

ORIGINAL ARTICLE

Length–biomass equations to allow rapid assessment of semi-aquatic bug biomass in tropical streams

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Abstract

Length–biomass equations are relatively easy and cost-effective for deriving insect biomass. However, the exact relationship can vary between taxa and geographical regions. Semi-aquatic bugs are abundant and are indicators of freshwater quality, but there are no studies investigating the effect of habitat disturbance on their biomass, although it is useful in assessing ecological processes. We identified the best-fit length–biomass models to predict the biomass of semi-aquatic bugs (Hemiptera, Gerromorpha) collected from streams in Sabah, Malaysia. We used 259 juvenile and adult semi-aquatic bugs to compare a range of plausible length–biomass functions, and to assess whether relationships differed across the following families and body forms: (1) Cylindrostethinae, Gerrinae, and Ptilomerinae, which are subfamilies within Gerridae consisting of small-to-large bugs that have long and slender bodies, (2) Halobatinae, a subfamily within Gerridae, consisting of small-to-medium bugs with wide heads and thoraxes as well as short abdomens, and (3) Veliidae, which are small bugs with stout bodies. Estimation used five fitting functions – linear regression, polynomial regression order 2, 3, and 4, and power regression – on the following groupings: three body forms combined; each body form with life stages (juvenile and adult) combined; and each body form with life stages separated. Power regressions were the best fit in predicting the biomass of semi-aquatic bugs across life stages and body forms, and the predictive power of models was higher when the biomass of different body forms was calculated separately (specifically for Halobatinae and Veliidae). Splitting by life stages did not always result in additional improvement. The equations from this study expand the scope of possible future ecological research on semi-aquatic bugs, particularly in Southeast Asia, by allowing more studies to consider biomass-related questions.

KEYWORDS

bioindicator, body forms, Gerromorpha, habitat disturbance, Hemiptera, indicator species, length–biomass relationships, life stages, power regression, Sabah, semi-aquatic bugs, water quality

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INTRODUCTION

The status of a particular assemblage or community in a habitat is often initially described in terms of abundance and richness – that is, by counting individuals and taxa. However, functional traits, such as feeding group, trophic level, body size, and biomass, are needed to assess the role that different taxa play in ecosystem processes (Dobson et al., 2006; de Bello et al., 2010; Sackett et al., 2010; Slade et al., 2011; Thompson et al., 2012; Jabiol et al., 2013; Luke, et al., 2014; Rousk, 2016). For instance, understanding how land-use change affects the biomass of insects can indicate the importance of their roles in dung burial and seed dispersal (Slade et al., 2011) and can also provide information about the amount of food available for predators (Turner & Foster, 2009; Kunin, 2019; Wagner, 2020). Therefore, as a complement to diversity, biomass data can be used as a tool to assess the ecological status of insects, habitats, and the value of conservation strategies. Despite this, biomass data can be difficult to collect. For example, a sensitive balance can be expensive, and accurate measurements for small-sized insects can be an issue for studies in the field (Rogers et al., 1977; Sample et al., 1993). Additionally, the sample size of ecological studies can be large, necessitating a great investment in time to weigh each individual. Furthermore, handling, and drying samples can cause damage, which can reduce the ability to carry out other work on the samples subsequently, such as identification and preparation of voucher specimens (Gruner, 2003). Hence, estimating biomass from body length measurements is much easier and quicker, as well as avoiding damage to specimens. Several studies have obtained predicted biomass using well fitted length–biomass regressions, with a small error when compared with measured biomass of the same samples (Rogers et al., 1977; Sample et al., 1993; Giustini et al., 2008; Wardhaugh, 2013; Kinsella et al., 2020), making such equations a feasible and useful alternative to drying and weighing all specimens for primary studies.

Differences in body form should be considered when carrying out biomass estimation (Schoener, 1980; Sample et al., 1993). Body form or shape is determined by the proportion of body length and width, and can differ across taxonomic groups, life stages, and geographical regions where samples were collected (Gowing & Recher, 1985). Different taxa can have different body forms, although insects often have a similar body form at the family level (Sample et al., 1993). In addition to the difference of body forms between taxa, the life stage of insects could be an important factor in determining the accuracy of biomass estimation (Rogers et al., 1977). This is because there is a stark difference in body form between juvenile and adult individuals of insect species with complete metamorphosis (Rogers et al., 1977). On the other hand, for insects that undergo incomplete metamorphosis, the same length–biomass equation used on both juvenile and adult individuals could produce reliable biomass estimates (Rogers et al., 1977), although studies confirming this across a

range of taxa are lacking. Furthermore, body forms can also be affected by geographical regions, related to adaptations within a particular taxonomic level, such as within the same order, to different climates and habitat conditions (Schoener, 1980). Because of this, a length–biomass equation developed for a taxon in one region may not always be suitable to estimate the biomass of that taxon in another region (Schoener, 1980). For example, power equations – $y = a(x)^b$, with y = biomass, x = insect body length, and a and b are coefficients – to estimate the biomass of terrestrial hemipteran insects in tropical rainforest in Costa Rica and temperate deciduous-conifer forest in the USA had different coefficients a and b (Schoener, 1980). The difference could be because the samples used from the tropical rainforest in Costa Rica consisted of hemipteran species that were longer and thinner (possibly because of a higher incidence of twig-mimicking insects) than those from the temperate deciduous-conifer forest in the USA (Schoener, 1980), affecting the resulting equations. Although this has not yet been specifically assessed, it is likely that a similar trend might also be seen at lower taxonomic levels. Therefore, for biomass estimates to be as reliable as possible, it is important to develop specific length–biomass equations for each insect family (Sample et al., 1993), body form (Schoener, 1980; Sample et al., 1993; Wardhaugh, 2013), life stage (Rogers et al., 1977), and also for different regions (Schoener, 1980; Gowing & Recher, 1985).

Gerromorpha is an infraorder of Hemiptera, which consists of semi-aquatic bugs that live on the surface of freshwater or marine ecosystems (Andersen, 1982), and can be found in all continents except Antarctica (Spence & Anderson, 1994). Semi-aquatic bugs are predator-scavengers that feed on invertebrates in the water and those falling from riparian vegetation (Spence & Anderson, 1994), and are prey for fishes (Foster & Treherne, 1981; Armisen et al., 2015) and other invertebrates such as back swimmers (Lang, 1980) and fishing spiders (Zimmermann & Spence, 1989). Semi-aquatic bugs undergo incomplete metamorphosis in which the development involves nymphal and adult stages (Andersen, 1982). Nymphs have a similar appearance to the adults but differ in body size and lack wings and reproductive organs (Andersen, 1982). There is sexual dimorphism within some species of semi-aquatic bugs, in which there are substantial differences in body size or body parts (Andersen, 1997). However, in other species, sexual dimorphism is much less marked, and sexes can be difficult to distinguish because, for example, the ovipositor or clasper is reduced (Andersen, 1982). Another characteristic of this group is the presence of wing polymorphism, meaning that there are winged (long or short) and wingless adult individuals (Andersen, 1982). Furthermore, wing emergence is associated with habitat permanence (such as ponds or streams) and quality (Spence, 1983, 1989; Cunha et al., 2020). For instance, higher abundances of winged bugs have been found in primary forests than in oil palm in the Amazon (Cunha et al., 2020).

There have been several studies investigating the impacts of habitat change in freshwater ecosystems on

semi-aquatic bugs (Ditrich et al., 2008; Dias-Silva et al., 2020a, 2020b; da Silva Giehl et al., 2020), which have shown that they are vulnerable and sensitive to habitat alteration (Cunha et al., 2015, 2020; Vieira et al., 2015; Cunha & Juen, 2017, 2020; Guterres et al., 2020, 2021; Sundar et al., 2021). For example, studies have found lower beta-diversity of semi-aquatic bugs in altered habitats compared to forests, perhaps because water temperature was higher and water pH was more acidic in altered habitats (Dias-Silva et al., 2020b). In contrast, a recent study in Brazil found that species richness of semi-aquatic bugs was higher in altered than undisturbed savanna streams, particularly in habitats characterised by wet and open soils on flat areas (da Silva Giehl et al., 2020). One possible reason for this discrepancy was the higher number of prey items in these habitats (da Silva Giehl et al., 2020). Currently, impacts of habitat disturbance are particularly severe in many tropical regions, where large areas are undergoing rapid land-use change for expansion of agriculture, urbanisation, and industrialisation (Hosonuma et al., 2012). Considering their importance and vulnerability, semi-aquatic bugs have the potential to act as bioindicators (Nummelin et al., 2007; Saha & Gupta, 2019), informing biodiversity conservation. However, no studies have yet focused on changes in their biomass as a result of habitat disturbance. Because there has been no work to quantify length–biomass equations for tropical members of this group, large-scale studies of habitat change effects on semi-aquatic bug biomass are difficult to conduct.

Among insect groups that have been processed for biomass estimation, equations obtained from terrestrial bugs across a variety of regions (Rogers et al., 1977; Schoener, 1980; Sample et al., 1993; Ganihar, 1997; Gruner, 2003), or from semi-aquatic bugs collected in a subtropical region (Smock, 1980), could potentially be applicable to tropical semi-aquatic bugs. However, even though these belong to the same order (Hemiptera), it is likely that semi-aquatic bugs have different body forms from their terrestrial counterparts, due to their adaptation to live on the water surface. In addition, tropical groups could vary compared to sub-tropical groups owing to the differing species found, which is associated with differences in climate and the types of stream ecosystems inhabited. Developing a reliable length–biomass equation for tropical Gerromorpha will allow greater exploration of the impacts of land-use change on this group and associated wider ecosystem functioning – something which diversity data alone cannot address.

In this study, we quantified the length–biomass relationship of semi-aquatic bugs from three groups (separated based on family and body form), collected across a land-use gradient in Sabah, Malaysian Borneo. We selected plausible fitting functions based on the likely relationship between length and volume and previous studies of length–biomass in terrestrial Hemiptera (Rogers et al., 1977; Schoener, 1980; Sample et al., 1993; Ganihar, 1997; Gruner, 2003) as well as aquatic and semi-aquatic insects collected in a subtropical region (Smock, 1980). Specifically, we assessed: (1) what is

the best model to predict the biomass of semi-aquatic bugs (Gerromorpha) from their body lengths? (2) Does the relationship change between juvenile and adult bugs? (3) Does the relationship change between different body forms of semi-aquatic bugs? And (4) do our selected best models predict biomass better than models constructed using general Hemiptera or subtropical semi-aquatic bugs, both obtained from published literature? By using semi-aquatic bugs collected from pristine and disturbed habitats, we ensured that a wide range of species were included and that the equations constructed from this study will allow a robust universal length–biomass estimation of semi-aquatic bugs. By facilitating the study of semi-aquatic bug biomass without the need for sophisticated equipment, we hope this work will facilitate the use of semi-aquatic bugs as important indicators of environmental health in tropical ecosystems.

MATERIALS AND METHODS

Collection sites

Data collection in the field took place in 2011–2013 in Sabah, Malaysian Borneo. Semi-aquatic bugs were collected with other freshwater invertebrates from stream sites within four major land-use types that are common within the region: old growth forest (OG), logged forest (LF), oil palm with forested riparian buffer strips (OPB), and oil palm without forested riparian buffer strips (OP) (Figure S1). The mean (\pm SE) altitude of all stream sites was 236 ± 26 m above sea level, and the mean slope of each catchment was $18.24 \pm 0.81^\circ$ (Luke et al., 2017). Study sites included streams within Danum Valley Conservation Area ($117^\circ 48.75'E$, $5^\circ 01'N$), Maliau Basin Conservation Area ($116^\circ 54'E$, $4^\circ 49'N$), and the SAFE (Stability of Altered Forest Ecosystems; Ewers et al., 2011) project sites in the Kalabakan Forest Reserve ($116^\circ 57'–117^\circ 42'E$, $4^\circ 38'–4^\circ 46'N$) (Luke et al., 2017). In total, 12 streams were sampled, including three in OG, four in LF, three in OPB, and two in OP. The collection sites were head-water streams that ranged in mean wetted width from 3.26 to 7.83 m, contained areas of riffle and pool habitats, and were dominated by rocky substrate. At streams that were surrounded by forested riparian buffer strips, and continuous logged or old-growth forest, the surrounding forest habitat varied in quality, with mean canopy openness values – measured using a spherical densiometer; see Lemmon (1956) for more details – ranging from 5.9 to 68.8%, and mean tree density values ranging from 0 to $38.36 \text{ m}^2 \text{ ha}^{-1}$. OP streams had oil palm planted to the edge of the streams – for more details see Luke et al. (2017).

Insect collection

Semi-aquatic bugs were sampled along a 200-m transect in each stream. Along each transect, we walked five 10-m

sub-transects (but were nine sub-transects in Gaharu and six in each Maliau and Selangan Batu) to collect the bugs. All semi aquatic bugs within the sub-transect were caught using hand-held nets and stored in 70% ethanol.

Insect identification and processing

All individuals were identified to family and classified to morphospecies level following Andersen (1982) with additional information from other key publications (Polhemus & Polhemus, 1988; Chen & Nieser 1992, 1993a,b; Nieser & Chen, 1992; Polhemus & Zettel, 1997; Chen & Zettel, 1998) and advice from taxonomic experts (see Acknowledgements for details). Each individual was also identified into one of the three distinct groups based on family and body form, consisting of both juveniles and adults: (1) *Cylindrostethinae*, *Gerrinae*, and *Ptilomerinae*, three subfamilies within the *Gerridae* family that consist of small to large bugs with slender bodies (in this study: adult lengths were 4–17 mm, widths 1–4 mm; juvenile lengths were 1–13 mm, widths 0.5–3 mm); (2) *Halobatinae*, a subfamily of the *Gerridae* that comprises bugs with wide heads and thoraxes and short abdomens (Andersen, 1982) (in this study: adult lengths were 3–5.5 mm, widths 2–3 mm; juvenile lengths were 1–3 mm, widths 0.25–2 mm); and (3) *Veliidae* which are small bugs with stout bodies (in this study: adult lengths were 1–3.5 mm, widths 0.5–1.5 mm; juvenile lengths were 0.5–3 mm, widths 0.25–1 mm) (Table S1, Figure 1). Juveniles in our samples are likely to include a range of instars, but we could not divide these specifically because the key identification guide by Andersen (1982) provided detailed descriptions for only the first few instars across families, and no other specific descriptions exist. We also did not divide adults into females and males due to feasibility issues. In particular, although a few species in this study could be easily separated by the presence of ovipositors or claspers, most others had reduced genital parts making this division difficult.

Biomass calculations

For biomass calculations, we selected a subset of semi-aquatic bug samples using a stratified random sampling method across the following characteristics: the three groups split based on families and body forms – (1) *Cylindrostethinae*, *Gerrinae*, and *Ptilomerinae*; (2) *Halobatinae*; and (3) *Veliidae* – adults / juveniles, and land use types. We also made sure that a spread of individuals from across all stream sites were chosen. We aimed to have a broad range of body lengths represented in each category. To achieve this, we selected 45 individuals within each body form group for each juvenile and adult life stage comprising short, medium, and long individuals (Table S1, Figure S2). So, in total we aimed to have 45 juveniles and 45 adults for each body form group. If the body was observed to be damaged on any specimen, the specimen was not used and another specimen was selected at random in the same category, choosing the same sample location as far as possible (Figure S3). Following this protocol and owing to low numbers (when substitution with a good specimen was not possible), only 34 adult individuals from the *Halobatinae* group were sampled (but 45 juveniles were used, as planned). Therefore, in total there were 259 individuals selected for biomass calculations. There were fewer morphospecies in the *Halobatinae* group (three and five morphospecies for juveniles and adults, respectively), which resulted in less variation (Table S1, Figure S2B), when compared with *Cylindrostethinae*, *Gerrinae*, and *Ptilomerinae* (eight morphospecies for each juvenile and adult covering all subfamilies in the group; Table S1, Figure S2A) or *Veliidae* (three and 11 morphospecies for each juvenile and adult; Table S1, Figure S2C). To assess length, individuals were first taken from the ethanol, dabbed dry, and their length was measured from the tip of the head to the end of the last segment of the abdomen, using a millimetre block with gradations to the nearest 1 mm. After selection,

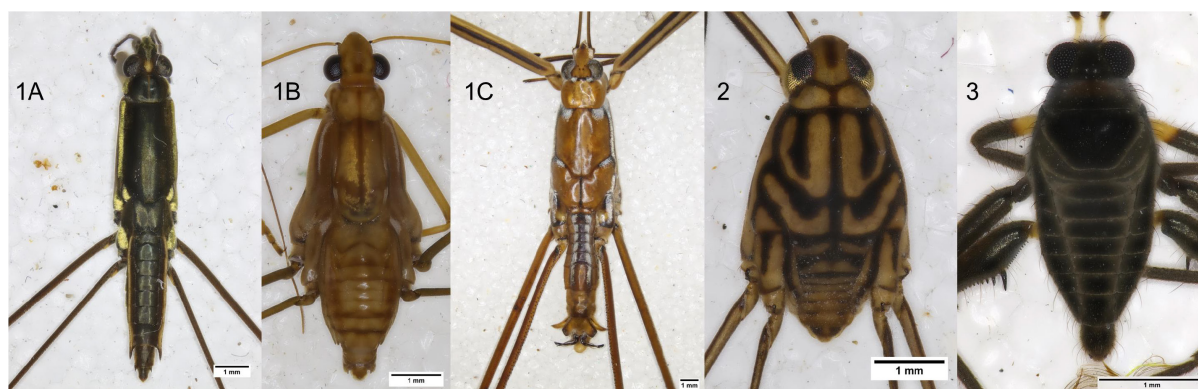


FIGURE 1 Example photos to show the families and body forms of semi-aquatic bugs analysed in this study. (1A) *Cylindrostethinae*, (1B) *Gerrinae*, and (1C) *Ptilomerinae* – with small to large slender bodies; in this study adult length of this first group was 4–17 mm and width 1–4 mm, juvenile length was 1–13 mm and width 0.5–3 mm. (2) *Halobatinae* – with small to medium bodies with wide head and thorax as well as short abdomen; in this study adult length was 3–5.5 mm and width 2–3 mm, juvenile length was 1–3 mm and width 0.25–2 mm. (3) *Veliidae* – with small stout bodies; in this study adult length was 1–3.5 mm and width 0.5–1.5 mm, juvenile length was 0.5–3 mm and width 0.25–1 mm. Photos courtesy of Matthew Hayes.

each individual was kept in a separate tube with ethanol. The resulting selection contained a good spread of replicates across the lengths available (Figure S2).

To measure the biomass of the 259 selected specimens, individuals were taken out from the ethanol, dabbed dry, and their length was remeasured to the nearest 0.25 mm from the same measurement locations as before, using underlaid graphing paper. Insects were then dried in batches on a Thermo Scientific hot plate at a temperature of 50 °C. Specimens were weighed every 2 h until the largest specimen's biomass was constant. As the largest specimens would take the longest to dry, this ensured that all specimens were dried to a constant weight. The average amount of time needed for the largest specimens to reach constant biomass was 4.5 h. All insects were then weighed when fully dry using a Sartorius balance (to a higher resolution; $d = 0.002$ mg).

Statistical analysis

All visualisations and analyses were done in R v.4.0.4 (R Core Team, 2021) with R Studio v.1.3.959 (R Studio Team, 2020). Analyses were carried out using basic R syntax and package *plotrix* (Lemon, 2006), whereas for visualisations, packages used were *tidyverse* (Wickham et al., 2019), *cowplot* (Wilke, 2020), *ggpubr* (Kassambara, 2020), and *gridExtra* (Auguie, 2017).

Comparisons of five fitting functions and curves across body form groups and life stages

Five functions were fitted to the length–biomass data and compared: linear regression, polynomial regression order 2, 3, and 4, and power regression. Power regression followed Sample et al. (1993). The equations used in this study are as follows:

Linear regression:	$y = a + b(x)$,
Polynomial regression order 2:	$y = c + a(x) + b(x)^2$,
Polynomial regression order 3:	$y = d + c(x) + a(x)^2 + b(x)^3$,
Polynomial regression order 4:	$y = e + d(x) + c(x)^2 + a(x)^3 + b(x)^4$,
Power regression:	$y = a(x)^b$,

with y = predicted biomass, x = body length of an individual insect, and a – e are coefficient parameters.

In this study, we aimed to construct universal length–biomass equations of semi-aquatic bugs which were collected from a gradient of land use. We therefore did not split our samples based on land-use types for any analysis. Fitting of functions was done on (1) combined body form groups (across life stages), (2) each body form group with life stages combined, and (3) each body form group with life stages separated. To obtain the values of coefficient b and the adjusted R^2 for the power regressions, we log-transformed both length and biomass variables and ran the 'lm' function. We then used $a = e^x$ to obtain the values of coefficient a , with x = the intercept value of the linear

regression run with the 'lm' function. We compared the adjusted R^2 values between the above three groupings of fitting functions to assess whether the biomass estimation was similar across body form groups and life stages.

Comparison of measured and predicted biomass

We assessed the biomass prediction of our best fitting length–biomass relationships on semi-aquatic bug samples (on each body form group as well as each life stage) by comparing them with the measured biomass in this study. We also made comparisons with the predictions derived from six published relationships. These included: five published length–biomass relationships for terrestrial Hemiptera collected from Costa Rica (Schoener, 1980), India (Ganihar, 1997), and USA (Rogers et al., 1977; Schoener, 1980; Sample et al., 1993; Gruner, 2003) and one relationship specific for semi-aquatic bugs collected from a subtropical region in North Carolina, USA (Smock, 1980) (Table 1). Family or species identities of the terrestrial Hemiptera used to develop the length–biomass relationships in Ganihar (1997), Gruner (2003), Rogers et al. (1977), Sample et al. (1993), and Schoener (1980) were not recorded in the publications, so we used a general equation of all Hemiptera combined from each of these studies to give biomass predictions. The species of aquatic and semi-aquatic insects in Smock (1980) were mentioned, and so, as well as using the general equation of all Hemiptera in the study, we also used equations developed specifically for semi-aquatic bugs: *Gerris remigis* Say and *Metrobates hesperius* Uhler (Gerridae), and *Rhagovelia obesa* Uhler (Veliidae) (Table 1).

In some of the studies, power regression equations were linearised (Rogers et al., 1977; Smock, 1980; Sample et al., 1993; Ganihar, 1997). When a linearised regression equation was used to predict biomass in the studies [$\ln(y) = \ln(a) + b \ln(x)$, with y = biomass, x = body length, and a and b coefficient parameters], we transformed the coefficient ' $\ln(a)$ ' to ' a ' [by $e^{\ln(a)}$] so it could be used in a power regression (Table 1).

We used paired Wilcoxon tests with Bonferroni correction for the comparisons between measured and predicted biomass, with predicted biomass obtained from the best fit equations of combined body form groups (across life stages) and each group with life stages combined, as well as equations in the six other studies. Wilcoxon tests were chosen because biomass data were not normally distributed.

RESULTS

What is the best model to predict biomass of semi-aquatic bugs from their body lengths?

Power regressions produced the highest adjusted R^2 values across all body form groups and life stages, except for juvenile and combined Veliidae (i.e., juveniles and adults grouped together), where polynomial regression

TABLE 1 Studies that estimated insect biomass using equations, from which coefficients were used to predict biomass of semi-aquatic bug samples in this study. Taxa investigated were terrestrial bugs collected from a range of countries across several climatic regions (Rogers et al., 1977; Sample et al., 1993; Ganihar, 1997; Schoener, 1980; Gruner, 2003), as well as semi-aquatic bugs from a sub-tropical region (Smock, 1980). These studies used either linearised [$\ln(y) = \ln(a) + b \ln(x)$] or power [$y = a(x)^b$] regressions. When a linearised regression was used, the coefficient ' $\ln(a)$ ' was transformed to coefficient ' a ' to be used in power regressions in this study

Reference	Location of insect collection	Taxa investigated in the study	Taxon selected for biomass prediction in this study	Coefficient parameter ' a ' ¹ or ' $\ln(a)$ ' ² and ' b ' in the study	Coefficient parameter ' a ' after being transformed from ' $\ln(a)$ '	Equation used for this study ³
Ganihar, 1997	Goa, India	Arthropods in 20 categories	Hemiptera (terrestrial bugs)	$\ln(a) = -3.8893 \pm 0.3387$, $b = 2.7642 \pm 0.3113$	$a = 0.020$	$y = 0.020x^{2.764}$
Gruner, 2003	Hawaii, USA	Arthropods in 14 orders	Heteroptera (terrestrial bugs)	$a = 0.0411$, $b = 1.9340$	Transformation not needed	$y = 0.041x^{1.934}$
Rogers et al., 1977	Washington, USA	Arthropods in nine groups	Hemiptera (terrestrial bugs)	$\ln(a) = -2.998 \pm 0.113$, $b = 2.270 \pm 0.081$	$a = 0.049$	$y = 0.049x^{2.270}$
Sample et al., 1993	West Virginia, USA	Insects in 13 orders	Hemiptera (terrestrial bugs)	$\ln(a) = -4.784 \pm 0.313$, $b = 3.075 \pm 0.147$	$a = 0.008$	$y = 0.008x^{3.075}$
Schoener, 1980	Costa Rica and Massachusetts, USA	Insects in eight orders	Hemiptera (terrestrial bugs) collected from the tropical rainforest in Costa Rica	$a = 0.027 \pm 0.419$, $b = 2.28 \pm 0.76$	Transformation not needed	$y = 0.027x^{2.280}$
Smock, 1980	North Carolina, USA	Aquatic and semi-aquatic insects in eight orders	Hemiptera	$\ln(a) = -3.461 \pm 0.311$, $b = 2.40 \pm 0.21$	$a = 0.031$	$y = 0.031x^{2.40}$
			<i>Gerris remigis</i>	$\ln(a) = -4.200 \pm 0.916$, $b = 2.60 \pm 0.45$	$a = 0.014$	$y = 0.014x^{2.60}$
			<i>Metrobates hesperius</i>	$\ln(a) = -4.080 \pm 0.525$, $b = 2.66 \pm 0.34$	$a = 0.016$	$y = 0.016x^{2.66}$
			<i>Rhagovelia obesa</i>	$\ln(a) = -4.791 \pm 1.018$, $b = 2.78 \pm 0.40$	$a = 0.008$	$y = 0.008x^{2.78}$

¹When the equation in the study was $y = a(x)^b$.

²When the equation in the study was $\ln(y) = \ln(a) + b \ln(x)$.

³With transformed coefficient parameter a , if needed.

TABLE 2 Equations and adjusted R^2 values of five fitting functions (linear regression, polynomial regression order 2, 3, and 4, and power regression) on semi-aquatic bugs belonging to three body form groups: (1) Cylindrostethinae, Gerrinae, and Ptilomerinae, (2) Halobatinae, and (3) Veliidae. Regressions were done on combined body form groups as well as on each group with life stages combined and separated. The regression for each group was developed using measurements from 45 juveniles and 45 adults for each body form group, except for Halobatinae, which was based on 45 juveniles and 34 adults due to a limited number of adult specimens. The highest adjusted R^2 value in each category is highlighted in bold.

Body form group	Life stage	Fitting function	Equation	Adjusted R^2
Gerromorpha	All taxa with all life stages combined	Linear regression	$y = -3.2 + 1.4x$	0.74
		Polynomial regression, order 2	$y = 0.83 - 0.52x + 0.13x^2$	0.85
		Polynomial regression, order 3	$y = -0.56 + 0.51x - 0.037x^2 + 0.0068x^3$	0.86
		Polynomial regression, order 4	$y = 0.51 - 0.6x + 0.27x^2 - 0.023x^3 + 0.00091x^4$	0.86
		Power regression	$y = 0.053x^{2.190}$	0.95
Cylindrostethinae, Gerrinae, and Ptilomerinae	Juvenile and adult combined	Linear regression	$y = -7.7 + 1.8x$	0.70
		Polynomial regression, order 2	$y = 2 - 0.87x + 0.15x^2$	0.79
		Polynomial regression, order 3	$y = -1.4 + 0.81x - 0.071x^2 + 0.0079x^3$	0.79
		Polynomial regression, order 4	$y = 4.3 - 3.5x + 0.87x^2 - 0.07x^3 + 0.0021x^4$	0.79
		Power regression	$y = 0.040x^{2.271}$	0.94
	Juvenile only	Linear regression	$y = -4.5 + 1.5x$	0.87
		Polynomial regression, order 2	$y = 1.1 - 0.64x + 0.15x^2$	0.94
		Polynomial regression, order 3	$y = -0.49 + 0.4x - 0.03x^2 + 0.0084x^3$	0.94
		Polynomial regression, order 4	$y = -2.5 + 2.2x - 0.52x^2 + 0.06x^3 - 0.0018x^4$	0.94
		Power regression	$y = 0.039x^{2.362}$	0.97
	Adult only	Linear regression	$y = -13 + 2.3x$	0.68
		Polynomial regression, order 2	$y = 14 - 3.4x + 0.26x^2$	0.75
		Polynomial regression, order 3	$y = -24 + 9.4x - 1.1x^2 + 0.042x^3$	0.77
		Polynomial regression, order 4	$y = 26 - 14x + 2.8x^2 - 0.22x^3 + 0.0064x^4$	0.77
		Power regression	$y = 0.030x^{2.349}$	0.86
Halobatinae	Juvenile and adult combined	Linear regression	$y = -0.65 + 0.55x$	0.83
		Polynomial regression, order 2	$y = -0.18 + 0.15x + 0.068x^2$	0.86
		Polynomial regression, order 3	$y = 0.077 - 0.18x + 0.19x^2 - 0.013x^3$	0.86
		Polynomial regression, order 4	$y = 0.061 - 0.15x + 0.17x^2 - 0.0091x^3 - 0.00033x^4$	0.86
		Power regression	$y = 0.072x^{2.218}$	0.92
	Juvenile only	Linear regression	$y = -0.35 + 0.38x$	0.75
		Polynomial regression, order 2	$y = -0.046 + 0.025x + 0.092x^2$	0.76
		Polynomial regression, order 3	$y = 0.29 - 0.59x + 0.43x^2 - 0.057x^3$	0.76
		Polynomial regression, order 4	$y = -0.23 + 0.65x - 0.62x^2 + 0.32x^3 + 0.048x^4$	0.75
		Power regression	$y = 0.068x^{2.300}$	0.85
	Adult only	Linear regression	$y = -1.2 + 0.7x$	0.67
		Polynomial regression, order 2	$y = -0.4 + 0.3x + 0.048x^2$	0.66
		Polynomial regression, order 3	$y = 5.4 - 4.2x + 1.2x^2 - 0.089x^3$	0.65
		Polynomial regression, order 4	$y = 140 - 140x + 53x^2 - 8.4x^3 + 0.5x^4$	0.67
		Power regression	$y = 0.141x^{1.704}$	0.68

(Continues)

TABLE 2 (Continued)

Body form group	Life stage	Fitting function	Equation	Adjusted R ²
Veliidae	Juvenile and adult combined	Linear regression	$y = -0.23 + 0.25x$	0.81
		Polynomial regression, order 2	$y = 0.095 - 0.15x + 0.1x^2$	0.87
		Polynomial regression, order 3	$y = -0.14 + 0.29x - 0.14x^2 + 0.041x^3$	0.88
		Polynomial regression, order 4	$y = 0.14 - 0.43x + 0.48x^2 - 0.18x^3 + 0.028x^4$	0.88
	Juvenile only	Power regression	$y = 0.041x^{2.320}$	0.87
		Linear regression	$y = -0.12 + 0.16x$	0.85
		Polynomial regression, order 2	$y = -0.036 + 0.048x + 0.034x^2$	0.86
		Polynomial regression, order 3	$y = 0.06 - 0.15x + 0.16x^2 - 0.023x^3$	0.86
		Polynomial regression, order 4	$y = -0.09 + 0.3x - 0.31x^2 + 0.17x^3 - 0.029x^4$	0.86
	Adult only	Power regression	$y = 0.037x^{2.322}$	0.81
		Linear regression	$y = -0.25 + 0.27x$	0.84
		Polynomial regression, order 2	$y = 0.067 - 0.11x + 0.094x^2$	0.87
		Polynomial regression, order 3	$y = -0.43 + 0.76x - 0.35x^2 + 0.069x^3$	0.87
		Polynomial regression, order 4	$y = 1.6 - 3.7x + 3x^2 - 0.98x^3 + 0.12x^4$	0.88
		Power regression	$y = 0.049x^{2.229}$	0.94

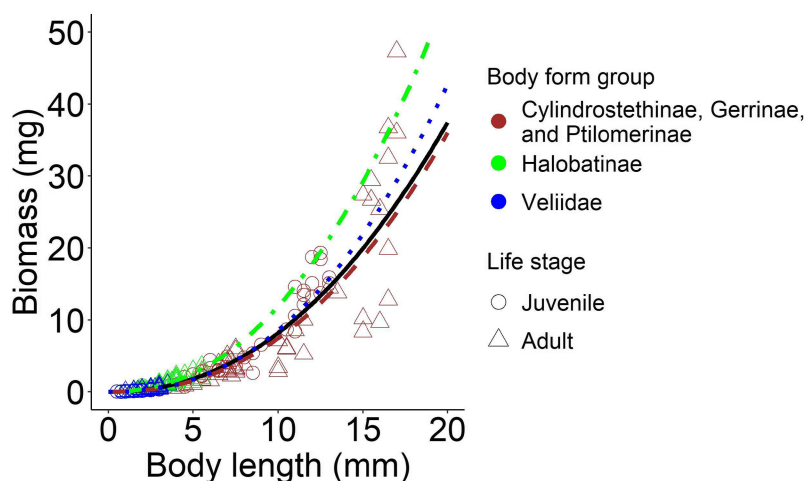


FIGURE 2 Four curves fitted on all juvenile and adult semi-aquatic bug samples, each with coefficients obtained from power regression fitted on: combined body form groups (black solid line; $y = 0.053x^{2.190}$); Cylindrostethinae, Gerrinae, and Ptilomerinae (brown dashed line; $y = 0.040x^{2.271}$); Halobatinae (green dashed-dotted line; $y = 0.072x^{2.218}$); and Veliidae (blue dotted line; $y = 0.041x^{2.320}$). All the curves, except the combined body form groups, were obtained from power regressions on each group with life stages combined.

had marginally higher adjusted R^2 value (Table 2, Figures S4–S13). In addition, power regression fitted on combined body form groups (across life stages) gave higher adjusted R^2 values in most cases (adjusted $R^2 = 0.95$), except when compared with juvenile Cylindrostethinae, Gerrinae, and Ptilomerinae, which had a slightly higher value as a single group (adjusted $R^2 = 0.97$) (Table 2).

Curves created using the power regression equations were similar between combined body form groups and each group with life stages combined, although group-specific curves were a better fit for Halobatinae and Veliidae (Figure 2). Even though adjusted R^2 values of

power regressions differed when combined and separated life stages were compared, the curves were similar for each group (Figure 3).

Does the relationship change between juvenile and adult bugs, and does the relationship change between body forms of semi-aquatic bugs?

Comparisons between measured and predicted biomass of juvenile and adult semi-aquatic bugs – with predicted

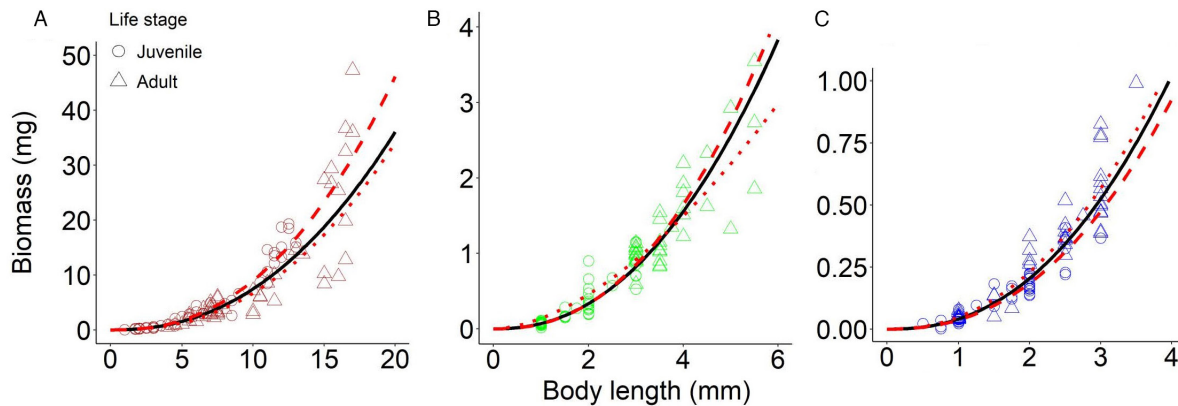


FIGURE 3 Three power regressions fitted on all juvenile and adult semi-aquatic bug samples of (A) Cylindrostethinae, Gerrinae, and Ptilomerinae, (B) Halobatinae, (C) and Veliidae. Curves for each body form group were created with coefficients obtained from power regression fitted on each group with life stages combined (black solid line; $y = 0.040x^{2.271}$ for Cylindrostethinae, Gerrinae, and Ptilomerinae, $y = 0.072x^{2.218}$ for Halobatinae, and $y = 0.041x^{2.320}$ for Veliidae); juvenile individuals only (red dashed line; $y = 0.039x^{2.362}$ for Cylindrostethinae, Gerrinae, and Ptilomerinae, $y = 0.068x^{2.300}$ for Halobatinae, and $y = 0.037x^{2.322}$ for Veliidae); and adult individuals only (red dotted line; $y = 0.030x^{2.349}$ for Cylindrostethinae, Gerrinae, and Ptilomerinae, $y = 0.141x^{1.704}$ for Halobatinae, and $y = 0.049x^{2.229}$ for Veliidae).

TABLE 3 Comparison of measured and predicted biomass of juvenile (J) and adult (A) semi-aquatic bugs from the three body form groups obtained in this study, using coefficients from power regression, fitted on combined body form groups ($y = 0.053x^{2.190}$, $n = 259$) and each group with life stages combined ($y = 0.040x^{2.271}$ for Cylindrostethinae, Gerrinae, and Ptilomerinae, based on $n = 45$ juveniles and 45 adults; $y = 0.072x^{2.218}$ for Halobatinae, based on $n = 45$ juveniles and $n = 34$ adults; and $y = 0.041x^{2.320}$ for Veliidae, based on $n = 45$ juveniles and 45 adults). Bold P-values indicate a significant difference between the measured and predicted biomass ($P < 0.05$).

Body form group	Life stage	Mean (\pm SE) biomass (mg)		Fitting function	W	P ^a
		Measured	Predicted			
Cylindrostethinae, Gerrinae, and Ptilomerinae	J	6.042 \pm 0.931	5.338 \pm 0.752	$y = 0.053x^{2.190}$	965	0.70
	J	6.042 \pm 0.931	4.863 \pm 0.699	$y = 0.040x^{2.271}$	920	0.46
	A	10.365 \pm 1.725	10.639 \pm 1.295	$y = 0.053x^{2.190}$	1107	0.45
	A	10.365 \pm 1.725	9.889 \pm 1.238	$y = 0.040x^{2.271}$	1053	0.75
Halobatinae	J	0.349 \pm 0.044	0.234 \pm 0.026	$y = 0.053x^{2.190}$	763	0.043
	J	0.349 \pm 0.044	0.324 \pm 0.037	$y = 0.072x^{2.218}$	979	0.79
	A	1.424 \pm 0.114	1.016 \pm 0.085	$y = 0.053x^{2.190}$	299	<0.001
	A	1.424 \pm 0.114	1.435 \pm 0.123	$y = 0.072x^{2.218}$	568	0.91
Veliidae	J	0.154 \pm 0.015	0.194 \pm 0.019	$y = 0.053x^{2.190}$	1263.5	0.042
	J	0.154 \pm 0.015	0.165 \pm 0.017	$y = 0.041x^{2.320}$	1033	0.87
	A	0.317 \pm 0.037	0.319 \pm 0.034	$y = 0.053x^{2.190}$	1102	0.47
	A	0.317 \pm 0.037	0.280 \pm 0.031	$y = 0.041x^{2.320}$	903	0.38

^aComparisons between measured and predicted biomass were based on Wilcoxon test with Bonferroni correction.

biomass obtained using power regression equations fitted on combined body form groups and each group with life stages combined – showed no significant difference in Cylindrostethinae, Gerrinae, and Ptilomerinae and adult Veliidae (Table 3). However, the measured and predicted biomass of Halobatinae (both life stages) and juvenile Veliidae differed significantly, when the predictions were made using the equation fitted on combined body groups (Table 3). On the other hand, there was no significant difference in each of the Halobatinae and Veliidae (across life stages) when biomass predictions used equations fitted on each group with life stages combined (Table 3).

Do our selected best models predict biomass better than models constructed using published general Hemiptera or subtropical semi-aquatic bugs?

Fitting functions from Rogers et al. (1977), Sample et al. (1993), Schoener (1980) as well as Smock (1980) on general Hemiptera, *G. remigis*, and *M. hesperius* were able to provide reliable biomass estimates of both juvenile and adult Cylindrostethinae, Gerrinae, and Ptilomerinae (shown by no significant difference between measured biomass and predictions derived from published equations).

TABLE 4 Comparison of measured and predicted biomass using coefficients from six studies: five on terrestrial Hemiptera (fitting function source 1–5) and one on semi-aquatic bugs from a sub-tropical region (fitting function source 6–9). The measured values for each body form group were based on 45 juveniles (J) and 45 adults (A), except for adult Halobatinae, which were based on 34 individuals. Bold P-values indicate a significant difference between the measured and predicted biomass ($P < 0.05$)

Body form group	Life stage	Mean (\pm SE) biomass (mg)		Fitting function	Fitting function source ^a	W	P ^b
		Measured	Predicted				
Cylindrostethinae, Gerrinae, and Ptilomerinae	J	6.042 \pm 0.931	7.741 \pm 1.236	$y = 0.020x^{2.764}$	1	1078	0.60
	J	6.042 \pm 0.931	2.289 \pm 0.300	$y = 0.041x^{1.934}$	2	730	0.022
	J	6.042 \pm 0.931	5.944 \pm 0.855	$y = 0.049x^{2.270}$	3	1014	0.99
	J	6.042 \pm 0.931	6.488 \pm 1.091	$y = 0.008x^{3.075}$	4	968	0.72
	J	6.042 \pm 0.931	3.352 \pm 0.483	$y = 0.027x^{2.280}$	5	807	0.098
	J	6.042 \pm 0.931	5.092 \pm 0.755	$y = 0.031x^{2.40}$	6	917	0.44
	J	6.042 \pm 0.931	3.678 \pm 0.569	$y = 0.014x^{2.60}$	7	791	0.074
	J	6.042 \pm 0.931	4.842 \pm 0.758	$y = 0.016x^{2.66}$	8	869	0.25
	J	6.042 \pm 0.931	3.216 \pm 0.515	$y = 0.008x^{2.78}$	9	743	0.029
	A	10.365 \pm 1.725	17.806 \pm 2.571	$y = 0.020x^{2.764}$	1	1272	0.036
	A	10.365 \pm 1.725	4.279 \pm 0.471	$y = 0.041x^{1.934}$	2	670	0.005
	A	10.365 \pm 1.725	12.083 \pm 1.512	$y = 0.049x^{2.270}$	3	1171	0.20
	A	10.365 \pm 1.725	16.146 \pm 2.503	$y = 0.008x^{3.075}$	4	1153	0.26
	A	10.365 \pm 1.725	6.832 \pm 0.858	$y = 0.027x^{2.280}$	5	842	0.17
	A	10.365 \pm 1.725	10.693 \pm 1.396	$y = 0.031x^{2.40}$	6	1076	0.61
	A	10.365 \pm 1.725	8.119 \pm 1.123	$y = 0.014x^{2.60}$	7	862	0.23
	A	10.365 \pm 1.725	10.851 \pm 1.526	$y = 0.016x^{2.66}$	8	1028	0.90
	A	10.365 \pm 1.725	7.427 \pm 1.077	$y = 0.008x^{2.78}$	9	808	0.099
Halobatinae	J	0.349 \pm 0.044	0.142 \pm 0.019	$y = 0.020x^{2.764}$	1	525	<0.001
	J	0.349 \pm 0.044	0.147 \pm 0.015	$y = 0.041x^{1.934}$	2	557	<0.001
	J	0.349 \pm 0.044	0.230 \pm 0.027	$y = 0.049x^{2.270}$	3	762	0.042
	J	0.349 \pm 0.044	0.074 \pm 0.011	$y = 0.008x^{3.075}$	4	241	<0.001
	J	0.349 \pm 0.044	0.128 \pm 0.015	$y = 0.027x^{2.280}$	5	496.5	<0.001
	J	0.349 \pm 0.044	0.162 \pm 0.020	$y = 0.031x^{2.40}$	6	610	0.001
	J	0.349 \pm 0.044	0.086 \pm 0.011	$y = 0.014x^{2.60}$	7	312	<0.001
	J	0.349 \pm 0.044	0.103 \pm 0.014	$y = 0.016x^{2.66}$	8	418	<0.001
	J	0.349 \pm 0.044	0.057 \pm 0.008	$y = 0.008x^{2.78}$	9	178.5	<0.001
	A	1.424 \pm 0.114	0.861 \pm 0.094	$y = 0.020x^{2.764}$	1	226	<0.001
	A	1.424 \pm 0.114	0.550 \pm 0.040	$y = 0.041x^{1.934}$	2	68	<0.001
	A	1.424 \pm 0.114	1.051 \pm 0.092	$y = 0.049x^{2.270}$	3	330	0.002
	A	1.424 \pm 0.114	0.537 \pm 0.066	$y = 0.008x^{3.075}$	4	103	<0.001
	A	1.424 \pm 0.114	0.587 \pm 0.051	$y = 0.027x^{2.280}$	5	93	<0.001
	A	1.424 \pm 0.114	0.798 \pm 0.074	$y = 0.031x^{2.40}$	6	187	<0.001
	A	1.424 \pm 0.114	0.478 \pm 0.048	$y = 0.014x^{2.60}$	7	68	<0.001
	A	1.424 \pm 0.114	0.594 \pm 0.062	$y = 0.016x^{2.66}$	8	114	<0.001
	A	1.424 \pm 0.114	0.352 \pm 0.038	$y = 0.008x^{2.78}$	9	20	<0.001
Veliidae	J	0.154 \pm 0.015	0.111 \pm 0.013	$y = 0.020x^{2.764}$	1	711.5	0.014
	J	0.154 \pm 0.015	0.126 \pm 0.011	$y = 0.041x^{1.934}$	2	851	0.19
	J	0.154 \pm 0.015	0.190 \pm 0.019	$y = 0.049x^{2.270}$	3	1261.5	0.044
	J	0.154 \pm 0.015	0.056 \pm 0.007	$y = 0.008x^{3.075}$	4	440	<0.001
	J	0.154 \pm 0.015	0.105 \pm 0.011	$y = 0.027x^{2.280}$	5	700	0.011

TABLE 4 (Continued)

Body form group	Life stage	Mean (\pm SE) biomass (mg)		Fitting function	Fitting function source ^a	W	p ^b
		Measured	Predicted				
	J	0.154 \pm 0.015	0.132 \pm 0.014	$y = 0.031x^{2.40}$	6	837	0.16
	J	0.154 \pm 0.015	0.069 \pm 0.007	$y = 0.014x^{2.60}$	7	495.5	<0.001
	J	0.154 \pm 0.015	0.082 \pm 0.009	$y = 0.016x^{2.66}$	8	585	<0.001
	J	0.154 \pm 0.015	0.045 \pm 0.005	$y = 0.008x^{2.78}$	9	398.5	<0.001
	A	0.317 \pm 0.037	0.211 \pm 0.026	$y = 0.020x^{2.764}$	1	722	0.018
	A	0.317 \pm 0.037	0.194 \pm 0.019	$y = 0.041x^{1.934}$	2	659	0.004
	A	0.317 \pm 0.037	0.318 \pm 0.035	$y = 0.049x^{2.270}$	3	1066	0.67
	A	0.317 \pm 0.037	0.115 \pm 0.015	$y = 0.008x^{3.075}$	4	467	<0.001
	A	0.317 \pm 0.037	0.177 \pm 0.019	$y = 0.027x^{2.280}$	5	602.5	<0.001
	A	0.317 \pm 0.037	0.228 \pm 0.026	$y = 0.031x^{2.40}$	6	760	0.041
	A	0.317 \pm 0.037	0.125 \pm 0.015	$y = 0.014x^{2.60}$	7	500	<0.001
	A	0.317 \pm 0.037	0.152 \pm 0.018	$y = 0.016x^{2.66}$	8	552	<0.001
	A	0.317 \pm 0.037	0.085 \pm 0.010	$y = 0.008x^{2.78}$	9	436	<0.001

^aFitting function from the following publication: (1) Ganihar, 1997; (2) Gruner, 2003; (3) Rogers et al., 1977; (4) Sample et al., 1993; (5) Schoener, 1980; (6) Smock, 1980 (on general Hemiptera); (7) Smock, 1980 (on *Gerris remigis*); (8) Smock, 1980 (on *Metrobates hesperius*); (9) Smock, 1980 (on *Rhagovelia obesa*).

^bComparisons between measured and predicted biomass were based on Wilcoxon test with Bonferroni correction.

(Table 4). None of the nine sources from the six studies could estimate the biomass of Halobatinae across all life stages (Table 4). In most cases, equations from the published studies could not provide reliable biomass estimates for both juvenile and adult Veliidae, with only equations from Gruner (2003) and Smock (1980) predicting biomass for the juveniles, and the equation from Rogers et al. (1977) for the adults (Table 4).

DISCUSSION

We found that power regression equations, particularly those constructed for specific body forms, produced good biomass estimates for semi-aquatic bugs across life stages (both juvenile and adult). Power regression has also been found to best predict insect biomass for a range of taxa, when compared with several different approaches (linear, logarithmic, and exponential) in other studies (Rogers et al., 1977; Smock, 1980; Ganihar, 1997). This finding is likely to be related to the scaling relationship between length and volume, as well as specific differences in how insects grow and assimilate new biomass. For example, mechanistic growth models produced for insects by Maino & Kearney (2015) – taken from 50 individual insects from seven orders, i.e., Coleoptera, Lepidoptera, Hemiptera, Hymenoptera, Orthoptera, Diptera, and Neuroptera – identified reduced investment in structural biomass over time in insects as they grow. Straus & Aviles (2017) found that allometric scaling between size and weight decreased as insects got larger, suggesting that larger insects have a lower tissue density, or more internal air spaces. Other factors may also influence the specific relationship between

length and biomass as insects grow, including levels of food resources which, when low, can result in insects using reserves to compensate for an increase in size (Maino & Kearney, 2015).

The curve fitted on combined body form groups was more similar to that fitted on the Cylindrostethinae, Gerrinae, and Ptilomerinae than on Veliidae and Halobatinae, probably because there were more insects in the Cylindrostethinae, Gerrinae, and Ptilomerinae group, and so they dominated the body length and weight ranges of all samples combined. This might also explain why group-specific curves fitted better for Halobatinae and Veliidae, which differ markedly in their shape from Cylindrostethinae, Gerrinae, and Ptilomerinae. As a result, biomass estimation using power regression equations fitted on combined body form groups performed well only for the Cylindrostethinae, Gerrinae, and Ptilomerinae across both life stages, but not for Halobatinae and Veliidae. On the other hand, power regression equations fitted specifically for each group with life stages combined could well estimate the biomass of each group across life stages. Therefore, studies working exclusively on semi-aquatic bugs should use body-form specific biomass equations. An alternative could be to include width measurements in the analysis, allowing differences in shape related to biomass to be better explained. This has been suggested in studies comparing taxa from different families (Sample et al., 1993), but our findings indicate that such an approach could also be useful for within-family studies, as seen with bugs in the Cylindrostethinae, Gerrinae, and Ptilomerinae that have a different body form to those in the Halobatinae, despite belonging to the same family (Gerridae).

When life stages were combined or separated for each group, all resulting curves for biomass estimates were similar, although with varying adjusted R^2 values. This indicates that an equation specific for the body form is sufficient for predicting the biomass of that group across life stages. This finding was also reported by Rogers et al. (1977), indicating that combining juvenile and adult stages in biomass calculations may be a tractable option across groups, particularly for insects that undergo incomplete metamorphosis, in which the juveniles and the adults have similar body forms. Although we did not consider the differing instars for juveniles in this study or differences between sexes, we would argue that, as the combined biomass predictions we obtained were good for both juveniles and adults of a particular body form and there were no obvious morphological differences differentiating instars or sexes in most cases in this study, the equation is most likely giving good predictions across a range of instars and both sexes. However, further investigations could provide more clarity in this by testing equations specific to particular instars and for males and females separately.

The length–biomass equations obtained from six other sources [Rogers et al., 1977; Schoener, 1980; Sample et al., 1993; Smock (1980) on general Hemiptera, *G. remigis*, and *M. hesperius*] could produce a good biomass estimate for both juvenile and adult Cyndrostethinae, Gerrinae, and Ptilomerinae, whereas coefficients from three other sources [Smock (1980) on *R. obesa*; Ganihar, 1997; Gruner, 2003] resulted in inconsistent biomass predictions for both juvenile and adult life stages. However, no sources could provide a consistent and reliable biomass estimate for both juvenile and adult Halobatinae and Veliidae samples in this study. Therefore, although fitting functions at the order or family level can be useful, attention should also be paid to any variation in body forms between samples belonging to the same family. If such variation exists, separating samples into different body form groups may be needed to obtain accurate biomass estimates.

In most cases, an equation obtained from one species can predict the biomass of other species belonging to the same body form group. For instance, equations obtained from *G. remigis* and *M. hesperius* (both Gerridae; Smock, 1980) provided good biomass estimates for juvenile as well as adult Cyndrostethinae, Gerrinae, and Ptilomerinae. However, it should be noted that, in another case, the equation obtained from *R. obesa* (Veliidae; Smock, 1980) could not provide a good estimate for either juveniles or adults within the Veliidae group. This might be owing to species-specific idiosyncrasies in shape.

Our results also indicate that body form is a more important factor for biomass estimation than geographical region. For example, biomass equations derived from *G. remigis* and *M. hesperius* (Smock, 1980) provided a good biomass estimate for both juvenile and adult Cyndrostethinae, Gerrinae, and Ptilomerinae in this study, even though collection site and climate (North Carolina, USA) were different from the specimens collected in this

study (Sabah, Malaysia). Therefore, length–biomass equations constructed in this study are likely to be applicable to predict the biomass of semi-aquatic bugs across regions.

This paper lays the groundwork for studying the biomass of this group in a relatively easy, cheap, and accurate way. We anticipate that surveys of semi-aquatic bugs have the potential to provide a tractable and cost-effective means of monitoring environmental change in tropical freshwater systems and hope that the relationships defined here will support further research in this area. To obtain the most accurate estimates for these studies, we recommend using length–biomass equations that are specific to different body forms for semi-aquatic bugs of all life stages.

AUTHOR CONTRIBUTIONS

Martina F. Harianja: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (lead); methodology (equal); project administration (lead); visualization (equal); writing – original draft (lead); writing – review and editing (lead).

Sarah H. Luke: Conceptualization (lead); data curation (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); supervision (lead); visualization (supporting); writing – review and editing (supporting).

Holly Barclay: Data curation (supporting); funding acquisition (equal); methodology (supporting); writing – review and editing (supporting). **Vun K. Chey:** Methodology (supporting); writing – review and editing (supporting). **David C. Aldridge:** Methodology (supporting); writing – review and editing (supporting).

William A. Foster: Methodology (supporting); writing – review and editing (supporting). **Edgar C. Turner:** Conceptualization (lead); formal analysis (equal); methodology (equal); supervision (lead); visualization (equal); writing – review and editing (supporting).

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DATA AVAILABILITY STATEMENT

The data that support the findings and analytic codes will be available in Zenodo Repository at 10.5281/zenodo.6659535

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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