Mayfly emergence production and body length response to hydrology in a tropical lowland stream

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Background. Hydrological impacts on aquatic biota have been assessed in numerous empirical studies. Aquatic insects are severely affected by population declines and consequent diversity loss. However, many uncertainties remain regarding the effects of hydrology on insect production and the consequences of energy transfer to the terrestrial ecosystem. Likewise, sublethal effects on insect morphology remain poorly quantified in highly variable environments. Here, we characterized monthly fluctuation in benthic and emerged biomass of Ephemeroptera in a tropical lowland stream. We quantified the proportion of mayfly production that emerges into the riparian forest. We also examined the potential morphological changes in *Farrodes caribbianus* (the most abundant mayfly in our samples) due to environmental stress.

Methods. We collected mayflies (nymphs and adults) in a first-order stream in Costa Rica. We compared benthic and adult biomass from two years' worth of samples, collected with a core sampler ($0.006m^2$) and a $2m^2$-emergence trap. The relationship between emergence and annual secondary production (E/P) was used to estimate the Ephemeroptera production that emerged as adults. A model selection approach was used to determine the relationship between environmental variables that were collected monthly and the emergent biomass. To determine potential departures from perfect bilateral symmetry, we evaluated the symmetry of two morphological traits (forceps and forewing) of *F. caribbianus* adults. We used Spearman's rank correlation coefficients (ρ) to examine potential changes in adult body length as a possible response to environmental stress.

Results. Benthic biomass was variable, with peaks throughout the study period. However, peaks in benthic biomass did not lead to increases in mayfly emergence, which remained stable over time. Relatively constant mayfly emergence suggests that they are aseasonal in tropical lowland streams. Our E/P estimate indicated that approximately 39% and 20% (for 2002 and 2003, respectively) of the nymph production emerged as adults. Our estimated proportion of mayfly production transferred to terrestrial ecosystems is high relative to reports from temperate regions. We observed a strong negative response of *F. caribbianus* body length to increased hydrology (Spearman: $\rho = -0.51$, $p < 0.001$), while slight departures from perfect symmetry were observed in all traits.

Conclusion. Our 2yr study demonstrates that there was large temporal variability in mayfly biomass that was unrelated to hydrological fluctuations, but potentially related to trophic interactions (e.g., fish predation). Body length was a good indicator of environmental stress, which could have severe associated costs for mayfly fitness in ecosystems with high temporal variation. Our results highlight the complex ecological and evolutionary dynamics of tropical aquatic insects, and the intricate connection between aquatic and terrestrial ecosystems.

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15   **Abstract**

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17   studies. Aquatic insects are severely affected by population declines and consequent diversity

18   loss. However, many uncertainties remain regarding the effects of hydrology on insect

19   production and the consequences of energy transfer to the terrestrial ecosystem. Likewise,

20   sublethal effects on insect morphology remain poorly quantified in highly variable environments.

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22   a tropical lowland stream. We quantified the proportion of mayfly production that emerges into

23   the riparian forest. We also examined the potential morphological changes in *Farrodes*

24   *caribbianus* (the most abundant mayfly in our samples) due to environmental stress.

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27   sampler ($0.006m^2$) and a $2m^2$-emergence trap. The relationship between emergence and annual

28   secondary production (E/P) was used to estimate the Ephemeroptera production that emerged as

29   adults. A model selection approach was used to determine the relationship between

30   environmental variables that were collected monthly and the emergent biomass. To determine

31   potential departures from perfect bilateral symmetry, we evaluated the symmetry of two

32   morphological traits (forceps and forewing) of *F. caribbianus* adults. We used Spearman's rank

33   correlation coefficients (ρ) to examine potential changes in adult body length as a possible

34   response to environmental stress.

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36   in benthic biomass did not lead to increases in mayfly emergence, which remained stable over

37   time. Relatively constant mayfly emergence suggests that they are aseasonal in tropical lowland

38   streams. Our E/P estimate indicated that approximately 39% and 20% (for 2002 and 2003,

39   respectively) of the nymph production emerged as adults. Our estimated proportion of mayfly

40   production transferred to terrestrial ecosystems is high relative to reports from temperate regions.

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43   all traits.

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45   biomass that was unrelated to hydrological fluctuations, but potentially related to trophic

46   interactions (e.g., fish predation). Body length was a good indicator of environmental stress,

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48   variation. Our results highlight the complex ecological and evolutionary dynamics of tropical

49   aquatic insects, and the intricate connection between aquatic and terrestrial ecosystems.

50

51   **Introduction**

52   Identifying factors that drive changes in natural communities has been a key issue in

53   ecology, because it allows us to understand current patterns and to predict community responses

54   to environmental change (Power et al., 1988; Resh et al., 1988). In freshwater ecosystems, much

55   attention is given to understand the impact of environmental variables on aquatic organisms at

56   multiple levels, from individuals to the community (e.g., Ardón et al., 2013; Klem & Gutiérrez-

57   Fonseca, 2017). Among a wide range of factors, hydrology has often been reported as most

58   prominent affecting aquatic biota (Ramírez & Pringle, 1998; Ríos-Touma, Encalada & Prat

59   Fornells, 2011). Thus, while it is well known that hydrology reduces populations by catastrophic

60   mortality, channel scouring, and resource redistribution; we know less about sublethal stresses

61   that elicit escape (e.g., early emergence) or generate morphological changes.

62   Insect emergence can be used as a reliable indicator of population success as it reflects

63   the influence of multiple environmental stressors that populations face during their larval

64   development. Temporal patterns of insect emergence are often synchronized and occur during a

65 limited period of time (e.g., Castro-Rebolledo & Donato-Rondon, 2015). However, hydrology

66 may strongly affect seasonality, magnitude, and timing of emergence (Whiles & Goldowitz,

67 2001; Lytle, 2002). This can have negative effects in aquatic-terrestrial energy fluxes, since

68 emerged aquatic insects provide significant subsidies for riparian food webs (Nakano &

69 Murakami, 2001). Thus, although emergent biomass may be a small fraction of benthic biomass

70 (Statzner & Resh, 1993), changes in the number and morphology (e.g., body length) of

71 individuals can have a large impact on the biomass and nutrient export to adjacent terrestrial

72 ecosystem (Small et al., 2013a; Kelly, Cuevas & Ramírez, 2015).

73   Individual body length and deviation from perfect bilateral symmetry are particularly

74 useful as measures of developmental stability (i.e., ability of an individual to adequately buffer

75 its developmental processes against environmental and genetic perturbations and ensure common

76 developmental outcomes under particular ecological and evolutionary conditions).

77 Environmental stressors affect body length by affecting growth or development rates, which

78 carries significant consequences for individual fitness and alters mortality rates and reproductive

79 success (Peckarsky et al., 2001; Dahl & Peckarsky, 2003). Bilateral symmetry is known for

80 many traits, and departure from symmetrical phenotypes has been linked to low success of

81 individuals in sexual competition (Møller, 1990; Santos, 2001; Jorge & Lomônaco, 2011).

82 Departures from symmetry are commonly grouped into three categories, based on frequency

83 distributions of the differences between the right and left sides of a structure: a) directional

84 asymmetry (i.e., greater development of a character on one side), b) antisymmetry (i.e.,

85 asymmetry without directional bias), and c) fluctuating asymmetry (i.e., random departure from

86 perfect symmetry of any bilateral anatomical character, showing a normal distribution with a

87 mean of zero). The first two cases of asymmetry are related to heritable variation for asymmetry,

88 while fluctuating asymmetry is commonly caused by environmental disturbance (Palmer &

89 Strobeck, 1986, 1992).

90   Tropical streams are highly variable in their environmental parameters, which has an

91 influence on aquatic biota (Jacobsen & Encalada, 1998; Ramírez, Pringle & Douglas, 2006).

92 Streams draining humid tropical rainforests often experience unpredictable hydrological events,

93 which may represent sources of stress to aquatic populations. This is especially true for tropical

94 mayflies, which may live relatively long-periods in the streams (range from 26 to 165 days,

95 Sweeney, Jackson, & Funk, 1995) compared to their lifespan as adults (range from 3 to 6 days,

96   Vásquez, Flowers, & Springer, 2009). Streams at La Selva Biological Station (LSBS) offer an

97   excellent opportunity to assess how environmental stressors influence aquatic biota, as they show

98   high interannual variability in their environmental variables (Ramírez, Pringle & Douglas, 2006;

99   Small et al., 2012; Gutiérrez-Fonseca, Ramírez & Pringle, 2018).

100   In this study, we examine the benthic and emerged biomass of Ephemeroptera in a small

101   tropical lowland stream. We quantified the biomass transfer to the terrestrial ecosystem by adult

102   emergence production (i.e., E/P ratio). We also assess whether environmental variability can

103   influence mayfly morphology (i.e., symmetry and body length). We approach our objectives in

104   four ways: first, we used data collected during two years (2002-2003) to determine temporal

105   patterns of emerged Ephemeroptera and the benthic standing-stock biomass. Then, we estimated

106   the E/P using adult biomass and nymph secondary production for each year. Second, we

107   identified which environmental variables are related to the emergence patterns of mayflies.

108   Third, we examined departures from perfect symmetry in adults of the Leptophlebiidae *Farrodes*

109   *caribbianus* (Traver) comb. nov. (Domínguez, 1999), the most abundant Ephemeroptera found in

110   our emergence trap. Fourth, we assessed potential changes in body length of *F. caribbianus* and

111   how they relate to the variation in rainfall. We focus on precipitation as a key factor impacting

112   macroinvertebrates, since previous studies have demonstrated their influence on LSBS streams

113   (Ramírez, Pringle & Douglas, 2006; Gutiérrez-Fonseca, Ramírez & Pringle, 2018). We

114   hypothesized large mayfly mortality due to drag forces experienced by individuals during floods,

115   as well as an increase in nymph development instability due to exposure to environmental

116   stressors during their lifespan. We expected to observe a peak in benthic and emergent biomass

117   during the low rainfall season, as well as changes in adult symmetry and body length.

118

119   **Materials & Methods**

120   **Study system**

121   This study was conducted at La Selva Biological Station (LSBS) (10º26' N, 84º01' W), a

122   1563 ha reserve in the Caribbean slope of Costa Rica, located in a gradient break between the

123   Cordillera Central and the coastal plain. The forest in LSBS is composed of mature and

124   secondary tropical rainforest (Holdridge, 1967). Long-term average annual precipitation (1963-

125   2016) is 4354 mm, ranging from 2809 mm in 1995 to 6165 mm in 1970 (available at

126   http://www.ots.ac.cr/meteoro/). The annual distribution is bimodal, with peaks of >400 mm/mo

127   occurring both in June-July and November-December. The period with low rainfall values is

128   February-April (Sanford et al., 1994).

129   We collected the Ephemeroptera samples (nymphs and adults) from Carapa, a first order

130   stream (1 m wide and 0.25 m deep) bordered with abundant riparian vegetation (canopy cover:

131   85%, Small et al., 2013b). We obtained the samples from an approximately 100 m reach, which

132   was representative of overall stream conditions. Within the study reach, channel substrate type

133   was consistently dominated by detritus and fine sediments (i.e., silt and clay). Long-term data

134   sets (1997-2011) show that discharge ranges from 0.011 to 0.027 m^3/s, stream temperature from

135   21.4 to 27.2 ºC and pH from 3.62 to 6.46, with low values occurring during the El Niño event of

136   1997-1998 (Small et al., 2012; Gutiérrez-Fonseca, Ramírez & Pringle, 2018).

137   Benthic macroinvertebrate assemblages in Carapa are diverse, and include several species

138   of dipterans, mayflies, caddisflies, odonates, beetles, and non-insects. Diptera dominates the

139   taxonomic richness, abundance, and biomass of insects. Odonata, Trichoptera, and

140   Ephemeroptera are also numerically important groups (Ramírez, Pringle & Douglas, 2006;

141   Gutiérrez-Fonseca, Ramírez & Pringle, 2018). Fish assemblages are dominated by the

142   insectivorous fish *Priapicthys annectens* (98% of abundance, Family Poeciliidae) (Small et al.,

143   2013b).

144

145   **Nymph biomass and production**

146   We used data of mayfly benthic standing-stock biomass from Gutiérrez-Fonseca,

147   Ramírez and Pringle (2018). Ephemeroptera nymphs were sampled monthly for two years (2002-

148   2003). Three core samples (0.006 m^2 each) were collected in runs with leaves as the dominant

149   substrate. All material enclosed into the core was removed to a depth of ~10 cm or until reaching

150   bedrock. Mayfly nymphs were removed from organic matter and preserved in 80% ethanol.

151   Biomass of individual nymphs was estimated by applying the length-mass relationship

152   developed by Benke *et al.* (1999), expressed as ash-free dry mass (AFDM) per area (m^2).

153   We were unable to calculate directly the annual secondary production (P) using samples

154   collected in our study, as we did not find nymphs of all size classes on all sampling dates.

155   Instead, we estimated P using the production-to-biomass (P/B) method (Benke, 1993). An

156   average P/B value for Ephemeroptera at La Selva (13.63 y^{-1}) was obtained from Ramírez &

157   Pringle (1998), and biomass (B) was from our benthic sampling. While this is an indirect

158   method, it should provide us with the best approximation given our limited information on

159   tropical Ephemeroptera.

160

161   **Adult emergence and biomass**

162   We used a 2m² (sampling area) emergence trap (BioQuip Products, Rancho Dominguez,

163   California) to sample mayfly adults continuously from July 2001 to February 2004. The trap was

164   suspended over the stream and covered the entire stream width, which allowed us to sample in

165   various microhabitats such as riffles, pools, runs and stream banks. Emerging insects were

166   collected weekly and preserved in 80% ethanol for subsequent taxonomic identification. A

167   modified handheld vacuum was used to remove emergent insects from the trap. The trap was

168   inspected often for maintenance (i.e., repair of holes and removal of spider webs). Mayfly

169   biomass was calculated by measuring the length of each individual and applying the length-mass

170   relationship developed by Sabo *et al.* (2002). Emergence biomass was expressed as mg AFDM

171   m² by taking the total biomass of each month adjusted by the trap area. Total annual biomass was

172   determined by adding all weekly samples for each year.

173

174   **Physicochemical variables and hydrology**

175   We measured eight physicochemical variables monthly, simultaneously with

176   Ephemeroptera collections. Nutrient concentrations (i.e., NO_3^--N, NH_4^+-N and $PO_4^{3-}-P$ as

177   soluble reactive P: SRP) were measured by collecting 2 filtered (0.45 μm Millipore filters) water

178   samples using new 125 mL bottles. Samples were kept frozen until analyzed. NO_3^--N, NH_4^+-N,

179   and SRP concentrations were measured using continuous-flow colorimetry and an Alpkem RFA

180   300 colorimetric analyzer. We used cadmium-reduction, phenol-hypochlorite, and ascorbic acid

181   methods for NO_3^--N, NH_4^+-N, and SRP, respectively (APHA, 1998). We measured stream

182   temperature, pH, and conductivity *in situ* with a handheld meter (Hanna Instruments,

183   Woonsocket, Rhode Island). The stream flow was measured with a Marsh–McBirney current

184   meter (Marsh McBirney Inc., Frederick, Maryland), and discharge was calculated using the

185   velocity–area method (Gordon et al., 2004). Monthly precipitation was recorded using data from

186   the meteorological station available at LSBS (OTS meteorological data,

187   http://www.ots.ac.cr/meteoro/).

188

Measurement of traits and body length of *F. caribbianus*

189

190   We measured the body length (not including cerci) of all individual mayflies to quantify

191   size variation patterns. To determine departures from symmetry, we measured the length of the

192   second segment of forceps (SF) on the right and left sides of males, as well as forewing area

193   (AFW) and forewing length (LFW) on the right and left sides of both males and females. Body

194   parts were removed with forceps, mounted on glass slides, and photographed with a

195   stereomicroscope (AmScope) and a microscope (Nikon Eclipse E400). Images were analyzed

196   with the free computer software ImageTool 2.0 (University of Texas Health Science Center, San

197   Antonio). All linear and area measurements were done with an accuracy of 0.01 mm and 0.01

198   mm^2, respectively. To avoid mistaking human error with potential asymmetries, we used one

199   image per individual to take three non-consecutive (i.e., in a random order) measurement of each

200   trait.

201

202   **Data analyses**

203   We used biomass to compare temporal patterns between benthic and emerging mayflies,

204   since biomass better reflects potential changes in production driven by environmental variability,

205   beyond other metrics such as abundance and diversity (Malison, Benjamin & Baxter, 2010).

206   Likewise, biomass estimates are widely used in analyses of food webs and secondary production.

207   To estimate the fraction of Ephemeroptera production that emerged as an adult and was exported

208   to riparian ecosystems, we calculated the ratio of adult emergence to nymph secondary

209   production (E/P) for each year.

210   A model selection approach based on Akaike's Information Criteria (AIC, Akaike, 1973)

211   was used to identify the best-fit model that included the environmental variables influencing

212   adult mayfly biomass. Model constructions and selections are described in more detail in

213   Gutiérrez-Fonseca, Ramírez and Pringle (2018). Briefly, we build linear regression models using

214   a forward selection procedure. We identified the model with the best quality (i.e., with a

215   minimum number of required explanatory variables) based on the lowest AICc (<2Δ). Model

216   averaging was used to draw conclusions when more than one model was included in the subset.

217   Multicollinearity among variables was assessed by calculating the variance inflation factor

218   (VIF). Environmental variables with a VIF>10 were identified and removed from the analysis

219   (O'Brien, 2007). Before building the models, we also excluded variables that were highly

220   correlated (|r| >0.60).

221   To examine the association between body length and hydrology, we first related monthly

222   average precipitation to discharge using Pearson correlation coefficients. Then, we determined

223   the relationship between body length and average precipitation in the 159 days before collecting

224   the emergence trap using non-parametric Spearman's rank correlation coefficients, due to the

225   non-normal distribution of the data. Spearman's rank correlations were calculated separately for

226   males (n=67), females (n=50) and both sexes combined (n=117). We used average precipitation

227   of the 159 days, as this timeframe coincides with the life cycle of *Thraulodes* sp. (same family,

228   Leptophlebiidae), which was estimated as the median days since an egg hatches until the

229   individual reaches adulthood (Jackson & Sweeney, 1995).

230   Analyses of potential departure from symmetry were performed on approximately 30

231   randomly selected individuals for each test, following the procedure recommended by Palmer

232   and Strobeck (1986, 2003) and Palmer (1994). We used the signed differences between right and

233   left (R−L) to distinguish fluctuating asymmetry (must be normally distributed with zero mean)

234   from antisymmetry and directional symmetry. The Shapiro-Wilks test was used to determine

235   whether the data were normally distributed, which would rule out antisymmetry. Directional

236   asymmetry is characterized by a normal distribution with a mean other than zero. Therefore, we

237   also conducted one-sample *t*-tests to determine if the mean of signed (R − L) differed statistically

238   from zero.

239   Analysis of potential fluctuating asymmetries were conducted for each trait using three

240   indices recommended by Palmer and Strobeck (2003): FA1, FA4a, and FA10a. FA1, calculated

241   as mean of |R-L|, is the recommended index because it is easy to understand. FA4a (0.798

242   $\sqrt{var(R-L)}$) has higher statistical power and represents the contribution of FA measurement error.

243   The FA10 index describes the average difference between sides after measurement error has

244   been partitioned out. This index was calculated using two-way mixed model ANOVAs with side

245   (Fixed), individual (Random) and their interaction (Side x Individual). Then, we used the mean

246   square (ME) to calculate FA10 as $0.798 \sqrt{2\sigma_i^2}$ (where $\sigma_i^2 = ME_{SxI} - ME_{error}$). Additionally, we

247   calculated measurement error as a percentage (ME3, according to Palmer & Strobeck, 2003) by

248   $ME_{error} / ME_{SxI} \times 100$. ME3 represents the mean difference between replicate measurements as a

249   proportion of mean difference between of the sides x individual interaction.

250   Pearson (r) and Spearman's rank (ρ) correlations were calculated with the *cor.test()*

251   function of the *stats* package, the two-way mixed model ANOVA with the *lmer()* function of the

252   *lme4* package (Bates et al., 2015), model averaging with the *AICcmodavg* package (Mazerolle,

253   2019) and graphics were produced using the *ggplot2* package (Wickham, 2016) in R version

254   3.6.3 (R Core Team, 2019). Raw data and code used in this study are available on a GitHub

255   repository: https://github.com/PEGutierrezF/mayfly_morphometry.

256

257   **Results**

258   **Precipitation and hydrology**

259   Monthly total precipitation showed a strong seasonal pattern during the study period.

260   The dry season had the lowest precipitations in March 2002 (140.8 mm) and April 2003 (45.3

261   mm). The wet season had maximum values in May 2002 (890.8 mm) and December 2003

262   (561.3 mm) (Fig. 1A). Discharge reflected the monthly average precipitation variability in the

263   two years of sampling (Pearson: r = 0.64, *p*< 0.001, Fig. 1B).

264

265   **Figure 1.** Temporal variability of precipitation in 2002 and 2003 (A), and relationship between

266   mean monthly precipitation and discharge (B). Grey shaded area represents the 95% confidence

267   intervals.

268

269   **Benthic and emergence biomass, and E/P patterns**

270   Mean annual biomass of nymphs was 1.74 mg AFDM/m^2 in 2002 and 2.22 mg

271   AFDM/m^2 in 2003. Benthic biomass peaked in March, July, and September 2002, and January,

272   and October 2003 (Fig. 2). Meanwhile, mean annual biomass of emerging adults was 0.76 mg

273   AFDM/m^2 in 2002 and 0.50 mg AFDM/m^2 in 2003. Monthly mayfly emergence was relatively

274   constant throughout the study periods compared to benthic biomass, except for a slight increase

275   in June 2002 (Fig. 2A). Total emergence was 9.14 mg AFDM m^{-2} y^{-1} and 5.99 mg AFDM m^{-2} y^{-}

276   ^{1}, while annual secondary production was 23.70 mg AFDM m^{-2} y^{-1} and 30.28 mg AFDM m^{-2} y^{-1}

277   for 2002 and 2003, respectively. Therefore, we estimated that total emergence production

278   represented 38.57% and 19.78% of nymph secondary production for 2002 and 2003,

279   respectively.

280

281   **Figure 2.** Temporal variability of benthic and emerging adult biomass during (A) 2002 and (B)

282   2003.

283   **Physicochemical characteristics and individual-level variation**

284   The AIC analysis used to determine the relative importance of environmental variables

285   on the biomass of emerging adults showed no support for any model. Therefore, none of the

286   variables related to nutrients, hydrology or physicochemistry that were evaluated in our study

287   explained the relatively constant patterns of mayfly emergence in our streams.

288   Body length of *F. caribbianus* was strongly influenced by average rainfall during the 159

289   days of nymph development. Spearman's rank correlations revealed a negative relationship

290   between average rainfall and body length of males (Spearman: $\rho = -0.45$, $p < 0.001$), females

291   (Spearman: $\rho = -0.64$, $p < 0.001$), and both sexes combined (Spearman: $\rho = -0.51$, $p < 0.001$, Fig.

292   3).

293

294   **Figure 3. Body length variability of *F. caribbianus* and precipitation.**

295   Relationship between *F. caribbianus* body length and average precipitation in the 159 days prior

296   to the sampling date. Each point represents an individual, including males and females. Gray

297   shaded area represents the 95% confidence intervals.

298

299   **Fluctuating asymmetry analysis in *F. caribbianus***

300   A total of 117 *F. caribbianus* (50 females and 67 males) were assessed (in groups of ~30

301   randomly selected individuals for each test) to determine departure from symmetry. Analyses of

302   trait value distribution satisfied the assumption of normality, so there was no evidence of

303   antisymmetry in any of the characters ($p > 0.05$, Table 1). Also, the *t*-tests revealed that mean (R-

304   L) was not significantly different from zero, which suggests that there was no directional

305   asymmetry ($p > 0.05$, Table 1).

306

307   **Table 1.** Test for normality and *t*-test for each trait. AFW: forewing area, LFW: forewing length;

308   SF: length of the second segment of forceps.

309

310   Two-way mixed model ANOVAs (Table 2) showed that the side x individual interaction

311   was always significant, while global error (ME_{error}) was found to be smaller than the error of the

312   interaction (ME_{SxI}) for all traits. Measurement error (ME3: $M_{error} / ME_{SxI} \times 100$) contributed 5.9–

313 19.6% of the total variance between-sides for each trait, which indicates that ~80% of the

314 measurement variation was reliable. Fluctuating asymmetries for each trait amounted to roughly

315 1.07% - 2.67% of the average size of the corresponding trait (FA1/mean). The estimates for

316 FA4a and FA10a varied from 0.013 to 0.088 and from 0.002 to 0.034, respectively.

317

318 **Table 2.** Result of the two-way mixed model ANOVAs performed for each trait with Side as a

319 Fixed component and Individual as Random component.

320 Indices of asymmetry: FA1, FA4a, FA10a and measurement error as percentage (ME3 as % of

321 ME$_{SxI}$). AFW: forewing area, LFW: forewing length, SF: length of the second segment of

322 forceps.

323 *p<0.05; [a] mean of the standard deviation of triplicate measurements on the right and left sides

324 (i.e., indicator of accuracy during photo measurement); [b] mean of the right and left side

325 measurements; [C] mean squared of the side × individual interaction; [d] mean squared of the

326 variance of the repeated measurements.

327

328 **Discussion**

329   Our two-year study showed high temporal variability of mayfly benthic biomass,

330 characterized by multiple peaks during the study period. Unexpectedly, these peaks in benthic

331 biomass did not translate into measurable increases in emerging adult biomass, which

332 represented a small fraction of the benthic biomass. Notably, while benthic and emergent

333 biomass were similar during most of the study period, the observed peaks in nymph biomass

334 were not associated to similar peaks in the emergence of adults. Peaks in benthic biomass

335 occurred in different periods of the year, which may suggest that different mechanisms control

336 population dynamics of benthic and emerging mayflies. E/P ratio varied between 38.57% in

337 2002 and 19.78% in 2003, and was higher than reports in previous studies for emerging aquatic

338 insects in temperate regions. We did not observe a relationship between hydrology and the

339 biomass of benthic and emergent mayflies, so our expectation that mayfly biomass would peak

340 in the dry season of La Selva was not supported by our findings. Looking more closely at *F.*

341 *caribbianus*, the most abundant mayfly collected in emergence traps, we found no evidence of

342 antisymmetry or directional symmetry, but we did find slight deviations from symmetry that

343 were larger than measurement errors, suggesting alterations in bilateral morphology of *F.*

344 *caribbianus*. We also found a strong negative relationship between body length and precipitation

345 variability at La Selva. This relationship was consistently significant for males, females, and both

346 sexes combined.

347   Our E/P estimation indicated that less than 40% (38.57% and 19.78% for 2002 and 2003,

348 respectively) of nymph secondary production was exported to the terrestrial ecosystem. This

349 potentially low value may have negative consequences on the riparian food web, as emerging

350 insects represent an important source of energy and nutrients in La Selva streams (N-flux: 0.40-

351 1.25 mg N m^{-2} d^{-1}, Small, Duff, et al. 2013). Surprisingly, our E/P estimates were equal to, or

352 exceeded, the values previously reported for single groups of aquatic insects, such as 19.3% for

353 *Hydropsyche angustipennis* and 27% for *H. pellucidula* (Trichoptera) in a Northern German

354 lowland stream (Poepperl, 2000a), and 16% for a limnephilid (Trichoptera) in an intermittent

355 wetland (Whiles, Goldowitz & Charlton, 1999). Our E/P estimates were also high when

356 compared to entire insect assemblages, such as 16.6% in desert streams (Jackson & Fisher, 1986)

357 and 18.3% in a northern German stream (Poepperl, 2000b). Our E/P estimates even exceed the

358 biomass export of aquatic vertebrates, such as salamanders (10%, Regester, Lips & Whiles,

359 2006). These elevated E/P ratios may be explained by the warm water temperatures (range: 21.4

360 to 27.2 °C, Gutiérrez-Fonseca, Ramírez & Pringle, 2018), and high biomass turnover rates of

361 fast-growing mayflies (P/B= 13.63, Ramírez & Pringle, 1998) that are characteristic of many

362 tropical streams.

363   Unlike the large fluctuations in emergence patterns observed in other studies (Masteller,

364 1993; Pescador, Masteller & Buzby, 1993; Castro-Rebolledo & Donato-Rondon, 2015; Yuen &

365 Dudgeon, 2016), providing support for seasonality in many tropical aquatic insects, the lack of

366 abrupt peaks in mayfly emergence found in our study suggests that mayfly emergence is

367 aseasonal. Fish predation on newly emerged adults may be a possible explanation for the

368 constant biomass of emerging mayflies in our study. Our focal stream is inhabited by the

369 insectivorous poeciliid, *P. annectens,* which is abundant (4-14 individuals/m^2, Small et al. 2013)

370 and could have negatively affected mayfly emergence. Previous studies have shown a reduction

371 of total emergence biomass of aquatic insects by 62 ± 8% (Merkley, Rader & Schaalje, 2015)

372 and 65% (Warmbold & Wesner, 2018) in mesocosms with a similar fish density (11.4 fish/m^2

373 and 7.8 fish/m^2, respectively) to our study stream. Field studies have also found that fish can

374 regulate the timing and duration of aquatic insect emergence (Moore & Schindler, 2010).

375   Therefore, it is not surprising for biotic control to be an important factor controlling insect

376   emergence in our tropical lowland streams, which harbor high fish diversity.

377   Hydrology has been recognized as a key factor controlling macroinvertebrate

378   assemblages in tropical streams (Flecker & Feifarek, 1994; Ramírez & Pringle, 1998; Molineri,

379   2010), with peaks in biomass expected during the dry season and high mortality during the rainy

380   season. However, we observed peaks in benthic biomass during both the dry and rainy seasons

381   in La Selva. Peaks during the rainy season might be caused by microdistributional changes in

382   macroinvertebrates driven by high floods (Lancaster & Hildrew, 1993; Lancaster, 1999). For

383   instance, mayflies have been observed to make small-scale refuge-seeking movements between

384   substrate layers during simulated floods (Holomuzki & Biggs, 2000). This type of mechanisms

385   could allow for high mayfly survival in streams such as Carapa, where the deep subsurface layers

386   may provide shelter and protection for insects year round, as proposed for similar streams

387   (Holomuzki & Biggs, 2000).

388   Our results show a strong influence of precipitation on the total body length of *F.*

389   *caribbianus*. Large individuals were negatively affected by precipitation, while small-sized

390   mayflies persisted during high rainfall events (Fig. 3). In tropical streams, two mechanisms have

391   been identified as potential ways in which insects respond to catastrophic floods. The first

392   mechanism proposes that small-sized individuals have better chances of surviving floods by

393   finding refuge in interstitial spaces (Townsend, Dolédec & Scarsbrook, 1997; Segura, Siqueira &

394   Fonseca-Gessner, 2013). The second hypothesis suggests that some aquatic insects (e.g.,

395   Odonata: Polythoridae) emerge, copulate and oviposit at the onset of the rainy season, so that

396   only small larvae (which can be protected by large logs and rocks) are present during periods of

397   frequent floods (Pritchard, 1996). Since mayflies in La Selva are multivoltine (Ramírez &

398   Pringle, 1998), we find more support for the first hypothesis through this study.

399   Asymmetrical traits have been used successfully as an early warning biomarker related to

400   developmental stress (Graham et al., 2010). Common stress sources that cause fluctuating

401   asymmetries in aquatic insects include water quality (Bonada & Williams, 2002), insecticide

402   (Mpho, Holloway & Callaghan, 2001), experimental food deprivation (Nosil & Reimchen, 2007)

403   and changes in the physical structure of riparian vegetation (Pinto et al., 2012). We found slight

404   variations from perfect symmetry in the wings and forceps of *F. caribbianus*, which suggests

405   some level of developmental instability of the nymph. However, given the limited number of

406 mayflies per period, we were unable to perform analyses under different levels of environmental

407 stressors (e.g., low, medium, and high hydrology). Therefore, although we found slight random

408 deviations from symmetry, they are not distinguishable from developmental noise (i.e., random

409 variation from symmetry caused by metabolic rates, concentrations of regulatory molecules,

410 diffusion, or thermal noise) without further study (Palmer & Strobeck, 2003).

411   Future climate change scenarios predict an increase in hydrological extreme events for

412 many regions (Christensen et al., 2013). Extreme precipitation events are expected to increase in

413 tropical regions (O'Gorman & Schneider, 2009), with potential negative effects on aquatic biota

414 and aquatic-terrestrial linkages. Increases in heavy precipitation events have already been

415 observed in the Caribbean slope of Costa Rica during the last decades (Aguilar et al., 2005; Rapp

416 et al., 2014; Sánchez-Murillo et al., 2017), where climate projections suggest an increase in mean

417 annual precipitation of between 10 to 50% (Alvarado et al., 2012). Therefore, large hydrological

418 variability can threaten the fitness of mayfly populations in La Selva, as well as in other tropical

419 regions.

420

421 **Conclusions**

422 Contrary to our expectations and patterns shown in literature, we found a lack of seasonality in

423 benthic biomass. Adult biomass was unrelated to peaks in benthic biomass, which makes us

424 wonder what is controlling adult biomass export in these systems if not hydrology (e.g. fish

425 predation). Based on our E/P ratios, Neotropical streams can provide larger subsidies to adjacent

426 terrestrial ecosystems than their counterparts in temperate regions. Departures from perfect

427 symmetry were evident in all the evaluated traits, which suggests developmental instability of

428 mayflies. Body length proved to be a better indicator of environmental stress, which could have

429 severe associated costs for mayfly fitness in ecosystems with high temporal variation. Further

430 research could quantify effects of body length reduction in mayfly fitness, energy and nutrient

431 export to riparian food webs, as well as the role of biotic control on mayfly biomass in tropical

432 lowland streams.

433

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440

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660

Figure 1

Temporal variability of precipitation in 2002 and 2003 (A), and relationship between mean monthly precipitation and discharge (B). Grey shaded area represents the 95% confidence intervals.

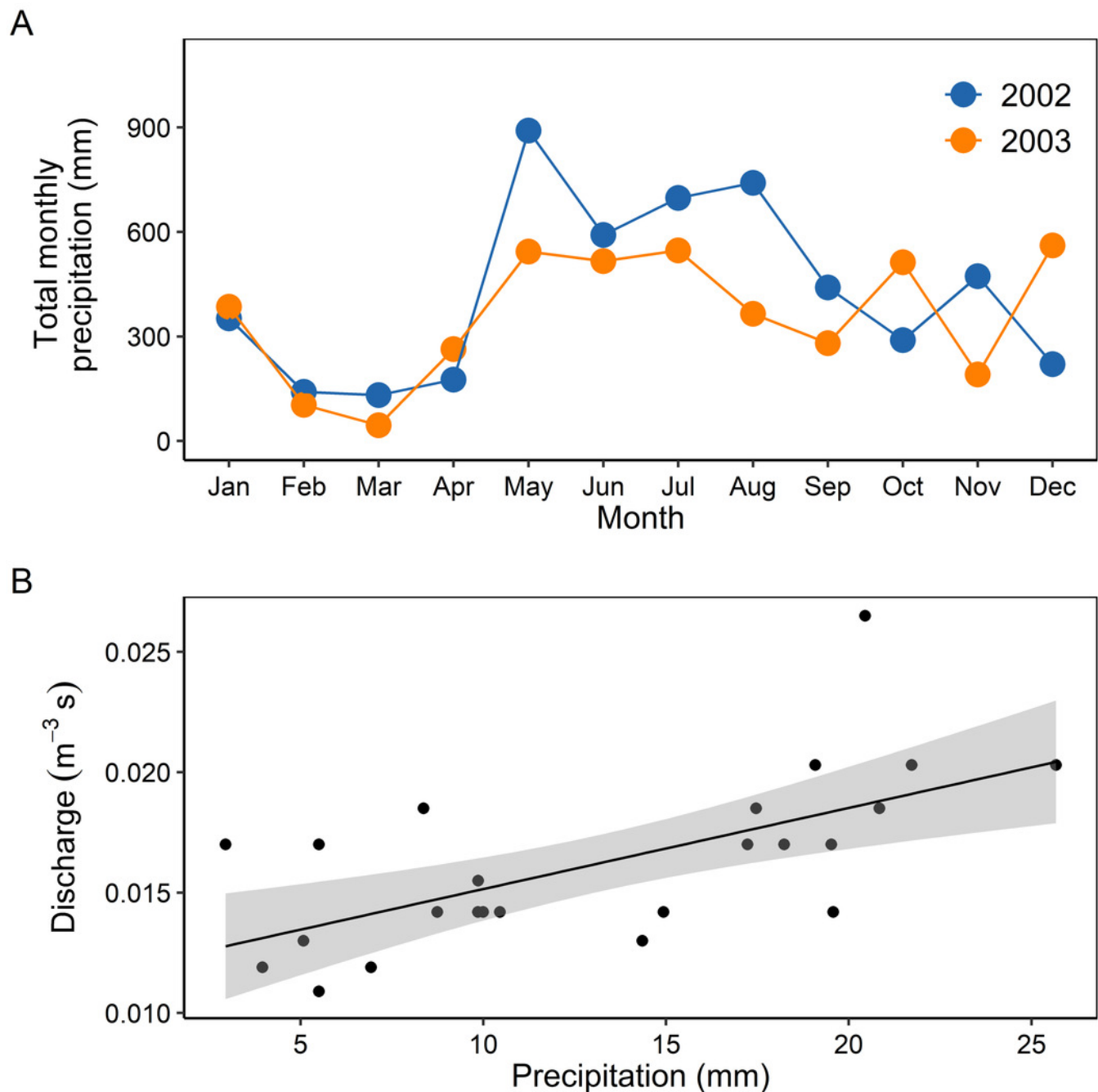



Figure 2

Temporal variability of benthic and emerging adult biomass during (A) 2002 and (B) 2003.

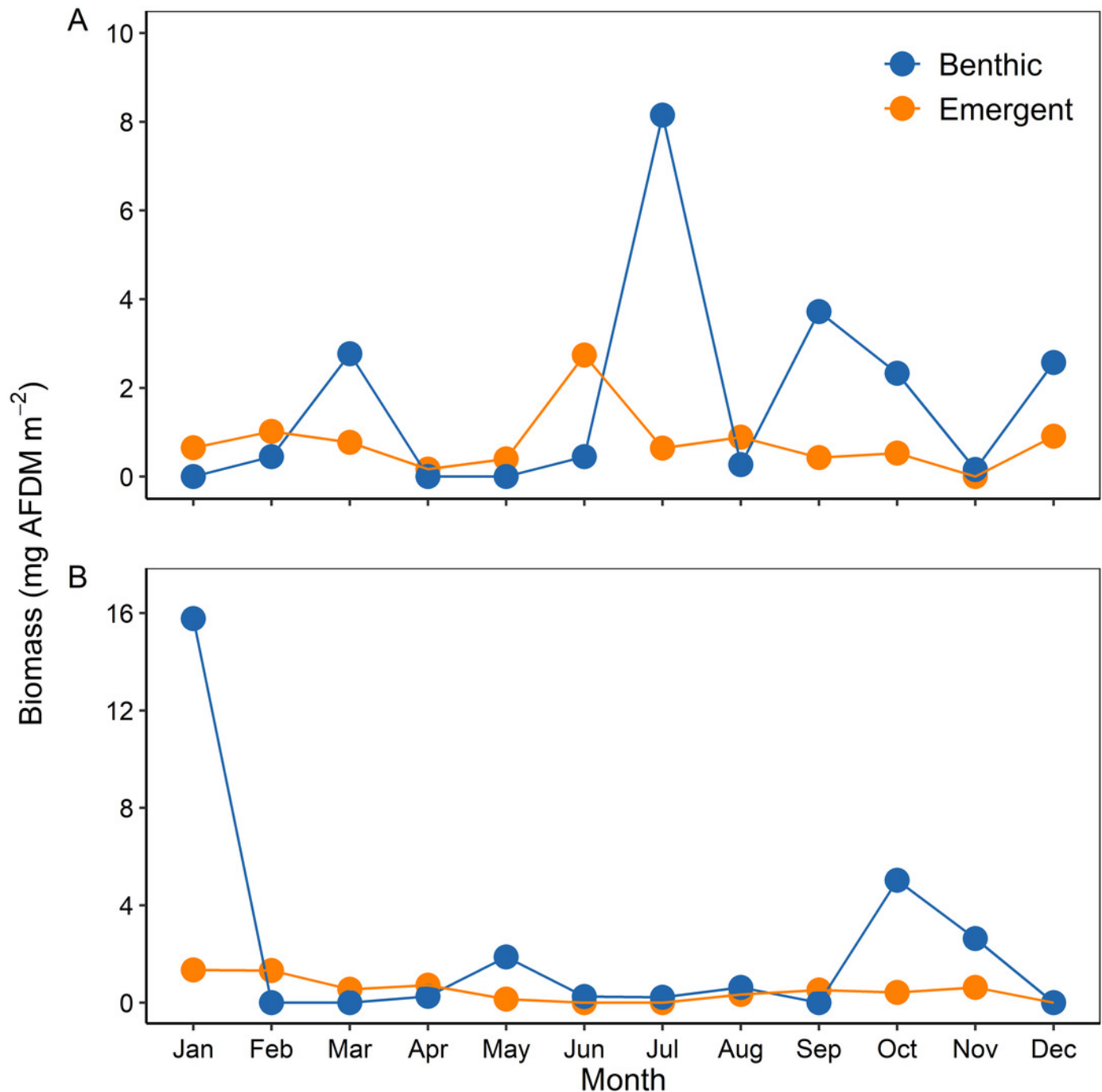
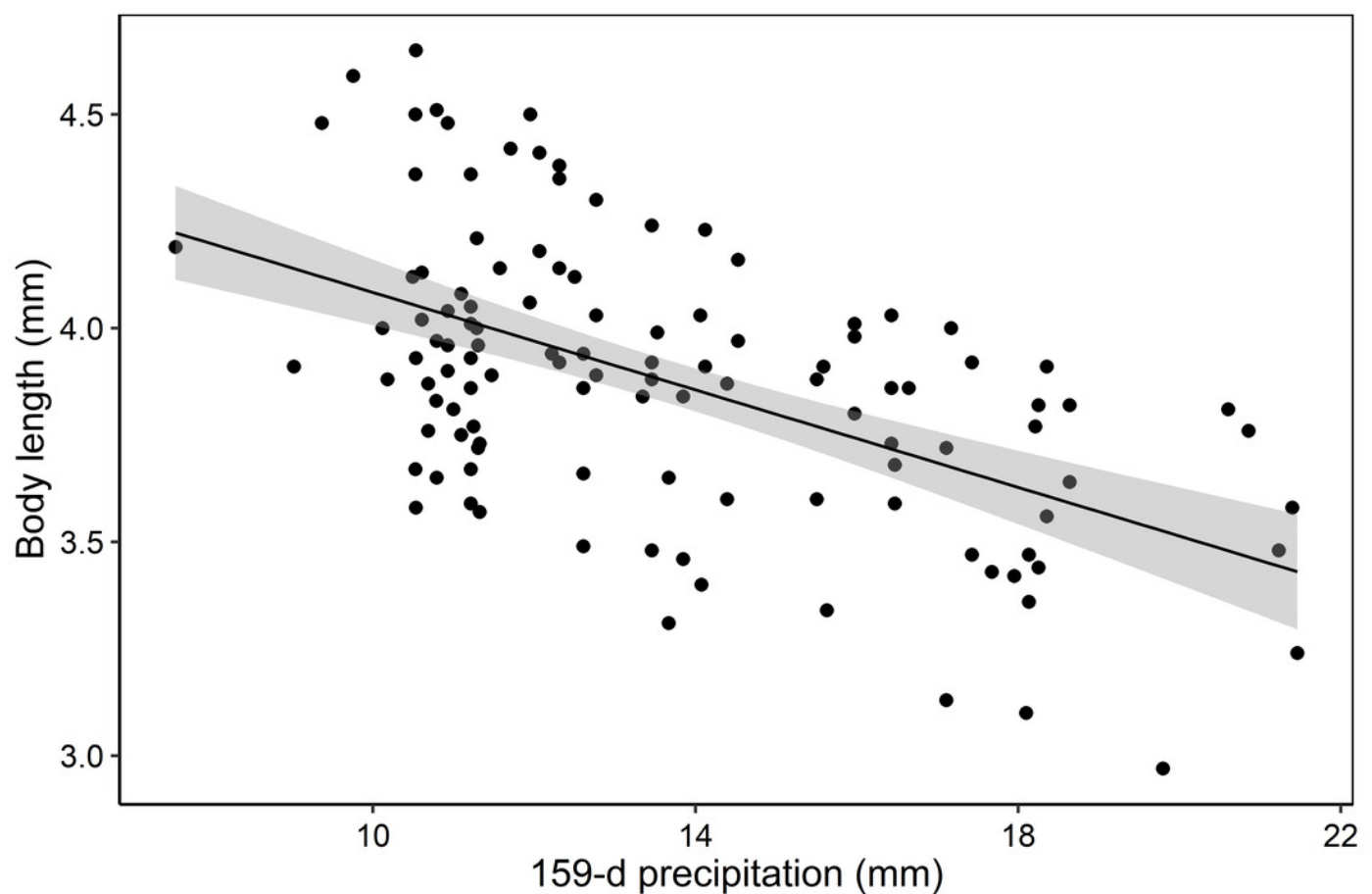



Figure 3

Body length variability of *F. caribbianus* and precipitation.

Relationship between *F. caribbianus* body length and average precipitation in the 159 days prior to the sampling date. Each point represents an individual, including males and females. Gray shaded area represents the 95% confidence intervals.

Test for normality and *t*-test for each trait. AFW: forewing area, LFW: forewing length; SF: length of the second segment of forceps.

	Trait	N	Mean (R-L)	SE	Normality test		*t*-tests	
					W	*P-Value*	*t*	*P-Value*
Male								
	AFW	31	0.00	0.082	0.975	0.685	0.065	0.984
	LFW	33	-0.013	0.083	0.969	0.473	-0.924	0.363
	SF	30	-0.004	0.016	0.955	0.235	-1.453	0.157
Female								
	AFW	28	0.028	0.077	0.981	0.862	1.958	0.061
	LFW	40	0.007	0.023	0.956	0.120	2.015	0.051

Result of the two-way mixed model ANOVAs performed for each trait with Side as a Fixed component and Individual as Random component.

Indices of asymmetry: FA1, FA4a, FA10a and measurement error as percentage (ME3 as % of ME_{SxI}). AFW: forewing area, LFW: forewing length, SF: length of the second segment of forceps.

*$p<0.05$; [a] mean of the standard deviation of triplicate measurements on the right and left sides (i.e., indicator of accuracy during photo measurement); [b] mean of the right and left side measurements; [c] mean squared of the side × individual interaction; [d] mean squared of the variance of the repeated measurements.

Trait	N	Mean error [a]	Mean (mm)[b]	Side		Individual		Side x Individual			ME_{error}[d]	FA1	FA4a	FA10a	ME3 (%)
				df	F	df	F	df	ME_{SxI}[c]	F					
Male															
AFW	31	0.03	4.41	1	18.90*	30	797.40*	30	3.98e-5	13.30*	2.99e-6	0.076	0.088	0.004	16.4
LFW	30	0.03	3.79	1	2.05	29	223.89*	29	3.33e-3	5.10*	6.52e-4	0.063	0.066	0.034	19.6
SF	30	0.006	0.45	1	10.70*	29	103.80*	29	4.25e-4	7.50*	5.67e-5	0.012	0.013	0.012	13.3
Female															
AFW	28	0.03	5.16	1	0.01	27	2163.2*	27	1.38e-5	16.9*	8.27e-7	0.065	0.062	0.002	5.9
LFW	28	0.02	4.01	1	8.66*	27	507.30*	27	2.35e-3	5.20*	4.51e-4	0.043	0.046	0.028	19.2