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Long-term stability of the hawkmoth fauna (Lepidoptera, Sphingidae) in a protected area of Brazilian Atlantic Rain Forest

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Abstract

Hawkmoths are an important component of tropical ecosystems, with significant roles as herbivores and pollinators. These moths can be used as indicators in biodiversity assessments because they can be easily sampled and identified. However, hawkmoths have seldom been surveyed over the long term for this purpose, especially in the Neotropical region. Considering that long-term datasets are of indisputable importance for understanding and monitoring temporal changes in biodiversity, this study assessed long-term changes in the hawkmoth fauna in a protected Atlantic Rain Forest area over a period of 64 years. We used historical and recent empirical datasets to ask whether faunal-diversity patterns and species composition have changed over time. We used individual- and sample-based rarefaction and extrapolation curves based on Hill number (diversity order of q = 0) to compare species richness, and the probability version of the abundance-based Chao-Jaccard index to assess beta diversity over time. To assess changes in faunal composition, we conducted a nonmetric multidimensional scaling analysis and performed an analysis of similarities to test whether the community composition has changed. Our results clearly showed long-term stability of the hawkmoth community over the 64 years, despite the growing human-induced landscape changes that occurred in the region surrounding the study area during the last 6 decades. This study emphasizes the importance of large remnants of Atlantic Forest for long-term maintenance of both functional diversity and ecosystem functioning.

Keywords Atlantic Forest · Brazil · Diversity · Insect conservation · Pollinator · Sphingid

Introduction

Human impacts on the environment have led to accelerated biodiversity loss, with harmful consequences to many ecosystem processes and functions (Vitousek et al. 1997; Butchart et al. 2010; Pereira et al. 2010; Dirzo et al. 2014). Tropical ecosystems face the most critical conditions because they hold most of the world biodiversity and are subject to the highest levels of anthropogenic degradation (Dirzo and Raven 2003; Bradshaw et al. 2009; Gibson et al. 2011). The Brazilian Atlantic Forest is one of the most diverse and threatened ecosystems in the world (Mittermeier et al. 1998; Myers et al. 2000). The Atlantic Forest harbors almost 16,000 plant species, more than 1360 species of non-fish vertebrates, and almost 300 species of mammals; for some groups, the number of endemic species to the Atlantic Forest exceeds 40% of the known world diversity (Myers et al. 2000; Stehmann et al. 2009; Paglia et al. 2012). However, its original area has been reduced to less than 16% and the remnant area is scattered in more than 245,000 fragments, most of them (> 80%) smaller than 50 ha (Ribeiro et al. 2009). Hence, in spite of the great importance of the Atlantic Forest for Brazilian and global biodiversity, its conservation status is critical (Myers et al. 2000; Ribeiro et al. 2009).

Due to the enormous and still poorly known diversity of insects in Brazil (Rafael et al. 2009), the actual rates of insect endemism in the Atlantic Forest are not available.

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However, the outstanding species richness of some insect groups, such as ants (800 species), click beetles (1000 species) and longhorn beetles (10,000 species), highlights the importance of this biome for the diversity and conservation of insects (Brown Jr. 1997). Considering that environmental complexity (or heterogeneity) is an important factor affecting animal diversity (Haddad et al. 2001; Brose 2003; Tews et al. 2004; Lassau et al. 2005; Ignatov et al. 2011), the progressive loss of the Atlantic Forest is an obvious threat to the underlying ecological processes involving insects, such as pollination services (Cruz-Neto et al. 2011; Gonçalves et al. 2014).

Hawkmoths (Sphingidae) are among the best-studied lepidopteran groups in the world (Kitching and Cadiou 2000). These moths comprise approximately 1400 species distributed worldwide (van Nieukerken et al. 2011), with the highest diversity concentrated in tropical areas (Kitching and Cadiou 2000). Hawkmoths are an important component of tropical ecosystems, with significant roles as herbivores and pollinators (Haber and Frankie 1989; Darrault and Schlindwein 2002; Johnson et al. 2017). Long-tongued hawkmoths are the sole pollinators of plant species with specialized flowers (i.e., long flower tubes), which rely completely on these moths for sexual reproduction (Nilsson et al. 1987; Martins and Johnson 2013; Johnson and Raguso 2016). A low abundance of long-tongued hawkmoths in the Atlantic Forest may lead to plant-pollinator mismatches (Moré et al. 2012), hindering plant reproduction (Amorim et al. 2014). Since herbivores and pollinators are important components of ecosystem functioning, the conservation of the functional diversity of the Atlantic Forest depends on the conservation of invertebrates such as hawkmoths.

Hawkmoths can be easily sampled and identified, facilitating their use as indicators in biodiversity assessments at local, regional and global scales (see Beck et al. 2006a, b, c, d; Amorim et al. 2009; Ignatov et al. 2011; Ballesteros-Mejia et al. 2013; Camargo et al. 2016). However, despite the indisputable importance of long-term assessments for understanding and monitoring temporal changes in biodiversity (Wolfe et al. 1987; Nilsson et al. 2008; Magurran et al. 2010), hawkmoths have seldom been surveyed for this purpose, particularly in the Neotropical region (Wolda 1983; Wolfe et al. 1987; Conrad et al. 2004; Magurran et al. 2010). The hawkmoth fauna of the Boraceia Biological Station (BBS), a conservation unit situated within the Serra do Mar State Park (SMSP), which is the largest remnant of Atlantic Forest in Brazil, was systematically surveyed in the late 1940s and early 1950s, based on monthly collections (Travassos Filho and Camargo 1958; but also see Duarte et al. 2008). Since then, the area surrounding the BBS has experienced pronounced human population growth and urbanization. The municipality of Salesópolis, where the BBS is located, has doubled in population. The metropolitan region of São Paulo city (which is among the 10 World's largest urban areas), located less than 50 km from the BBS (Fig. 1), has experienced an eightfold population growth, from 2.6 million in the 1950s to 21.2 million in 2017 (IBGE 2017; Prefeitura de São Paulo 2018, but see also Online Resource 1).

Although the BBS has been a protected area since the 1950s, we do not know whether the human-induced landscape changes over time in its adjoining region have affected its fauna. Also, among the widely known anthropogenic drivers related to insect declines, such as habitat loss and climate change (Dirzo et al. 2014), artificial light at night has been proven to be another challenge for nocturnal insects (MacGregor et al. 2015; Knop et al. 2017; Irwin 2018; Wilson et al. 2018). Especially for hawkmoths, which are long-distance flight insects that can migrate between adjacent habitats and biomes (see Janzen 1986, 1987; Amorim et al. 2009, 2014), surrounding disturbances may affect their faunal dynamics. In this context, the aim of the present study was to assess the long-term dynamics of the hawkmoth fauna in an Atlantic Rain Forest area over a period of 64 years. We used historical (1948–1950) and recent empirical (2012–2013) surveys to ask whether the (1) faunal diversity patterns and (2) species composition have changed over time.

Methods

Study site and data collection

The study was conducted in an area of highland Atlantic Rain Forest (Montane Ombrophilous Dense Forest) at the Boraceia Biological Station (BBS; 23°39′15″S, 45°53′22″W; 850 m a.s.l.) located in the municipality of Salesópolis, state of São Paulo, southeastern Brazil (Fig. 1). The climate is Subtropical humid (Cfa or Cfb according to Köppen 1948), and the BBS is among the wettest areas in Brazil with a mean annual rainfall above 3300 mm and not less than 60 mm per month, even during the driest months (DAEE 2016). The BBS lies within the nature reserve of the Companhia de Saneamento Básico do Estado de São Paulo (Sabesp), which covers an area of 16,450 ha. This reserve is part of the Serra do Mar State Park (SMSP), the largest remnant of Atlantic Forest in Brazil, with a total area of about 332,000 ha (Fundação 2017). The BBS has been maintained as a conservation unit since 1954 (Category ib: Wilderness Area, according to IUCN), 23 years before the establishment of the SMSP in 1977 (see Duarte et al. 2008 for further details).

The historical survey comprised 27 monthly trips for moth collection in light-traps between January 1948 and March 1950 (details in Travassos Filho and Camargo 1958;

Fig. 1 Location of the Boraceia Biological Station. **a** Brazil, with the state of São Paulo in dark gray. **b** State of São Paulo, showing the area detailed in c. **c** Location of the Boraceia Biological Station (star) in the Serra do Mar State Park (large outlined green area). Forest areas are represented in green, urban areas in dark gray and areas converted to pastures or agricultural landscapes in light gray. Numbers indicate the cities of São Paulo (*1*), Mogi das Cruzes (*2*), Biritiba-Mirim (*3*) and Salesópolis (*4*). The metropolitan region of São Paulo city includes the four cities. (Color figure online)
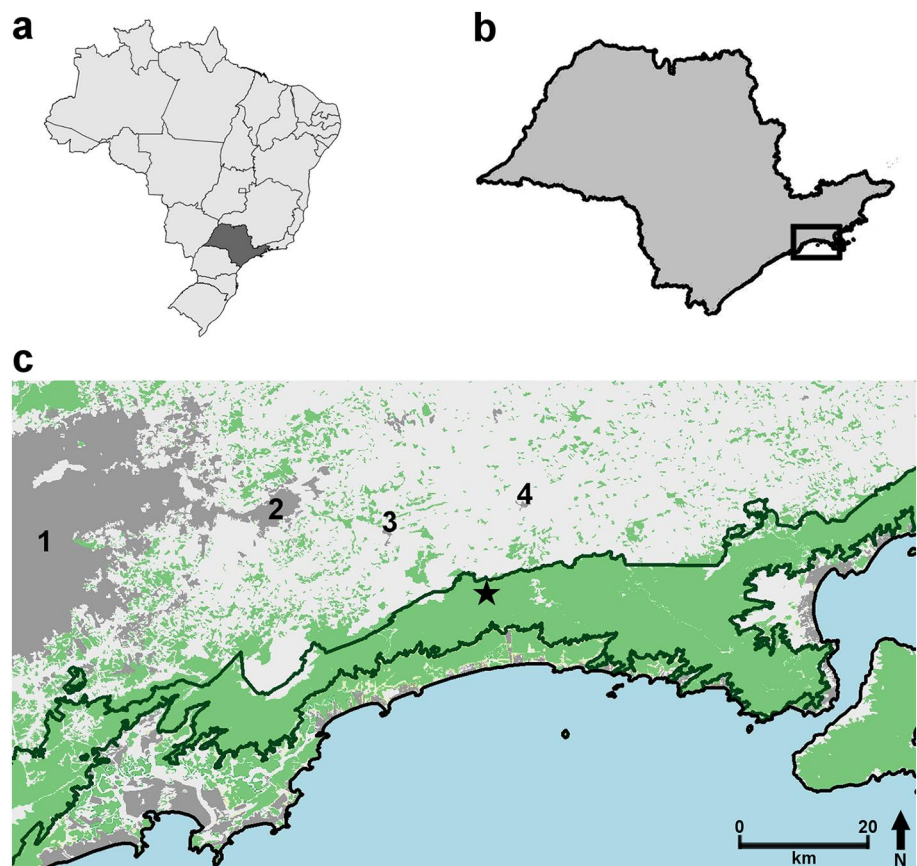
Duarte et al. 2008). Specimens from this survey are available at the Museu de Zoologia da Universidade de São Paulo (MZUSP), and a comprehensive list of the species collected at that time was provided by Duarte et al. (2008). We reassessed this collection at the MZUSP, to update the hawkmoth classification and also to correct possible misidentifications. We carried out genitalia examinations of a group of cryptic species of the genus *Manduca*, with the goal of generating a more accurate identification of the hawkmoth fauna from the historical survey.

The recent data collections were carried out monthly from July 2012 to June 2013, during four consecutive nights each month (except for December 2012, when only three nights of collections were performed) during the new-moon phase, totalling 47 nights of collection. Hawkmoth collections lasted for 12 h, from late afternoon (18:00 h) until the following morning (06:00 h), using eight outdoor lights (250 W mixed UV-rich bulbs) at one of the BBS buildings ("Residência dos Biologistas" *sensu* Travassos Filho and Camargo 1958, exactly the same location where specimens were collected in the historical survey). We sampled all individuals attracted to the light-traps, collecting them manually or with the aid of insect nets. Moths were killed with an injection of ammonia (10% aqueous solution), administered ventrally in the membrane between the thorax and abdomen. All hawkmoths collected were mounted, labelled and included in the Lepidoptera collection of the MZUSP. Moths were identified with reference to illustrations and identification keys (D'Abrera 1986; Kitching and Cadiou 2000; Moré et al. 2005; Martin et al. 2011; Kitching 2017), and also by examining the genitalia of cryptic species. We followed Kitching and Cadiou (2000) and Kitching (2017) for hawkmoth nomenclature and classification.

Diversity pattern analyses

To assess whether the diversity pattern changed over time (between historical and recent surveys), we followed Chao et al. (2014) to calculate individual- and sample-based rarefaction and extrapolation curves based on Hill number of the diversity order of $q = 0$ (species richness; see Chao et al. 2014). To make realistic comparisons between the assemblages of the two collection periods, we extrapolated the smallest reference sample (the historical survey for individual-based analysis and the recent survey for the sample-based analysis) up to the same number of individuals or samples in the largest reference sample. For the sample-based analysis, we used each monthly sample as a sampling unit (Amorim et al. 2009). To obtain 95% confidence intervals for all rarefied and extrapolated curves, we used a bootstrap

method with 200 replications (for details see Hsieh et al. 2016). Rarefaction and extrapolation analyses were performed using the *iNEXT* package in R programming language (Hsieh et al. 2016).

Sample incompleteness can affect the assessment of species richness (Gotelli and Colwell 2001), since richness is dependent on the abundance, which in turn is highly affected by short-term environmental conditions (see Yela and Holyoak 1997). Even though the historical survey had double the number of sampling months as those in the recent survey, it had only 35% of the number of hawkmoth individuals collected in 2012–2013 (see "Results"). This suggests that the abundance of hawkmoths collected in the historical survey may have been affected by factors such as fewer collection hours per night or even a selective collection of moths. Hence, in order to compensate for the effect of abundance on the assessment of diversity and improve the evaluation of the faunal changes over time, we calculated Fisher's α diversity index for each month of collection and used Student's *t*-test to compare the mean values of Fisher's α between the two survey periods (see Beck et al. 2006a). Finally, we also assessed the β-diversity as a measure of species turnover between the two periods. A reliable estimation of β-diversity must be independent of biases caused by sample size and unequal sampling effort (Barwell et al. 2015). In order to correct for these biases, we used the probability version of the abundance-based Chao-Jaccard index, which incorporates the effect of "unseen" shared species (Chao et al. 2005, 2006, but see also; Melo 2016). For computing $\beta_{Chao-Jaccard}$ we used the *dis.chao* function in the *CommEcol* package in R (Melo 2016). The $\beta_{Chao-Jaccard}$-diversity index varies from 0 to 1, in which values close to one indicate higher species turnover.

Faunal composition analyses

To assess changes in faunal composition between the two periods, we first conducted a nonmetric multidimensional scaling (NMDS) analysis using the Sørensen similarity index (Legendre and Legendre 1998). Since temperature and rainfall affect the hawkmoth abundance and species-richness distribution throughout the year (Amorim et al. 2009), in order to distinguish the wetter/warmer period (rainfall and temperature above 300 mm and 18 °C, respectively) from the less wet/colder one (rainfall and temperature below 200 mm and 16 °C), we treated each collection month as a separate sampling unit. For this purpose, we pooled the data from each year of the historical survey together by month. Therefore, each sampling unit corresponds to the total species composition recorded in a specific month regardless of the year of collection. Since the Sørensen coefficient takes into account only incidence data (presence/absence) and we intended to assess the long-term faunal change, this data

grouping provides a reliable comparison between the two periods (Legendre and Legendre 1998). In order to compute the NMDS ordination we used the *metaMDS* function in the *vegan* package in R (for details see Oksanen et al. 2017).

To assess how environmental variables affect the distribution of the hawkmoth fauna throughout the year, we fitted temperature and rainfall surfaces to the resulting NMDS ordination. For this, we used the *ordisurf* function in the *vegan* package in R (Oksanen et al. 2017), which applies generalized additive models in the *gam* function. This function uses thin-plate splines to fit smooth surfaces over the ordination diagram (Wood 2003), allowing better visualization of the effect of environmental variables on the hawkmoth distribution. However, since temperature and rainfall are highly correlated at the BBS (Pearson's correlation $r = 0.88$, $p < 0.0001$), we present only the results for rainfall, because the pattern observed is quite similar to that observed for temperature. In order to test whether seasonality affected the distribution of hawkmoth species throughout the year, we performed a permutational multivariate analysis of variance (PERMANOVA). This analysis tested for significant differences in the faunal composition between the wetter/warmer period and the less wet/colder one. We used the *adonis2* function in the *vegan* package in R to conduct the PERMANOVA test (Oksanen et al. 2017).

Finally, to test whether the community composition has changed over time, we performed an analysis of similarities (ANOSIM). We used the *anosim* function in the *vegan* package in R, which provides results that better reflect the pattern observed in the NMDS ordination (see Oksanen et al. 2017). ANOSIM statistics (R) generate values between 0 and 1, where a value close to 1 would indicate a distinct separation between the periods (historical and recent surveys) and a value close to 0 would suggest no separation (Clarke 1993). The significance of the R value was calculated by permutation of each month of collection (10,000 permutations), which generates a null distribution of R values (for details see Clarke 1993 and; Oksanen et al. 2017). All analyses were performed in R programming language (R Core Team 2017).

Results

Historical and recent surveys

In total, we recorded 76 hawkmoth species in the two sampling periods at the Boraceia Biological Station. Eleven species occurred exclusively in the historical survey, 14 were exclusive to the recent one, and 51 were recorded in both the historical and recent surveys (Online Resource 2). Only one genus (*Lintneria*) was exclusive to the historical survey, while five genera (*Hemeroplanes, Hyles, Isognathus, Madoryx* and *Orecta*) were recorded exclusively in the

recent survey. All the exclusive genera were represented by a single species, with the exception of *Hemeroplanes* which was represented by two species (Online Resource 2). During the historical survey, a total of 877 hawkmoths belonging to 62 species were collected. A detailed description of the hawkmoth fauna sampled during the historical survey was provided by Duarte et al. (2008), and an updated and corrected list of species is presented in Online Resource 2.

In the recent survey we sampled a total of 2509 moths belonging to 65 species, distributed in 22 genera, six tribes and three subfamilies (Online Resource 1). Four genera were responsible for 73% of all moths collected in this survey. *Xylophanes* was the most abundant genus, totaling about 29% of all moths collected, followed by *Erinnyis* (15%), *Adhemarius* (14.8%) and *Manduca* (14%). *Xylophanes* also showed the largest number of species (13 species, 20% of the total), followed by *Manduca* with eight species (ca. 12%). *Adhemarius eurysthenes* was the most abundant species, with 306 individuals collected in total (ca. 12%).

Diversity pattern analyses

Both the individual- and sample-based extrapolation curves showed no difference in species richness between the recent and historical surveys (Fig. 2). However, due to the differences in the number of individuals and sampling months between the historical and recent surveys, rarefied down (interpolation) curves showed an inverse pattern when comparing individual-based curves to sample-based ones (Fig. 2). The historical survey recorded 62 species with only 877 individuals sampled over 27 months, while the recent survey recorded 65 species with 2509 individuals over 12 sampling months. These differences caused the historical survey to show an apparently higher species richness in the individual-based rarefaction and a smaller species richness in the sample-based rarefaction. Nevertheless, Fisher's α diversity index showed no difference between the two surveys ($t = -1.283$; 37 d.f.; $p = 0.208$, Fig. 3), which also agreed with the very low species turnover ($\beta_{\text{Chao-Jaccard}} = 0.036$).

Fig. 2 Individual- and sample-based rarefaction (solid lines) and extrapolation (dotted lines) curves with Hill numbers of the diversity order of $q = 0$ for the recent (pink) and historical (blue) surveys of hawkmoths at the Boraceia Biological Station. The smallest reference samples (historical survey for the individual-based analysis and the recent survey for the sample-based analysis) were extrapolated up to the same number of individuals or months of collection as the reference sample. Shaded areas show the 95% confidence intervals for each sample after 200 bootstraps. (Color figure online)
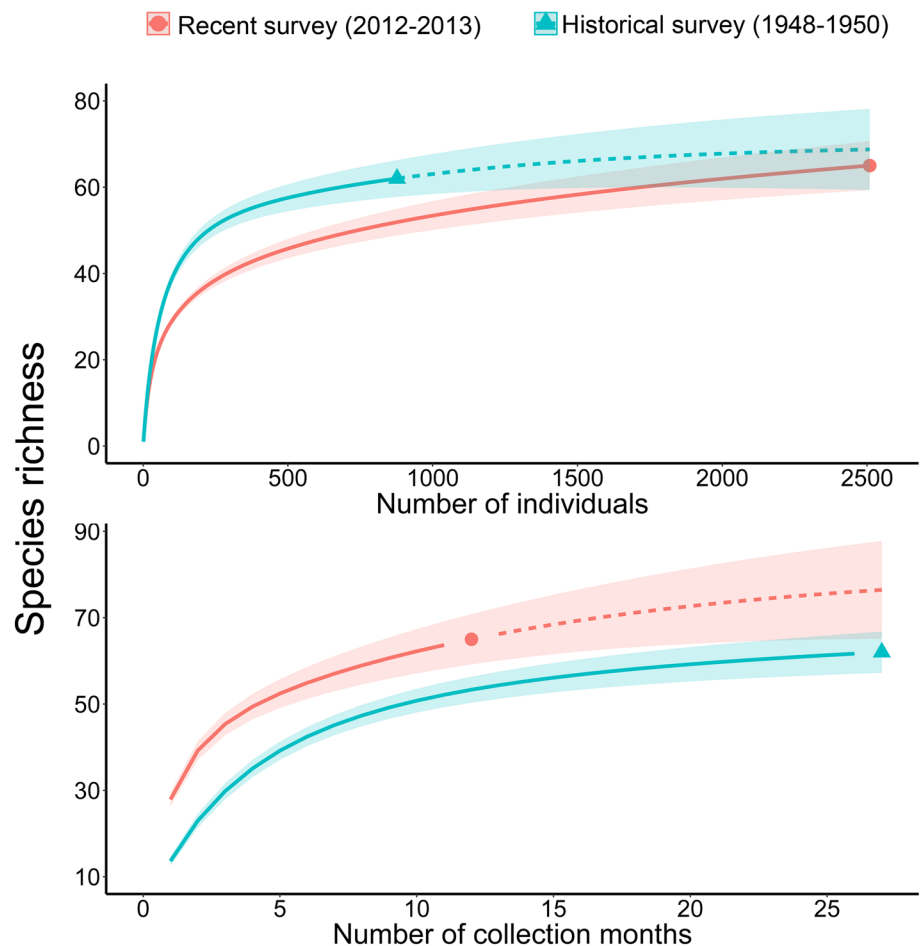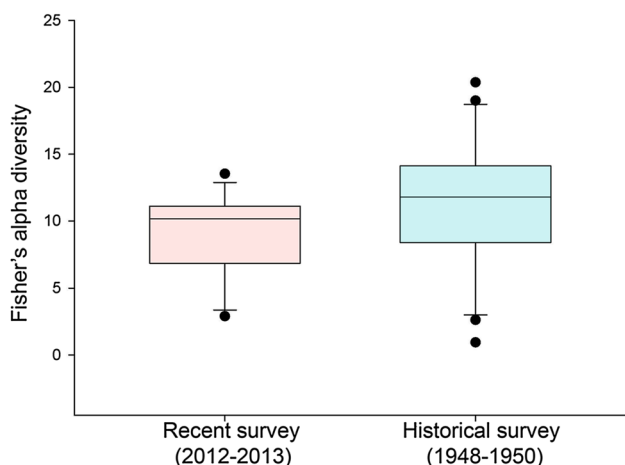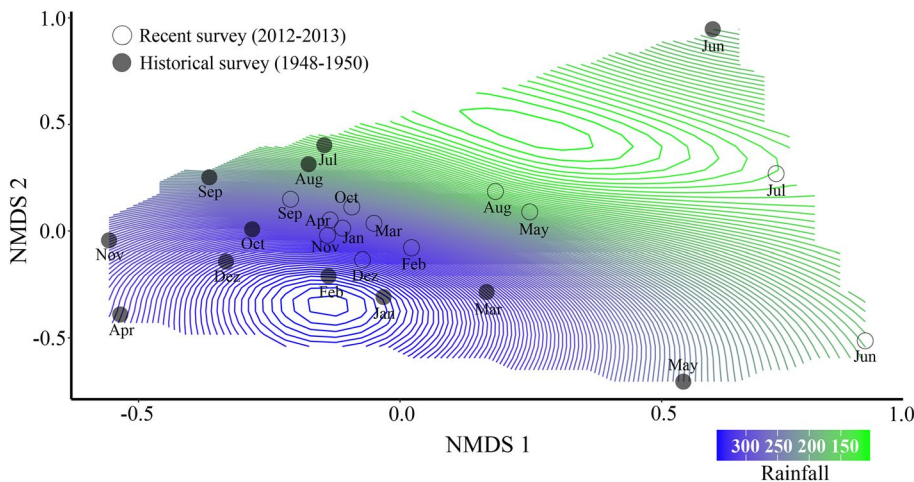
Fig. 3 Comparison of Fisher's α diversity index for each month of collection during the recent and historical surveys at the Boraceia Biological Station. Student's *t* test showed no difference between the two sampling periods ($t = -1.283$; 37 d.f.; $p = 0.208$). Boxes represent 25–75% percentiles, horizontal line inside each box represents the median, whiskers represent 10–90% percentiles, and dots represent outliers. (Color figure online)

Faunal composition analyses

For both the historical and recent surveys, the NMDS analysis revealed a clear separation between the drier/colder months (May to August) and the wetter/warmer ones (stress 0.17, $R^2 = 0.903$; Fig. 4). The PERMANOVA also confirmed the differences between seasons, independently of the survey period (Pseudo-$F = 2.06$, $p = 0.0072$, 10,000 permutations). However, we found no separation in the overall composition of the hawkmoth fauna between the historical and recent surveys (Fig. 4). The analysis of similarities (ANOSIM) also did not support a separation in the community composition of hawkmoths between the two surveys ($R = 0.17$, $p = 0.0009$, 10,000 permutations).

Discussion

To the best of our knowledge, this study is the first long-term assessment of hawkmoths in the Neotropical realm. Our results show a clear long-term stability of the hawkmoth community over a period of 64 years in an area within the largest remnant of Atlantic Forest in Brazil. Despite the growing human-induced landscape changes that have occurred in the region surrounding the study area (within a radius of less than 50 km) during the last 6 decades, the large size of the forest within which the BBS is located may have provided ideal conditions for long-term maintenance of the hawkmoth fauna. Since habitat disturbance is known to affect the species composition and community structure of hawkmoths in the Brazilian Amazon Forest (Camargo et al. 2016), it is reasonable to infer that small fragments of Atlantic Forest might be under similar pressure, resulting in loss of biodiversity and changes in the faunistic composition and community structure, as observed for other insect groups (Gonçalves et al. 2014; Justino et al. 2016; Shuey et al. 2017).

Forest size is an important determinant of hawkmoth species richness in the tropical rain forest of Southeast Asia (Beck et al. 2006c). In this region, habitat disturbance also affected the hawkmoth faunal composition, leading to a decrease in species of the subfamily Smerinthinae (which comprises short-distance flying species), while those of Macroglossinae (which includes long-distance flying species) showed the opposite pattern (Beck et al. 2006a). In Brazil, highly disturbed Atlantic Forest areas in the Northeast region also have some of the lowest hawkmoth species richness and diversity, compared to other tropical areas in South and Central America (see Amorim et al. 2009). Large remnants of Atlantic Forest are of higher conservation priority (Ribeiro et al. 2009) because they maintain important ecological processes capable of

Fig. 4 NMDS ordination for monthly species composition from historical and recent surveys of the hawkmoth community at the Boraceia Biological Station. Surfaces of rainfall were fitted over the NMDS ordination diagram (stress 0.17) to highlight the effect of rainfall on the distribution of hawkmoths throughout the year. (Color figure online)

long-term species maintenance (especially the most sensitive species, as was the case with the subfamily Smerinthinae in the Southeast-Asian hawkmoth assemblages studied by Beck et al. 2006a, c).

Of the eight species of Smerinthinae sampled at the BBS, 50% occurred in both periods of collection, one was exclusive to the historical survey (*Adhemarius gagarini*) and three were exclusive to the recent survey (*Adhemarius daphne, A. palmeri* and *Orecta lycidas*). Of the Macroglossinae, 73% occurred in both periods, while only 10.3 and 16.7% were recorded exclusively in the historical and recent surveys, respectively. A similar pattern was observed for species of the subfamily Sphinginae (which comprises the long-tongued and long-distance flying species). The very low Chao-Jaccard β-diversity index, which incorporates the effect of "unseen" shared species, indicated that this variation in species composition over time is related mainly to the occurrence of rare species that might have been present in both surveys but were recorded in only one of them. Some hawkmoth species are likely to have naturally low population densities (Janzen 1984; Kitching and Cadiou 2000) and also were recorded only as singletons (i.e., species represented by a single individual in the whole sample) in other Atlantic Forest areas in the Serra do Mar range (Laroca and Mielke 1975; Marinoni et al. 1999). Similar circumstances were observed for a temperate forest ecosystem in North America: of a total of 571 moth species collected, more than 100 were recorded only as singletons or uniques (i.e., species that occurred in only a single sample), even with a sampling effort as high as 52 nights and more than 14,000 specimens over 2 years (Summerville and Crist 2005).

Long-term changes in the composition of hawkmoth species in a Southeast-Asian tropical rain forest are likely to be related to climate change in addition to habitat disturbance (Beck et al. 2006a). Although we have no empirical support for the supposition that climate change can directly affect the hawkmoth fauna in the Atlantic Forest, it is well known that this factor strongly affects the distribution of plant communities in this biome (Salazar et al. 2007; Colombo and Joly 2010). Since vegetation is an important factor determining hawkmoth distribution and faunal composition (Ignatov et al. 2011; Beck et al. 2006c), the long-term stability of the hawkmoth fauna observed at the BBS highlights the importance of protecting large areas of mature Atlantic Forest such as the SMSP (Ribeiro et al. 2009).

Our results revealed a clear absence of long-term changes in both the faunal composition and species diversity of hawkmoths over a period of 64 years in a Brazilian Atlantic Forest area. Selective collection, however, was probably carried out during the historical survey, since the individual-based rarefaction curve showed a markedly faster increase in species richness, and the opposite pattern was observed for the sample-based rarefaction curve. Notwithstanding this possibility, the extrapolation curves and Fisher's α diversity index (which compensate for the effect of abundance on the assessment of diversity) showed that the alpha diversity has not changed over time. The sole differences observed are related to seasonality, which separated the hawkmoth fauna of the wetter/warmer period from the less wet/colder period in both surveys. Seasonality is an important factor affecting the annual distribution of other hawkmoth assemblages across the Americas (Janzen 1984; Haber and Frankie 1989; Amorim et al. 2009; Vieira et al. 2015; Sazatornil et al. 2016).

But what does the absence of long-term changes in both diversity and composition of hawkmoths (even at the subfamily level) entail for the ecosystem structure and functioning? Hawkmoths are part of complex mutualistic networks with the plants they pollinate (Johnson et al. 2017; Sazatornil et al. 2016), and some of these plants rely exclusively on long-tongued hawkmoths for sexual reproduction. Therefore, the long-term stability of hawkmoths may translate into the maintenance of both plant and pollinator functional diversity in the Atlantic Forest. Functional diversity is directly related to ecosystem functioning (Tilman et al. 1997; Gagic et al. 2015), and pollinator loss, especially the loss of long-tongued species, can lead to high and extreme pollen limitation, hindering the reproduction of both herbs and trees in the Atlantic Forest, including endemic species (Amorim et al. 2014).

In conclusion, even though we found no changes in the hawkmoth community over the last 6 decades, we do not know whether the hawkmoth-plant interaction network also remained stable over time (e.g., Burkle et al. 2013). The Brazilian population has quadrupled since the 1950s, especially in densely urbanized areas such as the metropolitan region of São Paulo city, which is located less than 50 km from the BBS. Human-induced changes in the abiotic conditions may affect both plant and pollinator phenologies (see Morellato et al. 2016), which in turn can also affect the structure of plant-pollinator mutualistic networks (MacGregor et al. 2015; Knop et al. 2017). Therefore, future studies might use the pollen load attached to the proboscis and body of the hawkmoths collected in the two surveys at the BBS, in order to assess whether the structure and functioning of the plant-pollinator interaction network have changed or remained stable over time.

Compliance with ethical standards

Conflict of interest We have no conflicts of interest to disclose. All necessary permits were obtained for the field study, which did not involve endangered or protected species.

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