Wachholtz

Rhainer Guillermo[1], Leandro Juen[2]

Odonate ethodiversity as a bioindicator of anthropogenic impact

Abstract: The increasing use of dragonflies and damselflies as models in studies on biodiversity in the last decades has unraveled several features of natural processes and mechanisms for species conservation. Nevertheless, biodiversity is a polysemic concept that resolves multiple dimensions that, together, enroll what we observe as species and lineages diversity. One of these dimensions is Ethodiversity, which may represent the individual diversity of behavioral traits and higher organization levels. Hence, measures of Ethodiversity may be used as indicator tools to measure such dimensions of biodiversity. However, we still lack methods and protocols to measure this diversity. Therefore, here we addressed whether damselfly behaviors may act as indicators of environmental impacts. We collected behavioral data of 120 males in two sites, one in an ecological reserve and another in an impacted habitat. Our results show differences in behavioral syndromes and behavioral integrity when comparing populations in impacted and conserved environments. In conclusion, we hope that these results stimulate future endeavors to create a methodological framework to assess behavioral diversity.

Introduction

Variations in individual behavior have been widely addressed as behavioral syndromes (Sih, Bell, & Johnson, 2004). Several studies have shown multiple factors that affect such syndromes, from the biotic to the abiotic environments (Bókony, Kulcsár, Tóth, & Liker, 2012; Sommer-Trembo et al., 2017). For instance, rural birds may exhibit different syndromes related to neophobia and risk-taking than to urban birds (Bókony et al., 2012). Likewise, abiotic habitat parameters may affect syndromes in a fish (Sommer-Trembo et al., 2017). More recently, such behavioral variations have been recognized as one of the multiple components of biodiversity (Cordero-Rivera, 2017), varying across populations (Michelangeli, Chapple, Goulet, Bertram, & Wong, 2019). This point of view regards behavior as a component of biodiversity, considering, for instance, unique behaviors and behavioral richness as an expression of genetic diversity and integrity. Although behavioral traits are plastic, their expression's heterogeneity and the integration of multiple traits into syndromes may be disrupted in impacted populations, and even lead to the local extinction of behaviors and syndromes. For instance, what was once considered a plastic trait, recent evidence has shown that can be lost - human impact may lead to the loss of antipredator responses in several animal lineages (Geffroy et al., 2020). Therefore, behavioral conservation is becoming a necessary approach for maintaining natural behaviors for population stability and biodiversity conservation (Berger-Tal et al., 2016; Merrick & Koprowski, 2017).

One focus of behavioral conservation is to assess how the environment may shape or impact animal behavior (Kühl et al., 2019; Merrick & Koprowski, 2017). In this context, it is urgent to address which components, traits, or syndromes of behavior are sensitive to environmental degradation and changes (Moule, Michelangeli, Thompson, & Chapple, 2016). Such sensitive traits may become indicators of environmental quality and provide cues that show us whether populations are well enough to keep their natural conditions (Carere et al., 2014). A homogenizing process of behavior at the population level and the loss of specific behaviors may become useful tools for population status assessments. Nevertheless, since the recent development of this field of knowledge, the study of Ethodiversity

1 Lestes Lab, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil.
rhainerguillermo@gmail.com
2 Laboratório de Ecologia e Conservação, Instituto de Ciências Biológicas, Universidade Federal do Pará, Rua Augusto Corrêa, 01, Guamá, 66075-110, Belém, PA, Brazil. leandrojuen@ufpa.br

(Cordero-Rivera, 2017) and behavioral conservation (Berger-Tal et al., 2016) requires good examples of how to address these issues (Cordero-Rivera & Zhang, 2018; Oliveira Roque et al., 2019).

Calopterygid damselflies have been widely used as models to study evolutionary and ecological questions (Córdoba-Aguilar, Ruiz-Silva, Munguía-Steyer, & Lanz-Mendoza, 2011; Gołab, Śniegula, Drobniak, Zajac, & Serrano-Meneses, 2013; Rhainer Guillermo-Ferreira, Therézio, Gehlen, Bispo, & Marletta, 2014), mostly because of their well-marked territorial and courtship behaviors (Guillermo-Ferreira & Del-Claro, 2011; Guillermo-Ferreira & Bispo, 2012; Guillermo-Ferreira, Gorb, Appel, Kovalev, & Bispo, 2015). Males of these damselflies often engage in aerial contests, exhibiting wing displays towards rivals (Guillermo-Ferreira et al., 2015), and remarkable courtship displays, when males dance for females (Guillermo-Ferreira & Bispo, 2012). Moreover, males may adopt alternative strategies, attempting to force copulation by harassing females (Cordero-Rivera, Andrés, Cordero, & Andrés, 2002; Córdoba-Aguilar & González-Tokman, 2011) and patrolling the area after females instead of defending a territory (Raihani, Serrano-Meneses, & Córdoba-Aguilar, 2008). Individual variation on such behaviors may then indicate behavioral syndromes in damselflies (e.g., Debecker, Sanmartín-Villar, de Guinea-Luengo, Cordero-Rivera, & Stoks, 2016).

Therefore, here we addressed whether populations of a damselfly exhibit different compositions of behavioral traits in impacted and conserved habitats. We hypothesized that a population that faces anthropogenic pressures has its behavior negatively affected compared to a population that occupies an untouched habitat. Considering that behavioral traits should be positively correlated across populations (Bell, 2005), under the hypothesis that there is an evolutionary constraint on behavioral traits, correlations between such behaviors should be impacted in an anthropic habitat. We expected that male damselflies in a conserved area would exhibit more integrated behaviors, showing a diverse state and the presence of behavioral syndromes. We assumed behavioral integration as a measure of behavioral syndromes, i.e., animals should exhibit correlated behavioral traits under natural conditions (Harris, Eroukhmanoff, Green, Svensson, & Pettersson, 2011). We consider that populations should have a more diverse scenario and higher behavioral integration under low human impact in a natural population since environmental changes may change the correlation between traits (Schlichting, 1989). Likewise, males in an impacted area might have lost such integration. Our predictions are based on the assumption that gene expression is adjusted for multiple behavioral traits, consequently, behavioral (i.e., phenotypic) integration should be maintained in all populations (Schlichting, 1989). Although high integration may be an ecological and evolutionary constraint, low integration may result in less adapted phenotypes due to a lack of coordination between behavioral traits (Schlichting, 1989).

Material and Methods

Fieldwork was conducted with males of the damselfly *Mnesarete pudica* Hagen in Selys (Zygoptera, Calopterygidae) at two sites in March and July 2010, during the dry season. One site was a stream located at the Ecological Reserve of the "Clube de Caça e Pesca Itororó de Uberlândia" (CCPIU), Uberlândia, Minas Gerais, Brazil (15º57'S, 48º12'W; altitude 863 m; 640 ha). This stream is part of a Vereda, a wetland habitat typical of conserved Neotropical Savannahs (Cerrado) (Guillermo-Ferreira, Vilela, Del-Claro, & Bispo, 2016). The stream had 1m from border to border and a 1.5m depth. Close to the stream, there was a large pond covered by macrophytes (*Eleocharis* and *Pontederia*). Around the stream and the pond, there is dense dry forest, typical of Cerrado. This area has a history of Cerrado conservation for more than 30 years and is considered a haven for odonates, where more than 40 species can be found, including new species (Rhainer Guillermo-Ferreira et al., 2016).

The other stream was on a farm in Assis, São Paulo, Brazil (22º38'S, 50º27'W; altitude 522 m), used as water source for the cattle. The latter was impacted by agricultural activities, cattle herding and had a shallow pond (10x10m area). This stream also was 1m wide, but shallower (~0.20m depth). Behavioral observations were made from 10:00 (when males begin to fight and court females) to 15:00 (when sexual and fighting activity declines). In the study, point one was classified as preserved named Reserve and point two as impacted. Both sites were visited in March and July, during the dry season with hot and sunny weather on all days of fieldwork.

Behavioral observations consisted of 15 hours of data gathering at each site (ca. 7–10 days of fieldwork on each site), using the *focal animal* method (Altmann, 1974), watching each focal male for 15 minutes and noting their behavior according to the categories described in Table 1. These categories are described following previous observations and descriptions for other odonate species (Corbet, 1999; Guillermo-Ferreira & Bispo, 2012; Guillermo-Ferreira et al., 2015). Data was collected as counts of the frequency of each behavior exhibited by the focal male. Males were marked with white correction fluid on the thorax and abdomen. Wings were not marked because wing pigmentation may influence male behavior and wing marking could disturb the focal male normal behavior (Rhainer Guillermo-Ferreira et al., 2014). When not engaged in one of the behavioral acts, the male was considered inactive, because the male remained immobile at the perch. We marked and observed 60 males in each site, totaling 120 males.

Thirty minutes after marking the animal, we made behavioral observations. The construction of an ethogram for each male allowed the evaluation of the behavioral differences between populations. Analyses were conducted to evidence the interpopulation differences in the behavioral acts related to male investment in fighting ("Contest" and "Chase"), mate searching ("Patrol"), and mate acquisition strategy ("Courtship" and "Harassment"). Males fight each other with elaborated aerial displays (Contests) or brief flights chasing each other (Chase) (Guillermo-Ferreira et al., 2015). Males also patrol the area searching for mates (Patrol) and eventually court females exhibiting their wing display (Courtship) or attempt to mate with females by grabbing their wings without any courtship (Guillermo-Ferreira & Bispo, 2012; Pena-Firme & Guillermo-Ferreira, 2020).

Statistical analyses

We estimated Spearman correlations between the five behavioral traits for each habitat to represent the behavioral syndromes. To assess whether habitats differed in the relationship between behavioral traits, we compared the correlation coefficients statistically using T tests (Zar 1999). We also estimated the overall level of phenotypic integration between the behavioral traits for the five behavioral traits (Harris et al., 2011; Schlichting, 1989). We estimated the integration levels in each population as the average of the absolute phenotypic correlations (Cane, 1993).

To assess the variation on the five behavioral traits, we ran a Principal Components Analysis (PCA) (Legendre & Legendre 2012). Data was transformed (log(x+1)) to for normalization. A covariance matrix was then used for analysis. We used the Broken stick criterion to select the axis number, where the selected axis has an autovalue larger than the value randomly stipulated by the Brock stick. Hence, a Permutational Multivariate Variance Analysis (PERMANOVA, Anderson 2001) was used to analyze behavioral traits differences using the transformed behavioral traits that were used in the PCA. As a Euclidian distance matrix, we assumed a significance value of *p<0.05* (Monte Carlo test) with 999 permutations. PCA and PERMANOVA analyses were performed in the R program (R Core Team, 2016) using the *vegan* package (Oksanen et al., 2016).

Results

Using Spearman's correlation test, we found positive pairwise correlations between harassment-courtship, harassment-contest, contest-courtship, courtship-chase, and chase-patrol in the Cerrado population (Table 1). In the impacted population, we found only one significant positive pairwise correlation between chase-patrol (Table 1). The Cerrado population showed a higher mean correlation (t-test, t = 0.518, p = 0.016). The Cerrado (conserved) habitat population showed a mean level of phenotypic integration of 0.228, while the impacted habitat population had a mean level of phenotypic integration of 0.199.

Table 1. Spearman's correlation test for behaviors exhibited by males of *Mnesarete pudica* on conserved and impacted habitats. Significant results marked with * (* = p<0.05, ** = p<0.01). The results for the impacted population are in the lower part, marked in gray; the results for the population in conserved area are in the upper part; N = 60 males in each treatment.

	Harassment	Courtship	Contest	Chase	Patrol
Harassment	–	0.405**	0.408**	0.140	0.084
Courtship	0.128	–	0.141	0.426**	0.270
Contest	0.125	0.149	–	0.054	−0.047
Chase	0.200	0.276*	0.242	–	0.308*
Patrol	0.163	0.121	0.172	0.415**	–

The PCA explained 47% of the variation on behavioral traits in its first axis. Following the Broken stick criterion, only the first axis of the PCA was retained for analysis. The variables that most contributed to this axis's formation were Chase and Patrol, both positively related to the first axis (Figure 1). Thus, males from the site Impacted had higher values of Chase and Patrol when compared to the Reserve site. Although contributing less to the formation of the axis (low loading values), the other variables were also positively correlated with the first axis. The Permanova confirmed this pattern observed in the PCA, showing that habitat modifies behavioral traits (pseudo F = 22.629; R^2 = 0.162; p = 0.001).
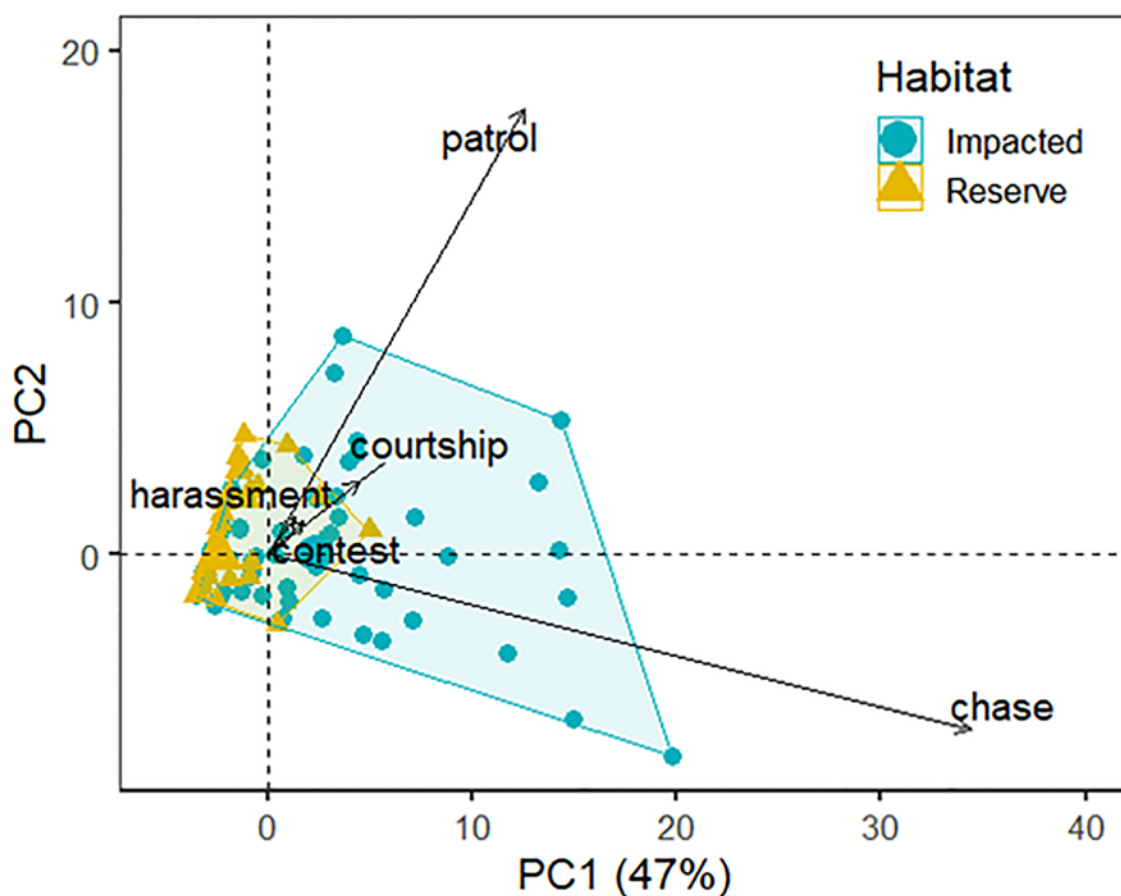

Figure 1. PCA analysis of the behavioral syndromes exhibited by males of *Mnesarete pudica* on conserved and impacted habitats. The value on axis 1 is the percentage of the variance explained.

Table 2 Behavioral traits that mostly contributed to form groups with the first two axes of the PCA. In bold the variables that most contributed to the formation of the axes.

	PCA 1	PCA 2
Harassment	0.235	
Courtship	0.574	
Contest	0.291	
Chase	**0.902**	
Patrol	**0.748**	
Broken stick	1.225	0.688
Autovalues	1.261	0.484
Explication (%)	46.9	

These relationships between traits seen in the pairwise correlations were confirmed by the projection of the factor-plane variables (Figure 1) with a principal component analysis (PCA). Data were log-transformed (so that it was on the same scale and no variable overly contributed). The PCA indicates that contest and harassment are within one behavioral syndrome (PC1); while males with another syndrome exhibit more patrolling flights, courtship displays and chase males away instead of aerial threat displays.

The results suggest that males of the two habitats (conserved or impacted) diverge in behavior (Figure 2). Males of the altered habitat exhibit more patrol and chase males more often than males in the conserved habitat. On the other hand, males in conserved areas exhibit more Harassment activities than males in altered areas (Figure 2).
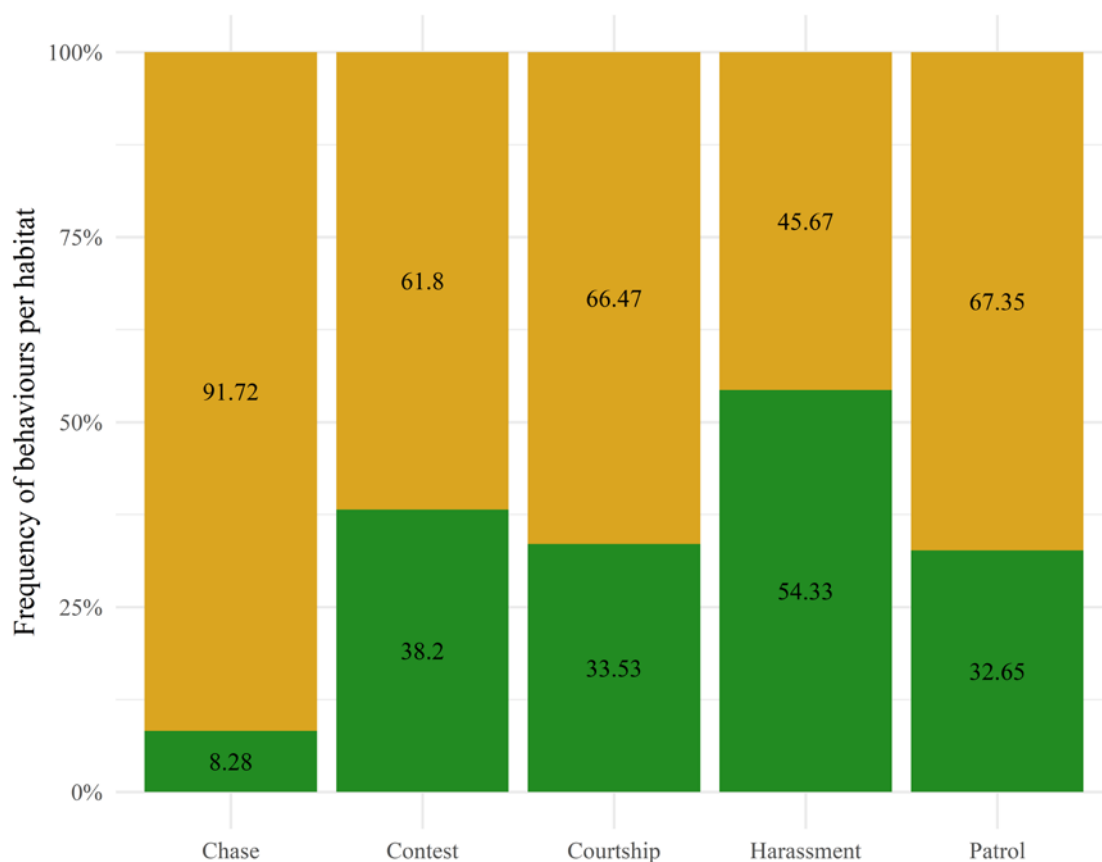
Figure 2. Frequency of the behavioral syndromes exhibited by males of *Mnesarete pudica* on conserved (green bars) and impacted habitats (yellow bars).

Discussion

The results show that behavioral traits, or syndromes, were not similar across populations. The occurrence of different behavioral syndromes may imply that selection may shape behavior according to an optimum in each habitat to maximize fitness. Some correlations may be favored in different habitats, whereas selection may result in maladaptive trait combinations (Sih, Bell and Johnson, 2004; Bell, 2005). For instance, the results show a lack of correlation between behavioral traits in an impacted habitat, while behaviors were mostly correlated in a conserved habitat. Lack of correlation contradicts the evolutionary constraint hypothesis, which states that behavioral traits should be positively correlated across populations (Bell, 2005; Schlichting, 1989). We argue here that the lack of correlation may result from the simplification of impacted environments, breaking stable evolutionary correlations between behavioral traits.

It is well known how impacted habitats may affect biodiversity. Nevertheless, how the behavioral dimension of this biodiversity is affected is still poorly known. We know that habitat may alter the covariation between behaviors (Dalesman, 2018) and reduce behavioral diversity (Kühl et al., 2019). When populations occupy novel environments, correlations between behavioral traits may change. Evidence suggests that genetic correlations that underlie behavioral syndromes may change rapidly when in a novel environment (Karlsson Green, Eroukhmanoff, Harris, Pettersson, & Svensson, 2016). The correlation between behaviors may also be influenced by the presence of different selective pressures, such as different species of predators, population densities and different habitat composition (Harris et al., 2011). Indeed, the Cerrado habitat here studied (the conserved habitat) had a richer community of predators and other odonate species, while the impacted habitat usually had higher population densities of *M. pudica*. A significant limitation of our study is that we sampled only two populations, hence, behaviors may have been affected by spatial effects and local factors. Another limitation is the fact that females were not surveyed. This of course might account for the fact that there was more courting behavior in the undisturbed habitat (i.e. if females were more scarce in the disturbed habitat). However, behavioral integrity is predicted to be constant across populations, independently of such variations.Further studies with a wider set of replicated populations are necessary to evaluate the generality of the methods applied for behavioral conservation and Ethodiversity measures. Behaviors are sensitive to anthropogenic changes in the environment since whenever the environment is heterogeneous, whether in time or space, selective pressures generate local adaptations increasing diversity (Cordero-Rivera, 2017). As examples, behavioral syndromes in song sparrows break down in impacted urban areas (Scales, Hyman, & Hughes, 2011); human impact may select for more aggressive owls in urban environments, breaking natural behavioral syndromes (Moroni, Crivelaro, Soares, & Guillermo-Ferreira, 2017); anthropization may change foraging behavior in lizards (Moule et al., 2016). Therefore, alterations of anthropic origin may affect the mating systems and these individuals' choices and, consequently, species richness, abundance and distribution in these altered places. Our results show that in impacted environments, *Mnesarete pudica* males invested more in the chase and patrol flights. These behaviors refer to investments in fights but also in search of mates. This difference in effort or energy in territory defense may be draining energy that could be invested in reproduction, on the other hand, this could also explain why individuals are spending most of their time looking for mates.

One of the most efficient ways to assess anthropogenic changes in aquatic systems is by using bioindicators, which are organisms or communities whose vital functions correlate closely with certain environmental factors, that any environmental change can lead to biological changes (Roque et al., 2014). Among these, Odonata stands out for their high sensitivity to environmental changes (Carvalho, Pinto, Oliveira Júnior, & Juen, 2013; Silva Monteiro Júnior, Couceiro, Hamada, & Juen, 2013ª; Oliveira-Junior et al., 2015; Oertli, 2008; Oliveira-Junior & Juen, 2019; Rodrigues et al., 2016). Within the order, there is a great distinction between the ecophysiological requirements of species (De Marco, Batista, & Cabette, 2015), however, distribution patterns and the response to habitat traits are varied depending on species specificity (Miguel, Oliveira-Junior, Ligeiro, & Juen, 2019; Oliveira-Junior et al., 2017; Pereira et al., 2018; Basto et al. 2021). There is even a ratio between the two Anisoptera / Zygoptera suborders as an index to assess environmental changes (Oliveira-Junior & Juen, 2019). Thus, the structure of the assembly can be changed due to changes in the environmental conditions and variables (Corbet, 1999; Silva Monteiro Júnior, Couceiro,

Hamada, & Juen, 2013b; Juen, Cabette, & De Marco, 2007; Juen & De Marco, 2011). In conclusion, our results indicate that behavior variations can also be used as a biomonitoring tool and as a strategy for Odonata conservation. We hope that these results stimulate future protocols of Ethodiversity measures.

Acknowledgements

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