Effectiveness of two sampling methods for social wasps in different ecosystems

Mateus Aparecido Clemente, Roger Guevara, Hugo Ribeiro Moleiro, Orlando Tobias Silveira, Marcos Magalhães de Souza & Edilberto Giannotti

1. Universidade Estadual Paulista, UNESP, Rio Claro, São Paulo, Brazil. 2. Instituto de Ecologia do México, INECOL, Xalapa, Veracruz, Mexico. 3. Museu Paraense Emilio Goeldi Belém, Pará, Brazil. 4. Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas, Inconfidentes, Minas Gerais, Brazil.

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Abstract. There are different methodologies used to make an inventory of social wasps. In general, these methods are divided into active search and passive collections. Each method has a different performance, depending on the environment in which the collection is being carried out. Thus, the choice for the proper methodology according to the study area will impact the success of sampled species. This study aimed to evaluate the performance of sampling methodologies for social wasps (active search and bait traps) in different phytophysionomies (Regenerating Cerrado, “Cerradão”, Restored Forest, Semideciduous Forest and Riparian Forest) in the state of São Paulo, Southeastern Brazil. The active search sampled a greater number of species, with the exception of the Regenerating Cerrado. Regarding abundance, the active search was also more efficient, except in “Cerradão”, where there was no difference between the methods, and in Regenerating Cerrado, where the traps sampled a higher number of individuals. The fact that none of the methods collected all the sampled species indicates that it is appropriate to use more than one collection method in order to obtain a sampling closer to the true richness of the studied sites.

Keywords: Active search; attractive traps; diversity; phytophysionomies; richness.

In zoological inventories, ecology or taxonomy of social wasps, insects popularly known in Brazil as “marimbondos” or “cabas”, different active and passive sampling methods are used for collection (Souza & Prezoto 2006; Souza et al. 2015; Somavilla et al. 2019).

The active methods are dependent of the collector presence in the field. There are different active sampling methods, such as: (1) Punctual sampling, when the collection is made in specific areas, such as floral resources (Marques et al. 2005; Hermes & Kohler 2006; Clemente et al. 2013); (2) Utilization of quadrants, in which an area is pre-determined before the collection and then carefully examined (Souza & Prezoto 2006); (3) Active search, when the collection is made by moving through an area in search for individuals or colonies, with the aid of an entomological net, with this methodology being the most used in social wasps samplings (Diniz & Katayama 1994; Silveira 2002; Elpino-Campos et al. 2007; Santos et al. 2007; Ribeiro-Junior 2008; Jacques et al. 2012; Silva 2012; Somavilla et al. 2014; Souza et al. 2020); (4) Active collection using a liquid bait of sucrose solution, with the aid of a pulverizer which sprays the solution over marked points on the vegetation of a trail (modified from Liow 2001; Lima et al. 2010; Tanaka-Junior & Noll 2011; Locher et al. 2014).

In the passive methods, traps are positioned and remain in field and therefore they have no direct interference of the collector; traps are methods in which an equipment is made to cease the insect’s movement, in a way that when an insect enters the trap, it can no longer escape (Almeida et al. 1998). These traps can be either flight interceptors, such as Malaise trap (Silveira 2002; Morato et al. 2008; Souza et al. 2015, Somavilla et al. 2019) or bait traps (Locher et al. 2014). The bait used in the traps can be of animal source, (such as sardines) (Silveira et al. 2005; Ribeiro-Junior 2008; Clemente 2009; Togni 2009), sugary liquids (Santos 1996; Ribeiro-Junior 2008; Souza et al. 2015), sodas (Wegner & Jordan 2005), chemical attractors (Lamdolt et al. 2000) and light traps used to attract species of Apoica (Neto et al. 1995).

Despite the rising number of studies regarding sampling methods of social wasps, there are few studies dealing with the efficiency of different methods when they are used in areas which have different ecosystems (Souza & Prezoto 2006). Considering that a species inventory of a given area is the first step for its conservation and the rational usage of its biotic resources (Melo et al. 2005), the definition of a sampling methodology is a strategic tool to obtain secure information of the biota, hence justifying studies with this theme.

Thus, the present study aims to assess the efficiency of different sampling methodologies for social wasps’ collection (active search and bait traps) in different ecosystems: Regenerating Cerrado, “Cerradão”, Restored Forest, Semideciduous Forest and Riparian Forest).

MATERIAL AND METHODS

The collects were made in five ecosystems of different phytophysionomies in the State of São Paulo (Figure 1).

(A) A fragment of Semideciduous Forest (Pagan & Leitão-Filho 1987), with approximately 230 ha, having parts of both Rio Claro and Araras municipalities (22°21’06” S, 47°29’07” W), located at 630 m of altitude. The area has a semi-dense...
canopy, with open canopy areas resulted from different levels of environmental stress, such as near sugar-cane plantations, fires and deforestation. The tree component has two floristically distinct stratifications: one from seven to 15 m high and another reaching 20 to 25 m high (PAGANO et al. 1987).

(B) An area of Restored Forest, located at the municipality of Iracemápolis (22°34′34″ S, 47°30′25″ W), at 624 m high. The restored area has approximately 20 ha and had its restoration process initiated in 1987 (Siqueira 2002). The area is surrounded by sugar-cane plantations and is possible to observe different levels of canopy stratification, with a mean of 10 m high.

(C) An area of Regenerating Cerrado (22°24′22″ S, 47°32′18″ W), at 650 m high. The area is a local public location, with the southern border being limited by the Universidade Estadual Paulista (UNESP) and the northeastern border limited by the Floresta Estadual Edmundo Navarro de Andrade (FEENA), a fragment of “Paludosa” forest (a swamp-like environment), and an urban neighborhood which causes intense pressure. This vegetation area has approximately 100 m², with a grove-like aspect, absence of underbrush and trees with height relatively uniform. The litter is scarce or inexistent, as the same as presence of seedlings (CARDOSO-LEITE et al. 2004).

(D) Riparian forest area (FRAGOSO 2005) located at the municipality of Itirapina, along the Lapa stream (22°22′16″ S, 47°47′16″ W), at 656 m of altitude, with an area of approximately 14 km². The study site, originally part of a larger fragment of Mesophile Semideciduous Forest, suffers from strong agricultural impact, which reduced its original area to a thin band of riparian forest surrounded by sugar-cane plantations.

(E) “Cerradão”(22°24′49″ S, 47°45′32″ W), located at 671 m high, at the municipality of Ipeúna, is a phytophysionomy which have some similarities with a “Cerrado” in a restricted sense. This area has a continuous canopy and arboreal coverage between 50% to 90%, directly influencing in luminosity conditions, thus favoring differentiations in the bushy and herbaceous strata. However, in the surrounding areas there are extensive sugar-cane plantations with burning practices during the pre-harvest, generating strong negative pressure towards the fragment. Additionally, a strong motorcycle traffic also occurs withing the fragment, causing damages to the ground and seedlings. Finally, several aggradations and tree falls were observed.

The field work was done from October 2011 to June 2013. During the whole year, in alternating months, two field trips were conducted in each area. The first trip of each month aimed to mount and set the traps and the second to disassemble and remove them. The first and second trip have a seven-days interval. Thus, each trap remained for 100 h in the field, totaling 10,080 h of passive collection per area.

Additionally, active searches were conducted during the same days of trap mounting/disassembling. The searching was compounded by two members which remained in field during seven hours/day, from 09 a.m. to 16 p.m., a time period with a higher foraging activity range of some groups of social wasps in different ecosystems (Santos & Presley 2010; Barrosa et al. 2014; Brito et al. 2020). Thus, a total of 168 h of sampling effort per area was done in a year. The searching for individuals were conducted in local trails of each area, with the verification of nest occurrence in tree holes, large-leaved plants and edifications. The collected wasps were killed in recipient containing ether and were posteriorly stored in 70% alcohol.

The bait-trap distribution was done by the following procedure: ten points, distanced 100 m from each other, were marked using plastic tape tied to trees, thus reducing the chance of pseudoreplication by collection of a single wasp population in different sampling unities. At each point, five traps with a height of 1.5 m from ground and five other traps at canopy level (around 5-9 m) were set. The traps were made with 2 L plastic bottles (Souza et al. 2015). At each bottle, four circular holes were done and 200 ml of concentrated passion fruit juice were added to be used as bait. The specimens were collected with a sieve and tweezers and fixed in 70% alcohol recipients of the universal collector type.

The sorting and pinning of specimens were done at the Rio Claro Campus of the UNESP. The species were identified by comparison with specimens deposited at the social wasp collection of the Zoology Department and by using identification keys to genera and species (Richards 1978; Cooper 1997; Carpenter & Marques 2001; Pickett & Wenzel 2007).

The software used in the analyses were PAST – v. 1.49 (Hammer et al. 2001), BioEstat v. 5.0 (Ayres et al. 2007) and R (R Development Core Team 2009).

The relative abundance of each species by each different method was measured by the ratio abundance of each species/total abundance. To verify which method was more efficient in each of the five sampled areas, the richness and abundance values were submitted to a linear mixed model test (lmm), aiming to verify the presence of significant differences. The type of vegetation, with five levels, and the sampling methods were set as fixed parameters. As each sampling point was repeatedly sampled, despite the method was changed, we used the identity of the points as a random parameter. It allowed us to infer the effects of microenvironments over the data variation in each kind of vegetation. For specific comparisons between sampling methods, we used the contrast based on the linear predictor of the model. To assess if the number of collections was enough to each sampling method (active search, bait traps) the estimators Jackknife 1 and 2 were applied.

RESULTS AND DISCUSSION

The mixed model revealed significant differences at the
species richness catches by each method among all the vegetation kinds ($\chi^2 = 19.4$, g.l. = 4, $p < 0.001$). In general, we determined that the active search has sampled a higher species richness, with exception of the Regenerating Cerrado, where the method resulted in 70% less species richness (mean = 3.5 species) in comparison to traps (mean = 6.3 species) at each sampling point $z = 2.8$, $p = 0.014$ (Figure 2).

In general, the abundance was more sampled by the active search as well, with higher absolute values of individuals (Figure 3). This difference was observed in three out of the five vegetation types ($z > 3.1$, $p < 0.004$): Restored Forest (172%), Semideciduous Forest (67%) and Riparian Forest (56%). On the other hand, there was no significant differences for the Cerradão, whilst for the Regenerating Cerrado we observed that traps resulted in a higher social wasp abundance (116%) in relation to the one resulted from active search ($z = 6.2$, $p < 0.001$).

For the Regenerating Cerrado, differing from remaining areas, the most effective sampling method was bait trap, collecting 17 species (80.35%), in comparison to the active search (13 species – 61.9%) (Table 1). The same was verified for the abundance, resulting in 203 individuals captured (68.35%), whilst only 94 were captured by active search (31.65%). When compared to other areas of less anthropic pressure of this study, the Regenerating Cerrado resulted in the highest abundance and the second highest social wasp richness among the sampled phytophysiognomies.

The Regenerating Cerrado has a degraded vegetation and a poor resource availability (Cardoso-Leite et al. 2004), hence turning the traps filled with passion fruit juice into attractive resources, what would explain the abundance values. Regarding species richness, the Regenerating Cerrado has in its surroundings a “Paludosa” forest and an area of eucalyptus planting in different successional stages (FEENA), which may house colonies of social wasps that may forage at the sampled area (Rodrigues & Machado 1982).

At the FEENA, Rodrigues & Machado (1982) made a twelve-year study and recorded 33 species and 10 genera of social wasps, demonstrating the expressive richness of this forest, close to studied site. Studies carried out in areas with a certain degree of impact, in Brazil, also resulted similarly (Marques et al. 1993; Lima et al. 2000; Marques et al. 2005; Alvarenga et al. 2010; Aluá et al. 2010; Santos & Presley 2010; De Souza et al. 2011; Bueno et al. 2019).

A total of 14 species were captured at the Cerradão, out of which 12 were captured actively (85.71%) in a total of 75 individuals (57.25%) (Table 1). This was the sole area in which bait traps and active search resulted in equal efficiency in relation to number of individuals collected, although it is concluded that for sampling richness, the active search was more effective. In relation to the phytophysiognomy, the Cerradão has a canopy predominantly continuous with coverage ranging from 50% to 90% (Ribeiro & Walter 1998), with canopy closure (Rizzini 1997), fact which directly affects the active collects made inside the vegetation. Thus, it was expected that in the Cerradão the number of collected species by active search would be similar to that of the Semideciduous Forest, as it really happened in the present study (Table 2).

Other works show that transition areas between Cerrado and forest, as well as in more bushy phytophysiognomies of Cerrado (such as Cerradão), usually have a higher richness of social wasps in relation to more open areas and adjacent agriculture areas. This may be explained by the larger structural complexity and availability of nesting sites (Santos et al. 2009; Ferreira et al. 2020).

At the Restored Forest, 15 species were recorded, out of which 13 (86.67%) were collected by active search and nine (60%) by passive methods (Table 1). With an entomological net, 102 individuals (62.58%) were collected, whilst 61 (37.42%) were captured by the bait trap (Table 1).

No studies were found dealing with social wasps in Restored Forests, however, due to the hydric resources close to the sampling area and a quite heterogenous vegetation, it was observed that this environment is capable of maintaining relatively high populations of these animals.

At the Regenerating Cerrado, the methodology of active searching resulted in being more effective to both species richness and abundance (Table 2). It may be explained by the large spacing between trees and absence of canopy closure, as it kept the trails always illuminated thus facilitating the visual location of the wasps (Raveret-Richter 2000).

At the Semideciduous Forest, 12 social wasp species were recorded, with 10 being collected by active search and eight (66.67%) by bait traps. In relation to abundance, 56 (73.68%) individuals were collected actively and 20 (26.32%) collected in traps (Table 1). This is the phytophysiognomy with the shortest proportion of collected species in relation to the estimation made to this ecosystem (Table 2).
Table 1. Absolute abundance (Ab) and relative frequency (F%) of wasp species sampled by active search (AS) and bait traps (BT) in the five studied areas.

| Sampled physiognomies | C | C. | C. | C. | C. |
|---|---|---|---|---|---|
|  | Ab | F% | Ab | F% | Ab | F% | Ab | F% | Ab | F% |
| Agelaia multiplex | 10 | 4.93 | 0 | 0.67 | 1 | 1.97 | 100 | 0.00 | 2 | 2.08 |
| Agelaia vicina | 17 | 5.74 | 1 | 0.67 | 1 | 0.17 | 100 | 0.00 | 2 | 2.08 |
| Apoica gelida | 16 | 7.88 | 1 | 0.67 | 1 | 1.79 | 100 | 0.00 | 2 | 2.08 |
| Brachygastra hectoriana | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Micronycteris marina | 2 | 0.99 | 1 | 1.06 | 1 | 1.79 | 100 | 0.00 | 2 | 2.08 |
| Micronycteris rufior | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Micronycteris tricolor | 1 | 0.49 | 1 | 1.06 | 1 | 1.79 | 100 | 0.00 | 2 | 2.08 |
| Polistes cinerascens | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polistes chrysothorax | 7 | 4.39 | 1 | 1.97 | 3 | 3.19 | 100 | 0.00 | 2 | 2.08 |
| Polistes versicolor | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia chrysothorax | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia fastidiosula | 1 | 4.93 | 0 | 0.67 | 1 | 1.79 | 100 | 0.00 | 2 | 2.08 |
| Polybia occidentalis | 7 | 3.45 | 3 | 3.19 | 2 | 3.57 | 100 | 0.00 | 2 | 2.08 |
| Polybia paulista | 1 | 0.49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia jurinei | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia sericea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia jurinei | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia jurinei | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia jurinei | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polybia jurinei | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 203 | 100 | 56 | 56.00 | 61 | 100.00 | 102 | 100.00 | 30 | 60.00 |

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general, a higher success during active search was obtained when the collectors got close to borders or opened areas, with higher luminosity, facilitating the visual location of the insects, as in Raveret-Richter (2000). The height of the trees and a too-closed canopy provide a low level of luminosity, hindering the collection with entomological net inside the phytophysognomy, as in other related studies (Diniz & Kitayama 1998; Lima 2008; Tanaka-Junior & Noll 2011; Souza et al. 2012; Togni et al. 2014). Regarding the low efficiency of bait traps in this ecosystem, it is probably explained by its considerable heterogeneity, providing abundant nectar resources, turning the baits into less attractive resources.

The work of Klein et al. (2015), carried out in deciduous forests of the State of Rio Grande do Sul, also reported a low species richness and abundance collected by bait traps when compared to more open environments, which are scarcer in resources, such as monoculture borders close to forests. Therefore, it is suggested that for these environments, other sampling methods should be used, such as Malaise trap (Somavilla et al. 2019).

The highest richness (23 species) was obtained at the Riparian Forest, with 21 (91.3%) collected by active search and 14 (60.87%) by bait traps. The same was observed for the abundance, in which 150 (60.98%) specimens were captured by active search and 96 (39.02%) were captured by traps (Table 1).

Different authors report an expressive richness of social wasps in areas of Riparian Forest when compared to other ecosystems (Diniz & Kitayama 1994; Clemente 2009; Henriques-Simões et al. 2011; Pereira & Antoniali-Junior 2011; Lócher 2012; Brunsmann et al. 2016). The Riparian Forests have a large vegetal complexity which may favor social wasps, as it provides a variety of physical supports to nest foundation, rises the amount and heterogeneity of feeding resources, impose less microclimate variability and rises the density of colonies (Lawton 1983; Diniz & Kitayama 1994; Souza et al. 2010). Furthermore, these ecosystems are close to hydric resources which may have affected the passive thermal regulation of the colonies. Yet, it is pointed out that the real species richness of this environment might be even higher, as both collection methods resulted in a relatively low proportion of estimated richness when compared to the Restored Forest and Regenerating Cerrado (Table 2).

Regarding social wasps collected in bait traps, 413 specimens (94.7%) belong to Epiponini, 16 (3.7%) to Miscicyttarini and seven (1.6%) to Polistini. In the active search, 328 specimens (68.76%) belong to Epiponini, 133 (27.88%) to Mischocyttarini and 24 (5.32%) to Polistini.

Thus, bait traps were more efficient for collecting wasps of the Epiponini. This result is contrasting to other ones reporting the active search as the most efficient method for Epiponini in comparison to bait traps (Souza & Prëzoto 2006; Klein et al. 2015). However, these works also resulted in the active search being the most effective to sample species richness of other tribes, as resulted in the present work.

Out of the 163 specimens of Mischocyttarus montei Zikán collected at the Restored Forest, 50 (92.6%) were obtained by active search and four (7.4%) by bait traps. A low-capture rate of Mischocyttarus species by bait traps were also reported by other studies, such as in Souza & Prëzoto (2006), whom worked in Cerrado Fields and Semideciduous Forests. In conserved Ombrophile Forests, no species of this genus was collected by passive methodology, where the bait used was the same as in the present study (Souza et al. 2012), probably due to the small number of individuals per colony (Giannotti 1998; Torres et al. 2011) hindering the sampling.

In relation to M. montei, Brunsmann et al. (2016) reported it as the most abundant species and its most efficient collection method was active search, highlighting the difficulty of collecting this genus in trap baits. Thus, it may be concluded that the use of bait traps as the sole method of sampling for social wasps might underestimate the real richness of the Polistini and Mischocyttarini of a given area.

Concerning the most abundant species by each ecosystem, the one with the highest number of collected specimens was the swarming wasp Agelaia pallipes Olivier at the Regenerating Cerrado, with 178 recorded specimens, with 117 (39.39%) by bait traps and 61 (20.53%) by active search.

Agelaia vicina de Saussure was the most representative in three other ecosystems: at Cerradão, with 20 (15.26%) sampled specimens by traps and 24 (18.32%) by active search; at the Semideciduous Forest, with 23 (30.26%) specimens being sampled by active search and nine (8.4%) by traps; and at the Riparian Forest, with 74 (30.08%) collected individuals by active search and 38 (15.44%) by traps.

At the Restored Forest, out of the 163 collected social vespids, 54 (33.12%) were specimens of M. montei, with 50 collected by active search and four by traps. The second-most numerous species was the swarming wasp Polybia chrysothorax Lichtenstein, with 41 individuals captured, out of which 39 were captured in bait traps.

In different studies, A. vicina was also the most abundant species (Souza & Prëzoto 2006; Ribeiro-Junior 2008; Silva 2012), possibly due to being the species which builds the largest colonies of Polistinae, as reported by Zucchi et al. (1995), whom recorded a colony with more than a million specimens of adults and nests with 7.5 million breeding cells. Oliveira et al. (2010) add that a high-growth ratio, a large population and an elevated number of queens is what

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**Table 2.** Estimative indexes of richness from Jackknife 1 and 2 using methods of Active Search (AS) and Bait Traps (BT) in five areas from the center-east of the São Paulo State. (RC – Regenerating Cerrado, C – Cerradão, RTF – Restored Forest, SDF - Semideciduous Forest and RPF – Riparian Forest).

| Richness | AS | BT | AS | BT | AS | BT | AS | BT |
|----------|----|----|----|----|----|----|----|----|
| **Estimator J1** | 13 | 17 | 12 | 9  | 13 | 9  | 10 | 8  |
| 18 | 22 | 18.66 | 12.33 | 16.33 | 9.4 | 15.83 | 13 | 32.66 | 21.71 |
| 20.4 | 24.4 | 23.46 | 13.93 | 17.93 | 13.4 | 19.96 | 17 | 41.46 | 26.95 |
| % estimated J1 | 72.99 | 77.27 | 64.30 | 72.99 | 79.60 | 95.74 | 63.17 | 61.53 | 64.29 | 64.48 |
| % estimated J2 | 63.72 | 69.67 | 51.15 | 64.60 | 72.50 | 67.16 | 50.10 | 47.05 | 50.65 | 51.94 |

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make nests with such greatness possible to exist. This factor certainly influenced the elevated occurrence of specimens of
this group in the active collections and in traps, highlighting
the importance of this tribe to the studied ecosystems.

All *Agelasia* species sampled were present in all collection
sites. Oliveira et al. (2010) suggest that *A. vicina* should be
considered in several environments as a key species, or in
other words, as defined by Pané (1969), a species in which
its population determines the stability (integrity and unaltered
consistency over time) of a community through its activities
and abundances.

Other *Agelasia* species are frequently among the most
abundant, be in more opened Cerrados, transition cerrado-
forest, forest borders (Ferreira et al. 2020) or inside forests
(Klien et al. 2015).

At the sampled phytophysiognomies, with exception of
Regenerating Cerrado, the active search with entomological
net resulted as the most efficient to sample species richness
of social wasps. In fact, bait traps collected almost exclusively
wasps tribe Epiponini, except for 23 individuals (5.3%)
belonging to other tribes.

In relation to abundance of collected individuals, there was
a higher efficiency rate of the active search in the Restored
Forest, Semideciduous Forest and Riparian Forest. At the
Cerradão, both methods resulted as equally efficient, whilst in
Regenerating Cerrado the traps resulted as the most efficient
method in this context.

The fact that neither of both methods could sample the total
of species sampled per each area demonstrated that these
strategies are complementary in any sampling and analyses
over the social wasp community in different ecosystems.
Thus, associating other methodologies, such as Malaise trap
(cf. Ferreira et al. 2020), might contribute to the rise of the
captured species number and for a sampling closer to the
real richness of these areas.

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