



Assessment of morphometric characters of the *Eurema Hecabe* (L.) and *Orthetrum brunneum* from Udaipur, India

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Abstract

The present study was carried out to investigate the morphometrics and length-length relationships (LLRs) of *Eurema Hecabe* (L.) and *Orthetrum Brunneum*. Animals were collected from various locations in Udaipur City, India. A total of 12 different parameters of the wing and body were taken into account. It was observed that total body length affects wing morphometry in both animals. The coefficient of determination (r^2) values were also determined. The allometric coefficient 'b' suggested positive allometric growth. The results of the present study are important in terms of the taxonomy tool used in the identification of species and subspecies.

Keywords: morphometric characters, *Orthetrum Brunneum*, *Eurema Hecabe* (L.)

Introduction

Morphometrics is the measurement and analysis of form and is one of the important tools for the identification of many groups of insects. Morphometrics is the technique used in the identification of animal and plant species by measuring and analysing individuals (Pimentel, 1979) ^[1]. Needless to say, morphometrics is not a coherent discipline. It is practised in many fields as anthropology (Oxnard, 1978) ^[2], cytology (Aherne & Dunnill, 1982) ^[3], entomology, geology, nematology, and paleobiology (Benson *et al.*, 1982) ^[4]. There is uniformity in morphometric studies using general statistical methods and some specialized techniques that have a wide range of applications.

Insect exoskeletons are hard and chitinous and protect animals from physical distortion as observed in soft-bodied animals, they can be easily measured. Insects are advantageous subjects for studying variation. Measurements of body parts, and especially wings, are used as a tool in the species identification of many insects (Sontigun *et al.*, 2017; Atchley *et al.*, 1976) ^[5-6]. Morphometric studies are equally important in research, as are life cycles and morphological trends of populations across regions (Hossain *et al.*, 2017) ^[7]. Azrizal-Wahid *et al.*, (2016) ^[8] investigated the wing and body morphometry in six species of *Eurema* butterflies of Malaysia Peninsula. Morphometric measurements of Afro-Asian genus *Trithemis* were studied by Outomuro *et al* (2013) ^[9], these have wide variety of habitat from forest to open country (Damm *et al.*, 2010) ^[10].

Descriptive statistical tools are widely applied to morphometric studies to reduce the potential for bias and errors and are used in conjunction with other taxonomic methods to observe phenetic and cladistic differences. (Prieto *et al.*, 2009) ^[11].

In the present study, the morphometric dimension of the different parameters of the body, the length-length relationship, and the growth patterns of the *Eurema hecabe* (L.) and *Orthetrum brunneum* were examined, and this report is the first from Udaipur, India.

Material and methods

Study area

Udaipur city is located in the southernmost part of Rajasthan, near the Gujarat border. It is surrounded by the Aravali Range, which separates it from the Thar Desert. Udaipur is located at 24.525049°N and 73.677116°E. The city is spread over an area of 64 km² and altitude is 598.00 m above sea level. Temperatures in the summer range from 23°C to 44°C, while in the winter they range from 5°C to 30°C, thus Udaipur has a hot, semi-arid climate.

Animals

The Common Grass Yellow Butterfly, *Eurema hecabe* (L.) (order Lepidoptera and family Pieridae), and the southern skimmer, *Orthetrum brunneum* (order Odonata and family Libellulidae), were used for this study. The animals were collected from different areas of Udaipur city (Government Meera Girls College, Dewali, MLS University Campus, Gulab Bagh).

Sampling and preservation

Eurema hecabe (L.) and *Orthetrum brunneum* were collected randomly using traditional methods (sweeping net and handpicking). Collected animals were placed in a plastic container containing chloroform-soaked cotton. Samples were then transferred to the Department of Zoology, Government Meera Girls College, Udaipur, for preservation and study.

Morphometric analysis

The twelve measurements were computed *viz.* Forewing Area (FWA), Forewing Perimeter (FWP), Forewing Length (FWL), Forewing width (FWW), Hindwing Area (FWA), Hindwing Perimeter (FWP), Hindwing Length (FWL), Hindwing width (FWW), Total Body Length (TBL), Head Length (HL), Thorax Length (TL), Abdomen Length (AL) (Figure. 1 and 2).

For the morphometric study, ImageJ software (1.52q) was used. Insect wings were traced on tracing paper and then digitised using a scanner at 300 dpi. A 5-centimeter straight line was also drawn on every sheet as a scale. After transferring the images, they were converted into grayscale

and inverted in order to achieve maximum clarity. In Image J, the measurement scale was set using the straight-line tool. After setting up the scale, measurements were done using a wand, line, and other tools.

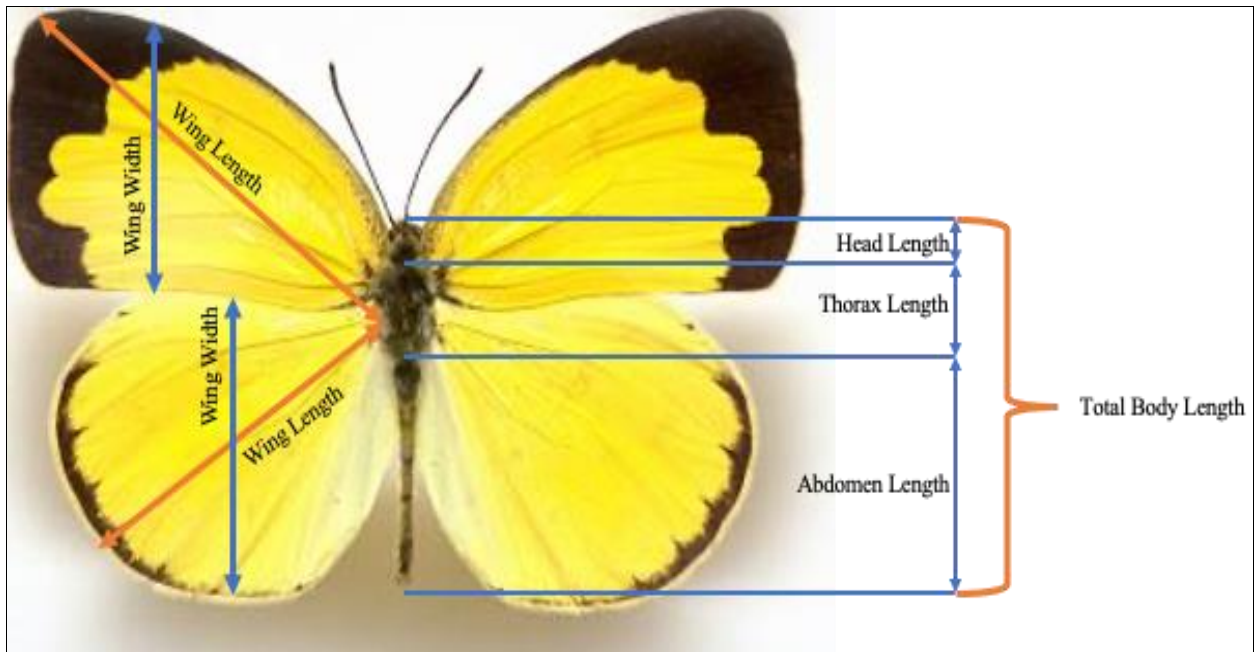


Fig 1: Measurement sites of the common grass yellow butterfly, *E. hecabe* (L.)

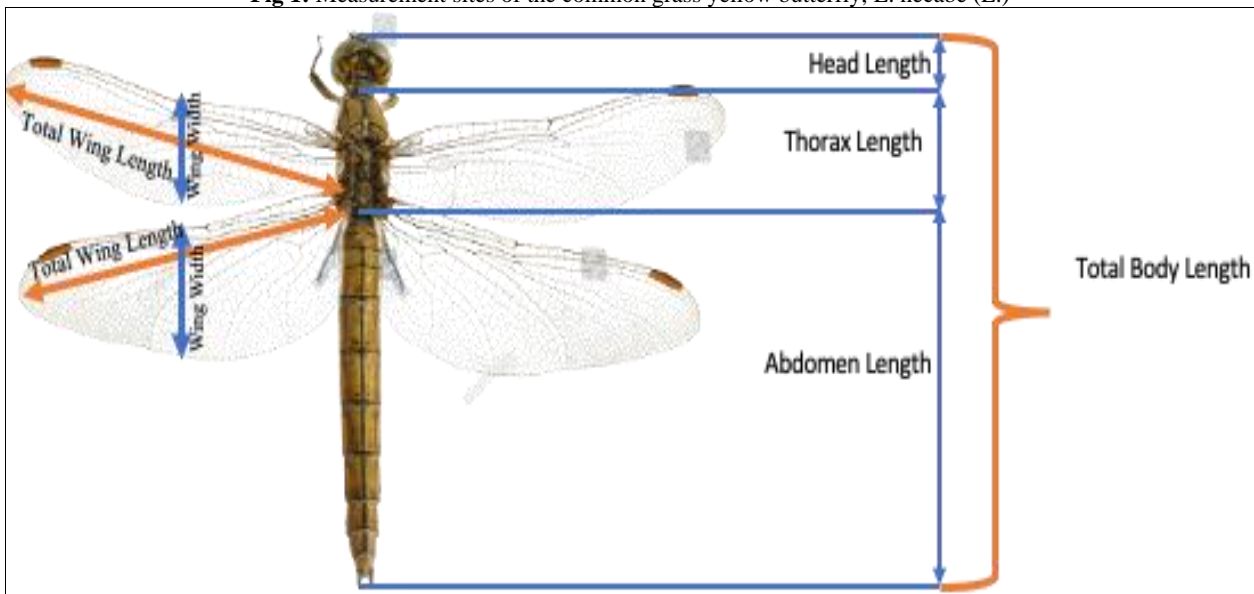


Fig 2: Measurement sites of the *Orthetrum brunneum*

Data analysis

The length-length relationship (LLR) was determined using the formula: $TL = a * L + b$, (Predicted variable (dependent variable) = slope * independent variable + intercept) where TL is the total length (cm), L is 12 different lengths (mm), "a" the intercept of the regression, and "b" is the regression coefficient (slope). The linear regression analysis was used to calculate the LLR regression parameters a and b. Furthermore, the 95% confidence limit (CL) of a and b and the co-efficient of determination (r^2) were estimated. The r^2 was used as an indicator of the quality of the linear regressions, and the t-test was applied to determine significant differences. The best model for LLRs was

chosen based on the maximum value of determination r^2 . The descriptive statistics were performed by SPSS 23, and these analyses were considered significant at 5% ($p < 0.05$).

Results

Morphometric analysis of 12 parameters of *Eurema hecabe* (L.) and *Orthetrum brunneum* were evaluated viz. Forewing Area (FWA), Forewing Perimeter (FWP), Forewing Length (FWL), Forewing width (FWW), Hindwing Area (HWA), Hindwing Perimeter (HWP), Hindwing Length (HWL), Hindwing width (HWW), Total Body Length (TBL), Head Length (HL), Thorax Length (TL), Abdomen Length (AL) (Table 1&2).

Table 1: Morphometric measurement of different parameters of the common grass yellow butterfly, *Eurema hecabe* (n= 20).

Parameter	Mean±SEM	Median	t-Value	Significance
Forewing Area (cm ²)	1.82±0.04	1.81	7.43	0.001
Forewing Perimeter (cm)	5.59±0.07	5.55	7.67	0.001
Forewing Length (cm)	1.88±0.03	1.84	2.34	0.29
Forewing Width (cm)	1.15±0.01	1.1475	2.49	0.02
Hindwing Area (cm ²)	2.06±0.04	2.056	1.13	0.26
Hindwing Perimeter (cm)	5.64±0.08	5.6425	8.02	0.001
Hindwing Length (cm)	1.75±0.03	1.76	7.6	0.001
Hindwing Width (cm)	1.38±0.04	1.4	6.46	0.001
Total Body Length (cm)	1.30±0.02	1.3	7.74	0.001
Head Length (cm)	0.10±0.0	0.1	4.7	0.001
Thorax Length (cm)	0.40±0.00	0.4	5.1	0.001
Abdomen Length (cm)	0.80±0.01	0.8	5.12	0.001

Table 2: Morphometric measurement of different parameters of the *Orthetrum brunneum* (n= 20).

Parameter	Mean±SEM	Median	t-Value	Significance
Forewing Area (cm ²)	2.44±0.14	2.215	3.19	0.005
Forewing Perimeter (cm)	7.64±0.20	7.4	3.11	0.006
Forewing Length (cm)	3.23±0.09	3.175	2.43	0.25
Forewing Width (cm)	0.84±0.03	0.78	1.21	0.24
Hindwing Area (cm ²)	2.89±0.20	2.59	4.36	0.001
Hindwing Perimeter (cm)	7.79±0.25	7.41	3.15	0.005
Hindwing Length (cm)	3.07±0.09	2.95	0.749	0.463
Hindwing Width (cm)	1.14±0.06	1.09	2.51	0.21
Total Body Length (cm)	3.82±0.08	3.8	10.69	0.001
Head Length (cm)	0.40±0.02	0.4	0	1
Thorax Length (cm)	0.94±0.02	0.9	10.25	0.001
Abdomen Length (cm)	2.48±0.05	2.45	10.25	0.001

In present study, the average TBL was 1.30 ± 0.02 cm for *Eurema hecabe* and for the dragonfly it was 3.82 ± 0.08 cm, and the median value of TLs was 1.3 cm for the butterfly and 3.8 cm for the dragonfly. The mean of Forewing Area of *Eurema hecabewas* 1.82 ± 0.04 cm² and the median value was 1.81 (Table 1). The mean of Forewing Area for dragonfly was 2.44 ± 0.14 cm² and the median value was 2.21 (Table 3). Hindwing area was slightly more than the forewing and was found to be 2.06±0.04. For dragonflies, the hindwing area was slightly larger than the forewing and was found to be 2.89±0.20 cm². Similar trends were observed for perimeter (Forewing: 7.64±0.20; Hindwing: 7.79±0.25). The perimeter for butterfly was Forewing: 5.59±0.07; Hindwing: 5.64±0.08), total wing

length (Forewing: 1.88±0.03; Hindwing: 2.06±0.04) and width (Forewing: 1.15±0.01; Hindwing: 1.38±0.04). Similar trends were observed in dragonflies for perimeter (Forewing: 7.64±0.20; Hindwing: 7.79±0.25), total wing length (Forewing: 3.23±0.09; Hindwing: 3.07±0.09) and width (Forewing: 0.84±0.03; Hindwing: 1.14±0.06). One-sample T-test results are also depicted in Table 1&2.

For *Eurema hecabe* total abdomen length was almost 61–62 percent of the total body length, the thorax length was 30-32 percent, and the head length ranged between 7-10 percent. Total abdomen length for the dragonfly was almost 64–65 percent of the total body length, thorax length was 24-25 percent, and the head length ranged between 10–11 percent.

Table 3: Descriptive statistics and estimated parameters of the length-length relationship of the common grass yellow butterfly, *Eurema hecabe* (n= 20)

Equation	Regression Parameter		95%CI of a		95%CI of b		r ²
	a	b	Lower	Upper	Lower	Upper	
TL=a*FWA+b	0.829	0.744	0.118	1.539	-0.183	1.671	0.239
TL=a*FWP+b	0.736	4.635	-0.652	2.123	2.824	6.445	0.061
TL=a*FWL+b	0.218	1.596	-0.399	0.835	0.791	2.401	0.028
TL=a*FWW+b	-0.61	1.226	0.414	0.293	0.765	1.687	0.007
TL=a*HWA+b	-0.189	2.302	-1.102	0.723	1.111	3.493	0.01
TL=a*HWP+b	-0.15	5.838	-1.637	1.337	3.896	7.779	0.002
TL=a*HWL+b	0.286	1.382	-0.318	0.89	0.594	2.17	0.049
TL=a*HWW+b	0.557	0.651	-0.189	1.303	-0.322	1.625	0.114

(Abbreviations a, intercept; b, slope; CI, confidence intervals, r², co-efficient of determination)

The regression parameters a and b of LLRs, their 95% confidence intervals (a and b), and coefficients of determination (r²) for the grass yellow butterfly and southern skimmer were determined (Table 2). For butterflies, the calculated allometric coefficient b ranged from a minimum of 0.651 for TL vs. Hindwing Width A, to

a maximum of 5.838 for TL vs. hindwing perimeter. (Table 2). For dragonflies, the calculated allometric coefficient b ranged from a minimum of 0.409 for TL vs. Forewing Width A, to a maximum of 6.548 for TL vs. hindwing perimeter (Tables 3 and 4). Regression plots are represented in Figures 3 and 4.

Table 4: Descriptive statistics and estimated parameters of the length-length relationship of the *Orthetrum brunneum*

Equation	Regression Parameter		95%CI of a		95%CI of b		r ²
	a	b	Lower	Upper	Lower	Upper	
TL=a*FWA+b	0.15	1.84	-0.73	1.03	-1.54	5.24	0.007
TL=a*FWP+b	0.23	6.74	-1.08	1.55	1.69	11.8	0.008
TL=a*FWL+b	0.274	4.27	-0.87	0.323	1.98	6.58	0.049
TL=a*FWW+b	0.113	0.409	-0.093	0.319	0.38	1.19	0.069
TL=a*HWA+b	0.281	1.82	-1.034	1.596	-3.221	6.862	0.011
TL=a*HWP+b	0.324	6.548	-1.28	1.929	0.395	12.7	0.01
TL=a*HWL+b	0.145	2.519	-0.464	0.753	0.185	4.85	0.014
TL=a*HWW+b	-0.153	1.724	-0.505	0.198	0.377	3.072	0.044

(n=20). (Abbreviations a, intercept; b, slope; CI, confidence intervals, r², co-efficient of determination)

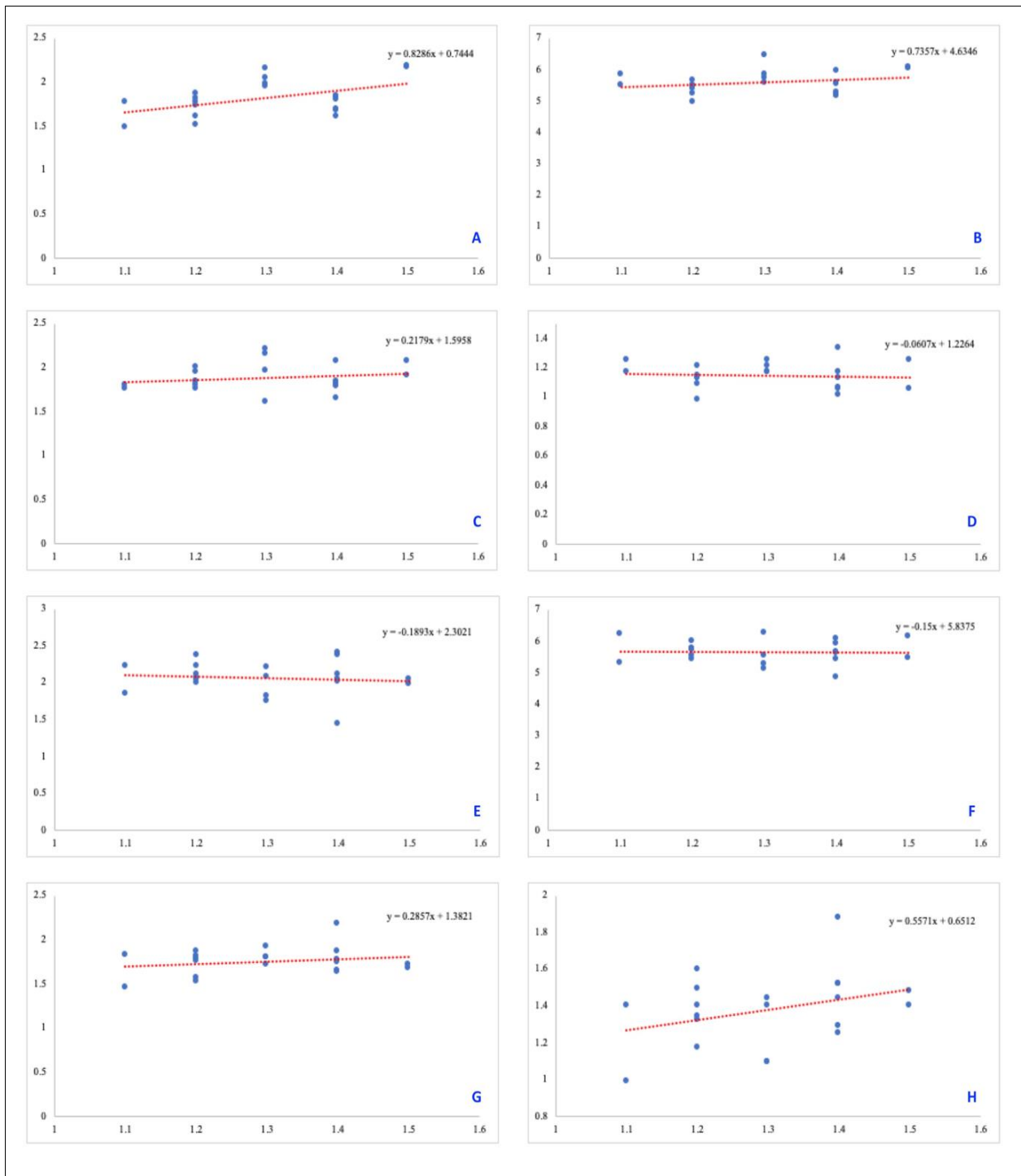


Fig 3: The regression plot between total body length (represented on the x axis) and wing morphometric parameters (represented on the y axis) for Grass Yellow Butterfly. (A) Forewing Area, (B) Forewing Perimeter, (C) Forewing Length, (D) Forewing Width, (E) Hindwing Area (F) Hindwing Perimeter, (G) Hindwing Length, (H) Hindwing Width.

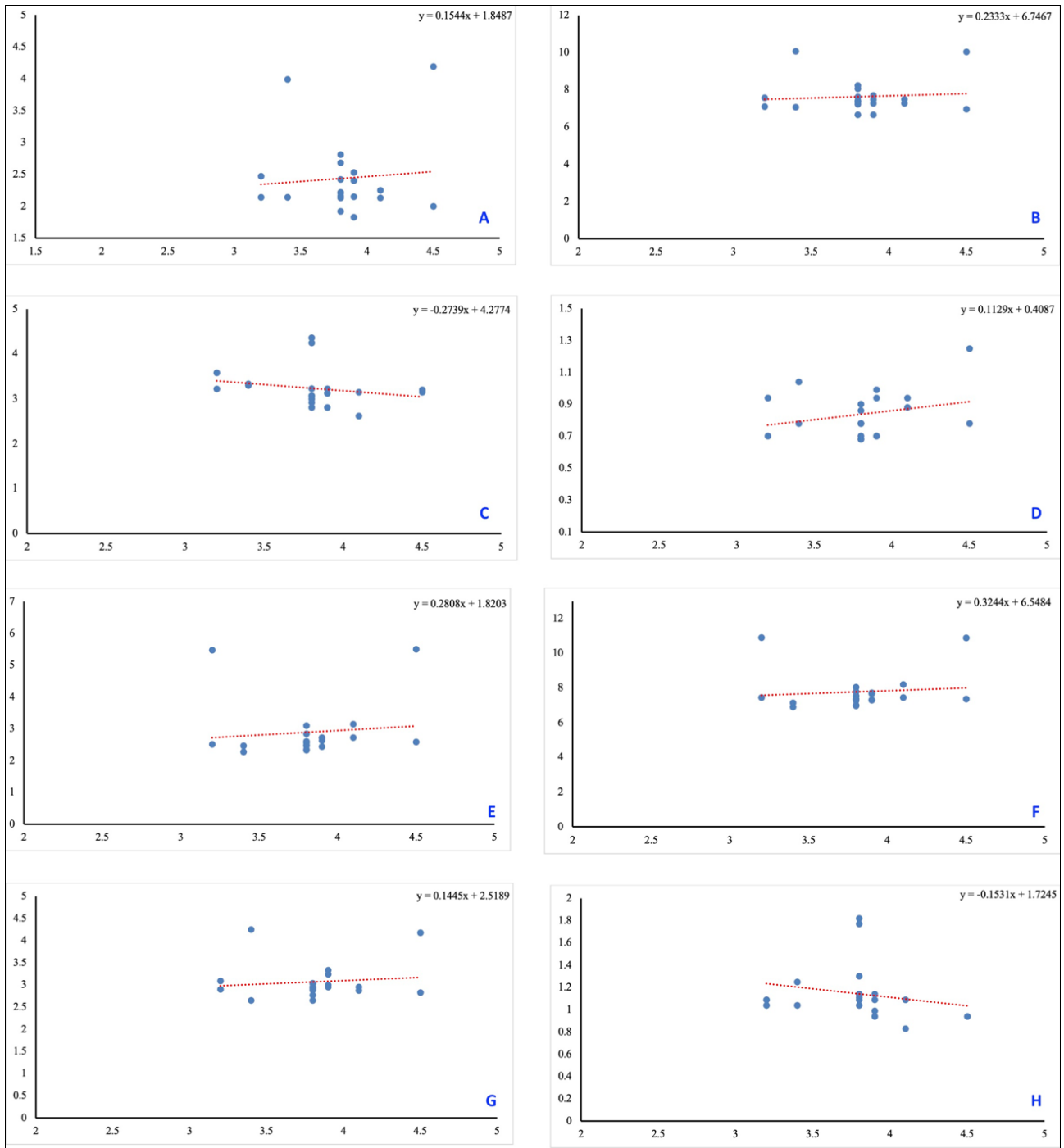


Fig 4: The regression plot between total body length (represented on the x axis) and wing morphometric parameters (represented on the y axis) for South Skimmer Dragonfly. (A) Forewing Area, (B) Forewing Perimeter, (C) Forewing Length, (D) Forewing Width, (E) Hindwing Area, (F) Hindwing Perimeter, (G) Hindwing Length, (H) Hindwing Width.

Discussion

All measurements are subject to error, which can be either instrumental or measurement error (depending on the observer); these are common errors, and it is impossible to control all sources of error in measurements; this is also true in morphometry research (Wolak *et al.*, 2012) [12].

Reproducibility of the results is warranted in every research domain whether it is social sciences, Life Sciences or Mathematics or latest domain that is AI (Fox *et al.*, 2020; Phexell *et al.*, 2019; Salganik *et al.*, 2020; DeBiasse & Ryan, 2019; Andrew *et al.*, 2015) [13-17]. Morphometric studies are not exceptions, but Johnson *et al.* (2013) [18] argues that in entomology reproducibility assessment of morphometric data is limited and is beyond the bounds of

possibility. Here many factors, including ecological niche, preclude the reproducibility of morphometric results especially wing size, stiffness and flexibility and associated flight behaviour (Combes & Daniel, 2003; Wootton, 1992; Suárez-Tovar & Sarmiento, 2016) [19-21]. Ellington (1984) [22] tried to parameterize the morphometric data (mass, body length, wing length, wing area, and wing mass) and wing shape and quantify the mechanical aspects of flight in five insect orders. To study the Scaling relationships and patterns of wing flexibility Combes & Daniel (2003) [19] studied the forewing flexural stiffness and venation patterns in six orders (16 species). Wing morphology is an evidential aspect to establish the relationship between closely related taxa and linkage between species genotype and its

environment (Tüzün, 2009; Ricklefs & Miles, 1994) [23-24]. How an individual defends, during the oncogenic development and later life, against environmental changes and fluctuations, and sustains selective pressures are the reasons for variations among individuals, this is true for the population as well (Digo *et al.*, 2015; Bashar, 2016; Mutanen *et al.*, 2007) [25-27]. The populace structure is influenced by ecological factors, both biotic and abiotic, leading to variation in the morphology of insects (Márquez & Kolasa, 2013) [28].

Diagnostic feature of dragonflies and butterflies is the wing shape and venation pattern (Smart, 1976) [29]. These can be visually distinguished between species and sexes, even on the hind wings and forewings, but there is greater variation in the forewings, which is likely due to the small sample size, which skewed the data (Benítez *et al.*, 2011) [30].

Forewings have more functionality during flight; lepidopteran females need to fly and find the optimal host plant to lay eggs, so they are longer winged than males (Betts & Wootton, 1988; Johansson *et al.*, 2009) [31-32]. For foraging and migration activities in butterflies Wing symmetry plays a major role.

On the family Pieridae butterflies much literature is not available but for other families the studies are available Mahdi *et al.* (2018) [33] studied the Lycaenidae and (Akand *et al.*, 2017) [34] studied Lycaenidae. Akand *et al.*, 2017 [34] investigated the morphometrics (lengths of the forewing, hindwing, body and antenna) of 44 species of butterflies of under the two subfamilies of Lycaenidae. Mahdi *et al.*, (2018) [33] in addition to total body length, wing morphometrics, they also measured the legs of *C. pandava* and *C. lajus*. Shah *et al* (2021) [35] reported morphometrics and length-length relationships of the common grass yellow butterfly.

Morphometrics is a common practice in fisheries, Length-Length Relationships and other parameters like fin formula, fin ray count etc are used to identify the fish species. M. Hossain *et al.* (2006) and Hossain (2010) [36,37] performed the morphometrics of Cyprinid fishes and reported maximum r^2 value was 0.936 for Total Length vs. Fork Length of *A. mola* and 0.998 for Total Length vs. Standard Length and Froese (2006) [38] reported allometric coefficient (b) values between 2.5 to 3.5. Hossain *et al.* (2006) [36], who recorded positive allometric growth in *A. mola* in the Mathabhanga River, southwestern Bangladesh.

Although the biological significance of allometries depends on the scaling relationship, both the slope (α) and the intercept (b) have biological significance. Regardless of body size, variations in the intercept of the allometry between insect species reflect variations in the proportionate size of the wing. On the other hand, discrepancies in the slopes of allometry among insect species reveal variations in how the relative size of the wing changes with body size. The link between size and form within and between species is thus captured by the slope and the intercept for morphological static allometries (Shingleton, 2010) [39].

Environmental conditions and fluctuations, geographical and climatic changes affect the wing shape in insect species (Stewart & Vodopich, 2018) [40]; observed in Dolichopodidae species wing shapes (Chursina & Negrobov, 2016) [41] and also in *Phlebotomus ariasi* (Prudhomme *et al.*, 2016) [42].

Larval development is also affected by environmental conditions (Abdul *et al.*, 2017) [43] body characteristics

(Oliveira-Junior & Juen, 2019) [44]. Shortess, (1929) [45] described changes in wing size and reticulation pattern of *Erythrodiplax Berenice* with reference to geographical location. Migratory and mating behaviours affect the wing shape and variations are observed in dragonflies.

Dragonfly populations exhibit hindwing shape variation associated with different migratory patterns and forewing shape variation associated with male mate guarding and mating displays.

Outomuro *et al.*, (2013) [10] suggest that male dragonflies use their shorter and broader forewings for sexual displays; likewise, sexual selection or differences in behaviours between the genders cause gender-based variation in monarch butterflies, in addition to differential resource allocation (Outomuro & Johansson, 2011) [46].

This study provides preliminary information for future researchers. Detailed studies are needed to probe the biology and physiology in depth.

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