Hydrology influences body length, but not benthic and emergent biomass of Ephemeroptera in a tropical lowland stream

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Background. Hydrological impacts on aquatic biota have been assessed in numerous empirical studies. Macroinvertebrate assemblages are severely affected by population declines and consequent diversity loss. However, many uncertainties remain regarding the effects of hydrology on insect production (biomass) 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 also examined the potential changes in morphology (i.e., departures from perfect symmetry and body length) in *Farrodes caribbianus* (the most abundant mayfly in our samples) due to environmental stress. **Methods.** We collected mayfly samples (nymphs and adults) in a first-order stream located on the Caribbean slope of Costa Rica. We compared benthic and adult biomass from two years' worth of samples, collected with a core sampler and a 2m^2-emergence trap. We also evaluated the relationship between emergent biomass and eight environmental variables that were recorded monthly. To determine potential departures from symmetry, we evaluated five morphological traits of *F. caribbianus* adults. In addition, we examined potential changes in adult body length as a possible response to environmental stress. **Results**. Benthic biomass was variable, with peaks throughout the study period. Peaks in biomass did not lead to increases in mayfly emergence, which remained stable over time. Our gross estimate comparing benthic and emerged biomass suggests that approximately 75% of the benthic biomass remained within the stream ecosystem. Relatively constant mayfly emergence suggests that mayflies are aseasonal in tropical lowland streams. While we found no evidence of departures from symmetry for any of the measured traits of *F. caribbianus*, hydrology did negatively influence adult body length (Spearman's r_s = - 0.51, p< 0.001). Our multi-year

study demonstrates that there is large temporal variability in mayfly biomass that is unrelated to hydrological fluctuations, but potentially related to trophic interactions (e.g. fish predation). Body length, and not bilateral asymmetry, proved to be a better indicator of environmental stress, which could have severe associated costs for mayfly fitness in ecosystems with high temporal variation.

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Abstract

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Methods. We collected mayfly samples (nymphs and adults) in a first-order stream located on the Caribbean slope of Costa Rica. We compared benthic and adult biomass from two years' worth of samples, collected with a core sampler and a 2m^2-emergence trap. We also evaluated the relationship between emergent biomass and eight environmental variables that were recorded monthly. To determine potential departures from symmetry, we evaluated five morphological traits of *F. caribbianus* adults. In addition, we examined potential changes in adult body length as a possible response to environmental stress.

Results. Benthic biomass was variable, with peaks throughout the study period. Peaks in biomass did not lead to increases in mayfly emergence, which remained stable over time. Our gross estimate comparing benthic and emerged biomass suggests that approximately 75% of the benthic biomass remained within the stream ecosystem. Relatively constant mayfly emergence suggests that mayflies are aseasonal in tropical lowland streams. While we found no evidence of departures from symmetry for any of the measured traits of *F. caribbianus*, hydrology did negatively influence adult body length (Spearman's $r_s = -0.51$, $p < 0.001$). Our multi-year study demonstrates that there is large temporal variability in mayfly biomass that is unrelated to hydrological fluctuations, but potentially related to trophic interactions (e.g. fish predation). Body length, and not bilateral asymmetry, proved to be a better indicator of environmental stress, which could have severe associated costs for mayfly fitness in ecosystems with high temporal variation.

Introduction

Identifying factors that drive changes in natural communities has been a key issue in ecology, because it allows us to understand current patterns and to predict community responses to environmental change (Power et al., 1988; Resh et al., 1988). In lotic environments, much attention is given to understand the impact of environmental variables on aquatic organisms at multiple levels, from individuals to the community (e.g., Ardón et al., 2013; Klem & Gutiérrez-Fonseca, 2017). Among a wide range of factors, hydrology has often been reported as most prominent affecting aquatic biota (Flecker & Feifarek, 1994; Ramírez & Pringle, 1998). Thus, while it is well known that hydrology reduces populations by catastrophic mortality, channel scouring, and resources redistribution; we know less about sublethal stresses that elicit escape (e.g., early emergence) or generate morphological changes.

Insect emergence can be used as a reliable indicator of population success as it reflects the influence of multiple environmental stressors that populations face during their larval development. Temporal patterns of insect emergence are often synchronized and occur during a limited period of time (e.g., Castro-Rebolledo & Donato-Rondon, 2015). However, hydrology may strongly affect seasonality, magnitude, and timing of emergence (Whiles & Goldowitz, 2001; Lytle, 2002). This can have negative effects in aquatic-terrestrial energy fluxes, since emerged aquatic insects provide significant subsidies for riparian food webs (Nakano & Murakami, 2001). Thus, although emergent biomass may be a small fraction of benthic biomass (Statzner & Resh, 1993), changes in the number and morphology (e.g., body length) of individuals can have a large impact on the biomass and nutrient export to adjacent terrestrial ecosystem (Small et al., 2013a; Kelly, Cuevas & Ramírez, 2015).

In addition to individual size, deviation from perfect bilateral symmetry is particularly useful as a measure of developmental stability (i.e., a process that buffers development against environmental and genetic disturbances), because the optimal phenotype is known for many traits (Palmer & Strobeck, 1986). Departures from symmetry are commonly grouped into three categories, based on frequency distributions of the absolute value of the differences between the right and left sides of a structure: a) directional asymmetry (i.e., greater development of a character on one side), b) antisymmetry (i.e., asymmetry without directional bias), and c) fluctuating asymmetry (i.e., random departure from perfect symmetry of any bilateral anatomical character, showing a normal distribution with a mean of zero). The first two cases of asymmetry are related to genetic changes, while fluctuating asymmetry (FA) is caused by environmental stressors. Environmental stressors can also affect body length by affecting growth or development rates, which carries significant consequences for individual fitness and alter mortality rates and reproductive success (Peckarsky et al., 2001; Dahl & Peckarsky, 2003).

Tropical streams are highly variable in their environmental parameters, which has an influence on aquatic biota (Jacobsen & Encalada, 1998; Ramírez, Pringle & Douglas, 2006). Streams draining humid tropical rainforests often experience unpredictable hydrological events, which may represent sources of stress to aquatic populations. This is especially true for tropical mayflies, that may live relatively long-periods in the streams (range from 26 to 165, Sweeney, Jackson, & Funk, 1995), compare to their lifespan as adults (range from 3 to 6 days, Vásquez, Flowers, & Springer, 2009). Streams at La Selva Biological Station (LSBS) offer an excellent opportunity to assess how environmental stressors influence aquatic biota, as they show high interannual variability in their environmental variables (Ramírez, Pringle & Douglas, 2006; Small et al., 2012; Gutiérrez-Fonseca, Ramírez & Pringle, 2018).

In this study, we examine the emerged and benthic biomass of Ephemeroptera in a small tropical lowland stream. We assess whether environmental variability can influence biomass export and mayfly symmetry. We approach our objectives in four ways: first, we used data collected during two years (2002-2003) to determine temporal patterns of emerged Ephemeroptera and the benthic standing-stock biomass. Second, we identified which environmental variables are related to the emergence patterns of mayflies. Third, we examined departures in the perfect symmetry of *Farrodes caribbianus* (Traver) comb. nov. (Leptophlebiidae, Domínguez, 1999) adults, the most abundant Ephemeroptera found in emergence traps. Fourth, we assessed potential changes in body length of *F. caribbianus* and how they relate to the variation in rainfall. We focus on precipitation as a key factor impacting macroinvertebrates, since previous studies have demonstrated their influence on LSBS streams (Ramírez, Pringle & Douglas, 2006; Gutiérrez-Fonseca, Ramírez & Pringle, 2018). We hypothesized a peak in benthic and emergent biomass during the low rainfall season, due to a decrease in the risk of mortality from drag during floods. We also hypothesized that potential changes in adult symmetry and body length would reflect nymph development instability due to exposure to environmental stressors during their lifespan, and their ability to buffer environmental disturbances.

Materials & Methods

Study system

This study was conducted at La Selva Biological Station (LSBS) (10º26’ N, 84º01’ W), a 1563ha reserve in the Caribbean slope of Costa Rica, located in a gradient break between the Cordillera Central and the coastal plain. The forest in LSBS is composed of mature and secondary tropical rainforest (Holdridge, 1967). Long-term average annual precipitation (1963-2000) is 4314 mm, ranging from 2809 mm in 1995 to 6164 mm in 1970 (available at http://www.ots.ac.cr/meteoro/). The monthly distribution is bimodal, with peaks of >400 mm/mo occurring both in June-July and November-December. The period with low rainfall values is February-April (Sanford et al., 1994).

Ephemeroptera samples (nymphs and adults) were obtained from an approximately 100m reach in the Carapa stream, which is a first order stream bordered with abundant riparian vegetation. The stream channel is about 1 m wide and 0.25 m deep. Dominant benthic substrata are detritus and sediments (i.e., silt and clay). Long-term data sets (1997-2011) show that discharge ranges from 0.011 to 0.027 m^3/s, stream temperature from 21.4 to 27.2 ºC and pH from

3.62 to 6.46, with low values occurring during the El Niño event of 1997-1998 (Small et al., 2012; Gutiérrez-Fonseca, Ramírez & Pringle, 2018).

Benthic macroinvertebrate assemblages in Carapa are diverse, and include several species of dipterans, mayflies, caddisflies, odonates, beetles, and non-insects. Diptera dominates the taxonomic richness, abundance, and biomass of insects. Odonata, Trichoptera, and Ephemeroptera are also numerically important groups (Ramírez, Pringle & Douglas, 2006; Gutiérrez-Fonseca, Ramírez & Pringle, 2018).

Nymph biomass

For comparative purposes, we used data of mayfly benthic biomass from Gutiérrez-Fonseca, Ramírez and Pringle (2018). Ephemeroptera nymphs were sampled monthly for two years (2002-2003). Three core samples (0.006 m^2 each) were collected in runs with leaves as the dominant substrate. All material enclosed into the core was removed to a depth of ~10 cm or until reaching bedrock. Mayfly nymphs were removed from organic matter and preserved in 80% ethanol. Biomass was estimated by measuring the length of each individual and applying the length-mass relationship developed by Benke *et al.* (1999).

Adult emergence and biomass

We used a 2m^2 (sampling area) emergence trap (BioQuip Products, Rancho Dominguez, California) to sample mayfly adults continuously from July 2001 to February 2004. The trap was suspended over the stream and covered the entire stream width, which allowed us to sample in various microhabitats such as riffles, pools and runs. Emerging insects were collected weekly and preserved in 80% ethanol for subsequent taxonomic identification. A modified handheld vacuum was used to remove emergent insects from the trap. The trap was inspected often for maintenance (i.e., repair of holes and removal of spider webs). Mayfly biomass was calculated by measuring the length of each individual and applying the length-mass relationship developed by Sabo *et al.* (2002). Emergence biomass was expressed as mg AFDM m^2 by taking the total biomass of each month adjusted by the trap area. Total annual biomass was determined by adding all weekly samples for each year.

Physicochemical variables

Eight physicochemical variables were measured monthly, simultaneously with Ephemeroptera collections. Nutrient concentrations (i.e., NO_3^--N, NH_4^+-N and PO_4^{3-}-P as soluble reactive P: SRP) were measured by collecting 2 filtered (0.45 µm Millipore filters) water samples using new 125 mL bottles. Samples were kept frozen until analyzed. NO_3^--N, NH_4^+-N, and SRP concentrations were measured using continuous-flow colorimetry and an Alpkem RFA 300 colorimetric analyzer. The Cd reduction, phenate, and ascorbic acid methods were used for NO_3^--N, NH_4^+-N, and SRP, respectively (APHA, 1998). Stream temperature, pH and conductivity were measured *in situ* using Hanna meters. Discharge was measured with a Marsh–McBirney current meter and was estimated using the velocity–area method (Gordon et al. 1992). Monthly precipitation was recorded using data from the meteorological station available at LSBS (OTS meteorological data, http://www.ots.ac.cr/meteoro/).

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

To determine departures from symmetry in *F. caribbianus*, a suite of five traits taken from three morphological characters were measured on the left and right sides of each adult (Table 1) after measuring body length (not including cerci, abbreviated to BL). Body parts were removed with forceps, mounted on glass slides, and photographed with a stereomicroscope (AmScope) and a microscope (Nikon Eclipse E400). Images were analyzed with the free computer software ImageTool 2.0 (University of Texas Health Science Center, San Antonio). All linear and area measurements were done with an accuracy of 0.01 mm and 0.01 mm^2, respectively. To avoid mistaking human error with potential asymmetries, all measurements were taken three times from the same image at a random order, without reference to the previous set of measurements.

Table 1. Codes of evaluated traits in *F. caribbianus* (Leptophlebiidae) adults.

Data analyses

We used biomass to compare between benthic and emerging mayflies, since biomass better reflects potential changes in mass production driven by environmental variability, beyond other metrics such as abundance and diversity (Malison, Benjamin & Baxter, 2010). Likewise, biomass estimates are widely used in analyses of food webs and stream productivity.

A model selection approach based on Akaike's Information Criteria (AIC, Akaike, 1973) was used to identify the best-fit model that included the environmental variables influencing biomass of adult mayflies. Model construction, variables standardization and subsequent tests are described in more detail in Gutiérrez-Fonseca et al. (2018).

The relationship between body length and average precipitation in the 159 days before collecting the emergence trap was calculated separately for males (n=67), females (n=50) and both sexes combined (n=117) using Spearman's rank correlation coefficients. We used average precipitation of the 159 days as this timeframe coincides with the life cycle of *Thraulodes* sp. (same family, Leptophlebiidae), which was estimated as the median days since an egg hatches until the individual reaches adulthood (Jackson & Sweeney, 1995).

Analyses of potential departure from symmetry were performed as suggested by Palmer & Strobeck (1986) and Palmer (1994). For each trait, the left measurements were subtracted from the right measurements and the absolute difference (|R–L|) was added to the dataset. To evaluate the potential presence of antisymmetry and directional asymmetry, a Shapiro-Wilks test was performed to determine whether the data was normally distributed, which would rule out antisymmetry. To test for any directional asymmetry, a Student's t-test was performed on the measurements of asymmetries; a mean equal to zero would rule out directional asymmetry. Two-way ANOVA mixed model with side (Fixed), individual (Random) and their interaction (Side x Individual) were used to verify any measurement errors (Palmer & Strobeck, 1986; Palmer, 1994). The F-ratio tested whether the true FA variance was statistically significant relative to measurement error. According to Palmer (1994), a significant result for Side x Individual would reveal the existence of FA.

We calculated the Spearman's rank correlations with the *cor.test()* function within the *stats* package and the two-way ANOVA with the *aov()* function within the *lme* package in R version 3.6.3 (R Core Team, 2019). Raw data and code used in this study are available on a GitHub repository: https://github.com/PEGutierrezF/mayfly_morphometry.

Results

Benthic and emergence biomass

Mean annual biomass of nymphs was 3.12 mg AFDM/m^2 in 2002 and 3.36 mg AFDM/m^2 in 2003. Benthic biomass peaked in March, July, September 2002 and January, May, October 2003 (Fig. 1). Meanwhile, mean annual biomass of emerging adults was 0.76 mg AFDM/m^2 in 2002 and 0.50 mg AFDM/m^2 in 2003. Monthly mayfly emergence was constant throughout the study periods, except for a slight increase in June 2002 (Fig. 1A). We estimated that emergence represented 24.4% and 14.9% of the total benthic mayfly for the 2002 and 2003, respectively.

Figure 1. Temporal variability of benthic and emerging adult biomass during A) 2002 and B) 2003.

Physicochemical characteristics and individual-level variation

The AIC analysis used to determine the relative importance of environmental variables on the biomass of emerging adults showed no support for any model. This trend was consistent for mayflies collected during 2002 and 2003.

Body length of *F. caribbianus* was strongly influenced by average rainfall during the 159 days of larval development. Spearman's rank correlations revealed a negative relationship between average rainfall and body length of males (Spearman's r_s = - 0.45, p< 0.001), females (Spearman's r_s = - 0.64, p< 0.001), and both sexes combined (Spearman's r_s = - 0.51, p< 0.001, Fig. 2).

Figure 2. Relationship between mayfly body length and average precipitation in the 159 days prior to the sampling date. Each point represents an individual, including males and females.

FA analysis in *F. caribbianus*

A total of 117 *F. caribbianus* (50 females and 67 males) were assessed to determine departure from symmetry. Analyses of trait value distribution satisfied the assumption of normality, so there was no evidence of antisymmetry in any of the characters (p> 0.05, Appendix 1). Also, the Student's t-test revealed no significant difference between right and left sides, which suggests that there was no directional asymmetry (p> 0.05, Appendix 1).

There was no evidence of FA, as each trait did not have significant differences in Side by Individual interactions in the two-way ANOVA mixed models (Table 2). Also, the measurement error was found to be negligible. Nevertheless, the individual level was significant in most cases, which suggests that the morphometric variability in insects was expressed in the body length of the individual rather in FA.

Table 2. Result of the two-way ANOVA performed for each trait.

Discussion

Our 2y-study showed high temporal variability of mayfly benthic biomass, characterized by multiple peaks during the study period. Unexpectedly, these peaks in benthic biomass did not translate into measurable increases in emerging adult biomass, which represented a small fraction of the benthic biomass. We did not observe a relationship between hydrology and the biomass of benthic and emergent mayflies, so our hypothesis that mayfly biomass would peak in the dry season of La Selva was not supported by our findings. Looking more closely at *F. caribbianus*, the most abundant mayfly collected in emergence traps, we found no evidence of asymmetries among the sampled periods for any of the measured morphometric characters. Nevertheless, we found a strong negative relationship between body length and precipitation variability at La Selva. This relationship was consistently significant for males, females, and both sexes combined.

Our gross estimation for comparing benthic and emergent biomass indicated that only 25% of benthic biomass was exported to the terrestrial ecosystem. This potentially low value may have negative consequences on the riparian food web, as emerging insects represent an important source of energy and nutrients in La Selva streams (N-flux: 0.40-1.25 mg N m^{-2} d^{-1}, Small, Duff, et al. 2013). Our estimations are more similar to those values previously reported for single groups of aquatic insects, such as 27% for a hydropsychid (Trichoptera) in desert streams (Jackson & Fisher, 1986) and 16% for a limnephilid (Trichoptera) in an intermittent wetland (Whiles, Goldowitz & Charlton, 1999). However, it is important to note that these studies measured productivity ($g/m^2/y$), while we reported standing-stock biomass (g/m^2). Notably, while benthic and emergent biomass were similar during most of the study period, the observed peaks in nymph biomass were not associated to similar peaks in the emergence of adults. These peaks occurred in different periods of the year, which may suggest there are various mechanisms at play that control population dynamics of benthic and emerging mayflies.

Unlike the large fluctuations in emergence patterns observed in other studies (Masteller, 1993; Pescador, Masteller & Buzby, 1993; Castro-Rebolledo & Donato-Rondon, 2015; Yuen & Dudgeon, 2016), providing support for seasonality in many tropical aquatic insects, the lack of abrupt peaks in mayfly emergence found in our study suggests that mayfly emergence is aseasonal. A possible factor explaining the stability in adult emergence is fish predation on newly emerged adults. For instance, Wesner (2016) showed that fish predation decreases the number of insects reaching riparian ecosystems by 40% on average, more than 2x stronger than their effects on the biomass of benthic prey. Our focal stream is inhabited by the insectivorous poeciliid, *Priapicthys annectens,* which is abundant (4-14 individuals/m^2, Small et al. 2013) and could have negatively affected mayfly emergence.

Hydrology has been recognized as a key factor controlling macroinvertebrate assemblages in tropical streams (Flecker & Feifarek, 1994; Ramírez & Pringle, 1998; Molineri, 2010). However, observed peaks in benthic biomass occurred in both dry and rainy seasons in La Selva. This may be due to high floods during the rainy season eliciting microdistributional changes in macroinvertebrates (Lancaster & Hildrew, 1993; Lancaster, 1999). Mayflies have been observed to make small-scale refuge-seeking movements between substrate layers during simulated floods (Holomuzki & Biggs, 2000). This type of mechanisms could allow for high mayfly survival in streams such as Carapa, where the deep subsurface layers may provide shelter and protection for insects year round, as proposed for similar streams (Holomuzki & Biggs, 2000).

Meanwhile, our results show a strong influence of precipitation on the total body length of *F. caribbianus*. Large individuals were negatively affected by precipitation, while small-sized mayflies persisted during high rainfall events (Fig. 2). In tropical streams, two mechanisms have been identified as potential ways in which insects respond to catastrophic floods. The first mechanism proposes that small-sized individuals have better chances of surviving floods by finding refuge in interstitial spaces (Townsend, Dolédec & Scarsbrook, 1997; Segura, Siqueira & Fonseca-Gessner, 2013). The second hypothesis suggests that some aquatic insects synchronize their oviposition to occur at the onset of the rainy season, and that only small larvae are present during periods of frequent floods (Pritchard, 1996). Since mayflies in La Selva are multivoltine (Ramírez & Pringle, 1998), we find more support for the first hypothesis through this study.

Asymmetrical traits have been used successfully as an early warning biomarker related to developmental stress (Graham et al., 2010). Our findings show no evidence of change in *F. caribbianus* symmetry for any individual metric during the sampling period (i.e., July 2001 to February 2004). Perhaps securing symmetry in traits that carry vital roles (e.g., forceps for reproduction and wings for dispersion and mating) is compensated by other morphometric characters, such as body length. Individuals from perturbed environments may develop symmetrical characters instead of an optimal body length as a trade-off for survival (e.g., Dobrin & Corkum, 1999).

Future climate change scenarios predict an increase in hydrological extreme events for many regions (Christensen et al., 2013). Extreme precipitation events are expected to increase in tropical regions (O'Gorman & Schneider, 2009), with potential negative effects on aquatic biota and aquatic-terrestrial linkages. Increases in heavy precipitation events have already been observed in the Caribbean slope of Costa Rica during the last decades (Aguilar et al., 2005; Rapp et al., 2014; Sánchez-Murillo et al., 2017), where climate projections suggest an increase in mean annual precipitation of between 10 to 50% (Alvarado et al., 2012). Therefore, large hydrological variability can threaten the fitness of mayfly populations in La Selva, as well as in other tropical regions.

Conclusions

Contrary to our expectations and patterns shown in literature, we found a lack of seasonality in benthic biomass. Adult biomass was unrelated to peaks in benthic biomass, which makes us wonder what is controlling adult biomass export in these systems if not hydrology (e.g. fish predation). Body length, and not bilateral asymmetry, proved to be a better indicator of environmental stress, which could have severe associated costs for mayfly fitness in ecosystems with high temporal variation. Further research could quantify effects of body length reduction in mayfly fitness, energy and nutrient export to riparian food webs, as well as the role of biotic control on mayfly biomass in tropical lowland streams.

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Table 1(on next page)

Codes of evaluated traits in *F. caribbianus* (Leptophlebiidae) adults.

Table 1. Codes of evaluated traits in *F. caribbianus* (Leptophlebiidae) adults.

Traits	Code
Area of the Fore Wing	AFW
Length of the Fore Wing	LFW
Area of the Hind Wing	AHW
Length of the Hind Wing	LHW
Second Segment of Forceps	SF

Table 2 (on next page)

Result of the two-way ANOVA performed for each trait.

Table 2. Result of the two-way ANOVA performed for each trait.

	Trait	N	Side		Individual		Side x Individual	
			F	*P-Value*	F	*P-Value*	F	*P-Value*
Male	AFW	31	0.15	0.70	25.26	**>0.001**	0.46	0.50
	LFW	31	0.06	0.81	12.43	**>0.001**	0.79	0.38
	AHW	27	0.09	0.76	10.34	**0.001**	0.02	0.88
	LHW	27	0.42	0.52	23.71	**>0.001**	0.18	0.67
	SF	66	0.60	0.44	15.59	**>0.001**	0.96	0.33
Female	AFW	30	0.01	0.96	0.92	0.34	0.09	0.77
	LFW	30	0.09	0.77	7.77	**0.005**	0.06	0.80
	AHW	41	0.01	0.97	0.38	0.56	0.01	0.91
	LHW	41	0.92	0.34	0.78	0.378	0.00	0.99

Figure 1

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

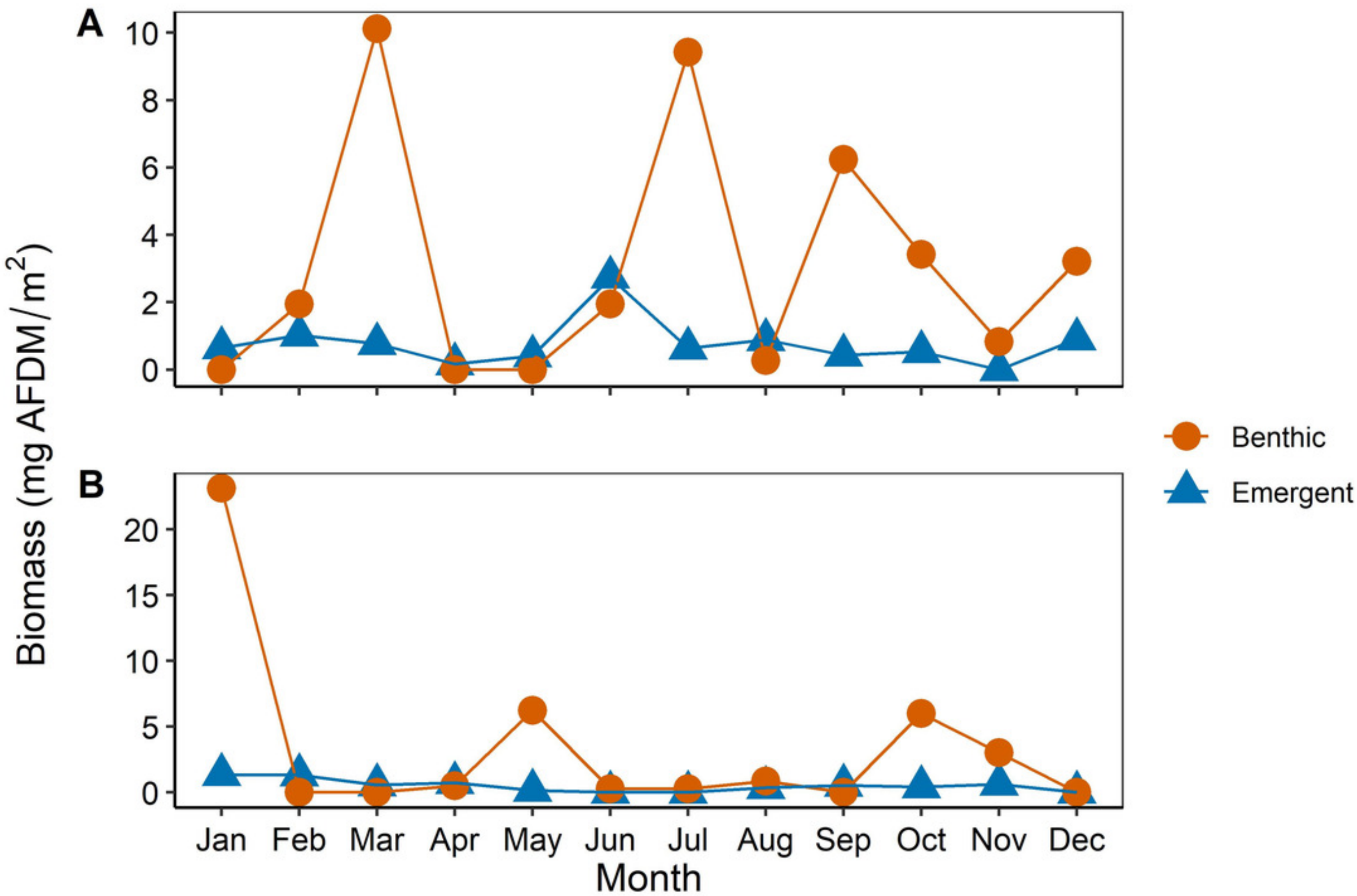

Figure 2

Relationship between mayfly body length and average precipitation in the 159 days prior to the sampling date. Each point represents an individual, including males and females.