Running head: The colourful side of the Amazon

# Title: Discolouring the Amazon Rainforest: How deforestation is affecting butterfly coloration

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## Abstract

Butterflies are among the most colourful organisms in the world and colour plays a central role in many of their life-history strategies. However, the efficacy of coloration strategies in these and other animals could be affected by sudden environmental changes, including anthropogenic disturbances such as habitat loss and fragmentation. Here we investigate the effect of forest disturbance gradients on the colours of fruit-feeding butterflies in the Amazon Rainforest. The disturbance gradients tested represented habitat-size (continuous primary forests versus forest fragments of 1, 10 and 100 ha) and succession gradients (continuous primary forests, 30 year old secondary forests, and three year old early successional forests). Using digital image analysis, we obtained intrinsic measures of butterfly colour patches corresponding to hue, saturation, brightness, in addition to measures of the contrast among patches and of the overall wing-colour diversity corresponding to 220 individuals, belonging to 60 species. Our results showed that butterflies in the secondary forest and continuous primary forest are more colourful than those found in early succession and fragments of forests. Individuals occurring in forests of early succession showed higher average values of hue and saturation, but lower brightness. Accompanying changes in colour composition, wing-colour diversity among species was lower in human-disturbed habitats, such as those of early forest succession and secondary forest. Forest fragments have smaller effects on butterfly colour composition, indicating that well-structured forest habitats can house butterfly assemblages with more diverse phenotypic features and colours. We show how high deforestation rates in recent years is linked with negative changes in functional coloration strategies (e.g. camouflage, warning colours), something that has to date been poorly explored or demonstrated for butterflies. Specifically, human interference has apparently placed butterflies are under strong selection for lower diversity in their colours and range of defensive strategies. Those species that are most colourful are the first to be locally extinguished, likely due to removal of native vegetation and increased exposure to predators, and more broadly owing to inhospitable environmental conditions. This illustrates an accelerated loss of local fauna and a "discolouration" of the Amazonian butterflies due to anthropogenic impacts.

Keywords: Anthropogenic gradients. Colour diversity. Conservation. Forest succession. Fruit-feeding butterflies. Tropical forest

## Introduction

Research on animal coloration is a classical issue that has long fascinated naturalists, including back to the great expeditions of influential naturalists during the 1800s (Bates 1863). This interest is partly due to the relevance of colours for understanding intra- and interspecific interactions, links to the abiotic environment, and the importance of such traits in illuminating our understanding of ecological and evolutionary processes (Stevens 2016; Endler and Mappes 2017). Through coloration, animals display broad repertoires in anti-predator and mating strategies, aiming to maximize survival and reproductive success (Cuthill et al. 2017).

Regardless of the specific strategies (e.g. anti-predatory or reproductive) employed by an organism, environmental characteristics work as key aspects selecting phenotypic patterns (Roslin et al. 2017). For example, recent research continues a long tradition in testing how visual signal transmission is related to the habitat in which animals occur and their contrast with the visual environment (e.g. Cheng et al. 2018;

Willmott et al. 2017; Walton and Stevens 2018). Therefore, environmental changes are expected to impact the adaptive value of individual coloration (Delhey and Peters 2017), in the same way that species assemblages from different habitats may house individuals with specific functional features and adaptations (Spaniol et al. 2019).

In butterflies, their evolutionary history and current selection pressures acting on phenotypic variation are printed on their wings, ranging from conspicuous colours or camouflage, to wing-eyespots and mimetic complexes (rings), which together are responsible for colour diversity in nature (Joshi et al. 2018). Environmental changes can affect colour patterns on butterfly wings in different ways. One of these is through the supply of host plants as food resources to immature individuals (Talloen et al. 2004). Caterpillar diet is often essential for chemical compound synthesis (e.g. flavonoids), which leads to diverse phenotypic expression, including of body and wing colours (Johnson et al. 2014). In addition, abiotic conditions may change wing patterns along different microhabitat gradients (Papageorgis 1975). Given that resource availability is linked to the abiotic conditions of each habitat, once plant community species composition is modified, more specialized butterflies (in terms of food resources) may disappear (Soga et al. 2015), or differ in colour pattern due to the nature and amount of available resources. A second way to understand the process of evolution and adaptation in butterfly colours consists of knowing how visual signals are transmitted when natural habitats are modified. Different communication strategies, including those used to mediate prey-predator and potential mating interactions may be affected by structural changes in the environment (Briolat et al. 2018; Dalrymple et al. 2018). Abiotic components (e.g. temperature, humidity, brightness), besides affecting animal thermoregulation, also have important roles determining energy and resource inputs in ecosystems (Dalrymple et al. 2018), impacting factors such as vegetation structure, visual backgrounds, and the light environment. In turn, in environments modified by anthropogenic impacts, the visual environment for signalling or against which animals would normally be hidden may change. This will affect the efficacy of colour strategies, either those relying on visual conspicuousness (e.g. mating and warning signals), or on concealment, affecting fitness.

In tropical forests, high biological diversity can lead to the coevolution of several adaptive strategies, including butterfly interactions with predators, competitors, mutualists, or even potential mates. This makes the tropical region not only rich in species but also in their phenotypic and behavioural diversity, including colour related strategies (Adams et al. 2014). The Amazon Rainforest is an ideal region to test the above issues because it is still largely preserved, yet is under great threat and suffering extensive deforestation, affording the potential to study organismal responses to human-induced environmental disturbance (Mesquita et al. 2015). In recent decades, this region has been widely transformed with high deforestation rates driven by the advance of agricultural frontiers, with concerning consequences for tropical biodiversity (Vieira et al. 2019). At present, public policies and interventions adopted are sparse and have promoted insufficient results for controlling the loss of huge areas of forest every year (Arima et al. 2014), which is accompanied by high species extinction rates (Stork 2010; Barlow et al. 2016). The immediate consequences of forest transformation are often unpredictable and require careful monitoring of ecological and evolutionary responses from different species (Caro et al. 2017).

Butterflies represent ideal study models for environmental assessments and allow relatively easy monitoring in nature (Freitas et al. 2014). These insects manifest a large repertoire of colours, which may be associated with the different habitats they occupy and different strategies for distinct signalling functions (Endler 1993; Dalrymple et al. 2015). Because butterflies play a diversity of ecological roles within complex networks, their responses can be extended to evaluate the effects of environmental degradation in the tropics (Spaniol et al. 2019). The fruit-feeding guild, in particular, comprises approximately 50-75% of all butterfly species belonging to the family Nymphalidae found in the Neotropics. This group is represented by four subfamilies: Charaxinae, Biblidinae, Satyrinae, and also a few tribes within Nymphalinae (Brown 2005). From the perspective of different coloration properties (hue, saturation, brightness, wing-colour diversity), our study aims to advance knowledge on how deforestation and habitat-size decrease may influence the colour patterns observed in those species that remain, and the potential effects on their anti-predator strategies. Environmental selection for specific colour combinations in species assemblages will be less intense in the primary forest, given that a variety of strategies and colour types should be able to thrive here with a greater range of visual backgrounds and light conditions. This should be reflected in greater colour diversity in more intact forest habitats, owing to a higher species richness and greater butterfly assemblages. In comparison, in forests of greater levels of fragmentation and earlier succession, fewer colour strategies may thrive, reducing overall species diversity, and this may be especially true for phenotypes that rely on specific linkages between colour phenotype and visual background or light conditions.

We analysed the coloration of butterflies found in different forest patches of different human impact, and classified butterflies based on putative defensive strategies (crypsis, transparency, warning signals, and eyespots). We hypothesized that fruit-feeding butterflies along anthropogenic gradients would be distinct with regards to their colour patterns, and that prevalent phenotypic characteristics should change according to habitat size and regeneration stage after a disturbance, including changes in the visual environment (light conditions, background contrast) (Fig. 1). From this viewpoint, we expect that: (i) with a decline of vegetation structure and changes in abiotic characteristics, as well as homogenization due to human activities will result in conspicuous butterflies being removed first, while broadly cryptic individuals (mainly brown winged butterflies) may persist by avoiding detection against generally brown substrates along the gradient. (ii) Colourful butterflies may be those most affected by forest disturbances, being the first to disappear due to increased exposure in more open habitats. This way, colour diversity and richness should be lower in disturbed habitats when compared to the preserved primary forest. As a counter-prediction, some types of intrinsically conspicuous coloration may be effective to a certain extent regardless of the visual environment (e.g. aposematism) and therefore persist even in degraded habitats. In contrast, specialist species with camouflage that is specifically effective on a limited range of backgrounds may decline if those backgrounds are removed.

## Materials and Methods

### Study area

Butterflies were sampled in areas of the Biological Dynamics of Forest Fragments Project (PDBFF) (2°21'36.14"S, 59°57'45.60" W), belonging to the National Amazon Research Institute (INPA). These are spread over 1000 km², 90 km north of Manaus, Amazonas State (AM), in a Brazilian Federal Protected Area created in the late 1970s to investigate the consequences of deforestation and forest fragmentation in the Amazon (Biereegaard et al. 1992). Three farms (Dimona, Porto Alegre, and Esteio) were defined as study areas. Each study area includes the following habitats: forest fragments of 1, 10, and 100 hectares, and habitats varying in succession, from in an initial stage of succession around the previous fragments, to secondary forests, and large extensions of primary continuous forest (Fig. 2). The initial succession comprised vegetation with approximately three years of regeneration, and isolated fragments from the other habitats. The secondary forest covers areas formerly used as cattle pasture, and which now have forests with 30 years of regeneration. Forest fragments are isolated patches of primary forest, structurally similar to the large areas of continuous forest (Laurance and Vasconcelos 2009). The primary continuous forest occupies extensive adjacent regions, and is used in this study as a control. In these landscapes, two distinct environmental gradients can be identified and monitored to understand the response of organisms to habitat variations: habitat-size and forest succession. We refer to the forest succession gradient as including the areas in initial succession, secondary forest and continuous forest; the habitat-size gradient, in turn, includes fragments of 1, 10 and 100 hectares, and the continuous forest as a control.

## Data sampling

Two field expeditions were performed between August and September 2015 and 2016, at the beginning of the dry season in the Amazon region. Each farm received a total of 12 sample units (SUs), each of them with five portable traps, containing attractive bait made with bananas fermented in sugar cane juice for 48 hours (Freitas et al. 2014). Sample units were installed on each farm, with three SUs in early successional sites, three in secondary forest sites, one for each forest fragment size (1, 10, and 100 hectares) and three points in the adjacent continuous forest, totalling 60 traps per farm. A minimum distance of 20 meters between neighbouring traps was observed, placed between 100 – 130 cm above the ground (Freitas et al. 2014). SUs were kept at least 500 meters from each other within each farm.

At each sampling occasion, traps remained exposed for eight consecutive days in each farm, being reviewed at 48h intervals to renew the bait, collect, identify, mark and release the captured butterflies. During the review process, two samplers equipped with entomological nets performed active samples of fruit-feeding butterflies around the traps, as long as they were visibly attracted by the bait. These combined methods allow us to complement the representation of butterfly species, including especially those species attracted but not caught by the traps (Checa et al. 2018). At least 10 individuals of each species were collected for subsequent lab measurement, and about three to four individuals per species were included, on average, for colour analysis (Fig. 3). Whenever possible, we selected the same number of males and females per species to account for sexual dimorphism.

### Colour measurements

The collected butterflies were fixed and deposited in an entomological collection (Laboratório de Ecologia de Interações - UFRGS) for gathering individual information. From 60 fruit-feeding butterfly species, we selected 220 individuals with well-preserved colour characteristics. Under natural light conditions, each individual was photographed in a ventral position. We took all pictures using a Nikon D5300 camera with 18-55 mm lens, accompanied with a scale bar and a grey card (18%) to correct for variation in light conditions among photographs (Stevens et al. 2007). The photographs were taken in RAW format and selected for appropriate exposure in RawTherapee software (version 5.3). All images were imported into the ImageJ program and through the MICA Toolbox add-on (version 1.22), and we generated multispectral images ".mspec" calibrated from the grey card (Troscianko and Stevens 2015). For every image, we drew “regions of interest” (ROI’s) around the wing areas that we wished to measure, based on the principle colours found on the wings. ROI’s were chosen based on identifying colour spots that could transmit visual information, such as wing areas with strong colour contrast with the wings as a whole. All photographed butterflies had their colour patterns quantified following a previous approach that made use of calibrated digital images (Stevens et al. 2007).

From the standardized images, we obtained colour data corresponding to reflectance in the three camera colour channels: red, green, and blue (RGB) on a scale of 0–255 for each colour channel. From these values, we were able to calculate several colour variables for each butterfly colour patch: hue (the colour type, e.g. orange or blue), saturation (colour ‘richness’ or intensity when compared to white light), brightness (the sum of the reflectance values along the spectral range, 300–700 nm), maximum contrast between patches (the greatest Euclidean distance measured between two patch colours on an individual in a colour space; see Data Analysis below), and wing-colour diversity (diversity of colours achieved on a single individual) (see Stevens et al. 2007). Finally, we present the frequency of four main putative anti-predator strategies: camouflage, transparency, warning colours, and wing eyespots. Each species and its type of defence was categorically classified by their general composition and likely interaction with the visual environment, observing the predominance of brown/cryptic colours, translucent wings, highly contrasting colours (for example, yellow, red, orange combined with black or white), and circular features on the wing resembling an eye. Brown/cryptic colours are categorized based on ecophysiological (thermoregulation) and defence (predation and detection avoidance) features. These colour patterns provide evidence on the habitat used by the species characterized by dark or light coloration, in which dark colours are essential for both camouflage and thermoregulation (Kingsolver 1985) in forested habitats, since dark colours increase radiant heat absorption. On the other hand, light colours are expected to be found in sites in which overheating is more likely (Ellers & Boggs, 2004), leading to higher chances of desiccation. Despite contrasting information about the functional role of eyespots, they can be characterized by the combination of ocellus on the ventral surface of wings, and have one of two anti-predator functions: (i) deflection, whereby a predator attack is redirected to non-vital body parts of the insect (many small ocellus occur on the border of the hind wing on the ventral surface) (Stevens, 2005); (ii) intimidation, in which the pattern of the wings may imitate a dangerous organism that is a threat to the predators themselves, such as the mimicry of owl eyes, or simply by generating a conspicuous display (one big spot on the hind wing in the ventral surface) (Stevens & Ruxton 2014). We appreciate that this is to a certain extent subjective but full categorisation of the type of defence each species primarily relies on methods beyond the scope of this study (requiring, for example, toxicity analysis and behavioural experiments).

**Data analysis**

We converted standardized RGB reflectance values to XY coordinates in a triangular colour space, whereby the centre of the space represents the achromatic point and the location around this corresponds to a given colour type and saturation. This approach is a common method used to characterise the nature of colour variation that exists, and quantify aspects of colour such as saturation and contrast, given a certain dimension of colour space, here based on three colour channels (see Stevens et al. 2009). We calculated saturation as the Euclidian distance from the centre of the colour space, whereby greater distances equate to higher levels of colour richness (Endler and Mielke 2005; Stevens et al. 2014). To calculate hue, we followed a range of past approaches in describing hue in the form of colour channels that describe the variation in colours present in the dataset (Komdeur et al. 2005; Spottiswoode and Stevens 2012; Stevens et al. 2014). This is not intended to directly mimic how visual systems process colour types with specific opponent colour channels, but is broadly similar in nature. To determine the most appropriate channels, a principal component analysis (PCA) was used to summarize the importance of each colour channel in butterfly phenotypic variation and to define the colour types that exist along relevant axes of variation in colour space. To eliminate achromatic variation and to analyse only colour differences, we removed brightness from the dataset by standardizing the colour values to proportions (Cuthill et al. 1999; Spottiswoode and Stevens 2012), and then from the resulting PCA derived colour channels that best described the variation in colour that existed among species and colour patches (Spottiswoode and Stevens 2012, Stevens et al. 2014). These colour channels define types of colour in the form of a ratio, which corresponds to an axis of variation running through the colour space. To quantify wing-colour diversity, we use the Mean Euclidean Distance between *x, y* colour space coordinates for all individuals within each treatment. Through a resampling analysis (random sampling with replacement) we corrected the differences in sample size among habitats in each gradient. The number of resampled butterflies was equal to the smallest group in the analysis (see Spottiswoode and Stevens 2012). For brightness, we calculated the average brightness in reflectance across the RGB channels.

All colour variables (hue, brightness, saturation, differences between patches, and colour diversity) were then individually analysed along the habitat-size and forest succession gradients, using generalized linear mixed models. For these analyses, we input a colour variable (the response) and environmental data (the explanatory variables) as fixed factors, and we also controlled for differences in abundance among species as a random factor. In order to make the coloration results independent of the butterfly assemblages in each habitat, we also controlled for the effects of species composition incorporating species identification in the generalized linear mixed models, and running it as random factor. We also present butterfly richness data for each habitat in a Venn diagram, and composition changes across habitats using a non-metric multidimensional scaling (NMDS) ordination, complemented with statistical testing by permutation via Adonis, using 999 permutations and Bray-Curtis index. All computations were performed with R, using PAVO and lmer4 statistical packages (Maia et al. 2013; Bates et al. 2015; R Core Team 2018). Sample coverage for each habitat was calculated to estimate the representativeness of fruit-feeding butterflies through the *q* statistics as proposed by Chao et al. (2013). This analysis was performer using the software *iNEXT* online (Hsieh et al. 2016).

## Results

From the 60 species sampled in field, 30 species (95 individuals) were found in primary forest, 33 (85) in secondary forest and 29 (229) in early successional habitats. Primary forest and early successional assemblages harboured most unique species, but just one species was common for both habitats. Eight species were common to all habitats across this gradient (Fig. 4), and several were found in at least two kind of habitats. In general, there were important changes in butterfly composition among the successional habitats (Fig 5). Forest fragments (1, 10, and 100 hectares) housed 10 species (18 individuals), 11 (17) and 13 (27), respectively (Fig 4). No significant changes were found in assemblage composition among the forest fragments (S1). The representativeness of fruit-feeding butterflies for each habitat as measured by sample coverage was respectively: 3 years = 0.97; 30 years = 0.87; Primary/Continuous Forest = 0.84, 01 hectare = 0.73, 10 hectares = 0.61, 100 hectares = 0.78.

First, we checked if there was structure in colour composition of the fruit-feeding butterfly assemblages along both habitat-size and forest succession gradients. From the achromatic central point (grey) in the colour space triangles, we observed a trend for longwave (LW) colours in all the habitats, and the colour values cross through the centre of the colour space from long to shortwave (SW) and mediumwave (MW) parts (Fig. 6). This was confirmed by Principal Component Analysis (PCA) using a covariance matrix with standardized values for each colour channel.

For the forest succession gradient, the first two axes of the PCA expressed 99% of all variation in butterfly colour. The first axis separated LW from the other colour channels, explaining 79% of the phenotypic patterns (i.e. LW / [SW+MW]). Larger values correspond to patches that are more red in colour, whereas lower values to patches that are more blue or green. The second axis contained 20% of the variation, separating SW from LW and MW (SW / [LW + MW]: PC1 coefficients: SW: 0.554; MW: 0.525; LW: -0.645. PC2: SW: 0.660; MW: -0.749; LW: -0.042). Larger values correspond to blue colours, and smaller values to those that are more yellow/brown. The first two axes of the PCA also expressed 99% of the phenotypic variation of the butterflies to the habitat-size gradient. In a similar pattern, the first axis separated LW from MW and SW, explaining 72% of the phenotypic patterns observed. The second axis contained 27% of the variation, separating SW from LW and MW (PC1 coefficients: SW: 0.553; MW: 0.483; LW: -0.677. PC2: SW: 0.634; MW: -0.771; LW: -0.031).

Significant changes were found along the forest succession gradient for the following colour variables: hue, saturation, brightness, and wing-colour diversity. Higher hue values were observed in primary forest regions (F-value = 6.60, P = 0.01). In other words, longer wavelength rich colours (LW) such as brown/red prevail in older forested areas regarding succession. For saturation, we detected lower values in areas of early succession (F-value = 4.48, P = 0.03). This means that butterflies are less colourful in recently disturbed habitats when compared with continuous forest. Brightness values were higher in the early succession and secondary forest habitats (F-value = 3.93, P = 0.05) (Fig. 5), in which butterflies with lighter colours are more common. The forest interior may be housing melanic patterns, phenotypically darker individuals. We found a larger wing-colour diversity for the continuous forest ($P < 0.05$ for 75.6% of resamples with N = 40) when compared to the treatments under succession. A similar pattern was also observed for forest patches ($P < 0.05$ for 67.1% of resamples with N = 7), which showed a lower colour diversity than continuous forest (Fig. 7). Larger forest habitats can house butterfly assemblages with more diverse phenotypic features and colours. Boxplots of the non-significant variables of colour for fruit-feeding butterflies are attached in the supplements (S2).

Among putative anti-predatory strategies using colour patterns (Fig. 8), camouflage appears as a predominant feature in all studied habitats. As the forest succession progresses, wing-eyespots become more important and appear frequently. Transparency seems to be an exclusive strategy for the well-preserved continuous forest. Thus, the co-occurrence of all different phenotypic manifestations is observed exclusively under areas of intact vegetation, and reinforces our results on colour diversity.

## Discussion

In agreement with our predictions, the colour composition of butterfly assemblages is in close association with habitat features. Several butterfly colour variables changed among habitats, especially along the forest succession gradient. The butterfly species composition followed the same patterns for this gradient, indicating segregation among butterfly communities in initial stage of succession, secondary forests, and primary continuous forest. Butterflies using conspicuous colour patterns seem to be the most affected by human-activities, appearing among the first to disappear from recently disturbed forest regions in the Amazon. On the other hand, this study also demonstrates the positive effect of natural regeneration in recovering colour composition and ecological structure in modified tropical forests (Crouzeilles et al. 2017). The maintenance of both primary and secondary forests in human-modified landscapes might be helpful for re-colonization of plant species and interaction networks (Pellissier et al. 2018; Rozendaal et al. 2019). In the same way, the regeneration of perturbed habitats over decades of succession may also assist in the maintenance of species composition, and of the diversity of protective coloration and signalling in butterflies associated with forest interiors. Our results are supported by the large sampling coverage calculated for all types of studied habitats in the Amazon.

Whatever the kind of interaction, environmental features can produce effects on species assemblages through individual colour composition, and this was observed for butterfly response to the forest degradation and habitats loss in the Amazon (Spaniol et al. 2019). Firstly, camouflage appears as a dominant feature against predation in many animal groups across the tropics, including birds and butterflies (Dalrymple et al. 2015). With a predominance of individuals with cryptic colours (especially browns) throughout the forest succession gradient, including in continuous forest, this study supports that idea, suggesting that camouflage is one of the main defence strategies for Lepidoptera and is a valuable defence even as the habitat composition is altered. This also suggests that generally dull cryptic colour patterns may enable concealment in a range of habitats. Moreover, a lower colour saturation of butterflies occurring in recently disturbed sites reveals that conspicuous individuals may be receiving greater predatory pressures, especially in open habitats. While one may expect that conspicuous markings may operate across a range of visual backgrounds, it is possible that these conspicuous species may become too exposed when contrasting with new backgrounds created by disturbances. Consequently, more colourful butterflies, with the exception of aposematic patterns, may be among the first to disappear locally soon after the deforestation process. The above mentioned results are corroborated with changes in beta diversity of fruit-feeding butterflies in the different habitats of the successional gradient. In this case, species composition is directly linked to changes in colour patterns in the Amazon region. In addition, habitat degradation may alter the composition of predators and allow a greater number of naïve predators into the forest area (Bruno and Cardinale 2008; Ciuti et al. 2012). This may result in greater attack rates on butterflies that normally can rely to a great extent on the learnt avoidance by predators of their conspicuous warning, startle, or mimicry signals. From a high diversity regional pool of species, selection may therefore lead to lower diversity in butterfly colours, as well as in their protective and signalling strategies, in degraded habitats.

Furthermore, we note that conspicuousness is not necessarily an exclusive feature of butterflies living in primary continuous forests. There is a remarkable variance in the colour properties we analysed (hue, brightness, saturation and colour diversity), showing that conspicuous and cryptic individuals even with patterns conferring different communication roles can make use of the same habitats. In addition, habitats in a preserved forest are capable of harbouring greater colour diversity and subsequently more anti-predator strategies. This may be possible because of the large supply of substrates such as leaves, trunks, branches, stones, with which the individuals can "interact" in terms of their phenotypic appearance (Pinheiro et al. 2016). Such environmental resources are not always readily available after a severe disturbance, where abrupt and intense changes can lead to modifications in the habitat structure and vegetation resilience trajectories (Jakovac et al. 2015). Differences may also be strongly controlled by changes in predator communities (Mappes et al. 2014).

Analysing the habitat-size gradient, we discovered that forest patches with different sizes might have smaller effects on butterfly assemblage colour composition. Our study areas comprise an already well-developed forest matrix, which may be permeable to many species, including forest specialists (Schtickzelle et al. 2007). Reduced forest patches do not necessarily lead to strong changes in vegetation composition or structural features (Brown and Hutchings 1997), which however are easily identified along the forest succession (Mesquita et al. 2015). Therefore, the supply of immature host plants, adult food and substrates may remain. Complementarily, the way visual signals are propagated may remain mostly unchanged since forest characteristics are substantially maintained. This means butterflies with distinct phenotypes may be able to move among forest patches with a relatively reduced risk of predation. Environmental conditions and resources such as leaves, twigs, trunks and light entry through the forest canopy may also remain available and relatively constant, helping colour patterns remain the same within forest habitats patches.

Our findings suggest that butterflies from a regional species pool are under selection for lower diversity in their colours as well as in their defence strategies due to human interference. The same has been observed for species richness and composition (Spaniol et al. 2019). Coloration is only one of several organismal traits offering quick responses to environmental changes, but this has been seldom applied in our quest to understand threats to biodiversity and processes that lead to species extinctions (Hook et al. 1997; Caro et al. 2017). We highlight the study of animal coloration as an excellent basis for the evaluation of environmental health and in the planning for management and conservation of forest ecosystems. Colour shifts are important markers of several anthropogenic changes (Caro et al. 2017), and by observing phenotypic aspects in animals, we can systematically monitor the performance of protective coloration and signalling under different environmental conditions. Certainly, the colour patterns observed here are not only due to increased predation but might have alternative explanations. Species composition changes could be caused by many drivers aside from predation rates on adults mentioned above. Changes could also be caused by decreased host plants, increased larval predation rates in more opened forest structures, increased parasitoid abundances, or increased predator populations (for both butterfly and caterpillar). Therefore, a next important step is to investigate whether the reported pattern reflects ecological filtering or adaptation processes.

At a time when deforestation rates are increasing again in the largest tropical forest in the world (INPE 2019), this study draws attention to the importance of maintaining well-preserved forest remains in the Amazon. In addition, forested habitats that have been recovering for 30 years (secondary forest) show increasing in colour diversity, allowing the movement and permanence of organisms near the primary forests, maintaining ecosystem services. Thus, the maintenance of legal reserves inside private properties and restoration programs in degraded areas with higher biological importance (Metzger et al. 2018; Vieira et al. 2019) should be encouraged, being key factors aiming conservation of biodiversity in tropical forests. We also suggest that the presented results and actions should be incorporated into public policy, reinforcing that information based on scientific research is crucial to the decision-making process. Once unplanned, forest conversion leads to constant uncertainties about the compliance with Brazilian environmental legislation, we provide strong evidence that a significant portion of the butterfly fauna may be facing disappear from tropical forests in the near future. This is just the visible part of the Amazonian fauna - in the literal sense of the word, where the most colourful butterflies are the first to be locally extinguished by removing the native vegetation. It is necessary to use pragmatism, and this insect group holds the public eye enabling effective warnings about the limits of forest exploitation. Otherwise, the largest rainforest in the world will be less colourful without some of its main inhabitants.

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## Figure captions

**Fig. 1** Conceptual framework and predictions on colour-habitat patterns for fruit-feeding butterfly communities in the Amazon Rainforest. From the regional species pool, we expect that butterfly assemblages under similar habitats in the intact forest can have a higher colour composition compared to degraded habitats, where selection pressures on phenotypes may change and select for a fewer colour patterns. Under human-induced changes, anti-predator strategies may display different performances, leading to differences in some colour properties. Colour richness and intensity (saturation), differences between colour patches, and wing colour diversity may decrease with a greater homogenization. In forests made more open and brighter, conspicuous colours that are highly contrasting with altered backgrounds may not have the same performance due to increased exposure of those butterflies to threats. Camouflage involving a resemblance to the remaining substrate after a disturbance, combined with the presence of wing-eyespots may increase the survival chances of some butterflies, avoiding detection or directing predator attacks to less vital parts of the body. Overall, we expect a shortage of colourful butterflies associated with deforestation in the Amazon Rainforest, such that the Amazonian fauna is currently undergoing a process of discolouration.

**Fig. 2** Map with the three study areas at the Biological Dynamics of Forest Fragments Project (PDBFF), indicating the location of Dimona, Porto Alegre and Colosso farms. White patches represent deforested areas initially used for grazing purposes. The green portion corresponds to primary intact forest areas, including all the forest fragments used for the experiments in each study area (1, 10, and 100 hectares). The inset reveals the habitat disposition on each farm: green is for primary forest (either continuous or in fragments), white for secondary forest (30 years of regeneration), and black for early succession patches (three years after a disturbance - around fragments).

**Fig. 3** We measured the colours of 220 individuals belonging to 60 species of fruit-feeding butterflies. (a) Transparent butterfly (*Cithaeria andromeda*) with camouflaged wings. Photograph by Iserhard CA. (b) Owl butterfly (*Caligo teucer*) with typical wing eyespot. Photograph by Spaniol RL. (c) The colourful *Prepona narcisus* with highly contrasting wings. Photograph by Spaniol RL.

**Fig. 4** Venn diagram comparing species richness among habitats that make up the a) Sucessional gradient and b) Habitat-size gradient.

**Fig 5.** Non-metric multidimensional scaling (*NMDS*) ordination of Bray-Curtis distance matrices for butterfly assemblage composition in samples from early successional habitats (three years after a disturbance), secondary forests (30 years of regeneration), and primary intact forest in the Amazon Rainforest. The diagram was constructed by using the 'ordispider' function of vegan package to illustrate the habitat and samples. Each dot represents the sample unit ensemble from one site. Statistical testing by permutation (999 times) via Adonis indicates differences in composition among the habitats: $R^2=0.14$, $P=0.001$.

**Fig. 6** Butterfly reflectance in a colour space triangle. Each dot represents the reflectance values for each individual observed along the forest gradients in the Biological Dynamics of Forest Fragments Project (BDFFP) areas: a) Forest Succession; b) Habitat-size. All dots were coloured according to the treatments identified through the legend. S = shortwave; M = mediumwave; and L = longwave refers to the wavelength according to the RGB reflectance values.

**Fig. 7** Boxplots of colour variables for fruit-feeding butterflies. Wing-colour diversity in the different habitats in areas in the Biological Dynamic of Forest Fragmentation Project (BDFFP) of the (a) habitat-size and (b) forest succession gradients. c) Hue; d) Saturation and e) Brightness in habitats of the forest succession gradient (Early Succession – three years after a disturbance, Secondary Forest – 30 years of regeneration and Primary Continuous Forest).

**Fig. 8** Main defence strategies manifested by butterflies (camouflage, transparency, warning-colour, and wing eyespots) for different habitats that make up the succession (a) and habitat-size (b) gradients in the Amazon Rainforest (for details see text).

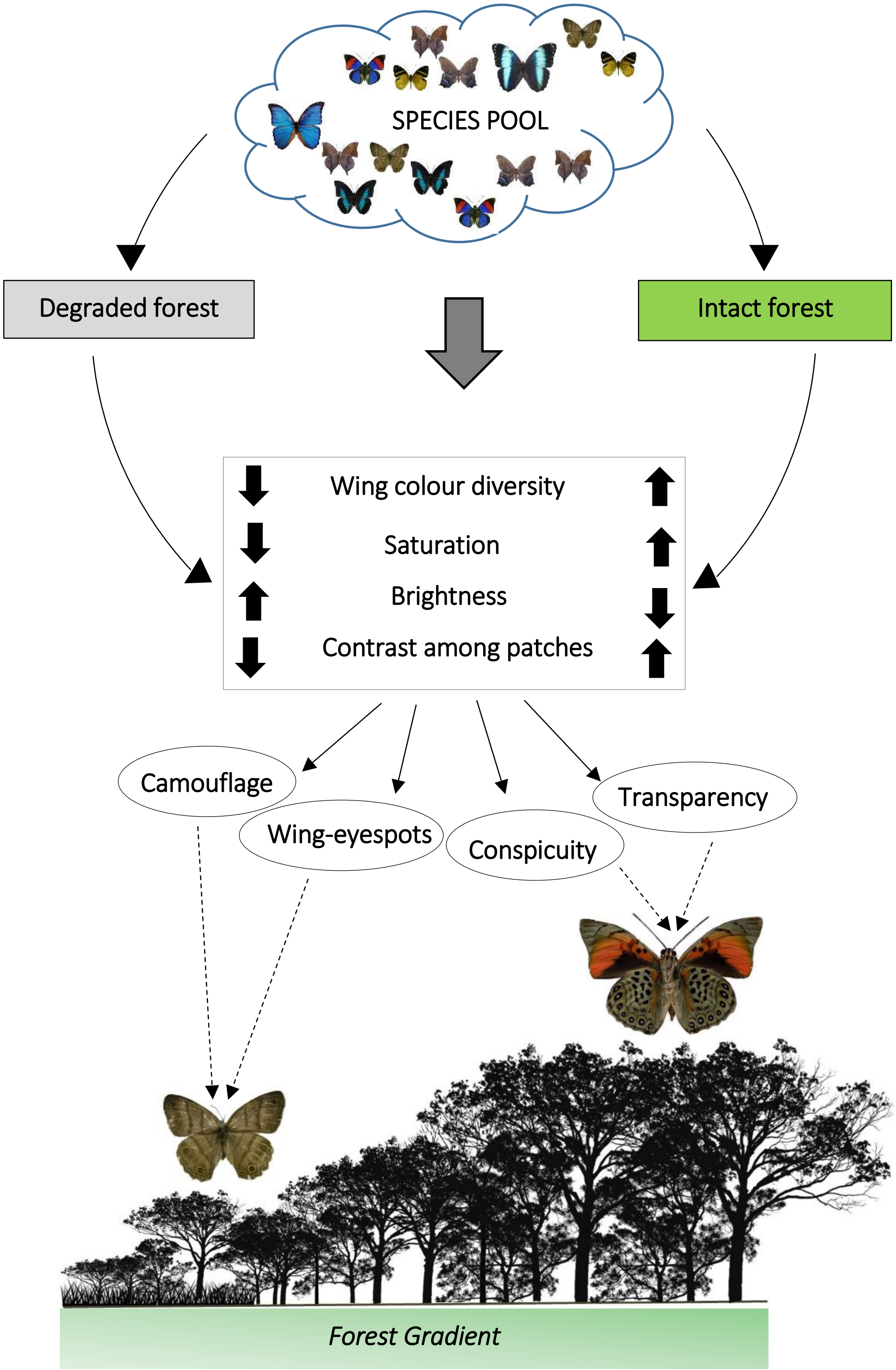
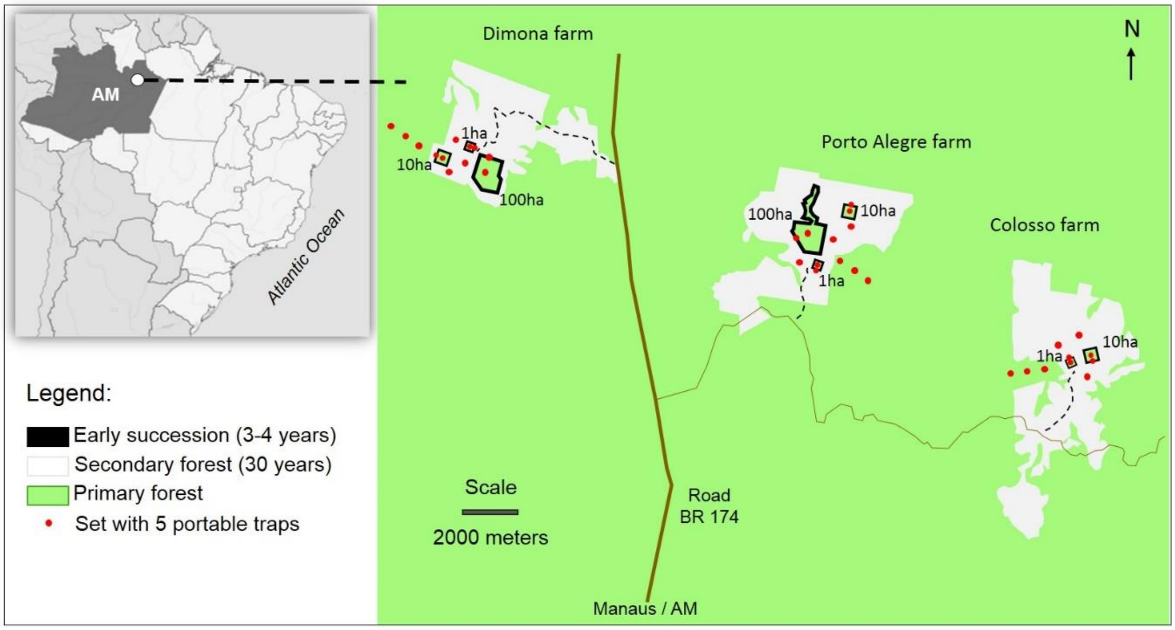

SPECIES POOL
Degraded forest
Intact forest
Wing colour diversity
Saturation
Brightness
Contrast among patches
Camouflage
Wing-eyespots
Conspicuity
Transparency
Forest Gradient

**Fig. 1**



AM
Atlantic Ocean
Dimona farm
1ha
10ha
100ha
Porto Alegre farm
100ha
10ha
1ha
Colosso farm
1ha
10ha
N
Legend:
Early succession (3-4 years)
Secondary forest (30 years)
Primary forest
Set with 5 portable traps
Scale
2000 meters
Road
BR 174
Manaus / AM

**Fig. 2**

A
B
C

**Fig. 3**

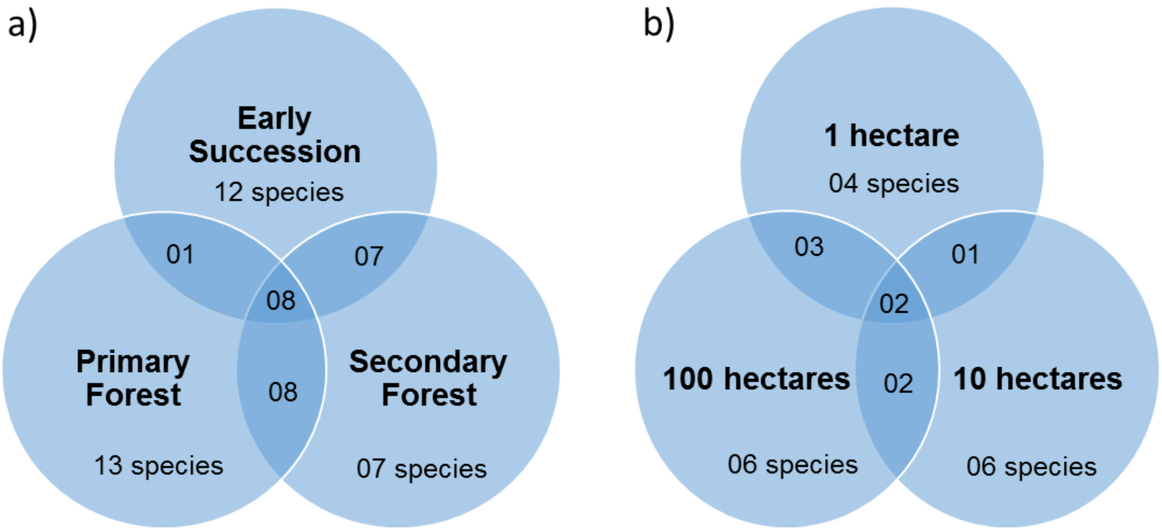

a)
Early Succession
12 species
01
07
08
Primary Forest
08
Secondary Forest
13 species
07 species
b)
1 hectare
04 species
03
01
02
100 hectares
02
10 hectares
06 species
06 species

Fig. 4

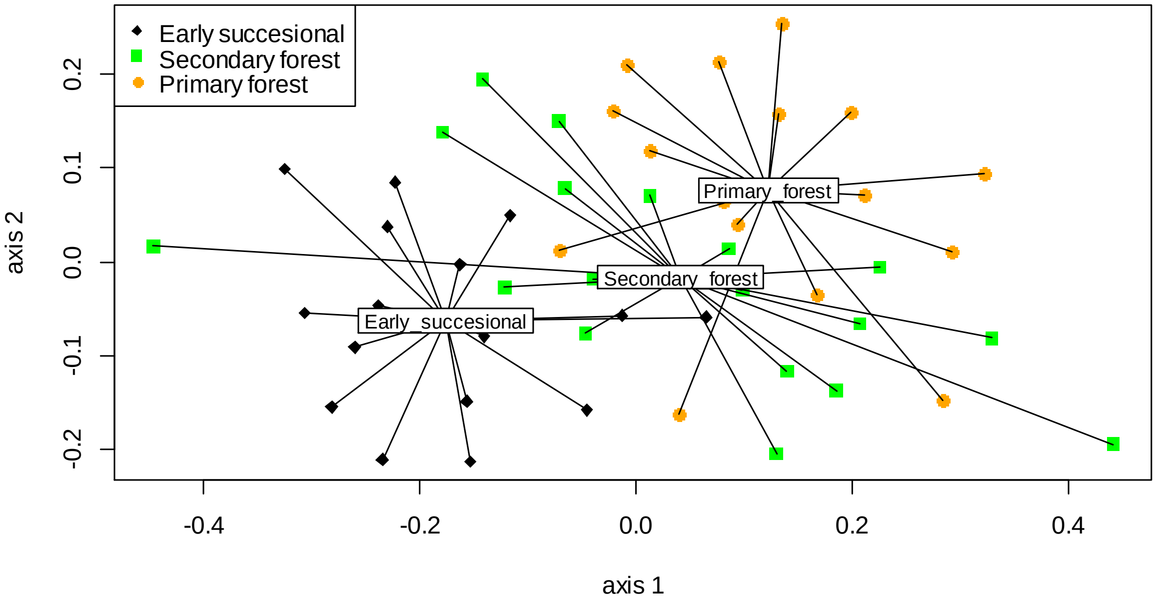

Early succesional
Secondary forest
Primary forest
Primary_forest
Secondary_forest
Early_succesional
axis 2
0.2
0.1
0.0
-0.1
-0.2
-0.4
-0.2
0.0
0.2
0.4
axis 1

Fig 5.

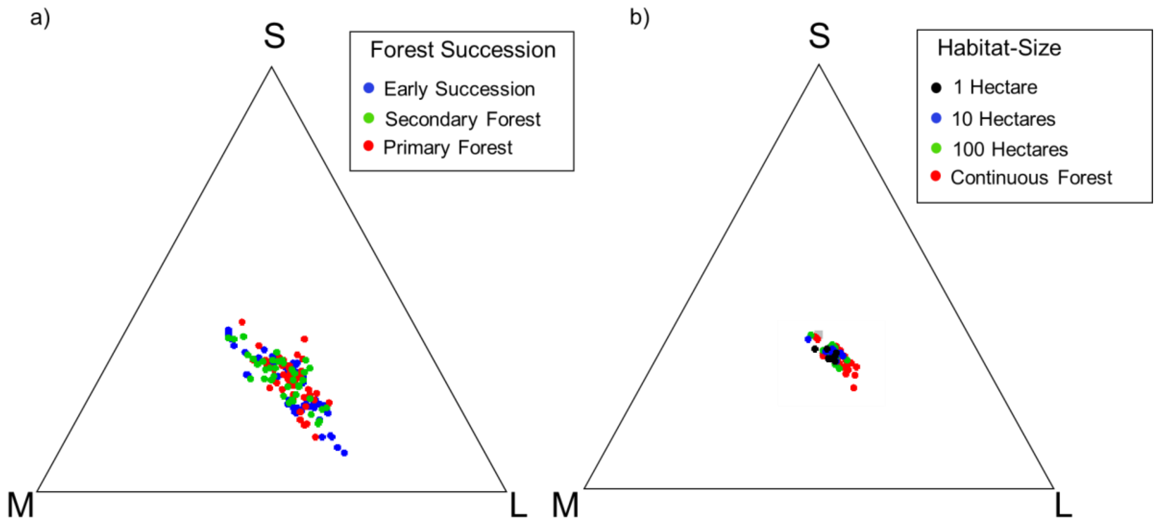

a)
S
M
L
Forest Succession
● Early Succession
● Secondary Forest
● Primary Forest
b)
S
M
L
Habitat-Size
● 1 Hectare
● 10 Hectares
● 100 Hectares
● Continuous Forest

**Fig. 6**

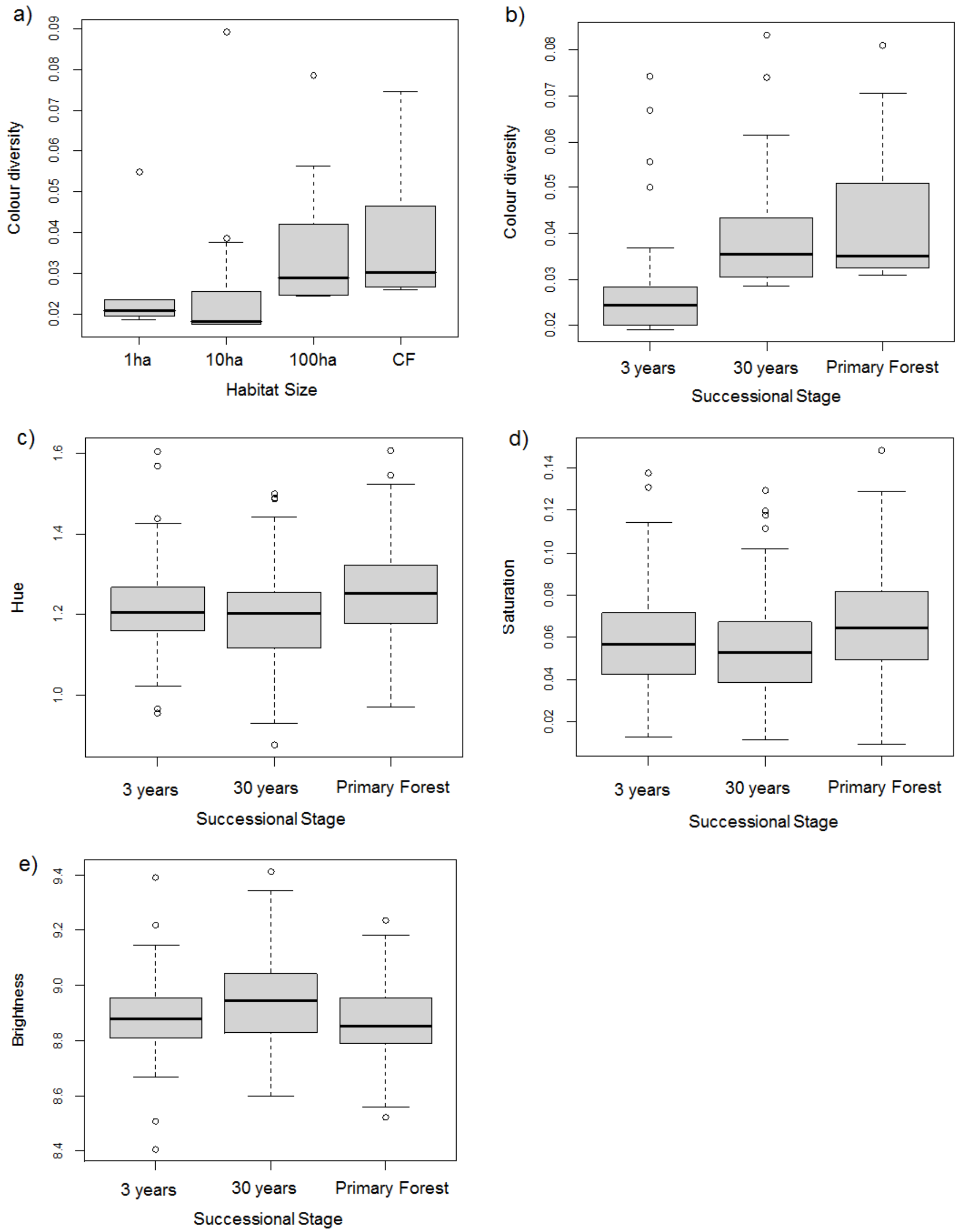

a)
Colour diversity
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
1ha
10ha
100ha
CF
Habitat Size
b)
Colour diversity
0.02
0.03
0.04
0.05
0.06
0.07
0.08
3 years
30 years
Primary Forest
Successional Stage
c)
Hue
1.0
1.2
1.4
1.6
3 years
30 years
Primary Forest
Successional Stage
d)
Saturation
0.02
0.04
0.06
0.08
0.10
0.12
0.14
3 years
30 years
Primary Forest
Successional Stage
e)
Brightness
8.4
8.6
8.8
9.0
9.2
9.4
3 years
30 years
Primary Forest
Successional Stage

**Fig. 7**

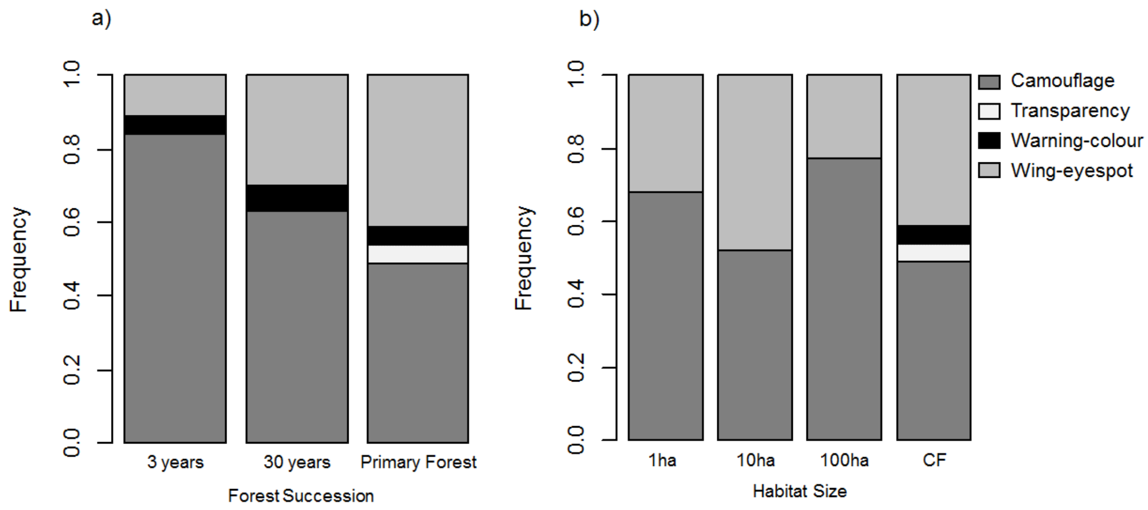

a)
b)
Frequency
0.0
0.2
0.4
0.6
0.8
1.0
3 years
30 years
Primary Forest
Forest Succession
1ha
10ha
100ha
CF
Habitat Size
Camouflage
Transparency
Warning-colour
Wing-eyespot

**Fig. 8**