Intraspecific competition along different life stages can stabilize coexistence among dragonflies and damselflies

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Conflict of Interest

The authors declare that they have no conflict of interest

Author´s contribution

GCP and VSS originally formulated the idea, GCP, FOZ, ES and VSS developed methodology, FOZ and GCP conducted the experiments, ES and GCP collected observational data, GCP conducted data analyses with the supervision of VSS, GCP and VSS wrote the manuscript with contributions of FOZ and ES.

Abstract

Biodiversity is sustained by stabilizing mechanisms of coexistence that inhibit species of competitive exclusion. Yet, in organisms with complex life cycles competitive dynamics may vary according to the life stage. Dragonflies are optimal organisms to test these ideas, as aquatic larvae are generalists in their feeding behavior, but adults have a wide variety of sexual behaviors. In this way, we aimed to understand how intra and interspecific interference may vary according to the life stage in tropical Odonata species. We hypothesized that competition in the larvae stage is mostly neutral, *i.e.* independent of species identities but responsive to total densities, while in adults, intraspecific interference may be more intense due to sexual selection mechanisms operating as deterministic stabilizing processes. Therefore, the study integrated two approaches, focusing on: 1. Laboratory experiments, where larvae of *Acanthagrion* (Zygoptera, Coenagrionidae) and *Erythrodiplax* (Anisoptera, Libellulidae) were allocated to four different treatments, with variation in total abundance and dominance of species; 2. Field observations, where we assessed adult co-occurrences and species interactions. Our results suggested a stronger intraspecific interference in both life stages. We found a spatial segregation in the larval stage decreasing interspecific interference and increasing cannibalism. Whereas adults were spatially segregated at fine scale, increasing the frequency of intraspecific interactions, especially in species with prominent territoriality. This study provided novel results on how coexistence mechanisms could be consistent along ontogenetic development broadening our understanding of processes sustaining biodiversity in tropical ecosystems.

Keywords: neotropical species, niche partitioning, ontogenetic development, reproductive interactions, species interaction

Introduction

Community ecology seeks to understand how species are able to coexist sharing limited resources, yielding our current and past patterns of biodiversity. Because biodiversity is the main actor of ecosystem processes that translate into services for human society (*e.g.*, nutrient cycling, clean water, and fisheries), understanding how species coexist is pivotal to forecasting and avoiding undesirable changes that may lead to negative environmental and societal consequences. Species can interact in both positive and negative ways. In this context, competition emerges as an important mechanism in shaping community composition, potentially leading to the competitive exclusion of the worst competitor. While competition among species has been studied for more than a century (Lotka 1914), theoretical and empirical advances are shedding new light on this important process regulating biodiversity (Chesson 2000; Kraft *et al.* 2015; Saito *et al.* 2018; Hülsmann *et al.* 2024).

Previous studies have demonstrated the effects of competition on the maintenance of biodiversity (*e.g.* Kraft *et al.* 2015) and the importance of a variety of mechanisms that reduce the impacts of competition and avoid exclusions. For instance, stabilizing mechanisms (Chesson 2000) are those that increase intraspecific competition, making species regulate their per capita growth rate more intensely than the growth rate of their competitors. Traditionally, such stabilizing mechanisms are linked to resource competition and the ways how species partition resources. For example, neotropical fish communities experience seasonal variation in food preferences reducing their diet breadth to specific food items when resources are scarcer, which increases their niche differences and the likelihood of coexistence (Neves *et al.* 2021).

Although trophic resources (*e.g*., prey and nutrients) have traditionally been the most studied, non-trophic resources can also mediate stabilizing processes. For instance, reproductive interactions may impact intraspecific competition, through either male-male or male-female interactions (*i.e*. Yamamichi *et al.* 2020; Gómez-Llano *et al.* 2021). As an example, territoriality can affect community dynamics directly or indirectly, as studies have shown that this behavior increases intraspecific competition and can negatively affect the survival rate of males and mating success (Svensson *et al.* 2018; Pinto *et al.* 2022). Additionally, behaviors involved in sexual conflict, such as male harassment may have negative effects on female's survival rate and fecundity (Takahashi and Watanabe 2010; Gomez-Llano *et al.* 2018). Both situations may control population growth as densities rise, enhancing the chances of intraspecific self-regulation and species coexistence (M'Gonigle *et al.* 2012; Kobayashi 2019).

Despite the potential impacts of reproductive behaviors on species coexistence, most of the theoretical and empirical studies are focused on organisms with relatively simple life cycles, such as annual plants (Kraft *et al*. 2015) and phytoplankton (Narwani *et al.* 2013), while studies in groups with complex life cycles (*i.e*. holometabolous) are rare (Gómez-Llano *et al.* 2021). Dragonflies and damselflies are optimal organisms to be studied in this regard as they are holometabolous organisms with aquatic larvae and winged terrestrial adults. While larvae of several groups can behave as equivalent generalist predators with simple foraging strategies that could lead them to have similar negative intra and interspecific per-capita effects (neutral dynamics, Siepielski *et al.* 2010), adults can be involved in complex intraspecific sexual interactions that could stabilize their coexistence (deterministic coexistence, Yamamichi *et al.* 2020; Yamamichi *et al.* 2023). Therefore, in organisms with complex life cycles, mechanisms of coexistence could vary along with ontogenetic development, potentially impacting broad patterns of coexistence and community patterns.

Furthermore, empirical studies on species coexistence are mostly done in temperate regions, while studies in tropical ecosystems are lacking. In these ecosystems, species diversity is notably higher in several taxonomic groups posing an additional challenge for understanding how these complex systems harbor numerous species within the same habitat. Additionally, tropical ecosystems with warmer climates should harbor species with faster metabolism and, consequently community assembly could be more stochastic (Saito *et al.* 2021). Studies on organisms with complex life cycles in tropical ecosystems can therefore help us elucidate competition patterns in communities with high diversity and potentially under higher influence of stochastic processes.

We aimed to understand how intra and interspecific interference vary according to the life stages in tropical Odonata to identify the stabilizing mechanisms that may promote coexistence. We hypothesized that competition in the larvae stage is neutral, because equivalent generalist predators would have similar negative intra and interspecific per-capita effects. While in adults, intraspecific interference should be more intense due to sexual selection mechanisms (*i.e.*, territoriality) operating as deterministic stabilizing processes. The study integrated two approaches, focusing on: 1) laboratory experiments with larvae of two taxa, *Acanthagrion* (Zygoptera, Coenagrionidae) and *Erythrodiplax* (Anisoptera, Libellulidae), to assess total mortality rate, predation, and the number of remaining larvae under different abundance conditions (high and low) and dominance scenarios; and 2) field observations with adults, to evaluate Odonata adult abundance and species-specific antagonistic and sexual interactions in a community composed of five different species—two Zygoptera and three Anisoptera. In the experimental approach, significant effects of total abundances on demographic properties would represent taxa-independent density-dependent effects, indicating neutrality; on the other hand, to the effect of dominance (either *Acanthagrion* or *Erythrodiplax*) would indicate distinct effects between intra- and interspecific interference on mortality. For the observational approach, a higher frequency of intraspecific than interspecific interactions with increasing density would be interpreted as stronger intraspecific rather than interspecific negative density-dependency, a mechanism for potential stable coexistence.

Methods

Observational approach

We conducted field observations in a headwater stream in São Carlos (São Paulo State, Brazil, 21° 59' 15.749" S 47° 52' 32.428" W). Previously, to determine adult Odonata community composition in this site, we sampled individuals freely and identified them to genus level using taxonomic keys (Garrison *et al.* 2006; Garrison *et al.* 2010). After, to assess adult abundance and species interactions we used the fixed area scanning method as it minimizes double counting individuals, as short time intervals cover small sections (De Marco Júnior *et al.* 2015). As such, the local stream was divided into 16 five-meter sections and each section was observed for two minutes by two trained researchers to account for the abundance of each species. After that, we selected the five-meter sections with the highest total abundances and we observed intra and interspecific interactions for five minutes, separated in three categories: 1) interspecific (individuals of one species displacing or chasing individuals of another species); 2) Intraspecific male-male (contests between males of the same species); 3) Intraspecific male-female (mating behavior, *i.e.* tandem, post-copulatory guarding). When any species was not recorded during the abundance survey, but was later observed interacting, we adopted the following protocol: if the species was interacting with another species, we recorded its abundance as one (minimum abundance for that interaction to occur at least once); if it was interacting with another individual of the same species, we recorded its abundance as two (minimum abundance for the interaction to occur at least once). Field observations were carried out weekly, between November, 2022 and March, 2023, totaling 15 samplings.

We also measured environmental variables in each sampling date. Air temperature was measured using an electronic thermometer. Water temperature, dissolved oxygen and pH were measured using a multiparameter probe. Rainfall data for the previous 3 days and on the day of sampling were compiled from the INPE website.

Experimental approach

We sampled Odonata larvae using a D net in the same stream where we conducted the observational method (21° 59' 15.749" S 47° 52' 32.428" W) and additionally, in a second stream of similar dimensions (21°58'26.3"S 47°52'14.4"W), also in São Carlos, Brazil. Species of the genus *Acanthagrion* (Zygoptera, Coenagrionidae) and *Erythrodiplax* (Anisoptera, Libellulidae), were found in large quantities at these sites, therefore, we selected them for the experiment. We took the collected larvae to the laboratory for screening and identification of genera using taxonomic keys (Hamada *et al.* 2018). Subsequently, larvae were measured from the top of the head to the end of the last abdominal segment using a millimeter scale to select individuals of similar sizes and to estimate larval biomass.

The selected larvae were placed into sterilized polypropylene boxes containing 6 liters of dechlorinated water, properly oxygenated, with 6 polypropylene black straws (a refuge simulating a plant root), in environmental temperature and lightness, according to the following orthogonal experimental design: two levels of total abundance (high abundance with 12 individuals and low abundance with 6 individuals in total) and two levels of relative density, alternating the dominant species (66% of one species and 33% of the other) (Siepielski *et al.* 2010). A total of four treatments were outlined (Fig. 1): 1) high abundance and dominance of *Acanthagrion*, 2) low abundance and dominance of *Acanthagrion*, 3) high abundance and dominance of *Erythrodiplax* and 4) low abundance and dominance of *Erythrodiplax.* The individuals were fed daily with six larvae of other aquatic insects highly abundant in the sampling sites (mostly Chironomidae and Simuliidae) per box. This meant that each Odonata on the low-density treatments had a potential prey per day. Data on mortality, remaining larvae and spatial distribution (larvae in straws) were collected daily, for 10 consecutive days, between December, 2022 and January, 2024, totaling 6 replicates for each treatment.

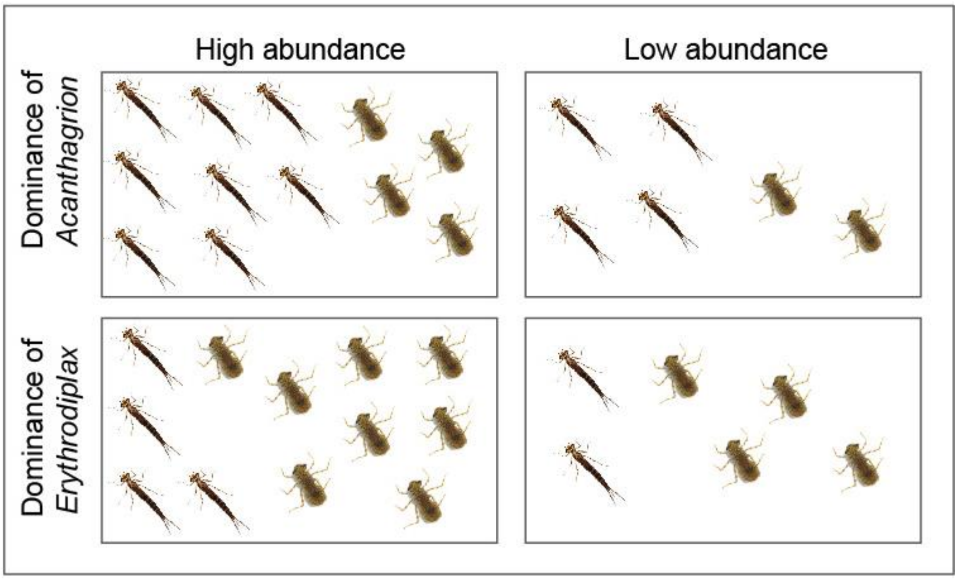

Figure 1. Experimental design for a pair of dragonfly and damselfly species. The scheme configures a 2x2 crossed orthogonal design, with two levels of total densities and alternation in the relative density of species. The number of replicates was six for each pair of species.

Data analyzes

Observational approach

Species in a community can share their niches temporally and spatially. In the observational approach, we considered the diel activity as a temporal niche and the sections occupied by species in each day as a spatio-temporal niche. To determine the temporal and spatio-temporal niche overlap of Odonata adults we applied the Pianka overlap index, using a matrix of abundance per section and sampling dates. This index is calculated by the following equation:

$$O_{jk} = O_{kj} = \sum P_{ji} \times P_{kj} \Big/ \sqrt{\sum (P_{ji}^2) \times (P_{ik}^2)} \quad \text{(eq.1)}$$

where, O_{jk} is Pianka's measure of niche overlap between species *j* and *k*; P_{ji} is the proportion of resource *i* in the total resources used by species *j*; P_{ik} is the proportion of resource *i* in the total items used by species *k*. Values range from 0 to 1, with 0 being completely separated niches and 1 being complete niche overlap (Pianka 1973). For the temporal analysis, the summed abundance in all sections were used for each date (15 temporal replicates) and for the spatio-temporal scale, the abundance in each section and on each date (75 spatial-temporal replicates) were used. We compared the Pianka's index to a randomized null model, where 5,000 randomizations were performed using the Ra3 algorithm (shuffle the resource utilization of each species holding species niche breadth and zero states, Winemiller and Pianka 1990). This is the null model algorithm recommended for discrete resources and analyses of niche overlap (Gotelli *et al.* 2015). We then estimated the effect size of the index as the difference between the empirical means and the simulated means divided by the null model standard deviations. Positive values were interpreted as a tendency towards niche overlap, and negative values as a tendency towards niche differentiation.

We performed analyses of covariance (Ancova) to compare the effects of intra- and interspecific densities on species interactions. Specifically, we compared the number of total interactions observed (n=75, 15 days x 5 stretches per day) (response variable) among intra and interspecific interaction (explanatory variable), using the abundance of the respective intraspecific and interspecific individuals per observation as a covariate. We run individual Ancovas for each taxon separately.

To assess whether species distribution could be influenced by environmental variables related to water and air parameters, we performed a Redundancy Analysis (RDA). The RDA is a multivariate extension of a multiple regression to estimate the linear relationships between an explanatory matrix and a response matrix based on minimum squares and residual calculations. We performed this analysis twice with different explanatory matrices, but with the same species abundance matrix: 1) RDA with the water parameters matrix containing the variables water temperature, dissolved O2, % dissolved O2 and pH as matrix explanatory. 2) RDA with the air parameters matrix containing the variables air temperature, average rainfall for the 3 days prior to collection and the sum of rainfall up to 12pm on the day of collection.

Experimental approach

To compare the differences of larvae competition and spatial distribution in each treatment, the total mortality ratio per species and spatial distribution ratio per species were used. The total mortality ratio of each species and the spatial distribution ratio were calculated using the following formula:

$$Total\ Mortality\ ratio = \frac{n\ larvae\ initial\ -\ n\ remaining\ larvae}{n\ larvae\ initial}$$

$$Spatial\ distribution\ ratio = \frac{n\ total\ larvae\ on\ straws}{n\ larvae\ initial}$$

Within total mortality, two categories were recorded: number of dead larvae (number of larvae found whole and dead in each box) and number of preyed larvae (missing larvae). The average of these variables for each treatment (total mortality ratio, spatial distribution ratio, dead larvae and preyed larvae) were analyzed using a three-way ANOVA, with the genus identity, total abundances (low and high total abundance) and dominance (*Acanthagrion* and *Erythrodiplax*) as independent variables (Siepielski *et al.* 2010). In every Anova that returned a significant result, we tested for pairwise differences in treatments using Tukey HSD (Honestly Significant Difference) test. To evaluate whether the larvae biomass varied between treatments in the beginning of the experiments we used body size data to estimate biomass according to the length-mass equations for Calopterigydae (Benke *et al.* 1999) and Libellulidae (Dekanová *et al.* 2022). Then, we performed a three-way ANOVA, with biomass as response variable and species identity, total abundances and dominance as independent variables.

All analyzes were performed using R software (Team 2013) and the packages EcoSimR and vegan.

Results

Observational Approach

We identified 5 morphotypes in adult Odonata communities, separated into four genera: *Acanthagrion* sp. (Zygoptera, Coenagrionidae), *Oxyagrion* sp. (Zygoptera, Coenagrionidae), *Erythrodiplax* sp1. (Anisoptera, Libellulidae), *Erythrodiplax* sp2. (Anisoptera, Libellulidae) and *Micrathyria* sp. (Anisoptera, Libellulidae). *Acanthagrion* sp. and *Erythrodiplax* sp2. were the most abundant, with 466 and 116 records, respectively. The other species were recorded 63 times (*Oxyagrion* sp.), 19 times (*Micrathyria* sp.) and 9 times (*Erythrodiplax* sp1.). Moreover, we recorded 691 interactions spread between interspecific interactions, male-male intraspecific interactions and male-female intraspecific interactions (Figure S1). *Acanthagrion* sp. interacted 65 times with others species and 124 times with individuals of the same species, where 86 times there were male-male interactions and 38 male-female interactions; *Erythrodiplax* sp2. interacted 122 times with others species and 183 times with individuals of the same species, where 177 times there were male-male interactions and 6 male-female interactions; *Erythrodiplax* sp1. interacted 99 times with others species and 8 times with individuals of the same species, where 8 times there were male-male interactions and zero male-female interactions; *Oxyagrion* sp. interacted 66 times with others species and 6 times with individuals of the same species, where 5 times there were male-male interactions and 1 male-female interactions; *Micrathyria* sp. interacted 17 times with others species and 1 times with individuals of the same species, where 1 times there were male-male interactions and 0 male-female interactions. All interspecific interactions occurred between males.

Pianka's index for temporal niche overlap (15 replicates) resulted in a mean value for all pairwise comparisons of 0.68. In the null model, the mean of the simulated index, that is, the overlap rate expected by chance, is 0.53, while the limits of variation were 0.48 to 0.60. Thus, the observed temporal overlap was higher than expected by chance (Standardized Effect Size=4.32, p=0.0002). Similarly, in the spatio-temporal comparison, the observed index was 0.31, higher than expected by chance in comparison with the null model which had threshold values of 0.22 and 0.27 (Standardized Effect Size=4.59, p=0.0002). The results for the pairwise Pianka's niche overlap indices (Table 1) showed that on the temporal scale, all species presented positive values, indicating a tendency for temporal niche overlap, while on the spatio-temporal scale the species pairs *Acanthagrion* sp. and *Oxyagrion* sp., and *Erythrodiplax* sp1. and *Micrathyria* sp. showed negative effects, indicating that these pairs tend to avoid each other in the finer spatial scale. Thus, the results indicate a tendency towards high overlap in the use of temporal and spatio-temporal resources, with the exception of the pairs *Acanthagrion* sp. and *Oxyagrion* sp. and *Erythrodiplax* sp1. and *Micrathyria* sp. which tend to occur on the same day, but occupying different sections.

Table 1. Standardized effect size of Pianka index of niche overlap in comparison to null models. Positive values were interpreted as a tendency towards niche overlap, and negative values as a tendency towards niche differentiation.

Genus 1	Genus 2	Temporal scale	Spatio-temporal scale
Acanthagrion sp.	*E*rythrodiplax sp2.	1.7394	1.1912
Acanthagrion sp.	*Erythrodiplax* sp1.	0.9084	0.1660
Acanthagrion sp.	*Micrathyria* sp.	1.2530	0.2525
Acanthagrion sp.	*Oxyagrion* sp.	2.5922	-1.2758
*E*rythrodiplax sp2.	*Erythrodiplax* sp1.	0.3581	2.0381
*E*rythrodiplax sp2.	*Micrathyria* sp.	2.0255	4.1782
*E*rythrodiplax sp2.	*Oxyagrion* sp.	2.1485	4.3892
*E*rythrodiplax sp1.	*Micrathyria* sp.	2.1007	-0.9157
Erythrodiplax sp1.	*Oxyagrion* sp.	0.9807	1.2749
Micrathyria sp.	*Oxyagrion* sp.	1.2302	1.4705

The analyses of covariance indicated differences in the frequencies of intra- and interspecific interactions, as well as a significant effect of the covariate of intra- and interspecific abundance, for all species (Table 2, Figure 2). In other words, the frequencies of intra- or interspecific interactions differ and are related to their respective abundance. Furthermore, the frequency of interactions differed between the types of interaction (intra and inter), with a higher frequency of interspecific interaction for the species *Oxyagrion* sp., *Micrathyria* sp. and *Erythrodiplax* sp1. (rare species throughout the study), and a higher frequency of intraspecific interactions for the species *Acanthagrion* sp. and *Erythrodiplax* sp2. (most abundant species). Despite this higher intraspecific interaction frequency for *Acanthagrion* sp., interactions increased with abundance at a similar rate between inter and intra (equal slopes but different intercepts). However, the statistical interaction between abundance and type of interaction was significant for *Erythrodiplax* sp2., indicating different slope between the intra- and interspecific interactions, with a marked increase in intraspecific interactions with increasing population density (Table 2 , Figure 2). It is also worth noting that for the three rarest species (*Oxyagrion* sp., *Micrathyria* sp. and *Erythrodiplax* sp1.), the intraspecific slope showed accentuated trends, but with non-significant results.

The RDA with the water and air parameters, water temperature, air temperature, dissolved O2, % of dissolved O2 and pH, average rainfall of the 3 days preceding the observations and the accumulated rainfall until the beginning of the observations did not show significant associations with community composition.

Table 2. Results of the Ancova test showing the F value and P value for each species. Values in bold represent significant results (p < 0.05).

		Acanthagrion sp.		*Mycrathiria* sp.		*Erythrodiplax* sp1.		*Erythrodiplax* sp2.		*Oxyagrion* sp.	
	DF	F value	P	F value	P	F value	P	F value	P	F value	P
Abundance	1	8.522	**0.004**	14.244	**0.001**	31.819	**<0.001**	4.382	**0.039**	14.088	**<0.001**
Interaction type	1	9.103	**0.003**	5.043	**0.038**	5.718	**0.021**	14.872	**<0.001**	12.554	**<0.001**
Abundance:interaction type	1	0.342	0.560	0.828	0.375	1.292	0.261	56.911	**<0.001**	1.119	0.295

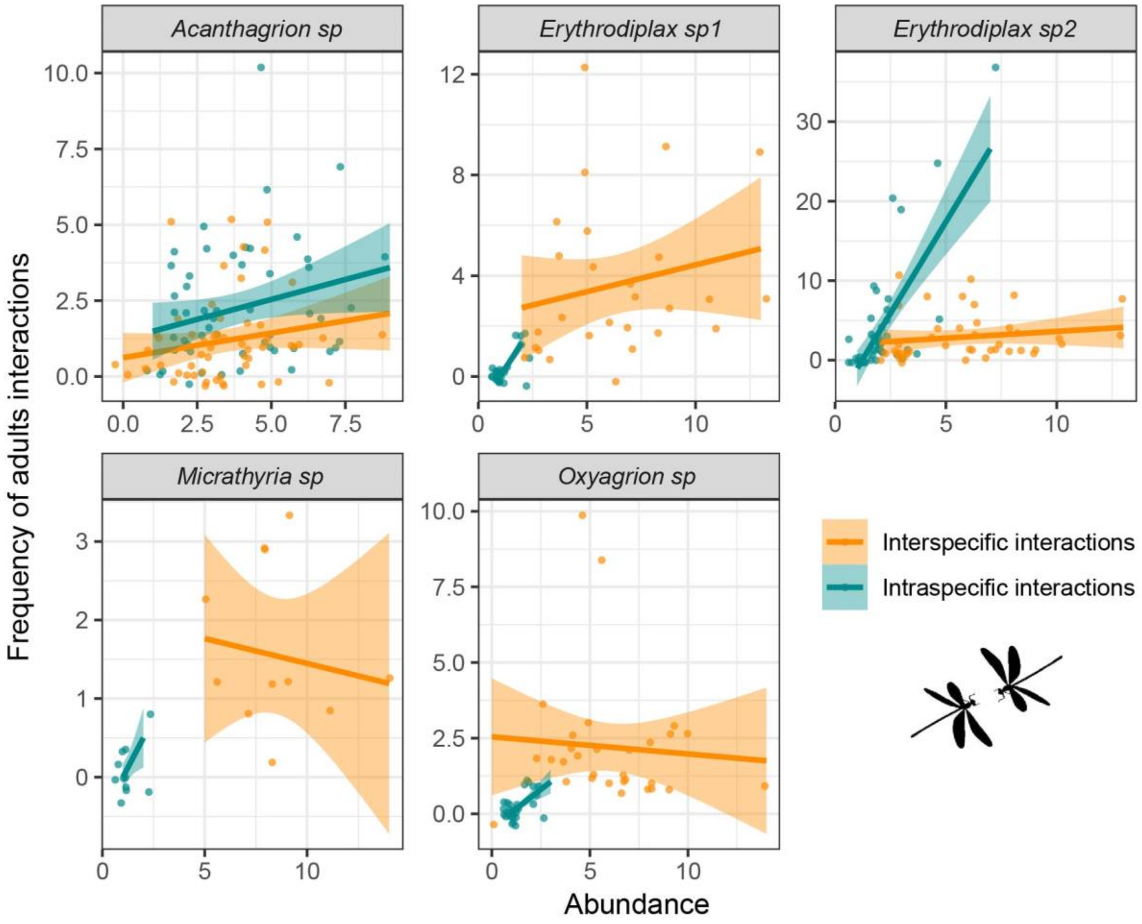

Figure 2. Linear regression showing differences between intra and interspecific interactions in relation to their respective abundances.

Experimental approach

The analysis of variance indicated differences between treatments for all parameters evaluated: total mortality ratio, number of preyed larvae and dead larvae (Table 3). Specifically, the total mortality ratio of *Acanthagrion* in treatments with *Acanthagrion* dominance was marginally higher in relation to the mortality ratio of *Erythrodiplax* in treatments with *Acanthagrion* dominance (Figure 3A; *ad hoc* Tukey, p=0.08), regardless of abundance. For the number of dead larvae, we found a higher mortality of *Erythrodiplax* in treatments with *Erythrodiplax* dominance compared to the number of dead larvae of *Erythrodiplax* in treatments with *Acanthagrion* dominance (Figure 3B; *ad hoc* Tukey, p=0.032) The number of dead larvae of *Erythrodiplax* in treatments with *Erythrodiplax* dominance was higher than the number of dead larvae of *Acanthagrion* in treatments with *Erythrodiplax* dominance (Figure 3B; *ad hoc* Tukey, p=0.054). Additionally, we found a higher predation as cause of mortality in treatments with high abundance compared to low abundance treatments (Figure 3C; *ad hoc* Tukey, p=0.0018). Larvae of *Acanthagrion* in treatments with *Acanthagrion* dominance were more preyed upon than *Erythrodiplax* in treatments with *Acanthagrion* dominance (Figure 3C; *ad hoc* Tukey, p=0.008) Also, larvae of *Acanthagrion* in treatments with *Acanthagrion* dominance were more preyed upon than *Acanthagrion* in treatments with *Erythrodiplax* dominance (*ad hoc* Tuckey; p=0.042). Thus, all effects suggest a higher intraspecific interference in relation to interspecific for all parameters evaluated.

We found that the spatial distribution ratio was different among genera (Table 3), with *Acanthagrion* larvae occupying more the straws during the experiment for all treatments (Figure S2). Finally, we observed significant differences in the individual biomass based on genus and dominance. Specifically, *Acanthagrion* were larger than *Erythrodiplax* and treatments with *Erythrodiplax* dominance exhibited higher biomass of both taxa (Table 3, Figure S3).

Table 3. Results of the Anova test showing the F value and P value of total mortality rate, preyed larvae, death larvae, spatial distribution rate and biomass. Values in bold represent significant results (p < 0.05).

		Total mortality ratio		Predated larvae		Dead larvae		Spatial distribution ratio		Individual biomass	
	DF	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
Genus	1	2.02	0.1633	2.77	0.1036	0.3883	0.536706	64.45	< **0.001**	19.5672	< **0.001**
Abundance	1	0.9666	0.3314	11.10	**0.0019**	1.55	0.219884	0.741	0.3945	1.3955	0.24446
Dominance	1	0.2983	0.5879	0.55	0.4634	0.8738	0.355518	0.3774	0.5425	23.1879	< **0.001**
Genus:Abundance	1	0.0119	0.9135	0.8562	0.360363	0.0971	0.756972	0.4715	0.4963	0.4508	0.50582
Genus:Dominance	1	4.31	**0.0444**	9.90	**0.00312**	9.71	**0.003387**	0.3848	0.5386	3.7510	0.05986
Abundance:Dominance	1	0.5847	0.4489	0.5479	0.463481	0.00	1.00	0.4781	0.4933	0.7175	0.40200
Genus:Abundance:Dominance	1	0.9666	0.3314	0.3082	0.581867	0.8738	0.355518	0.0814	0.7768	0.2388	0.62778

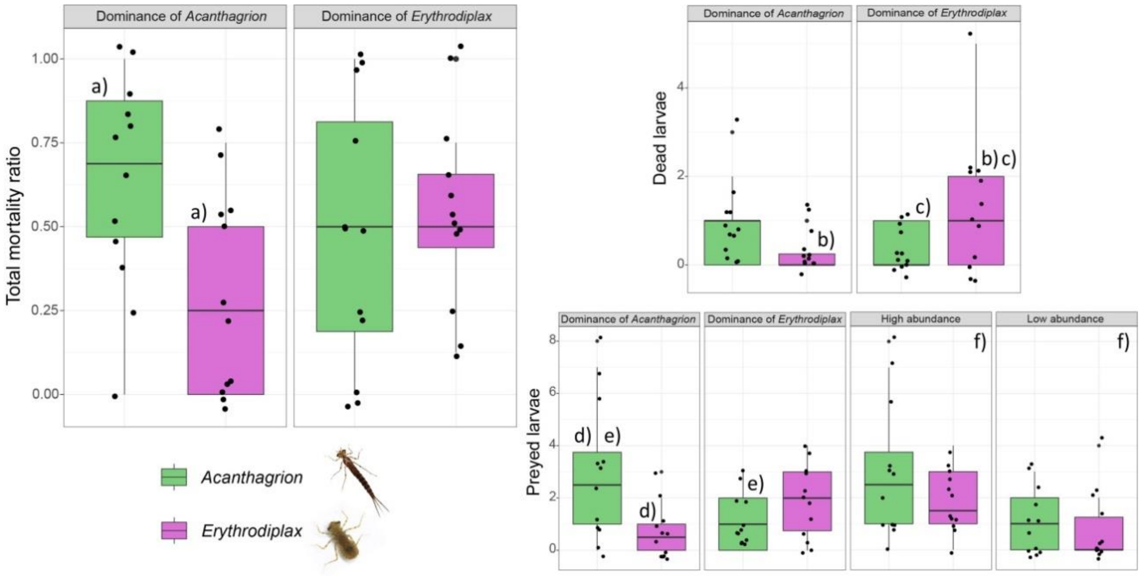

Figure 3. Boxplot showing significant differences for the total mortality ratio, preyed larvae and dead larvae between treatments. Equal letters indicate significant differences between treatments ($p < 0.05$).

Discussion

One of the main goals of community ecology is to understand the maintenance of biodiversity through mechanisms that enable species to coexist in local communities. Whereas competition may imply competitive exclusion of the weaker competing species, stabilizing mechanisms balance this equation increasing intraspecific competition in relation to interspecific (Chesson 2000). Although some empirical studies have been conducted in light of the Modern Coexistence Theory, there is a scarcity of studies carried out with species with a complex life cycle aiming at understanding how the ecological differences surrounding these stages may promote species coexistence (Gómez-Llano *et al.* 2021). While in the larval stage individuals focus on competition for resources and survival, in the adult stage the main goal is to maximize individual reproductive success through different reproductive strategies. Our results suggest that different mechanisms decrease interspecific interference and control population densities in both life stages. In the larval stage, intraspecific competition and cannibalism were found to be density-dependent, while in adults a higher rate of intraspecific interactions associated with sexual selection may act as a stabilizing mechanism, increasing intraspecific regulation. Spatial niche segregation should reinforce such mechanisms and was observed for both life stages due to differences in habitat preferences in larval stage, while in the adult community it occurred within streams at fine spatial scale for *Acanthagrion* sp. and *Oxyagrion* sp., and also for *Erythrodiplax* sp1. and *Micrathyria* sp. Our study adds another piece to the complex puzzle of tropical biodiversity, indicating that at fine resolutions, stabilizing deterministic mechanisms can indeed maintain high biodiversity even in dynamic tropical ecosystems.

For adult dragonflies and damselflies, our results showed that both intra and interspecific interactions can be density-dependent. We found higher male-male and male-female intraspecific interactions for the two most abundant species *Acanthagrion* sp. and *Erythrodiplax* sp2., suggesting a higher intraspecific interference for these species. For Odonata, this effect is magnified with reproductive intraspecific interactions. Regarding *Acanthagrion*, studies have shown that males are non-territorial, adopting sit-and-wait or actively searching for females as mating strategies. Thus, male-male interactions occur when one male chases the other or faces off against the other (Guillermo-Ferreira and Del-Claro 2012; de Almeida *et al.* 2022). Male-female interactions can occur before, during and after copulation. For this group oviposition guard can be frequent, when males hold the female during oviposition to prevent them from copulating with other males (Guillermo-Ferreira and Del-Claro 2012). Considering this complex reproductive system, the energetic cost for both males and females to increase individual reproductive success can be enormous, reducing the time and frequency of interaction with other species. Thus, our results indicate that reproductive interactions may act as stabilizing mechanisms, increasing intraspecific interactions in relation to interspecific interaction and potentially enhancing coexistence with other species. Furthermore, in the absence of courtship behaviors male harassment negatively affect females survival rate and fecundity (Takahashi and Watanabe 2010; Gomez-Llano *et al.* 2018). Such effects may control population growth at the expense of higher individual success, increasing the chances of intraspecific self-regulation and species coexistence (Yamamichi *et al.* 2020).

Additionally, *Erythrodiplax* sp2. also presented higher male-male interactions, showing a pronounced increase with increasing population density in relation to interspecific interactions. Species of the *Erythrodiplax genus* are mostly territorialists, where males defend a mating area from other males to attract females (Pinto *et al.* 2022). This behavior was observed during field samplings, where males pursued and chased other males that approached their area, and then returned to the same spot. Previous studies have demonstrated that male-male contests can reduce the lifespan of males or limit access to females, which can, directly or indirectly, affect the per capita growth rate (Svensson *et al.* 2018; Pinto *et al.* 2022). However, more studies are needed to understand whether the negative effects of territoriality on the per capita growth rate are sufficient to control population growth, and thus promote coexistence. On a different perspective, territoriality could influence coexistence by generating a spatial separation, where males are more aggressive towards conspecifics invading their territories, increasing the distance between conspecifics neighbors and allowing heterospecifics to occupy territories between conspecifics (Mikami *et al.* 2004). This may be a possible explanation for the co-occurrence of two morphotypes of the genus Erythrodiplax, given that both species exhibit territorial behavior. However, *Erythrodiplax* sp1. did not presented a high frequency of intraspecific interactions even though it showed territoriality behavior. This may occur due to the low abundance of the species and, therefore, lower probability of encountering conspecifics, which may mean that sexual selection mechanisms also operate depending on density.

While for Odonata adults mechanisms related to sexual selection can affect the intensity of interactions, in the larval stage these mechanisms are absent. Thus, considering the behavior of generalist predators we expected to find neutral mechanisms guiding the structuring of the community, dependent on abundances, but without distinction between the two genera (Siepielski *et al.* 2010). However, our results showed a strong influence of dominance (the identity of the most abundant species) in larvae mortality, regardless of the abundances. Our findings indicate a tendency towards greater intensity in the intraspecific competition of *Acanthagrion* larvae, since their total mortality ratio was higher when dominant. Regarding *Erythrodiplax*, the total mortality ratio was higher, although not statistically significant, when dominant than when rare (in dominance of *Acanthagrion*). This result may also signal a higher intraspecific effect on population density for *Erythrodiplax*. According to McPeeck (2012) competition model, intraspecific density-dependence may promote species coexistence, even though these species share the same resources. This occurs because each species controls its own population density, preventing just one species from dominating resources (McPeek 2012). Moreover, the presence of spatial niche segregation may explain the coexistence between the pair of studied species. Our results showed that *Acanthagrion* larvae and *Erythrodiplax* larvae occupied different locations in the experimental arenas. While *Erythrodiplax* larvae were found more frequently at the bottom of the boxes, *Acanthagrion* larvae were found predominantly in the straws. Previous studies have shown that there is a difference in habitat preference between larvae of the Coenagrionidae family and the Libellulidae family, with Coenagrionidae larvae (including the genus *Acanthagrion*) preferring more complex environments, with the presence of structures such as macrophytes (Tavares *et al.* 2018). Our results indicate that these habitat preferences are not only associated with an environmental selection, but also with reducing interspecific interference.

Additionally, several factors can affect Odonata mortality in the larval stage, controlling population density, such as competition, intraguild predation and cannibalism (Fincke 1994). We evaluated the differences between the numbers of dead and preyed larvae for the different treatments. Our results showed a strong influence of dominance for both parameters. Specifically, we found the highest number of dead larvae of *Erythrodiplax* when dominant. This may suggest that intraspecific resource competition is a strong factor influencing the dynamics of this population, enhancing conspecific deaths by stress or starvation until low densities stabilize (Fincke 1994). On the other hand, for *Acanthagrion*, the number of preyed larvae was higher when dominant compared to other treatments, suggesting a strong effect of cannibalism for this group. Cannibalism can enhance population maintenance by increasing predator growth—allowing cannibals to survive under adverse ecological conditions—and reducing direct competition for resources (Block and Stoks 2004). Previous studies have suggested that cannibalism is a significant factor influencing Odonata mortality in situations of high conspecific density (Wildy *et al.* 2001; Clark *et al.* 2021), as observed in our results. Some factors, such as food limitation (Wildy *et al*. 2001) and low habitat heterogeneity (Block and Stoks 2004), may intensify cannibalism, which could have occurred in our experimental design, also amplifying larval competition. Cannibalism may also be more intense when there is a significant size difference between larvae. Our results showed a significant difference in biomass between the genera *Acanthagrion* and *Erythrodiplax*, as well as significant differences in treatments with *Erythrodiplax* dominance. However, these results do not explain the occurrence of cannibalism in

Acanthagrion when dominant. On the other hand, the observed biomass differences between genera could have led to intraguild predation, although this did not significantly impacted larval mortality in our study. A possible explanation is that the variation in larval biomass was small, eliminating the size-dependent effect (larger larvae preying on smaller ones), thereby maintaining similar competitive abilities between the genera and reducing intraguild predation (Woodie and Anderson 2024).

Despite the clear evidences we have about the stronger intra than interspecific effects on Odonata competition, defining if species are able to coexist is a daunting quest. Empirical studies that seek to understand how interactions can affect the coexistence of species generally use the per capita growth rate as the fitness component. Here, we analyzed mortality ratio as the main population parameter to evaluate competition patterns in the larval stage, however we cannot estimate the impact of this competition on per capita growth rate. Furthermore, for the adult community we have the frequencies of interactions, but not their consequences on population growth. We estimate that high frequency interactions represent more time and energy spent on that type of interaction, which would consequently reduce interspecific interactions. Moreover, although our results present evidence of intraspecific density-dependence in the larval stage, our sampling design disregards the impact of predation by other species. Previous studies have shown that fish are one of the main predators of Odonata larvae and predation can have a stabilizing effect on the population growth rate, impacting population density and consequently reducing interspecific competition for resources (Chase *et al.* 2002).

Conclusion

The presence of stabilizing mechanisms can favor the coexistence of competing species, balancing inter and intraspecific effects. Higher intraspecific competition proved to be an important mechanism to decrease interspecific interactions and control larvae population density, which may occur due to spatial niche segregation or the presence of behaviors related to sexual selection. We conclude that several mechanisms may act together to structure the Odonata community in both life stages. Our study provided novel results on how coexistence mechanisms could vary along with ontogenetic development broadening our understanding of processes sustaining biodiversity in tropical ecosystems.

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Supplementary material

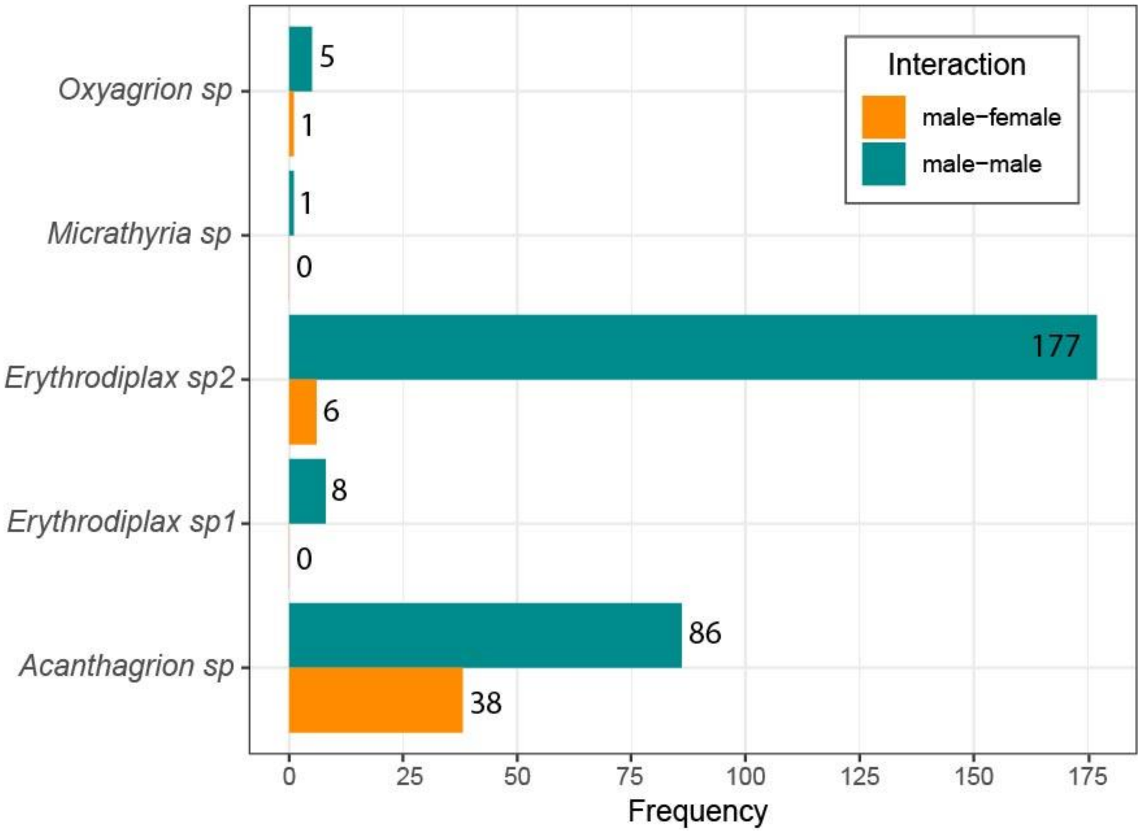

Figure S1. Number of intraspecific male-male and male-female interactions between Odonata adults.

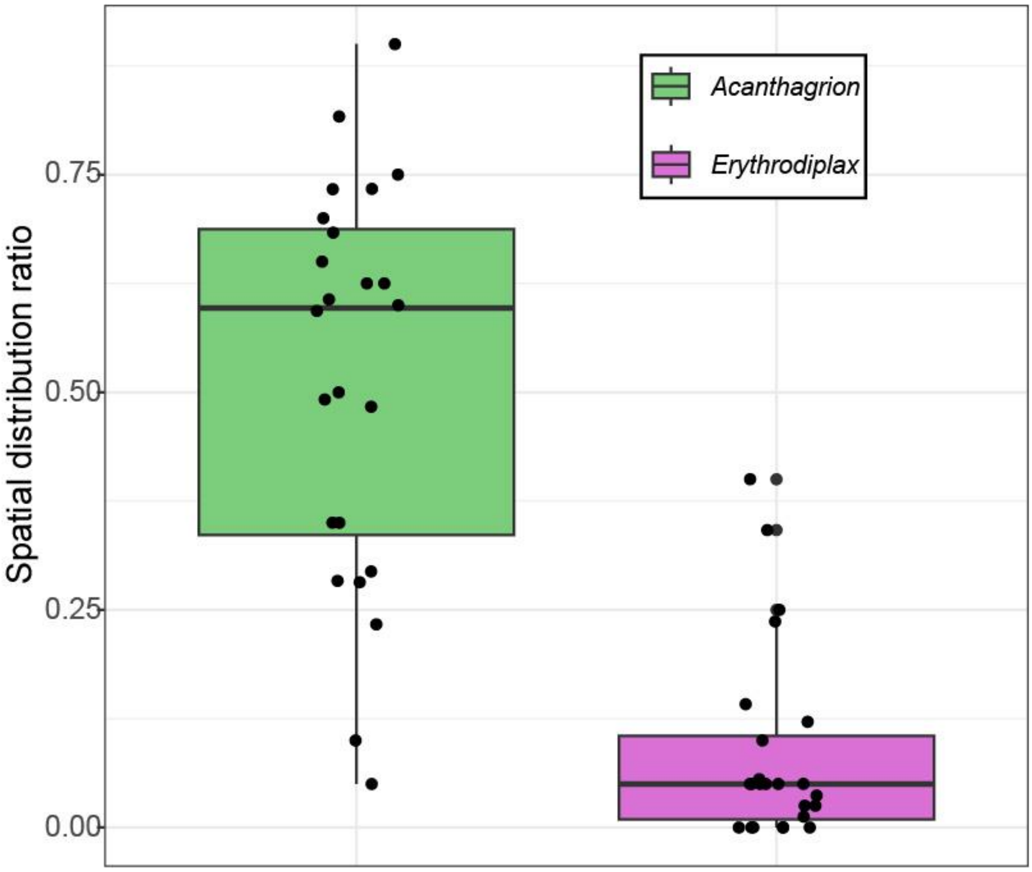

Figura S2. Spatial distribution ratio among *Acanthagrion* and *Erythrodiplax* larvae.

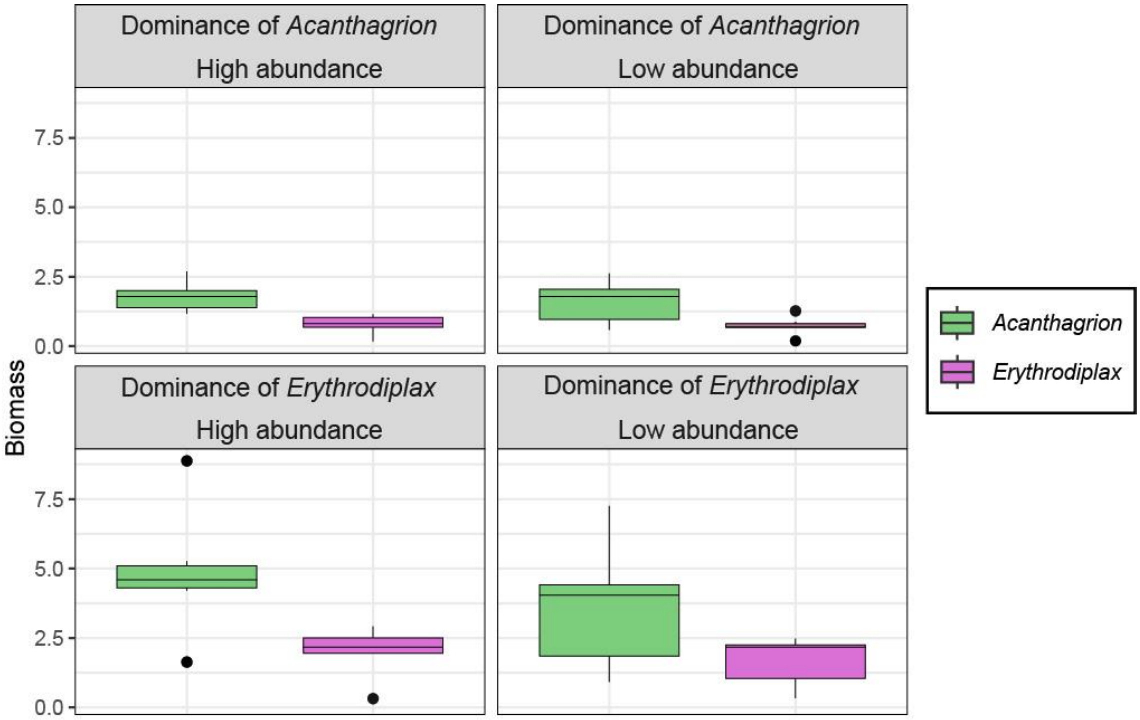

Figure S3. *Acanthagrion* and *Erythrodiplax* biomass for each treatment.