



Original Articles

Bird based Index of Biotic Integrity: Assessing the ecological condition of Atlantic Forest patches in human-modified landscape



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ABSTRACT

Wooded biomes converted to human-modified landscapes (HML) are common throughout the tropics, yielding small and isolated forest patches surrounded by an agricultural matrix. Diverse anthropogenic interventions in HMLs influence patches in complex ways, altering natural dynamics. Assessing current condition or ecological integrity in these patches is a challenging task for ecologists. Taking the Brazilian Atlantic Forest as a case study, we used the conceptual framework of the Index of Biotic Integrity (IBI), a multimetric approach, to assess the ecological integrity of eight small forest patches in a highly disturbed HML with different configurations and histories. The IBI was developed using bird assemblages found in these patches, and its performance was compared with analytical approaches commonly used in environmental assessment, such as general richness and Shannon's diversity index. As a first step, the IBI procedure identifies an existing gradient of human disturbance in the study region and checks which biotic characteristics (candidate metrics) vary systematically across the gradient. A metric is considered valid when its' relationship with the gradient provides an ecological interpretation of the environment. Then, the final IBI is elaborated using each valid metric, obtaining a score for each site. Over one year of sampling, 168 bird species were observed, providing 74 different bird candidate metrics to be tested against the disturbance gradient. Seven of them were considered valid: richness of threatened species; richness of species that use both “forest and non-forest” habitats; abundance of endemics, abundance of small understory-midstory insectivores, abundance of exclusively forest species; abundance of non-forest species, and abundance of species that forage exclusively in the midstory stratum. Each metric provided complementary information about the patch's ecological integrity. The resulting IBI showed a significant linear relationship with the gradient of human disturbance, while total species richness and Shannon's diversity index did not. Application of numerical approaches, such as total species richness and Shannon's diversity, did not distinguish ecological traits among species. The IBI proved better for assessing and interpreting ecological and environmental condition of small patches in highly disturbed HML. The IBI framework, its multimetric character, and the ease with which it can be adapted to diverse situations, make it an effective approach for assessing environmental conditions in the Atlantic Forest region, and also for many other small forest patches in the tropics.

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1. Introduction

1.1. Forest patches in human-modified landscapes

Tropical forests are critical for the maintenance of Earth's biodiversity and are subject to unprecedented destruction by human activities (Dirzo and Raven, 2003; Lambin et al., 2003; Wright, 2010; Haddad et al., 2015). Because most tropical forests are located within countries with ongoing economic development, tropical forests continue to be converted to human-modified landscapes at a rapid pace (HMLs; Lambin et al., 2003; Wright, 2005, 2010). Well known consequences of conversion of contiguous forest into fragments are: species loss (Turner, 1996; Dirzo and Raven, 2003; Ferraz et al., 2003; Anjos, 2006; Anjos et al., 2011), biodiversity homogenization (Lôbo et al., 2011), loss of functional diversity (Newbold et al., 2013; Bregman et al., 2014) and the spread of invasive species (Saunders et al., 1991; Goosem, 2000; Acurio et al., 2010; Tabarelli et al., 2012).

The Brazilian Atlantic Forest is the second largest biome in South America (Galindo-Leal and Câmara, 2003). Although considered a Biodiversity Hotspot (Myers et al., 2000; Mittermeier et al., 2005), only 16% of the original cover remains (Ribeiro et al., 2009, 2011). The forest now occurs largely as small and isolated patches (Ribeiro et al., 2009) with different origins, history of human influence (e.g., Prevedello and Vieira 2010; Melo et al., 2013; Ferraz et al., 2014), and highly varied environmental conditions (e.g., Ferraz et al., 2014). As a result, there are strong concerns about the state of Atlantic Forest biota, but lack of detailed knowledge of biological responses to various human influences limits our ability to mitigate biodiversity declines.

Past studies emphasized that landscapes that have >20% forest cover are most suitable for biodiversity conservation in the Atlantic Forest (e.g., Pardini et al., 2010; Martensen et al., 2012; Banks-Leite et al., 2014; Magioli et al., 2015). In contrast, little attention is given to forest patches in highly disturbed and deforested HMLs. Nonetheless, these patches can serve as ecological corridors and stepping-stones (Boscolo et al., 2008; Uezu et al., 2008; Rocha et al., 2011), provide viable habitat for some forest species (Ferraz et al., 2012), and harbor other threatened ones (Willis and Oniki 2002; Ribon et al., 2003; Antunes 2005; Magioli et al., 2016). Because of these findings, further investigations of the species requirements in small patches of HMLs have been encouraged (e.g., Tabarelli et al., 2010; Melo et al., 2013). This knowledge gap limits environmental assessments of patches, which undermine support for future conservation planning and human impact mitigations (i.e., within the Environmental Impact Assessment procedure, see Glasson and Salvador, 2000; Lima et al., 2010; Silveira et al., 2010; Koblitiz et al., 2011; Sánchez and Croal, 2012; Alexandrino et al., 2016).

Bird communities are often the focus of researchers assessing environmental conditions of patches, as they serve as bioindicators (Anjos and Boçon, 1999; Anjos, 2004; Piratelli et al., 2005; Magalhães et al., 2007; Cavarzere et al., 2009; Manhães and Loures-Ribeiro, 2011; Pereira and Azevedo, 2011; Arendt et al., 2012). The diverse ecological niches of birds (Sekercioglu, 2006, 2012) makes them an appropriate proxy for biodiversity and a descriptor of the existing ecological integrity (Temple and Wiens, 1989; Stotz et al., 1996; Byron, 2000; Sekercioglu, 2006; Johnson, 2007; Chambers, 2008). However, results from some widely used approaches (e.g., richness, species diversity, species composition) may fail to properly assess environmental conditions of patches in HMLs (see Metzger, 2006; Vasconcelos, 2006; Silveira et al., 2010; Alexandrino et al., 2016). Therefore, testing analytical approaches used in environmental assessments is an instructive way to provide knowledge for professionals involved with conservation actions (Verdade et al., 2014) and is a key goal of our paper.

1.2. Index of biotic integrity

The “integrity” concept first invoked by Aldo Leopold (1949), refers to an ecosystem that is not altered as a result of human actions. It is the condition and character of living systems that are the product of evolutionary and biogeographic processes (Angermeier and Karr, 1994; Karr, 1996; Karr and Chu, 1999). The Index of Biotic Integrity (IBI) assumes that any ecological system (i.e., natural or disturbed) has biotic elements (e.g., communities and populations) and ecological processes (e.g., intraspecific and interspecific interactions). Thus, the condition measurement of a given system, in comparison to an undisturbed correspondent, will identify the changes suffered in the biotic elements and ecological processes (Karr, 1991, 2006; Angermeier and Karr, 1994; Westra, 2005).

The IBI was originally created to assess the biological condition of aquatic ecosystems based on fish assemblages (Karr, 1981). Since then, this approach has been widely used to assess streams and rivers worldwide, examining fish (e.g., Karr et al., 1986; Lyons et al., 1995; Karr, 2006; Pinto and Araújo, 2007; Casatti et al., 2009; Costa and Shulz, 2010), aquatic macroinvertebrates (e.g., Kerans and Karr, 1994; Fore et al., 1996), aquatic plants (e.g., Grabas et al., 2012; Rooney and Bayley, 2012) and coral reefs (Jameson et al., 2001). Assessments of terrestrial ecosystems using the IBI have mainly been done in the northern hemisphere using invertebrates (e.g., Kimberling et al., 2001; Karr and Kimberling, 2003) and birds (e.g., Bradford et al., 1998; O'Connell et al., 2000; Bryce et al., 2002; Glennon and Porter, 2005; Bryce, 2006; see Ruaro and Gubiani, 2013 for a further review).

The IBI is designed to measure multiple biological dimensions of complex ecosystems (Karr and Chu, 1999; Karr 2006). The first step is to identify measurable biological attributes that change consistently along a gradient of human disturbance in the study region (Dale and Beyeler, 2001; Niemi and McDonald, 2004). This gradient is identified by taking into account multiple environmental variables that are expected to influence the living biota. Each biological attribute is a candidate metric, and those with a clear relationship with the gradient are valid metrics. Taxonomic richness, species composition and abundance, functional groups, and other biological attributes (from one or more taxonomic groups) are evaluated as potential metrics in an integrative IBI (e.g., O'Connell et al., 2000; Bryce et al., 2002; Glennon and Porter, 2005; Karr, 2006; Mack, 2007; Wilson and Bayley, 2012; Ruaro and Gubiani, 2013; Medeiros et al., 2015). Sites with minimal (or absent) disturbance are deemed to have integrity (Karr, 1981, 2006; Karr et al., 1986; Karr and Chu, 1999). The gradient should ideally encompass a range of sites with little or no influence of human disturbance up to highly disturbed sites. When rigorously employed, this procedure reveals the reference score for each valid metric (i.e., the observed metric value at minimally disturbed sites), which will be used to calculate the final metric score for each study site. The sum of metric scores defines the IBI for each study site (e.g., Karr, 1981, 2006; Bradford et al., 1998; O'Connell et al., 2000; Bryce et al., 2002; Glennon and Porter, 2005) and produces a measure that reflects how much each site deviates from the state of integrity.

Few researchers have applied this approach to assess tropical forest integrity (e.g., Anjos et al., 2009; Bochio and Anjos, 2012; Medeiros et al., 2015). The complexity of small Atlantic Forest patches (e.g., Ferraz et al., 2014) and the high bird diversity in this biome (Goerck, 1997; Lima, 2013) led us to initiate an assessment process using the principles of IBI, focusing on birds. Birds exhibit numerous ecological characteristics, which can be converted into multiple candidate metrics (e.g., foraging guild, habitat preference, etc.) useful for evaluating forest integrity.

Therefore, we aimed to develop a bird-based IBI to assess the current condition (as a divergence from integrity) of forest patches

in highly disturbed HML of the Atlantic Forest Biome. We focused on small patches with distinct configurations, with interior portions experiencing different rates and modes of deforestation and secondary re-growth. This situation is regarded to be difficult to evaluate with ornithological data (e.g., Alexandrino et al., 2016), but is commonly faced by ecologists, conservationists and managers conducting environmental impact assessments (e.g., Metzger, 2006; Straube et al., 2010; Koblitz et al., 2011; Ferraz et al., 2014). We evaluated IBI performance by comparing our results with measurements used in community ecology, such as species richness and Shannon diversity, which are often used by decision makers worldwide (Byron, 2000; Chambers, 2008). In Brazil, for example, these measures are mandated components of environmental impact assessments (e.g., IBAMA Normative Instruction n.146/2007). Our hypothesis is that a multimetric IBI created with empirically defined metrics will provide a more nuanced understanding of the relationship between human disturbance and biological condition than conventional measures such as species richness or Shannon's diversity index.

2. Material and methods

2.1. Study area and sampling design

Our study area, in the Corumbataí River Basin of eastern São Paulo State of Brazil (22°04'46"/22°41'28"S and 47°26'23"/47°56'15"W), is in the interior forest sub-region of the Atlantic Forest Biome (Silva and Casteleti, 2003) (Fig. 1a). Originally covered by semi-deciduous seasonal forest and sparse savannah woodlands (Cerrado Biome), years of human modification have converted this region into small to medium urban sites, surrounded by an agricultural mosaic. Within this 1710 km² region, cattle pasture (44% of the area located largely in the north) and sugar cane (26% mostly in the south) forms the main agricultural matrix. Only 11% is native forest which occur in small forest patches (Fig. 1b) (Valente and Vettorazzi, 2003). The climate is subtropical (i.e., Cwa climate on Köppen classification, see Alvares et al., 2013) with rainy (October–February) and dry (March–September) seasons. The moderately hilly topography (Garcia et al., 2006) is characteristic of the agricultural landscapes found in southeastern Brazil (e.g., Ferraz et al., 2014).

Human-modified landscapes are complex systems created when diverse human activities are imposed on natural ecosystems. Landscape composition, configuration and history of fragmentation are factors that may influence bird occurrence in highly disturbed patches in HMLs (e.g., Fahrig, 2003; Boscolo and Metzger, 2009; Metzger et al., 2009; Pardini et al., 2009; Prevedelo and Vieira, 2010; Lira et al., 2012; Martensen et al., 2012; Zurita and Bellocq, 2012). Study designs within HMLs should isolate key environmental variables to uncover ecological patterns within those landscapes. Therefore, our sampling design used five focal landscapes located in the river basin (see Ferraz et al., 2014). Each 16 km² focal landscape was 70% agricultural matrix (sugar cane or pasture) and at least 10% native forest. From this landscape, we selected eight forest patches (3–115 ha, ~530–650 m above the sea level) as described by Alexandrino et al. (2016). The forest patches were selected considering the predominant age of each patch (i.e., for how long the major amount of the patch is present in the landscape) through historical image analyses (from 1962, 1978, 1995, 2000 and 2008). First, we checked in the field which patches had forest cover matching with that of the 2008 satellite imagery. We also assessed the expected age of succession of the forest (e.g., Chazdon et al., 2007) and field accessibility. Thus, four old patches (the major part of the patch is present on the river basin since 1962 or 1978 images) and four new patches (the major part of the

patch was absent in the 1962 and 1978 images with regeneration started mainly in or after 1995) were selected, with two old and two new in each matrix category (pasture at north and sugarcane at south) (Fig. 1c). We based our patch selection on the historical fragmentation/re-growth and matrix type, as these characteristics are considered factors that drive differences in bird communities in forest patches (Metzger et al., 2009; Prevedelo and Vieira, 2010; Lira et al., 2012).

2.2. Bird surveys

Point count bird surveys (Bibby et al., 2000) were positioned randomly inside the patches at a minimum distance of 200 m among them to avoid overlap of the samples (e.g., Uezu et al., 2008; Penteado et al., 2014) (Fig. 1c). The numbers of points were proportionally allocated by forest patch area. A total of 48 point counts were used, each visited monthly from November 2011 to November 2012 (12 visits total). Trained observer conducted all surveys using ten-minute sampling periods with unlimited radius. Only birds heard or seen in the interior or forest edge (up to canopy) were recorded (e.g., Anjos et al., 2004; Uezu et al., 2005; Alexandrino et al., 2016). A mean of six point counts were conducted each field day between sunrise and 11:00 am. Relative abundances were based on the Punctual Abundance Index (PAI) (Bibby et al., 2000; Vielliard et al., 2010), a common approach based on the total number of visual and/or auditory contacts for each species in the point count, divided by the number of samples made. The result is a non-dimensional number that allows species comparisons across sample sites and/or time. To compute the relative abundance of each bird functional group (i.e., candidate metrics, see next item), we summed the contact number obtained for each species belonging to the group, and then calculated PAI for the respective group. All nomenclatures and taxonomic order follows that proposed by the Brazilian Ornithological Records Committee (Piacentini et al., 2015).

2.3. Development of the bird-based index of biotic integrity (IBI)

Development of a regional IBI depends on identification of a gradient of human disturbance across the selected study sites (i.e., in our case the bird point count locations), and collection of biological data for each site. Because diverse human activities (agriculture, urbanization, transportation corridors, etc.) are present in most HMLs, the definition of the gradient should encompass the full range of those diverse actions (Karr, 2006). One approach for defining that gradient is to rank ecosystem service provisioning for each study site, because these services depend on existing biological conditions (Wu, 2013). Thus, we used the rank of ecosystem service provisioning for each 1 ha of our patches (thus, also for each of our bird point counts, see Fig. 1) provided by Ferraz et al. (2014). They used environmental metrics that represent the history of degradation and re-growth of each forest patch (i.e., mean forest age) and landscape features (i.e., local forest neighborhood dominance, forest proximity, and forest contiguity) to define a rank that ranged from 3 (low ecosystem service supply) to 13 (high ecosystem service supply) in the studied patches. All these environmental factors affect biodiversity in secondary forest (e.g., Pardini et al., 2005; Santos et al., 2008; Melo et al., 2013), and also influence the occurrence of forest birds in patches (see Alexandrino et al., 2016). We assume higher values of ecosystem service provisioning to be associated with lower human disturbance, while lower values equate to higher disturbance. Therefore, this rank represents a valid gradient of disturbances for our point count sites.

Next we identify measurable characteristics (metrics) of bird communities that might be expected to change systematically across the gradient of human disturbances. Existing knowledge and

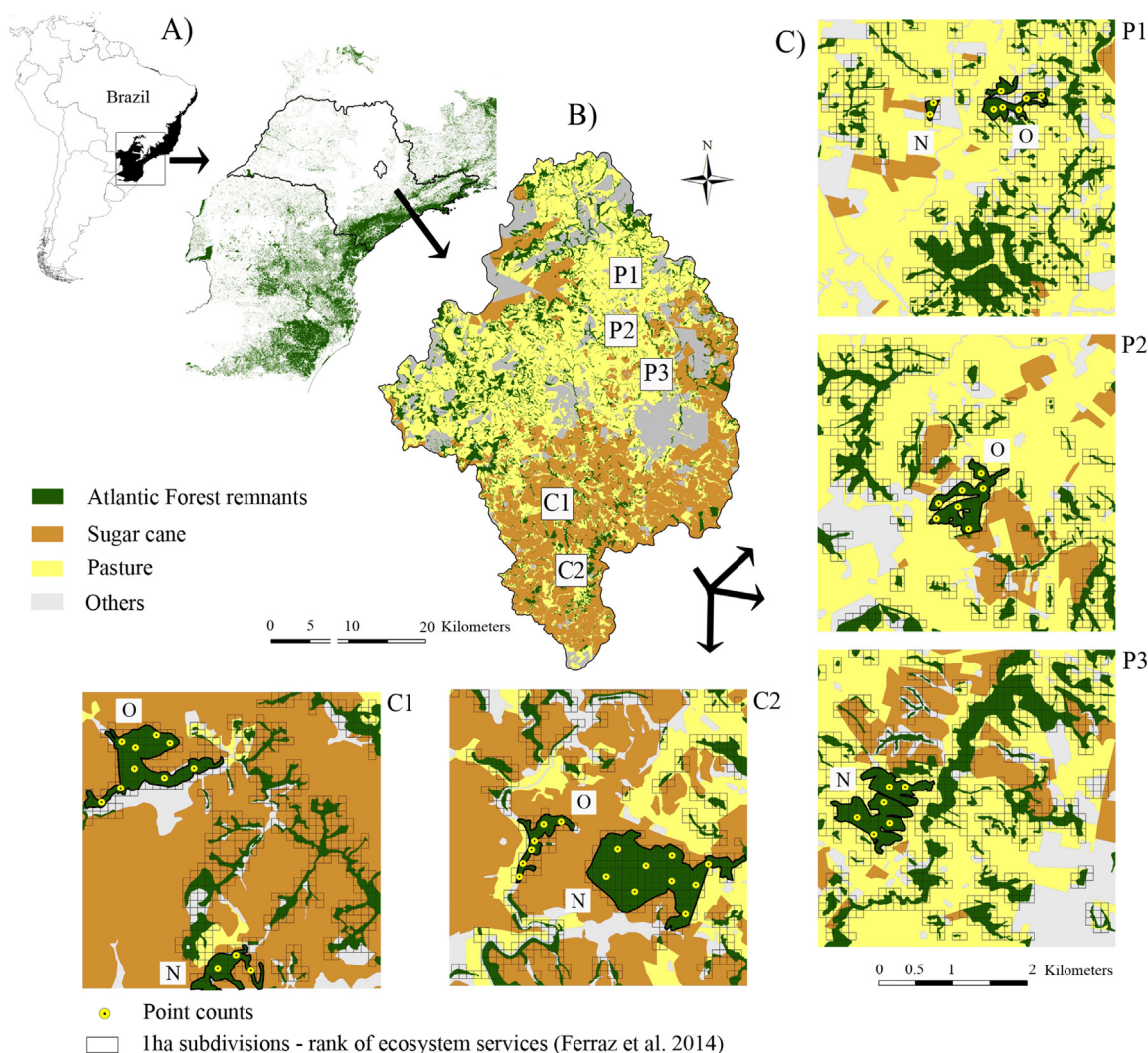


Fig. 1. A) Corumbataí River Basin is located at São Paulo State, southeast Brazil, inside the original Atlantic forest cover (in black in the South America map), where few forest patches are currently found. B) Main land use type that surrounds forest patches and location of each five focal landscapes (16 km²) used for patch selection. C) Eight forest patches selected for bird survey (outlined in black and with bird point counts inside) in the focal landscapes. The letters beside the selected patches indicate their predominant age (N – patch predominantly new, O – patch predominantly old). Focal landscape names starting with “P” mean pasture matrix and “C” mean sugarcane matrix. Land use follows Valente & Vettorazzi (2003). Squares of 1 ha have the rank of ecosystem services provisioning provided by Ferraz et al. (2014), which was used here to build our gradient of human disturbances.

understanding of bird communities provides a guide to the selection of potential metrics (O’Connell et al., 2000; Bryce et al., 2002; Glennon and Porter, 2005; Bryce, 2006; Ruaro and Gubiani, 2013). More detailed guidance on the principles of metric selection can be found in Karr (1991, 2006) and Karr and Chu (1999). We used attributes that represent the existing functional characteristics of the bird assemblages, classifying each species according to three natural history components.

1) *Habitat of occurrence*. In a comprehensive analysis of the natural history of Neotropical birds, Parker III et al. (1996) assign all species to one of three categories: forest (F), non-forest (NF) and aquatic habitats (A). A species may occur in more than one habitat. Thus, seven categories are employed for this study (see Alexandrino et al., 2013, 2016): F – forest, NF – non forest, A – aquatic, F-NF – forest and non-forest, F-A – forest and aquatic, NF-A – non forest and aquatic, and A-F-NF – aquatic, forest and non forest.

2) *Foraging stratum*. Again using Parker III et al. (1996) primary classifications were T – terrestrial, U – understory, M – midstory, C – canopy, Ae – aerial, W – water. When species occupied more than one we classified their foraging range (e.g., up to understory = T-U, up to midstory = T-M, understory to canopy = U-C).

3) *Foraging guild*. Preferred foods for each species were determined using a comprehensive literature survey of 248 sources that is updated regularly (see Sekercioglu et al., 2004; Sekercioglu, 2012). These bird diet classification methods are described extensively in Kissling et al. (2012). We identified the food item consumed by each bird and then ordered the items by priority diet. Seven food categories were used, non-reproductive plant material, seeds, fleshy fruits, nectar, invertebrates (arthropods, insects, aquatic invertebrates), carrion (carcasses, garbage, offal), fish, and other vertebrates. Then, the species’ primary diet choice was used to classify it into one of the following foraging guilds: herbivorous, granivorous, frugivorous, nectarivorous, insectivorous, scavenger, piscivorous, carnivorous. We considered omnivores to be species that routinely feed on four or more food groups.

Personal expertise improved the last two classifications (foraging stratum and foraging guild). Also, we excluded from our analysis two foraging strata (i.e., species able to feed in the ground and in the aerial stratum “T-Ae” and species able to feed into the water and in the aerial stratum “W-Ae”), and one foraging guild (i.e., herbivorous) because we did not have species exclusively belonging to these categories.

The species richness and PAI of all categories of habitat of occurrence, foraging stratum and foraging guild, obtained from each point count were used as candidate metrics. In addition, we also tested the richness and PAI from specific groups already considered vulnerable to human disturbances (named as Potential Indicator group), such as Atlantic Forest endemics and threatened species (Goerck 1997; Ribon et al., 2003; Anjos et al., 2010). We used endemism information from Bencke et al. (2006) and threat status from Silveira et al. (2009) a São Paulo State Red List that follows IUCN criteria on a local scale. Forest insectivores (i.e., F and F-NF species) that forage in the lower strata are widely considered sensitive to habitat loss and fragmentation (Sekercioglu et al., 2002; Sekercioglu, 2012), thus we also tested them as a candidate metric. Because of the high variety of these species in the Neotropics (Stotz et al., 1996), we first identified which insectivorous species performed better as indicators. To do this we used average mass (i.e., up to 30 g and up to 60 g) and foraging stratum (i.e., up to understory stratum, and up to midstory stratum) to classify our insectivorous birds into the groups: small understory insectivores (e.g., species that forage up to understory stratum and with average mass up to 30 g), small understory-midstory insectivores (e.g., species that forage up to midstory stratum and with average mass up to 30 g), small-medium understory insectivores (e.g., species that forage up to understory stratum and with average mass up to 60 g), and small-medium understory-midstory insectivores (e.g., species that forage up to midstory stratum and with average mass up to 60 g). General understory insectivores and general understory-midstory insectivores (i.e., all average mass) were also considered. To identify these insectivorous groups we classified birds based on data provided by bird captures in the same patches (Luz, 2013), and complemented this data with the literature (e.g., Reinert et al., 1996; Sick, 1997; Piratelli et al., 2001; Willis and Oniki 2001; Sekercioglu et al., 2004; Dunning, 2007; Faria and Paula, 2008).

We started with 74 candidate metrics: Richness and PAI for each of seven habitat categories of occurrence, 14 foraging strata, eight foraging guilds, endemics, threatened species and six insectivorous groups. All of these passed through the three sequential steps of analysis to identify potential final metrics. In our first step, as the number of candidate metrics was high, we selected only metrics with a significant linear model with human disturbance ($\alpha=1\%$, $R^2 > 0.2$) (e.g., Mack, 2007; Wilson and Bayley, 2012). By using linear regression, we provide both quantitative and visual displays of empirical pattern across our disturbance gradient. As a second step, among the insectivorous groups, we selected the metric with a significant relationship and the highest R^2 to be the best representative of this group. We then checked for redundancy among the remaining metrics, assuming richness and PAI of one trait category (e.g., richness and PAI of forest specialist species, richness and PAI of endemics) to be biologically redundant. We favored PAI over richness because richness by itself does not distinguish between exceptionally abundant species and those that are extremely rare (Magurran, 2004). The bird relative abundance measurement indicates to what degree each forest patch can support each bird functional group, which improves the environmental assessment (Anjos, 2004; Johnson, 2007; Vielliard et al., 2010). In the last step, we selected final metrics with complementary ecological information about the habitat under study that make ecological sense. To do this, we checked the observed data of all remaining metrics in order to identify whether any of them had a non-reliable relationship. We then ran the Jaccard's index of species similarity among all metrics (i.e., the best of insectivorous groups and the other selected metrics) to check how complementary each metric was to each other (Magurran, 2004). We considered acceptable the maximum value of 20% similarity. Finally, we selected the metrics that provide more consistent ecological information about the habitat according to their species composition.

These steps of selection follow similar procedures used by IBI developers in terrestrial ecosystems (e.g., O'Connell et al., 2000; Kimberling et al., 2001; Bryce et al., 2002; Karr and Kimberling, 2003; Glennon and Porter, 2005; Bryce, 2006; Mack, 2007; Wilson and Bayley, 2012) and recommended by the IBI creator (Karr, 1981, 2006; Karr et al., 1986). They suggest a prudent analysis of the data relationship under the biological concept, looking for clear signals of relationship between the biotic candidate metrics and the gradient of human disturbance, rather than selecting metrics using only statistical methods that may select the metrics automatically, as stepwise or multiple regression.

Finally, we built our IBI for each point count from the final valid metrics. First, for each valid metric, we identified its reference value, which was the highest observed data among the point counts. After, each observed metric value at each point count was weighted to its reference value and multiplied by 10 to obtain a scale of 0 (i.e., worst score) to 10 (i.e., best score). For those metrics that have negative response with the gradient of human disturbances we made the reverse score by subtracting 10 from the raw score in order to convert the 10 in the worse score and 0 the best. Then, the IBI for each point count was calculated summing each weighted selected metric (i.e., values from 0 to 10) multiplying by 10 and divided by the number of valid metrics used (e.g., Bryce et al., 2002; Bryce 2006).

Ideally, the IBI framework considers data from minimally disturbed or undisturbed sites as the reference condition (e.g., Karr, 1981; Karr et al., 1986; Anjos et al., 2009; Medeiros et al., 2015). However, mature forests patches, or at least larger and regular shaped patches with advanced secondary growth and less disturbed (i.e., that could be used as reference) are nonexistent in our highly agricultural landscape (e.g., Ferraz et al., 2012, 2014). There is also no reference patch located in similar landscapes nearby. These impediments led us to use as reference the best results that we have of each metric. The approach we used here to overcome this problem has also been successfully used in other IBI studies, such as Karr et al. (1986) assessing streams in the Midwestern U.S. using fishes, Bryce et al. (2002) and Bryce (2006) assessing forests and riparian corridors in Oregon, U.S. using birds, and Kimberling et al. (2001) and Karr and Kimberling (2003) using terrestrial insects in the Pacific Northwest U.S. to assess shrub-steppe habitats.

In order to evaluate the results of the final IBI to identify forest integrity, we compared their performance against classical measurements often used by ecologists, such as total species richness and Shannon's diversity index (Magurran, 2004; IBAMA Normative Instruction n.146/2007; Straube et al., 2010; Vielliard et al., 2010). To do so, we ran three independent linear regressions in each of the final IBI, total species richness and Shannon's diversity index as dependent variables, and the gradient of human disturbances as the independent variable. We compared their performances through the R^2 value. All statistical analyses were performed using R software (R Development Core Team, 2014).

3. Results

3.1. Metric selection

One-hundred sixty-eight species were observed in 95 h and 40 min of sampling (Appendix 1 in Supplementary material). From these, after our three sequential steps of analysis, seven final metrics were selected: richness of threatened species, PAI (i.e., Punctual Abundance Index) of endemic species, PAI of small understory-midstory insectivores (PAI und-mid-ins <30 g), richness of species that use both "forest and non-forest" habitats (F-NF), PAI of exclusively forest species (PAI F), PAI of non-forest species (PAI NF) and PAI of species that forage exclusively in the midstory stratum

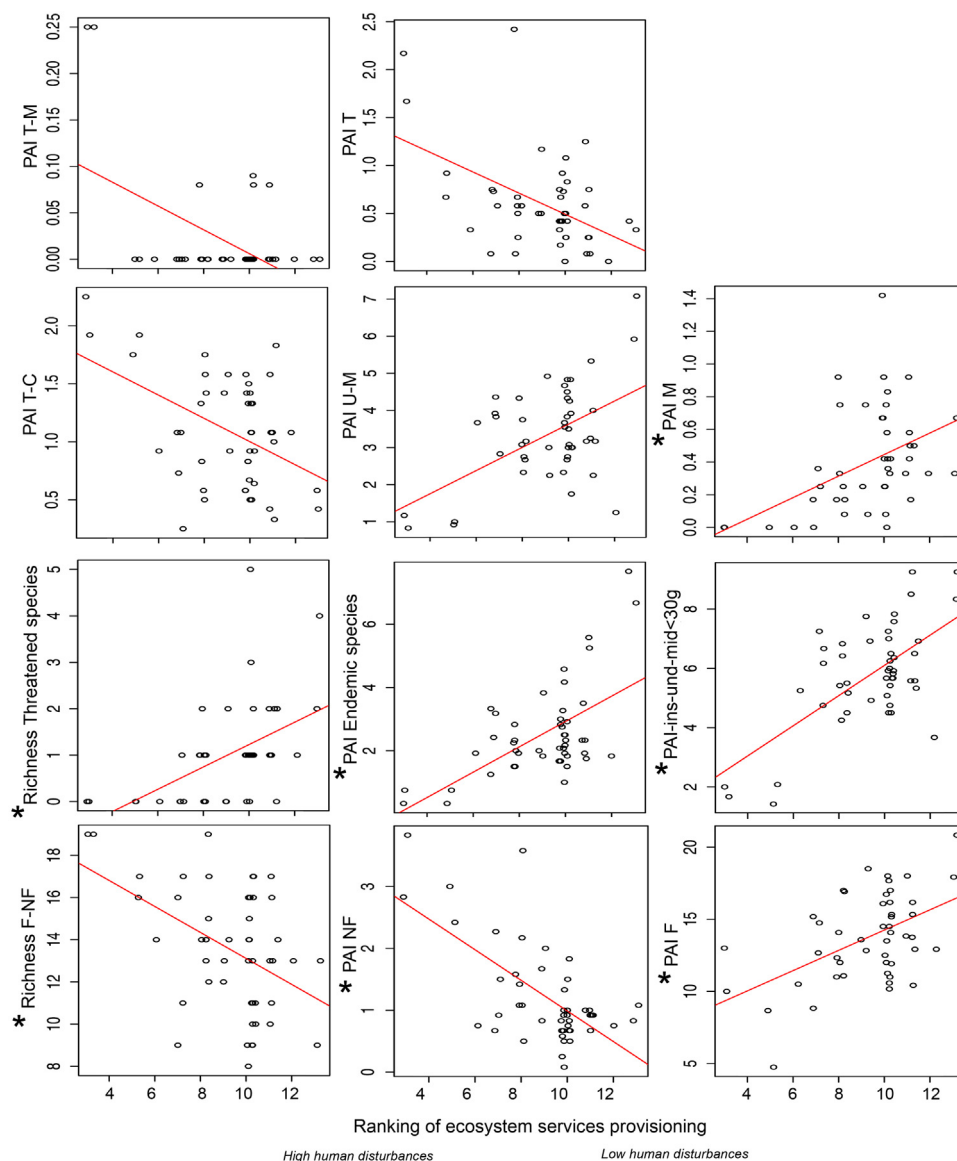


Fig. 2. Linear relationship between the 11 remaining metrics against the ranking of ecosystem service provisioning (provided by Ferraz et al., 2014) which represent the existing gradient of human disturbances on each 1 ha of the studied patches, where each of our bird point counts were located. These metrics were selected after the second step of our IBI metrics selection procedure. Only seven of them were selected as final metrics of our IBI (see Table 1). Higher values of the rank mean fewer disturbances on the bird point count, while lesser values mean high disturbances. * indicates the seven metrics that are included in the final IBI (see next steps of selection).

(PAI M). Five selected metrics showed a positive relationship with the gradient of human disturbances, while two showed a negative relationship (Fig. 2). These relationship differences were caused by the species composition in each final metric (see Appendix 2 in Supplementary material).

To reach these final metrics, each selection step helped us to refine our metric choice. In our first step, we found 22 metrics to be significantly associated with the gradient of human disturbance (i.e., $R^2 > 0.2$ and $P < 0.001$) from 74 metrics tested (Table 1, see Appendix 3 in Supplementary material for the relationship results of the other unselected metrics). In the second step, among those 22, we excluded three richness metrics (i.e., Richness of non-forest species, Richness species that forage exclusively in the midstory stratum, and Richness of endemic species) that were biologically redundant with their respective PAI. Also in this step, PAI of small understory-insectivores was selected as the best metric of the nine remaining insectivorous groups (Table 1). In the last step, our careful analysis of each remaining metric revealed that some metrics did not have a reliable relationship with the gradient or did

not provide logical biological information about forest integrity or did not provide complementary ecological information useful for the final IBI. Thus, the following were excluded:

- PAI of species that forage from ground to midstory (PAI T-M): The relationship with the gradient was based only in six non-zeros values (i.e., while 42 were zeros values) (Fig. 2). Besides this metric was composed by only one non-forest species (Eared Dove – *Zenaida auriculata*), which not include complementary ecological information for the final IBI, since this species was also present in other selected metric (i.e, PAI of non-forest species – PAI NF).

- PAI of species that forage from the understory to midstory (PAI U-M): This metric showed species similarity higher than 20% with PAI of small understory-midstory insectivores (Table 2). Considering the specificity of insectivorous groups and the existing literature reporting their sensitivity to forest fragmentation (Stouffer & Bierregaard 1995, Stratford and Stouffer 1999; Sekercioglu et al., 2002; Sekercioglu, 2012; Stratford and Stouffer, 2015; Anjos et al., 2015) this group likely provides a better way to interpret the forest integrity rather than the PAI U-M group, which also gath-

Table 1
Steps for selection of bird metrics for inclusion in the IBI. Step 1: we selected 22 candidate metrics that had a significant relationship with the rank of ecosystem provisioning ($P < 0.001$ and $R^2 > 0.2$) (see Appendix 3 in Supplementary material for regression results of all 74 candidate metrics). Step 2: we identified redundant metrics and selected the best insectivorous group metric by evaluating R^2 values. Step 3: we selected metrics for inclusion in the IBI on the basis of metric data quality, the complementary ecological information among them, and the potential to interpret the forest quality through species composition. A brief explanation of our decision is provided in parentheses.

Candidate metrics	Step 1 (* $P < 0.001$)		Step 2	Step 3 – Final metric decision	
	R^2	Relationship	Redundancy analysis		
Habitat of occurrence	Richness NF	0.24	negative	Redundant with PAI NF Metric excluded (less ecological information aggregated in comparison to PAI NF)	
	Richness F-NF	0.20	negative		
	PAI F	0.25	positive		Metric selected
	PAI NF	0.43	negative		Redundant with Richness NF Metric selected (more ecological information aggregated in comparison to Richness NF)
Foraging stratum	Richness M	0.25	positive	Redundant with PAI M Metric excluded (less ecological information aggregated in comparison to PAI M)	
	PAI T	0.23	positive	Redundant with Richness M	
	PAI M	0.22	positive		
	PAI T-C	0.20	negative		
	PAI T-M	0.26	negative		
	PAI U-M	0.27	positive		
Potential Indicators	Richness Endemic species	0.30	positive		Redundant with PAI Endemic species. Metric excluded (less ecological information aggregated in comparison to PAI of endemics species)
	Richness Threatened species	0.26	positive	Redundant with Richness of endemic species	
	PAI Endemic species	0.34	positive		
			Metric selected Metric selected (more ecological information aggregated in comparison to Richness of endemic species)		
Insectivorous groups	Richness gen-und-mid-ins	0.21	positive	Insectivorous groups selection	
	Richness und-mid-ins<30g	0.21	positive		
	Richness und-mid-ins<60g	0.20	positive		
	PAI gen-und-ins	0.32	positive		
	PAI gen-und-mid-ins	0.40	positive		
	PAI und-ins<30g	0.35	positive		
	PAI und-mid-ins<30g	0.42	positive		Best (Higher R^2 value)
	PAI und-ins<60g	0.33	positive		Metric selected (more ecological information aggregated in comparison to PAI U-M)
PAI und-mid-ins<60g	0.40	positive			

PAI = Punctual Index of Abundance (Vielliard et al., 2010). NF = exclusively non-forest species, F-NF = species that use both “forest and non-forest” habitats, F = exclusively forest species, M = species that forage exclusively in the midstory stratum, T = species that forage exclusively on the ground, T-C = species that forage from ground to canopy, T-M = species that forage from ground to midstory, U-M = species that forage from the understory to midstory, Endemic species = Atlantic forest endemics, Threatened species = Threatened species in the Sao Paulo state, Brazil. gen-und-ins = general understory insectivores, gen-und-mid-ins = general understory-midstory insectivores, und-ins < 30 g = small understory insectivores (body mass up to 30 g), und-mid-ins < 30 g = small understory-midstory insectivores (body mass up to 30 g), und-ins < 60 g = small-medium understory insectivores (body mass up to 60 g), und-mid-ins < 60 g = small-medium understory-midstory insectivores (body mass up to 60 g).

ered species with different foraging guild and body mass. Because of this, we excluded PAI U-M.

– PAI of species that forage exclusively on the ground (PAI T) and PAI of species that forage from ground to canopy (PAI T-C): The

species composition in both metrics did not allow us to assume a common response of all species across the gradient. For example, PAI T had forest species that require less disturbed forest habitat and streams (e.g., Sharp-tailed streamcreeper – *Lochmias nematura*,

Table 2

Jaccard index (results multiplied by 100) of species similarity among the 11 selected metrics in the step 2. These results indicate the complementary aspect among the metrics and indicate which of them were too similar (20% was the accepted species similarity limit).

	Richness F-NF	PAI F	PAI NF	PAI M	PAI T	PAI T-C	PAI T-M	PAI U-M	Richness Threatened species	PAI Endemic species
PAI F	0.00									
PAI NF	0.00	0.00								
PAI M	0.00	4.44	0.00							
PAI T	7.69	4.72	20.93	0.00						
PAI T-C	2.22	4.12	16.67	0.00	0.00					
PAI T-M	0.00	0.00	3.23	0.00	0.00	0.00				
PAI U-M	8.51	12.77	0.00	0.00	0.00	0.00	0.00			
Richness Threatened species	7.50	5.38	0.00	9.09	3.57	0.00	0.00	4.35		
PAI Endemic species	0.00	18.68	0.00	4.76	0.00	0.00	0.00	13.33	4.00	
PAI und-mid-ins<30g	4.84	20.00	8.93	9.68	6.25	0.00	0.00	27.78	2.70	14.29

considered regionally medium sensitivity to human disturbance and dependent of small streams, Sick, 1997; Alexandrino et al., 2016), however, it also had non-forest synanthropic species (e.g., Black vulture – *Coragyps atratus*) (see Appendices 1 and 2 in Supplementary material). Besides, both metrics were composed mainly by non-forest species. Excluding these species in both metrics caused them to lose their significant relationship with the gradient (PAI T: $R^2 = 0.045$, $P = 0.149$ /PAI T-C: $R^2 = 0.105$, $P = 0.024$). Moreover, PAI T had also high species similarity with the non-forest species metric (PAI NF) (Table 2). Thus, the ecological integrity interpretation by PAI T-C and PAI T metrics should be actually unfounded, as they did not include complementary ecological information for the final IBI.

3.2. Final IBI

Through our final seven selected metrics, IBI scores for point count sites ranged from a low biological condition of 1.3 to a high of 7.4 on point counts with the best ecological integrity (mean = 4.37, Std.dev = 1.29, var = 1.68). The IBI showed that integrity increased with decreasing human disturbance (i.e., high positive linear relationship with the gradient of human disturbances ($R^2 = 0.648$, $P < 0.001$). Meanwhile total species richness ($R^2 = 0.0082$, $P = 0.541$) and Shannon's diversity index ($R^2 = 0.0285$, $P = 0.251$) did not have a significant relationship with the gradient (Fig. 3), indicating their inability to effectively assess the ecological integrity of our patches.

4. Discussion

4.1. Selected metrics

The final metrics incorporated three complementary classes of ecological information, which supported by previous knowledge, allow us to describe the current condition of the forest patches (Fig. 4). The resulting multimetric IBI expresses that condition, including the extent to which it diverges from a state of "biological integrity".

Using bird point count data, three habitat-of-occurrence metrics (i.e., PAI F, PAI NF and Richness F-NF) effectively measured the general level of human disturbance of the forest patches structure in our landscape. The positive association of the forest species (PAI F) with the gradient of human disturbances was due to the species' preference for mature forest or forest sites in advanced successional stages (e.g., Imbeau et al., 2003; Uezu et al., 2008). Corroborating that, these species are often found in large Atlantic Forest reserves (e.g., Develey and Martensen, 2006; Cavarzere et al., 2009; Antunes et al., 2013). Thus, forest portions with high number of forest individuals are indicative of better forest habitat structure. The species considered "forest and non-forest" (Richness F-NF) are those able to occur in both habitats (Parker et al., 1996), indicating their higher tolerance to impoverishment of mature forest and their possibility to occur in early stages of secondary growth (e.g.,

Imbeau et al., 2003; Uezu et al., 2008). Because this metric shows a negative relationship with the gradient, we interpret this to mean that occurrence is related to forest structure typically found at the edge of small patches (i.e., we consider as edge the forest patch perimeter which has an immediate contact with the matrix, where forest in early-successional stages typically occurs, with a higher density of shrubs, vines and herbaceous plants (see Fonseca and Rodrigues, 2000; Harper et al., 2005)), which has little integrity (Candido Jr. 2000; Harper et al., 2005; Chazdon et al., 2009; Gardner et al., 2009).

The non-forest species (PAI NF) also show a negative relationship with the gradient, acting as another indicator of poor forest structure. As the forest structure and stratification increases in the secondary growth (e.g., Tabarelli and Mantovani, 1999; Guariguata and Ostertag, 2001; DeWalt et al., 2003), a non attractive habitat for small near-ground non-forest species develops (e.g., some sparrows, finches, seedeaters), limiting their use of the patch interior (e.g., Stouffer and Bierregaard, 1995; Candido Jr. 2000; Imbeau et al., 2003; Uezu et al., 2008). In the case of large non-forest species (e.g., vultures, hawks, falcons, doves), as well as swallows, they occur only in the high canopy of forest interior. These species might use the top-canopy as a stopping point during their movement in the HMLs (e.g., Piratelli et al., 2005). These facts explain the low PAI for non-forest species in sites with high forest integrity. In other tropical patches located in HMLs, similar trends with non-forest and forest species were also reported (e.g., Sekercioglu 2002; Sodhi et al., 2005; Buechley et al., 2015).

The foraging stratum metric (PAI M) and the understory insectivorous group metric (PAI ins-und-mid<30g) were positive indicators of the midstory and understory forest strata quality. PAI M was composed by four forest insectivorous species, such as Robust Woodpecker *Campephilus robustus*, Olivaceous Woodcreeper *Sittasomus griseicapillus*, Rufous-tailed Jacamar *Galbula ruficauda* and Euler's Flycatcher *Lathrotriccus euleri*. Woodpeckers and woodcreepers are specialized to climb trunk and twigs, searching for arthropods and larvae in cracks and holes (Willis, 1979; Sick, 1997). Rufous-tailed Jacamar and Euler's Flycatcher use perches in the midstory to capture winged insects in the air or gleaning from the vegetation (Sick 1997; Pinheiro et al., 2003; Gabriel and Pizo 2005). Their foraging and nesting cavities are associated with the midstory stratum (Sick 1997; Sekercioglu et al., 2002; Sekercioglu, 2006). Because forest stratification is favored over time in secondary growth (e.g., Tabarelli and Mantovani, 1999; Guariguata and Ostertag, 2001; DeWalt et al., 2003), the elevated abundance of midstory birds may be an indication of the high quality of the stratum (e.g., Willis, 1979; Giraudo et al., 2008).

In the case of small understory insectivores, the arthropod supply and distribution may influence their occurrences in the forest (e.g., Blake and Hoppes, 1986; Zanette et al., 2000; Vargas et al., 2011). Others suggested that microhabitat characteristics, such as vegetation density, understory structure, leaf litter depth,

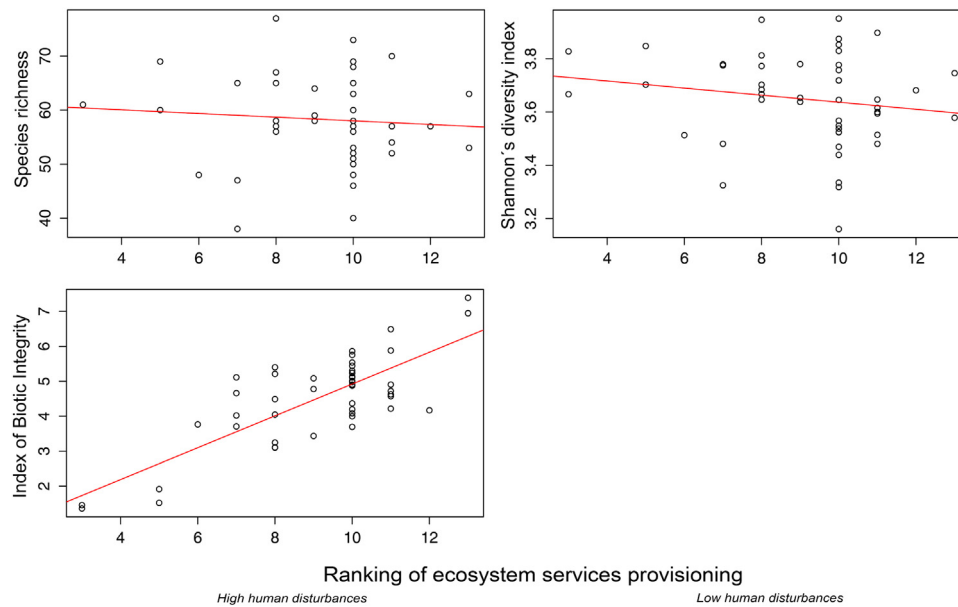


Fig. 3. Linear relationship between species richness, Shannon diversity, and our bird based Index of Biotic Integrity and the rank of ecosystem service provisioning (provided by Ferraz et al., 2014), representing the existing gradient of human disturbances on each 1 ha of the studied patches where each of our bird point counts were located. Higher values of the rank mean fewer disturbances on the bird point count, while lesser values mean high disturbances.

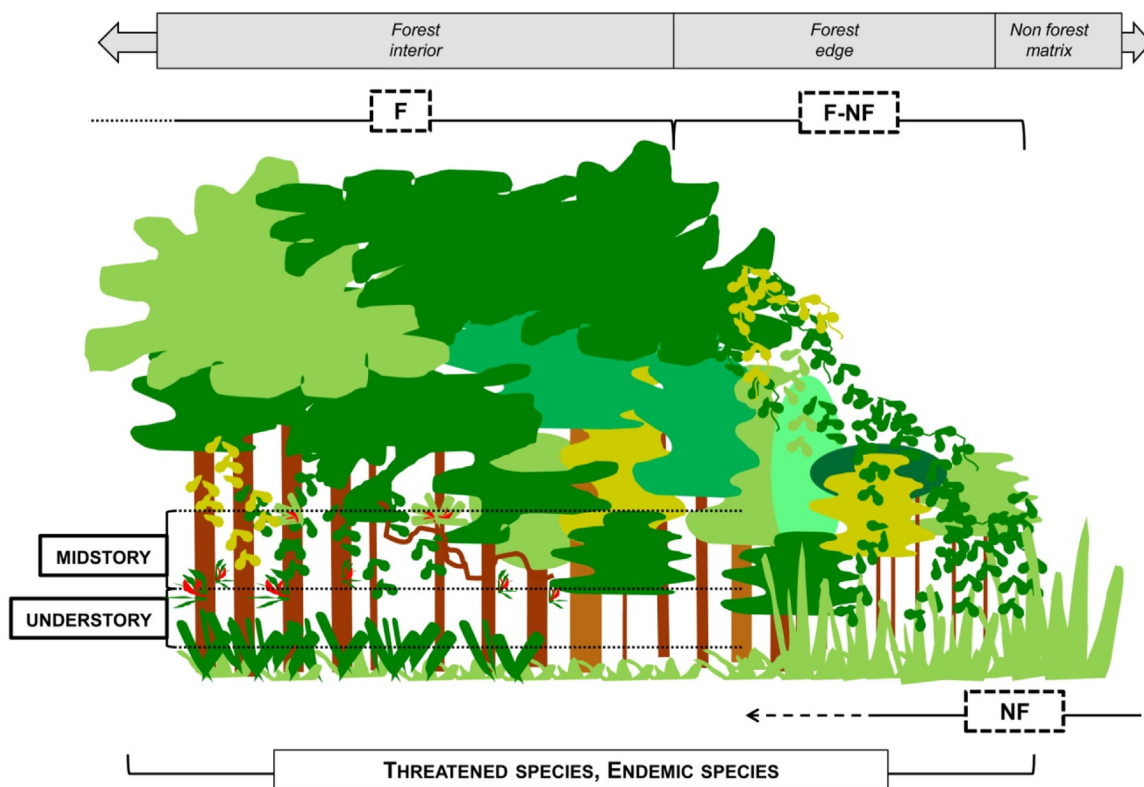


Fig. 4. Illustration exemplifying the forest habitat integrity represented by each selected bird metric for the IBI (in boxes). Each habitat of occurrence metric (dashed outline boxes) represents the general forest conditions of the patch, while foraging stratum metrics (solid outline boxes) represent the understory and midstory forest stratum quality. Threatened and endemic species metrics (inferior box) are an indication of the patch suitability for biodiversity conservation, another ecological value to be considered. The illustration follows the assumptions of successional stages and the edge effects often observed in the secondary growth patches located in HMLs in the Atlantic Forest domain (see Fonseca and Rodrigues 2000; Guariguata and Ostertag 2001; Harper et al., 2005).

and microclimatic condition, such as humidity, are main factors (Karr and Freemark, 1983; Karr and Brawn, 1990; Manhães and Dias, 2011; Stratford and Stouffer, 2013). Another factor that may determine species maintenance in patches is their ability to disperse through a non-forest matrix (Sekercioglu et al., 2002;

Antongiovanni and Metzger, 2005). However, as few species of small understory insectivores of Atlantic Forest have had their responses to the HMLs investigated (e.g., Uezu et al., 2005; Martensen et al., 2008; Boscolo and Metzger, 2009; Hansbauer et al., 2010; Zurita and Bellocq, 2012; Ferraz et al., 2012), it is difficult to

generalize the findings for all species of this group. Therefore, at this moment, we interpreted that the positive relationship of relative abundance of small understory-midstory insectivores (body mass up to 30 g) with the gradient is an indicator of either the in situ food availability or microhabitat conditions of the lower forest strata, as well as the landscape characteristics. Although there are uncertainties, our results demonstrate that small understory insectivores may reflect forest conditions even in highly degraded landscapes.

Although some endemic and threatened species are included in other metrics (see Appendix 2 in Supplementary material), they also act as an extra ecological value to be considered in the evaluation process of the studied patches (e.g., Karr and Chu, 1999). Both are sensitive to forest fragmentation (Goerck, 1997; Ribon et al., 2003; Anjos et al., 2010). Because of this, bird conservation plans commonly take into account the occurrence of these species to point out important areas to be protected (e.g., Bencke et al., 2006). Thus, presence of endemic and threatened species in patches of HMLs may be an indication of the existence of high integrity. Also these species suggest the patch suitability for biodiversity conservation.

No foraging guild metric showed a highly significant relationship with the gradient of human disturbances. Although bird trophic categories are normally used to evaluate and interpret the anthropogenic disturbances to tropical forests (e.g., Dale et al., 2000; Anjos, 2004; Gray et al., 2007; Giraudo et al., 2008; Lobo-Araújo et al., 2013) and the bird responses to forest succession (e.g., Aleixo, 1999; Pearman, 2002; Modena et al., 2013), usage by itself was not a good indicator of environmental conditions in our patches.

4.2. Evaluating Atlantic Forest patches: The IBI vs. classical measurements

The goal in development of an IBI is to incorporate a number of biological attributes of a place into the assessment process rather than depend on a single measure (e.g., richness or Shannon diversity). This leaves the questions: is our IBI an improvement over more conventional measures?

Species richness is the simplest and perhaps most widely employed measure of biological variability in ecosystems (Krebs, 1999; Magurran, 2004). Shannon diversity index accounts for both species abundance and evenness (Ludwig and Reynolds, 1988; Krebs, 1999; Magurran, 2004). Both measurements have been commonly used to compare and evaluate habitats (e.g., Tejeda-Cruz and Sutherland, 2004; Sodhi et al., 2005; Manu et al., 2007; Munro et al., 2011; Buechley et al., 2015) where different values between the communities are generally considered indicative of varying environmental conditions. In the Atlantic Forest biome this procedure has been widely used in studies of birds in large forest reserves (e.g., Antunes, 2005; Giraudo et al., 2008) and in the comparison between forest patches with different sizes and degrees of isolation (e.g., Anjos, 2001; Pozza and Pires, 2003; Piratelli et al., 2005; Lobo-Araújo et al., 2013). However, our results show that in small forest patches in a HML with high heterogeneity, the Shannon's diversity index and general bird species richness do not provide significant differences commensurate with the existing human disturbance level in each forest patch. This happens because in sites with low integrity a replacement of some species for another with distinct ecological function and habits may occur (e.g., generalist birds replacing specialists, see Antunes, 2005).

A focus on species composition and the relative abundance of functional groups adds additional information for interpretation of biological conditions (Byron, 2000; Chambers, 2008; Straube et al., 2010). This method can assess whether all ecological functions expected for an environment with integrity are being occupied, and by which species. However, it is not a simple task to identify

which functional groups are experiencing species compositions or relative abundance changes in response to differences in environmental conditions. For example, studies performed in HMLs that have considered few environmental variables are still questionable whether other source of variability could be the main driver of the observed changes in species composition in the functional groups (e.g., Anjos 2001; Piratelli et al., 2005; Lobo-Araújo et al., 2013).

We demonstrate here that an environmental assessment of small patches in highly disturbed HMLs using indexes such as richness and Shannon's diversity do not reliably capture the richness of biological conditions across a gradient of human impacts (Angermeier and Karr, 1994). In contrast, multiple metrics allow the final index to focus on both biotic elements and the processes that generate and maintain those elements (Angermeier and Karr, 1994). Systematic use of the IBI protocol assures that the monitoring and assessment process is likely to discover, understand, and explain many of the patterns and characters of biotic communities for each patch under assessment. Thus, the multimetric nature of IBI makes it adaptable to diverse environmental situations (Karr and Chu, 1999; Karr, 2006).

The existing Atlantic Forest located in HMLs are composed mainly by mature forest (e.g., Tabarelli and Mantovani 1999; Anjos et al., 2004; Anjos, 2006), early to late secondary forest (e.g., Ribon et al., 2003; Piratelli et al., 2008; Teixeira et al., 2009; Ferraz et al., 2012, 2014), small patches of assisted regenerating forests (e.g., Rodrigues et al., 2009; Brancalion et al., 2013), agroforestry patches (i.e., agricultural crops cultivated with forest association, see Sambuichi and Haridasan, 2007; Pardini et al., 2009), or patches composed by mixed native and exotic trees (i.e., abandoned plantations of *Pinus* and *Eucalyptus* with forest strata naturally regenerated, see Silva et al., 1995; Evaristo et al., 2011; Ferraz et al., 2014). Each patch is influenced by diverse human impacts depending on the surrounding land use and degree of human occupancy (e.g., Prevedello and Vieira, 2010; Melo et al., 2013). Examples of such influences include fires in sugar cane plantations (e.g., Martinelli and Filoso, 2008), cattle trampling in cow pastures (e.g., Pereira et al., 2015), and illegal hunting and logging in patches with high human accessibility (e.g., Aleixo, 1999; Cullen Jr. et al., 2001). Except for mature forest, all the above patch conditions were present in our study area. Therefore, considering our findings, the IBI adaptability and the Atlantic Forest overview, we advocate the use of IBI for assessment of small patches in HMLs throughout this biome (e.g., Medeiros et al., 2015).

Although we focused on the Atlantic Forest biome, many other tropical forests around the world face similar issues (Myers et al., 2000; Sodhi et al., 2005, 2006) that also demand assessments for future conservation and management plans (e.g., Lamb et al., 2005). Thus we deduce that the IBI is also a promising method for many other patches of tropical biomes in HMLs.

4.3. Final considerations about the IBI application

The conceptual foundation of the IBI approach has now been used to monitor and assess biological conditions of streams and rivers, wetlands, coral reefs, and terrestrial landscapes in at least 70 countries (Karr, unpublished data), including national and international efforts such as implementation of the Clean Water Act in the United States and the Water Framework Directive in the European Union (Ruaro and Gubiani, 2013). Although the use of IBIs in assessing tropical forests is new (e.g., Anjos et al., 2009; Medeiros et al., 2015), it has immediate potential application in countries that have well-founded environmental policy. For example, in Brazil, the Environmental Impact Assessment (EIA) program requires that forest patches in HMLs have environmental conditions assessed if any human activities will impact the region (CONAMA Resolution 001/86; CONAMA Resolution 237/1997; Glasson and Salvador,

2000; Sánchez, 2006; SMA Resolution 49/2014, this last for São Paulo state). Many of these EIAs are performed in highly disturbed HMLs (Alexandrino et al., 2016). The multidisciplinary characteristic of an EIA ensures that various environmental and biotic data be collected from the forest patches, which may facilitate the preparation of an IBI.

Since the first IBI definitions in Karr (1981), this multimetric assessment method has been improved by subsequent research (see Karr, 2006; Ruaro and Gubiani, 2013 for reviews of those advances). Advances include adjusting the metric selection process as well as the protocol for integrating information of multiple metrics to suit diverse ecological contexts and focal taxonomic groups (plants, invertebrates, birds, fish). The scale of the study, the number of sites investigated, the environmental conditions considered within the human gradient, and the endpoints of applying the IBI, are sources of variation among IBI studies. Also, the number of metrics in an IBI varies considerably among study systems (e.g., Bradford et al., 1998; O'Connell et al., 2000; Bryce et al., 2002; Glennon and Porter, 2005; Bryce, 2006). Thus, we emphasize that our forest scores should not be used in comparison with other locations and studies. However, we encourage other researchers to plan their own IBI analysis using the background we provide here, tailoring them to local needs.

This effort to adapt the IBI concept to Brazilian Atlantic Forest suggests both the utility of the IBI concept and the value to be derived from future studies. For example, which taxa and functional groups provide rigorous indicators of anthropogenic impacts in the Atlantic Forest biome (e.g., Medeiros et al., 2015)? What is the minimum sampling effort required to yield solid and interpretable results about the environmental conditions? These and other questions will provide insight about how to efficiently assess patches and plan proper biodiversity conservation actions in Atlantic Forest in HMLs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2016.10.023>.

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