*: 3.15 ± 0.25 mm, *Y*: 3.19 ± 0.25 mm and *L. huashen* (14.40 ± 2.21 $mm^3$/egg; *X*: 3.01 ± 0.15 mm, *Y*: 3.08 ± 0.14 mm). Conversely, the explosive-breeding burrowing species *G. molossus* produced high numbers of moderate-volume eggs (3.60 ± 1.45 $mm^3$/egg; *X*: 1.87 ± 0.24 mm, *Y*: 1.92 ± 0.36 mm). Intermediate egg volumes were recorded in *P. megacephalus* (2.24 ± 0.42 $mm^3$/egg), *H. erythraea* (2.14 ± 0.48 $mm^3$/egg), and *H. nigrovittata* (1.64 ± 0.26 $mm^3$/egg). *M. heymonsi* produced the smallest eggs among all evaluated taxa (0.57 ± 0.26 $mm^3$/egg; *X*: 1.01 ± 0.15 mm, *Y*: 1.11 ± 0.14 mm). Clutch volume also varied among species. *G. molossus* had the largest estimated total clutch volume (22,539.11 $mm^3$), followed by *L. huashen* (6,204.62 $mm^3$) and *H.*

*nigrovittata* (1,914.90 mm³). The estimated total clutch volumes of *H. erythraea*, *Q. fasciculispina*, *P. megacephalus*, and *M. heymonsi* were 1,067.47, 694.84, 273.52, and 224.89 mm³, respectively.

**Conclusion**: Reproductive output parameters in tropical Thai anurans demonstrate explicit life-history trade-offs dictated by microhabitat stability, deposition site hydrodynamics, and maternal physical capacity. Stream-associated taxa invest in low-fecundity, high-volume eggs enriched with energy reserves to prevent hydrodynamic displacement in fast-flowing lotic environments. Conversely, species utilizing temporary rain pools adopt an *r*-selected reproductive strategy, prioritizing high numerical fecundity and smaller egg volumes to facilitate rapid embryonic development and maximize overall offspring survival before pool desiccation. Establishing these baseline empirical parameters provides critical quantitative inputs for population matrix models, demographic assessments, and evidence-based amphibian conservation planning in tropical forest ecosystems.

**Keyword**: Amphibian ecology, reproductive strategy, snout – vent length (SVL), fecundity, life history traits, tropical forest

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## Introduction

Amphibians are integral components of tropical forest ecosystems, functioning as key energetic links in food webs and sensitive bioindicators of environmental health. A fundamental aspect of their life history is reproductive output, primarily dictated by clutch size (the number of eggs produced per oviposition event) and clutch volume (Duellman & Trueb, 1994; Vitt & Caldwell, 2014; Rongchapho *et al*., 2021). These traits directly reflect an individual's energy allocation strategy, fecundity, and overall fitness, ultimately influencing population dynamics and community structure (Duellman & Trueb, 1994; Wells, 2007). In female anurans, reproductive investment is frequently constrained by physical capacity, where larger body size (Snout – vent length, SVL) often correlates with increased egg production or larger egg volumes, representing a trade-off critical for understanding species-specific life-history strategies.

Thailand is situated within a global biodiversity hotspot, harboring a species-rich and functionally diverse anuran fauna. Despite this remarkable diversity, baseline ecological and natural history data for many tropical species remain sparse or fragmentarily documented. While reproductive biology has been extensively studied in temperate regions, empirical data regarding clutch dimensions, volume variations, and female body size relationships for Indochinese anurans remain surprisingly limited. In Thailand, the anuran fauna is currently represented by 202 species across seven families (Bufonidae, Ceratobatrachidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae, and Rhacophoridae, accounting for approximately 2.5% of global anuran diversity (Frost, 2026). Despite this rich phylogenetic diversity, comprehensive baseline data on reproductive investment parameters – such as clutch size variations, individual egg dimensions, and volumetric properties – remain remarkably sparse for most Indochinese species. This knowledge gap severely hinders our ability to assess population stability, predict responses to habitat alteration, and formulate targeted conservation management plans (Duellman & Trueb, 1994; Wells, 2007).

To address these empirical baseline gaps, this study examines the reproductive traits of seven native Thai anuran species representing diverse families and microhabitats: *Quasipaa fasciculispina* (Dicroglossidae), *Leptobrachium huashen* (Megophryidae), *Glyphoglossus molossus* (Microhylidae), *Microhyla heymonsi* (Microhylidae), *Hylarana erythraea* (Ranidae), *H. nigrovittata* (Ranidae), and *Polypedates megacephalus* (Rhacophoridae). Specifically, we

investigated (1) variations in clutch size and clutch volume across these species, and (2) the relationship between female body size (SVL) and reproductive investment parameters. Providing these fundamental natural history data contributes essential baseline information needed to advance tropical anuran ecology and inform evidence-based amphibian conservation strategies in Thailand.

## Material and Methods

### 1. Egg specimens

Egg specimens and gravid females representing seven native anuran species across five families were collected from northern, northeastern, and eastern Thailand (Figure 1; Table 1).

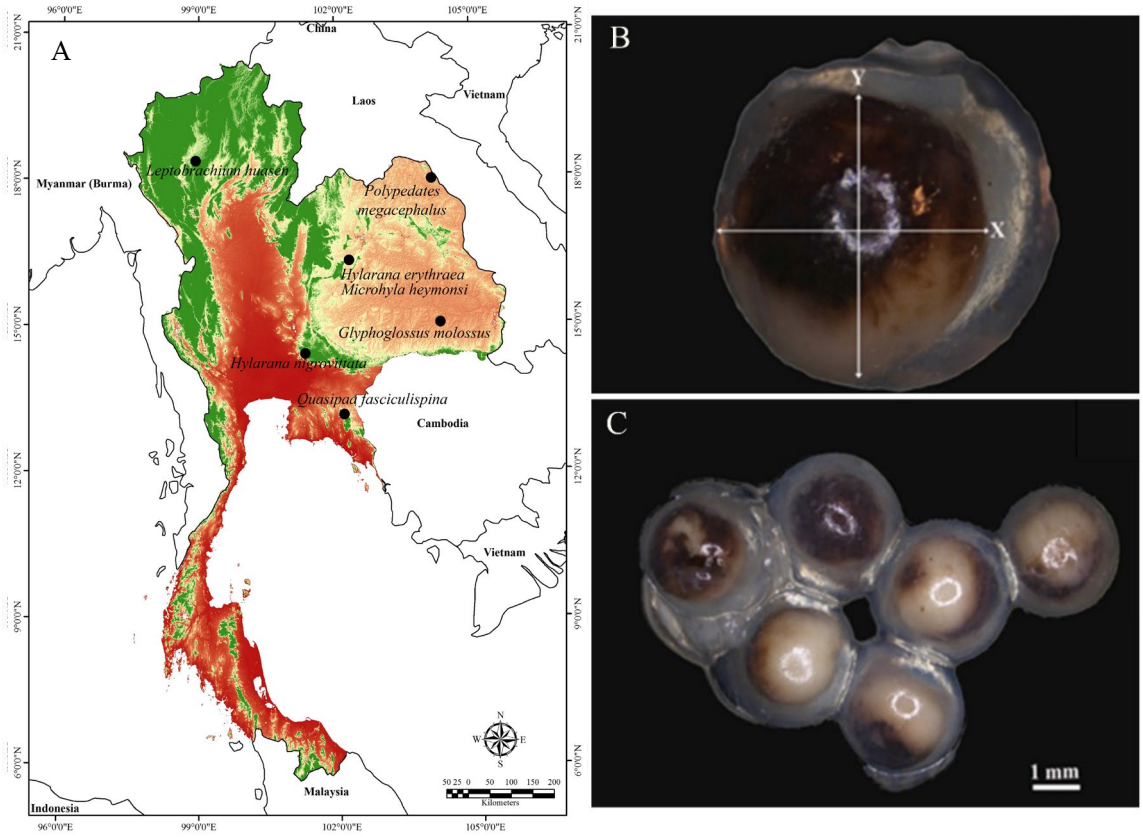



**Figure 1** Sampling locations and egg morphological measurements of seven Thai anuran species: (A) Geographic map of Thailand showing egg collection sites; (B) Linear egg dimensions showing length (*X*-axis, measured along the groove) and width (*Y*-axis, measured perpendicular to the groove); and (C) Representative egg mass clutch showing preserved egg specimens (scale bar = 1 mm).

The specific sampling locations, and number of gravid females collected per species comprised: Chiang Mai Province in northern Thailand; *L. huashen*, *n* = 1 female); Khon Kaen University, Khon Kaen Province; *M. heymonsi*, *n* = 3 females; *H. erythraea*, *n* = 3 females; and *P.*

*megacephalus*, *n* = 1 female), Si Sa Ket Province; *G. molossus*, *n* = 1 female), and Chaiyaphum Province; *H. nigrovittata*, *n* = 1 female) in northeastern Thailand; and Khao Soi Dao Wildlife Sanctuary, Chanthaburi Province in eastern Thailand; *Q. fasciculispina*, *n* = 1 female). The collected females were temporarily housed in plastic bags or aquariums overnight, and their eggs were collected the following day. These egg specimens were preserved in a solution of 10% formalin and 70% alcohol, cataloged, and deposited in the herpetological collection of the Thailand Natural History Museum (THNHM), located at the National Science Museum in Pathum Thani Province, central Thailand.

**Table 1** Summary of sampling locations, geographic coordinates, and number of gravid female specimens (*n*) collected across seven Thai anuran species. Classification followed Frost (2026).

| Region/Species | Family | *n* | Collection Site | Geographic Coordinate |
|---|---|---|---|---|
| **Northern Thailand** | | | | |
| *Leptobrachium huashen* | Megophryidae | 1 | Doi Inthanon National Park, Ban Luang Subdistrict, Chom Thong District, Chiang Mai Province | 18°35'15.0"N, 98°29'12.0"E |
| **Northeastern Thailand** | | | | |
| *Microhyla heymonsi* | Microhylidae | 3 | SC.03 Building, Khon Kaen University, Nai Mueang Subdistrict, Mueang District, Khon Kaen Province | 16°28'34.8"N, 102°49'33.1"E |
| | | | San Chao Pho Mo Din Daeng, Khon Kaen University, Nai Mueang Subdistrict, Mueang District, Khon Kaen Province | 16°28'36.8"N, 102°49'55.3"E |
| | | | Staff Dormitory, Khon Kaen University, Nai Mueang Subdistrict, Mueang District, Khon Kaen Province | 16°28'49.2"N, 102°49'38.7"E |
| *Hylarana erythraea* | Ranidae | 3 | San Chao Pho Mo Din Daeng, Khon Kaen University, Nai Mueang Subdistrict, Mueang District, Khon Kaen Province | 16°28'36.8"N, 102°49'55.3"E |
| *Glyphoglossus molossus* | Microhylidae | 1 | Kanthararom Market, Dun Subdistrict, Kanthararom District, Si Sa Ket Province | 15°06'10.9"N, 104°34'16.5"E |
| *Polypedates megacephalus* | Rhacophoridae | 1 | Wisit Subdistrict, Mueang District, Bueng Kan Province | 18°22'11.8"N, 103°39'08.6"E |
| **Eastern Thailand** | | | | |
| *Quasipaa fasciculispina* | Dicroglossidae | 1 | Khao Soi Dao Wildlife Sanctuary, Sai Khao Subdistrict, Soi Dao District, Chanthaburi Province | 13°06'11.1"N, 102°11'40.7"E |
| **Central Thailand** | | | | |
| *H. nigrovittata* | Ranidae | 1 | Wang Takrai Waterfall, Sarika Subdistrict, Mueang District, Nakhon Nayok Province | 14°20'10.3"N, 101°18'22.3"E |

**2. Egg-mass counts and measurements**

The shapes of the eggs were estimated by measuring the differences between two key dimensions (Rongchopho *et al*., 2021). These dimensions, *X* and *Y* (Figure 1), were measured on each egg using an ocular micrometer attached to a stereomicroscope. The egg length (*X* dimension) was measured along the groove, while the width (*Y* dimension) was measured perpendicular to the groove.

**3. Statistical analysis**

To determine individual egg volume, calculations were performed using the formula for the volume of a sphere:

$$V = 4/3 * \pi * r^3$$

where:

V is the volume

$\pi$ is the mathematical constant pi (approximately equal to 3.14159)

r is the radius of the sphere

To determine the radius of each egg, the average of the two measured dimensions (*X* and *Y*) was divided by 2. This value was then used in the volume formula to calculate the volume of each individual egg. By analyzing both the dimensional differences and the calculated volumes, the researchers were able to gain valuable insights into the shape and size variations within the egg population.

## Results and Discussion

**1. Clutch size**

Clutch size varied substantially across the seven studied anuran species (Table 2; Figure 2), ranging from a minimum of 42 eggs in *Q. fasciculispina* ($n$ = 1) to a maximum of 6,269 eggs in *G. molossus* ($n$ = 1). Among species evaluated with multiple sample observations ($n$ = 3), *H. erythraea* exhibited a highly consistent clutch size with a mean of 498.00 ± 36.39 eggs (456 – 520 eggs), whereas *M. heymonsi* demonstrated greater intraspecific variability, yielding a mean clutch size of 395.33 ± 116.76 eggs (292 – 522 eggs; including a female weighing 1.28 g with a SVL of 23.00 mm that produced 522 eggs).

For species evaluated with single sample observations ($n$ = 1), moderate clutch sizes were recorded in *L. huashen* (431 eggs) and *P. megacephalus* (122 eggs). In contrast, *Q. fasciculispina* recorded the smallest clutch size among all observed species at 42 eggs, whereas *H. nigrovittata* and *G. molossus* deposited markedly larger clutches containing 1,171 eggs and 6,269 eggs, respectively (the latter produced by a large gravid female weighing 52.78 g and SVL of 63.25 mm).

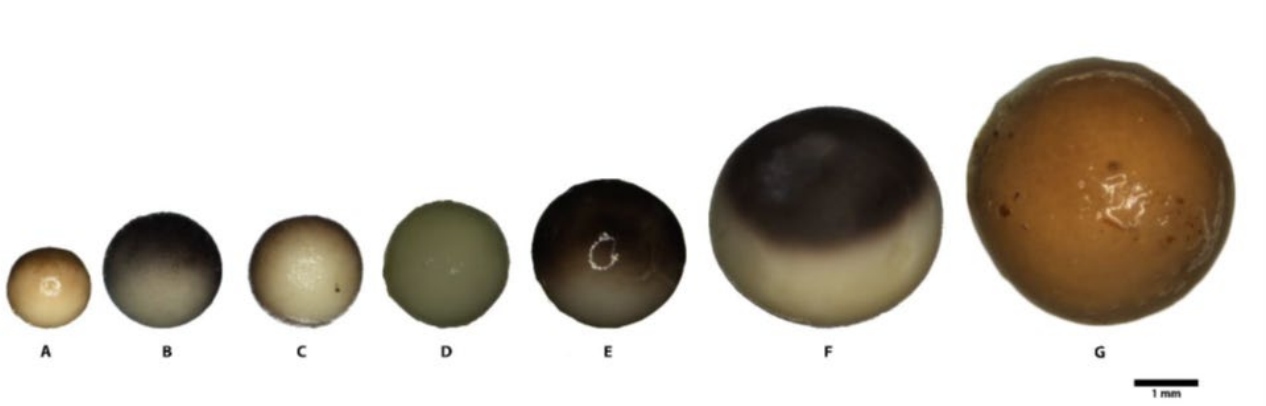



**Figure 2** Comparative egg morphology and size variation among seven Thai anuran species: (A) *Microhyla heymonsi*; (B) *Hylarana nigrovittata*; (C) *H. erythraea*; (D) *Polypedates megacephalus*; (E) *Glyphoglossus molossus*; (F) *Leptobrachium huashen*; and (G) *Quasipaa fasciculispina* (scale bar = 1 mm).

**Table 2** Summary of clutch size, linear egg dimensions (*X*- and *Y*- axes), and calculated individual egg volume among seven anuran species from Thailand. Values for species with multiple clutches ($n = 3$) are presented as mean ± standard deviation (SD) alongside the overall range.

| Species | Clutch Size | X-Dimension (mm) | Y-Dimension (mm) | Volume ($mm^3$)/egg | Volume ($mm^3$)/clutch |
|---|---|---|---|---|---|
| *Quasipaa fasciculispina* | 42, | 3.15 ± 0.25 | 3.19 ± 0.25 | 16.54 ± 3.59 | 694.84 |
| | $n = 1$ | (2.60 – 3.60), $n = 42$ | (2.70 – 3.80), $n = 42$ | (9.20 – 24.43), $n = 42$ | |
| *Leptobrachium huashen* | 431, | 3.01 ± 0.15 | 3.08 ± 0.14 | 14.40 ± 2.21 | 6,204.62 |
| | $n = 1$ | (2.65 – 3.50), $n = 431$ | (2.65 – 3.08), $n = 431$ | (9.79 – 22.45), $n = 431$ | |
| *Glyphoglossus molossus* | 6,269, | 1.87 ± 0.24 | 1.92 ± 0.36 | 3.60 ± 1.45 | 22,539.11 |
| | $n = 1$ | (1.30 – 2.50), $n = 6,269$ | (0.60 – 4.20), $n = 6,269$ | (1.15 – 8.18), $n = 6,269$ | |
| *Microhyla heymonsi* | 395.33 ± 116.76 | 1.01 ± 0.15 | 1.11 ± 0.14 | 0.57 ± 0.26 | 224.89 |
| | (292 – 522), $n = 3$ | (0.58 – 1.96), $n = 1,186$ | (0.77 – 1.85), $n = 1,186$ | (0.10 – 3.93), $n = 1,186$ | |
| *Hylarana erythraea* | 498.00 ± 36.39 | 1.59 ± 0.11 | 1.61 ± 0.12 | 2.14 ± 0.48 | 1,067.47 |
| | (456 – 520), $n = 3$ | (1.27 – 2.33), $n = 1,494$ | (1.20 – 2.13), $n = 1,494$ | (1.06 – 6.65), $n = 1,494$ | |
| *H. nigrovittata* | 1,171, | 1.46 ± 0.07 | 1.45 ± 0.07 | 1.64 ± 0.26 | 1,914.90 |
| | $n = 1$ | (1.13 – 2.13), $n = 1,171$ | (1.20 – 1.73), $n = 1,171$ | (0.76 – 5.08), $n = 1,171$ | |
| *Polypedates* | 122, | 1.62 ± 0.12 | 1.69 ± 0.10 | 2.24 ± 0.42 | 273.52 |
| *megacephalus* | $n = 1$ | (1.12 – 1.65), $n = 122$ | (1.42 – 1.92), $n = 122$ | (0.73 – 3.29), $n = 122$ | |

Methodologically, accurately quantifying massive egg clutches in explosive-breeding ranid and microhylid species (*e.g.*, *G. molossus* with >6,000 eggs or *H. nigrovittata* with >1,000 eggs) presents technical challenges due to three-dimensional egg mass overlapping and dense jelly capsules. Traditional counting techniques, such as chemical dissociation or manual pipetting, can be tedious or impair embryo

viability, whereas image analysis of compressed masses can obscure individual eggs along grid lines. In our study, physical separation and standardized counting techniques (similar to the non-destructive compartmentalized tray and digital imaging protocols outlined by Swierk, 2015) were essential to ensure exact fecundity counts across both low-fecundity and high-fecundity clutches without causing mechanical damage to egg capsules or embryos (Rongchapho *et al*., 2021).

The broad divergence in clutch sizes observed among these tropical anurans reflects diverse reproductive strategies, microhabitat adaptations, and evolutionary trade-offs between numerical egg output, individual egg investment, and maternal body capacity (Roff, 1992; Brown & Shine, 2009; Sheridan, 2009; Malagoli *et al*., 2021). Beyond a simple linear trade-off between egg size and number, life-history theory increasingly recognizes clutch size as a non-linear maternal effect that directly influences embryonic microenvironment dynamics, incubation success, and subsequent hatchling phenotypes (Brown & Shine, 2009). The remarkably high clutch size in *G. molossus* (6,269 eggs) is typical of explosive-breeding microhylids adapted to monsoon-driven ephemeral rain pools. In such hydrologically unpredictable microhabitats, laying massive quantities of eggs represents an *r*-selected strategy that maximizes larval survival before rapid pool desiccation occurs. This extreme fecundity is structurally facilitated by female body cavity volume; the evaluated *G. molossus* female was the largest specimen in this study (52.78 g, 63.25 mm SVL). Positive correlations between maternal physical dimensions and clutch size are well documented in anurans, as larger body cavity volumes accommodate expanding ovaries (Roff, 1992; Wells, 2007; Brown & Shine, 2009; Rongchapho *et al*., 2021). For example, in another large-bodied dicroglossid from northeastern Thailand, *Hoplobatrachus rugulosus*, a female measuring 85.0 mm SVL produced a substantial clutch of 2,649 eggs deposited in temporary or permanent ponds, further illustrating how large maternal body size enables high numerical fecundity in open aquatic habitats (Rongchapho *et al*., 2021).

In contrast, *Q. fasciculispina* produced the lowest egg count (42 eggs). Species of *Quasipaa* inhabit fast-flowing mountain streams and exhibit specialized lotic breeding behaviors, depositing large, yolk-rich eggs attached underneath rock surfaces to prevent current displacement (Inthara *et al*., 2009; Chuaynkern *et al*., 2011, 2018; Ngo *et al*., 2013; Oonraun *et al*., 2015). This low fecundity illustrates the fundamental life-history trade-off where maternal investment favors fewer, larger, and energetically resilient offspring to ensure hatchling survival in lotic conditions, in contrast to high fecundity open-water discroglossids such as *H. rugulosus* (2,649 eggs; Rongchapho *et al*., 2021) or stream-dwelling

*Limnonectes gyldenstolpei* (193.30 ± 163.95 eggs, female SVL 50 mm; Unsrisong *et al*., 2002)

For species with multiple sample observations, *H. erythraea* displayed low clutch size variability (498.00 ± 36.39 eggs). Interestingly, higher average clutch sizes for *H. erythraea* have been recorded in other regional populations, such as Sakaerat in Nakhon Ratchasima Province, Thailand (1,074 ± 73.0 eggs, $n = 52$), as well as in Singapore (1,072 ± 74.7 eggs, $n = 20$; and 955 ± 51.8 eggs, $n = 48$) (Sheridan, 2009). As demonstrated by Sheridan (2009), anuran species inhabiting seasonally constrained environments (such as central/northeastern Thailand, with a 6-month rainy season) tend to produce nearly twice as many eggs per clutch early in the breeding season compared to populations in equatorial, aseasonal environments (*e.g.*, Singapore, with year-round rainfall) to compensate for shortened breeding windows. Although maternal SVL and mass for *H. erythraea* were not recorded prior to oviposition in our study, low intraspecific variability within a localized population strongly correlates with adaptation to stable, permanent, or human-modified lentic habitats (*e.g.*, agricultural ponds and roadside ditches). Global anuran reproductive modes (comprising over 42 distinct modes) reflect complex trade-offs between oviposition site selection, environmental hydroperiod, and parental care (Wells, 2007; Vassilieva *et al.*, 2017; Malagoli *et al*., 2021). These range from open aquatic bodies to highly restricted phytotelmata sites (*e.g.*, bromeliad leaf-tanks, *Nepenthes* pitchers or tree hollows), where spatial boundaries, hypoxia, and detrital accumulation constrain clutch size. For example, the obligate phytotelm-breeding microhylids like *M. arboricola* deposits small clutches attached to tree hollow walls containing only 5 – 37 eggs (16 ± 8 eggs), contrasting sharply with open-pond microhylids (Vassilieva *et al*., 2017). Species utilizing permanent open-water bodies encounter consistent hydrological conditions and predictable resource availability, enabling females to maintain steady physiological conditions and uniform energetic allocation to clutches (Duellman & Trueb, 1994; Wells, 2007; Rongchapho *et al*., 2021; Malagoli *et al*., 2021).

Conversely, *M. heymonsi* demonstrated greater intraspecific variability (395.33 ± 116.76 eggs, [292 – 522]). Despite its small body size (*e.g.*, a female of 23.00 mm SVL and 1.28 g mass yielding 522 eggs), *M. heymonsi* maintains high numerical fecundity relative to body mass, representing an opportunistic adaptation for open ephemeral puddle spawning. Our recorded mean clutch size for *M. heymonsi* aligns closely with observations from Sakaerat, Thailand (402 ± 16.2 eggs, $n = 161$), but is substantially higher than values reported for Singapore populations (236 ± 7.25 eggs, $n = 105$; and 246 ± 7.82 eggs, $n = 137$) (Sheridan, 2009). Sheridan (2009) noted that female body size in *M. heymonsi* does not vary across latitudinal/seasonal

gradients (Thailand vs. Singapore); thus, the increased clutch size in seasonal Thai populations represents a direct physiological adaptation to maximize single-event reproductive output within a constrained breeding window. Furthermore, open-water spawners like *M. heymonsi* produce vast numbers of small eggs (396 – 420 eggs per clutch; Vassilieva *et al*., 2017), whereas specialized arboreal congeners like *M. arboricola* (5 – 37 eggs) and *M. borneensis* (37 – 48 eggs) undergo partial embryonization and exhibit extreme clutch size reduction adapted to spatial limits in phytotelmata (Das & Haas, 2010; Vassilieva *et al*., 2017).

Intermediate clutch sizes were recorded in forest and stream-associated taxa, including *H. nigrovittata* (1,171 eggs), *L. huashen* (431 eggs), and *P. megacephalus* (122 eggs). In comparison, another megophryid, *L. sylheticum* from South Asia, exhibits considerably higher fecundity ranging from 1,950 to 2,430 eggs per clutch ($n$ = 2) deposited by larger females (63.9 – 68.3 mm SVL) (Al-Razi *et al*., 2021). *L. sylheticum* females produce large egg masses with individual egg diameters averaging 1.66 ± 0.05 mm ($n$ = 15) attached to substrates in low-current stream sections. The single clutch observation of *P. megacephalus* in our study (122 eggs) reflects an alternative life-history strategy, where energy is heavily allocated toward constructing protective form nests above water bodies to reduce aquatic predation and early embryonic desiccation. Previous extensive studies by Sheridan (2009) in Sakaerat, Thailand, documented larger clutch sizes for *P. megacephalus* (477 ± 20.7 eggs, $n$ = 36; and 500 ± 19.2 eggs, $n$ = 115) compared to Singapore (261 ± 12.3 eggs, $n$ = 90; and 229 ± 8.18 eggs, $n$ = 51). Sheridan (2009) demonstrated that in *P. leucomystax*, increased clutch size and clutch volume in seasonal Thailand are strongly driven by larger female body size (SVL 78.8 – 83.0 mm in Thailand vs. 62.7 – 64.7 mm in Singapore). This indicates that while relative reproductive output per unit body volume remains constant across regions, seasonal selection favors larger female body size to enable larger single-clutch output during abbreviated rainy seasons.

Furthermore, as conceptualized by Brown & Shine (2009), clutch size operates as a critical maternal effect driving reproductive decisions such as communal oviposition, delayed maturation thresholds, and female body size evolution. In egg-laying ectotherms, larger egg aggregations alter embryonic incubation microenvironments through thermal retention, communal respiratory dynamics, or localized moisture preservation (Brown & Shine, 2009). For example, in foam-nesting rhacophorids like *P. megacephalus* or open-water egg mass depositors like *H. rugulosus* and *G. molossus*, larger egg clutches provide collective physical protection against desiccation and thermal fluctuations, which enhances individual egg survival beyond initial maternal yolk provisioning (Brown &

Shine, 2009; Rongchapho *et al*., 2021). Collectively, these findings illustrate that tropical anuran clutch size is dictated by a dynamic balance between maternal body size, phylogenetic history, microhabitat hydroperiods, deposition site constraints, and the evolutionary benefits of egg aggregation (Brown & Shine, 2009; Sheridan, 2009; Vassilieva *et al*., 2017; Al-Razi *et al*., 2021; Rongchapho *et al.,* 2021).

**2. Egg size**

Egg dimensions showed clear interspecific variations in overall length (*X*-dimension) and width (*Y*-dimension) across the seven studied anuran species (Figure 2; Table 2), reflecting distinct life-history trade-offs shaped by microhabitat preferences. *Q. fasciculispina* recorded the largest individual egg dimensions, exhibiting a mean length of 3.15 ± 0.25 mm (2.60 – 3.60 mm, $n$ = 42) and a mean width of 3.19 ± 0.25 mm (2.70 – 3.80 mm, $n$ = 42). Similarly large eggs were documented in *L. huashen*, which measured an average length of 3.01 ± 0.15 mm (2.65 – 3.50 mm, $n$ = 431) and an average width of 3.08 ± 0.14 mm (2.65 – 3.08 mm, $n$ = 431). *G. molossus* exhibited moderate-to-large egg dimensions with a mean length of 1.87 ± 0.24 mm (1.30 – 2.50 mm, $n$ = 6,269) and a mean width of 1.92 ± 0.36 mm (0.60 – 4.20 mm, $n$ = 6,269). Intermediate egg dimensions were observed in *P. megacephalus* (1.62 ± 0.12 mm, 1.12 – 1.65 mm; width: 1.69 ± 0.10 mm, 1.42 – 1.92 mm, $n$ = 122), *H. erythraea* (length: 1.59 ± 0.11 mm, 1.27 – 2.33 mm; width: 1.61 ± 0.12 mm, 1.20 – 2.13 mm, $n$ = 1,494), and *H. nigrovittata* (length: 1.46 ± 0.07 mm, 1.13 – 2.13 mm; width: 1.45 ± 0.07 mm, 1.20 – 1.73 mm, $n$ = 1,171). Conversely, *M. heymonsi* produced the smallest eggs among all evaluated taxa, exhibiting a mean length of 1.01 ± 0.15 mm (0.58 – 1.96 mm, $n$ = 1,186) and a mean width of 1.11 ± 0.14 mm (0.77 – 1.85 mm, $n$ = 1,186). Overall, species breeding in fast-flowing mountain streams (*Q. fasciculispina* and *L. huashen*) invested in significantly larger individual eggs, whereas opportunistic species utilizing standing water or temporary rain pools (*M. heymonsi* and *H. erythraea*) favored smaller egg sizes.

The results for individual egg size (*X*-dimension length and *Y*-dimension width) highlight distinct evolutionary trade-offs in maternal resource allocation and microhabitat adaptation across the seven studied tropical anuran taxa. Standard life-history models assume a simple linear trade-off where finite reproductive resources are partitioned between egg size and egg number (Smith & Fretwell, 1974; Roff, 1992; Brown & Shine, 2009). However, empirical evaluations demonstrate that the relationship between clutch size and offspring size can operate non-linearly, as egg size interacts with physical nest dynamics,

maternal body cavity constraints, and environmental hydration (Brown & Shine, 2009). While egg size in many ectotherms scales with maternal SVL (Roff, 1992), egg dimensions in anurans frequently decouple from female body size and are driven by microhabitat hydrodynamics (Howard, 1978; Kuramoto, 1978; Sheridan, 2009; Brown & Shine, 2009; Rongchapho *et al*., 2021). For instance, Sheridan (2009) demonstrated that egg diameter in *P. megacephalus* remained consistent (1.76 – 1.90 mm) despite major latitudinal differences in female body size between Thailand and Singapore. Similarly, in *H. rugulosus*, egg length (1.81 ± 0.23 mm) and width (1.79 ± 0.24 mm) exhibit spherical morphology that matches the intermediate egg dimensions of open-water pond spawners like *G. molossus* (1.87 x 1.92 mm), despite *H. rugulosus* females possessing considerably larger SVL (85.0 mm; Rongchapho *et al*., 2021). In our study, variations in egg dimensions were strongly dictated by microhabitat hydrodynamics and environmental stability rather than maternal physical dimensions alone.

Stream-adapted species, specifically *Q. fasciculispina* (3.15 x 3.19 mm and *L. huashen* (3.01 x 3.08 mm), produced the largest individual egg dimensions among all evaluated taxa. In lotic ecosystems, high-velocity currents, lower ambient water temperatures, and elevated mechanical stress present severe constraints for embryonic survival. Depositing larger, yolk-rich eggs yields bigger, energetically resilient hatchlings with enhanced swimming capabilities or specialized anatomical structures to resist stream displacement (Ngo et al., 2013; Malagoli *et al*., 2021). Within the genus *Leptobrachium*, *L. huashen* produces exceptionally large eggs (3.01 – 3.08 mm) relative to its congener *L. sylheticum* (1.66 ± 0.05 mm egg diameter; Al-Razi *et al*., 2021). This dimensional variation highlights distinct reproductive strategies within Megophryidae, where *L. huashen* allocates energy into highly provisioned, low-fecundity individual eggs adapted to high-energy mountain torrents, whereas *L. sylheticum* yields smaller individual eggs packaged into vastly larger total clutches (up to 2,430 eggs) in lowland forest streams (Al-Razi *et al*., 2021). Because maternal abdominal volume imposes a physical ceiling on overall reproductive mass, allocating substantial energy into large, high-quality eggs necessitates a significant reduction in numerical clutch size, as evidenced by the small clutch of 42 eggs in *Q. fasciculispina* (Ngo *et al*., 2013; Brown & Shine, 2009).

Conversely, open-pond microhylids like *M. heymonsi* exhibited the smallest egg dimensions (1.01 x 1.11 mm). In comparison, other open-water breeding Indochinese microhylids also deposit small eggs (*e.g.*, *M. minuta*: 0.9 ± 0.03 mm;

*M. butleri*: 1.2 ± 0.33 mm; *M. mukhlesuri*: 1.2 ± 0.07 mm; Vassilieva *et al*., 2017). As opportunistic, *r*-selected species utilizing ephemeral rain pools and temporary roadside puddles, open-pond microhylids face hydroperiod desiccation rather than hydrodynamic stream stress. Minimizing individual egg dimensions allows females – even those with small body sizes (*e.g*., 23.00 mm SVL) – to maximize numerical fecundity. Smaller eggs also undergo accelerated embryonic development rates, facilitating rapid hatching (at early Gosner (1960) stages 20 – 21) before breeding sites desiccate (Vassilieva *et al*., 2017). In stark contrast, specialized phytotelm-dwelling microhylids such as *M. arboricola* (1.6 ± 0.16 mm egg diameter) and *M. borneensis* (2.3 ± 0.31 mm egg diameter) invest in significantly larger eggs with prolonged embryonic development (hatching at advanced Gosner (1960) stages 25 – 26 with substantial yolk reserves (Vassilieva *et al*., 2017).

Species with intermediate egg dimensions, such as *P. megacephalus* (1.62 x 1.69 mm), *H. erythraea* (1.59 x 1.61 mm), *H. nigrovittata* (1.46 x 1.45 mm), and *G. molossus* (1.92 x 1.93 mm), represent distinct reproductive strategies tailored to standing water bodies, explosive ephemeral spawning, or terrestrial-aquatic interfaces. In particular, the foam-nesting tree frog *P. megacephalus* mitigates aquatic egg predation and desiccation by depositing eggs within protective foam matrices suspended above water, balancing moderate egg dimensions with structural parental investment. Overall, these patterns confirm that anuran egg dimensions are strongly constrained by deposition site stability and microhabitat hydrodynamics, driving tropical species along an evolutionary continuum between high-fecundity/small-egg strategies and low-fecundity/large-egg adaptations (Brown & Shine, 2009; Ngo *et al*., 2013; Vassilieva *et al*., 2017; Al-Razi *et al*., 2021; Malagoli *et al*., 2021; Rongchapho *et al*., 2021).

**3. Egg volume**

Calculated individual egg volumes varied considerably among the seven anuran species examined (Table 2). *Q. fasciculispina* had the largest mean individual egg volume (16.54 ± 3.59 $mm^3$/egg, range 9.20 – 24.43 $mm^3$/egg, $n$ = 42), followed by *L. huashen* (14.40 ± 2.21 $mm^3$/egg, range 9.79 – 22.45 $mm^3$/egg, $n$ = 431). *G. molossus* had a mean individual egg volume of 3.60 ± 1.45 $mm^3$/egg (range 1.15 – 8.18 $mm^3$/egg, $n$ = 6,269). Intermediate values were recorded in *P. megacephalus* (2.24 ± 0.42 $mm^3$/egg, range 0.73 – 3.29 $mm^3$/egg, n = 122), *H. erythraea* (2.14 ± 0.48 $mm^3$/egg, range 1.06 – 6.65 $mm^3$/egg, $n$ = 1,494), and *H. nigrovittata* (1.64 ± 0.26 $mm^3$/egg, range 0.76–5.08 $mm^3$/egg, n = 1,171). *M. heymonsi* had

the smallest mean individual egg volume (0.57 ± 0.26 $mm^3$/egg, range 0.10–3.93 $mm^3$/egg, $n$ = 1,186).

Estimated total clutch volume also differed among species (Table 2). *G. molossus* had the largest estimated total clutch volume (22,539.11 $mm^3$), followed by *L. huashen* (6,204.62 $mm^3$) and *H. nigrovittata* (1,914.90 $mm^3$). The estimated total clutch volumes of *H. erythraea*, *Q. fasciculispina*, *P. megacephalus*, and *M. heymonsi* were 1,067.47, 694.84, 273.52, and 224.89 $mm^3$, respectively. Notably, the species with the largest individual eggs did not have the largest total clutch volume. *Q. fasciculispina* had the largest mean individual egg volume but a relatively small clutch, whereas G. molossus had the greatest clutch size and total clutch volume. These results demonstrate differences in the relative contributions of individual egg volume and clutch size to total reproductive volume.

Individual egg volume varied markedly among the seven anuran species examined, with the largest mean values recorded in *Q. fasciculispina* and *L. huashen* and the smallest in *M. heymonsi*. Egg volume, estimated from egg dimensions, provides a useful quantitative measure for comparing egg size among species and can contribute to assessments of reproductive investment (Duellman & Trueb, 1994; Wells, 2007; Rongchapho *et al*., 2021). However, egg volume should not be considered a direct measurement of energetic content or maternal energy expenditure because it does not account for differences in yolk composition, water content, or other egg components.

The estimated total clutch volumes further illustrate the differences between investment in individual eggs and total clutch output. *Q. fasciculispina* had the largest mean individual egg volume (16.54 $mm^3$/egg), but its clutch contained only 42 eggs, resulting in an estimated total clutch volume of 694.84 $mm^3$. In contrast, *G. molossus* had a substantially smaller mean individual egg volume (3.60 $mm^3$/egg) but produced 6,269 eggs, resulting in the largest estimated total clutch volume (22,539.11 $mm^3$). Similarly, *L. huashen* had the second-largest mean individual egg volume and an estimated total clutch volume of 6,204.62 $mm^3$ based on 431 eggs. These observations illustrate the importance of considering both egg size and clutch size when comparing reproductive output among anuran species. The pattern is consistent with the commonly discussed trade-off between egg size and egg number in reproductive allocation (Smith & Fretwell, 1974; Roff, 1992). Nevertheless, because most species in this study were represented by only one clutch, the observed differences should be interpreted as patterns among

the examined clutches rather than definitive evidence of species-level reproductive strategies.

The relatively large mean egg volumes of *Q. fasciculispina* and *L. huashen* may be relevant to their reproductive environments. Larger eggs may contain greater reserves for embryonic development, potentially providing advantages under certain environmental conditions. In stream environments, for example, greater yolk reserves could potentially support embryonic development under conditions of water movement and mechanical disturbance (Ngo *et al.*, 2013; Malagoli *et al.*, 2021). However, the present study did not directly examine yolk content, embryonic development, hatchling size, or survival. Therefore, the proposed relationship between larger egg volume and adaptation to flowing-water environments remains a hypothesis rather than a demonstrated mechanism. Within Megophryidae, the egg dimensions and volume recorded for *L. huashen* may also provide a basis for comparison with other stream-breeding species, such as *L. sylheticum*, for which smaller egg diameters and larger clutch sizes have been reported (Al-Razi *et al*., 2021). Such comparisons should, however, consider differences in measurement methods and sampling designs.

In contrast, *M. heymonsi* had the smallest mean individual egg volume (0.57 $mm^3$/egg), while its mean clutch size was 395.33 ± 116.76 eggs across three clutches. This combination of relatively small eggs and comparatively numerous eggs may indicate a different pattern of reproductive allocation from that observed in species with larger eggs. However, the available data do not establish whether small egg size is an adaptation to temporary breeding habitats or whether it is associated with faster embryonic development. Comparisons with phytotelm-breeding microhylids, such as *M. arboricola* and *M. borneensis*, may provide additional context for understanding variation in egg size among microhylid frogs. Some species occupying specialized breeding sites exhibit distinctive reproductive and developmental characteristics, including extended embryonic development and specialized larval feeding modes (Das & Haas, 2010; Vassilieva *et al*., 2017). Nevertheless, these comparisons should be made cautiously because reproductive modes and developmental environments may differ substantially among species.

The remaining species had intermediate mean individual egg volumes, ranging from 1.64 $mm^3$/egg in *H. nigrovittata* to 2.24 $mm^3$/egg in *P. megacephalus*. Their estimated total clutch volumes also varied, reflecting differences in both individual egg volume and clutch size.

For example, *H. erythraea* had a mean clutch size of 498.00 ± 36.39 eggs across three clutches and an estimated total clutch volume of 1,067.47 mm³. In comparison, the examined clutch of *P. megacephalus* contained 122 eggs and had an estimated total clutch volume of 273.52 mm³. The foam-nesting reproductive mode of *P. megacephalus* may provide protection for eggs during early development, but the present data do not demonstrate a direct relationship between foam nesting and egg volume.

Overall, the results demonstrate substantial interspecific variation in individual egg volume and estimated total clutch volume among the seven anuran species examined. These differences provide useful baseline information for understanding variation in reproductive characteristics among Thai anurans. However, the limited number of clutches examined for most species restricts conclusions about intraspecific variation and the relationship between egg size and reproductive ecology. Further studies involving more females and populations, maternal body-size measurements, and direct assessments of egg composition and developmental outcomes are needed to clarify the relationships among egg volume, clutch size, maternal investment, and breeding environments.

## Conclusion

The current study presents quantitative empirical findings on clutch sizes, egg dimensions, and clutch volumes for seven anuran species native to Thailand: *Q. fasciculispina*, *L. huashen*, *G. molossus*, *M. heymonsi*, *H. erythraea*, *H. nigrovittata*, and *P. megacephalus*. Reproductive output parameters in tropical Thai anurans demonstrate explicit life-history trade-offs dictated by microhabitat stability, deposition site hydrodynamics, and maternal physical capacity. Stream-associated taxa invest in low-fecundity, high-volume eggs enriched with energetic reserves to prevent hydrodynamic displacement in fast-flowing lotic environments. Conversely, species utilizing temporary rain pools adopt an *r*-selected reproductive strategy, prioritizing high numerical fecundity and smaller egg volumes to facilitate rapid embryonic development and maximize overall offspring survival before pool desiccation. Establishing these baseline empirical parameters provides critical quantitative inputs necessary for population matrix models, demographic assessments, and evidence-based amphibian conservation planning in tropical forest ecosystems.

The empirical baseline parameters established in this study offer immediate utility for demographic modeling and local conservation planning. Building upon these empirical findings,

future research could explore broader applications, including:

1. Ecological Modeling: Incorporating species-specific clutch volume and size parameters into hydrological and climate change models to predict breeding success under shifting microhabitat hydroperiods.

2. Ex-situ Conservation: Utilizing volumetric and reproductive output data to optimize captive breeding protocols and egg-rearing programs for threatened Indochinese lineages.

3. Environmental Assessment & Monitoring: Applying egg morphology and clutch metrics as baseline bioindicator benchmarks for ecotoxicological monitoring and ecological damage assessments in wildlife enforcement contexts.

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