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Cover: Whale Shark *Rhincodon typus* and Reef - made with poster colours. © P. Kritika.

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A comparison of four sampling techniques for assessing species richness of adult odonates at riverbanks

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Abstract: Members of the insect order Odonata are known as good ecological indicators. Many are sensitive to habitat modifications and are easily monitored for use in environmental assessment studies. Rapid assessments rely on efficient sampling techniques. However, there is limited information available on sampling techniques for adult odonates, and protocols require evaluation. To do this, we standardized counting methods during sampling of odonates from August to November 2016 at the Mula River, Pune, India. We used four counting techniques; full-width belt transect (FWBT), full-circle point count (FCPC), half-width belt transect (HWBT), and half-circle point count (HCPC). For HWBT and HCPC areas facing the river were sampled, and for each technique we took multiple temporal replicates. We compared species detected per unit time, species detected per unit area, new species detected per unit time, and new species detected per unit area. Additionally, we compared species estimates. With HCPC we detected the maximum number of species and new species per unit area, whereas FWBT returned maximum coverage of recorded species. We recommend our proposed techniques be considered in the future across various habitats to decide the most suitable sampling strategy for the different habitats or situations.

Keywords: Dragonfly, ecological assessment, point count, species estimates, transect, urban wetland.

सारांश: चतुर या कुळामधील कीटक हे चांगले पर्यावरण सूचक म्हणून ओळखले जातात. पर्यावरणातील बदलांचा अभ्यास करण्यासाठी चतुर हे खूप महत्वाचे मानले जातात. त्यांचा अभ्यास करण्यासाठी वापरल्या जाणाऱ्या पद्धती या खुप कमी असून त्यांची पुरेशी माहिती अजून उपलब्ध नाही. त्यासाठी प्रमाणबद्ध उपाय उपलब्ध नाहीत. यासाठी आम्ही चतुरांची गणती करण्यासाठी पद्धतशीर तंत्र शोधण्याचा प्रयत्न केला आहे. हा अभ्यास २०१६ च्या ऑगस्ट ते नोव्हेंबर या महिन्यात मुळा नदीकाठी करण्यात आला. यामध्ये आम्ही ४ पद्धती - Full width Belt transect, Full circle point count,half width belt transect and half circle point count यांचा वापर केला. यामध्ये HWBT and HCPC या पद्धती नदीकडे तोंड करुन odonates ओळखण्यात व मोजण्यात आले. अभ्यासातील त्रुटी टाळण्यासाठी प्रत्येक पद्धत ही अनेकदा वापरली गेली. आम्ही चतुरांच्या प्रजातींची (species) प्रत्येक मिनीट व क्षेत्रफळानुसार तुलना केली तसेच सापडलेल्या नवीन प्रजातींची प्रत्येक मिनीट व क्षेत्रफळानुसार तुलना केली. यातून आम्हाला HCPC ही पद्धत क्षेत्रफळानुसार सर्वात जास्तं संख्येने प्रजाती तसेच नवीन प्रजाती शोधत आहे हे लक्षात आले. FWBT ही पद्धत जास्तीत जास्तं नोंदवलेल्या प्रजातींचे coverage देत आहे. आमच्या अभ्यासानुसार, चतुरांचा अभ्यास करण्याची चांगली पद्धत ठरवण्यासाठी ह्या पद्धती वेगवेगळ्या अधिवासात तसेच परिस्थितीत वापरल्या जाव्यात.

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Author contributions: AD collected and analyzed data, and wrote the initial draft of the manuscript. PK conceptualized the study, analyzed data, and helped with writing and editing the manuscript. AP helped with logistics for the project, provided lab facilities, and edited the manuscript.

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INTRODUCTION

Odonata depend on freshwater ecosystems to complete their life cycle, as their larvae are aquatic (Corbet 1962). This dependency on freshwater ecosystems makes odonates good aquatic and terrestrial bio-indicators (Corbet 1962; Simaika & Samways 2011, 2012; Stoks & Cordoba-Aguilar 2012; Monteiro-Júnior et al. 2014; Chovanec et al. 2015). Assessment of odonates primarily deals with sampling adults and is highly recommended (Kutcher & Bried 2014; Valente-Neto et al. 2016), because in many cases collecting and identifying adults is easier than finding larvae or exuviae, especially in the case of bio-monitoring projects (Córdoba-Aguilar & Rocha-Ortega 2019) except for gomphids (da Silva-Méndez et al. 2022). Identification keys and field guides for adult odonates are mostly available, while larval identification is more problematic for many species, as the Indian Odonata literature lacks larval and exuvial identification keys (Kumar & Khanna 1983). Another aspect is the availability of comparable data over more extensive spatial coverage. Adult odonate data can be relatively easily obtained for comparison purposes, making adult sampling more popular than larval or exuvial or combined sampling.

Odonates play a crucial role as predators in freshwater ecosystems. They are very useful as ecosystem service providers, especially in urban wetlands which are the freshwater ecosystems available in human-modified landscapes (Bolund & Hunhammar 1999; Angold et al. 2006; Suhling et al. 2015, Córdoba-Aguilar & Rocha-Ortega 2019). Studying such freshwater ecosystems is vital, as they may provide information about understanding the pace of urbanization and species losses (McKinney 2008; Johnson et al. 2013, Córdoba-Aguilar & Rocha-Ortega 2019), aiding conservation management.

Various methods have been employed to study odonates, such as collecting individuals with sweep nets, and using lights and malaise traps, line transects, and point counts (Almeida et al. 2013; Bried & Ervin 2006; Bried et al. 2012; Patten et al. 2015). Currently, relatively little published literature is available on standardized methods for sampling adult odonates. Taking transect surveys on a fixed route is the most popular method (Córdoba-Aguilar & Rocha-Ortega 2019). Quadrangular or rectangular survey plots have been used by some ecologists. For ponds and wetlands, sweep-nets have been used (Oertli 2008). Point counts (PC) have been used especially across pond ecosystems. Distance sampling methods have been used in rainforest ecosystems with scattered water resources (Oppel 2006). Random visual scanning method and visual scanning following a transect have been suggested for counting adult odonates (Sutherland 2006). For sampling adult odonates at rivers and streams, transects along the riparian zone are suggested (Panzer et al. 2005; Smallshire & Beynon 2010). Aerial netting is more useful when specimen collection is the primary aim. For systematic sampling, to come up with diversity indices and species estimates, non-invasive methods such as transects are expected to be more useful (Oppel 2006). Presently available sampling protocols have seldom been critically evaluated to identify the most efficient protocol that captures a reliable estimate of the species richness in a habitat.

In the present study, we tried to standardize a method of counting adult odonates at the riverbanks of a tropical urban river. We compared four different sampling techniques to check the best method which provides a complete assessment of the species richness of the selected urbanized site. This short-term study provides a baseline for future research on counting adult odonates.

MATERIALS AND METHODS

Sampling Methods

The study area was the riparian zone of the Mula River, Aundh, Pune, Maharashtra, India (18.5687 N, 73.8198 E, 551 m). The study site is present in the city and is much disturbed by humans. They carry out activities like cattle grazing nearby, and polluting the river by washing their clothes, among other activities. The study duration was four months from August 2016 to November 2016. We used direct observations for the identification of adult odonates at the site with the aid of binoculars. We identified species using field guides (Subramanian 2005) and referred to previously published material from the Pune area (Kulkarni & Subramanian 2013; Koparde 2016). The habitat sampled consisted of the stream bed and marginal vegetation. We used a transect length of 300 m, as at the study site it was the length that we could walk continuously without any breaks in the transect due to water level, mud, garbage, and uneven terrain. We standardized point count timing to two mins after a pilot survey. During our pilot sampling, we observed, while walking the transects, odonates aggregated in high numbers in the area facing the river rather than inland. We observed a similar pattern while conducting point counts. Therefore, we decided

Table 1. Details of four sampling techniques evaluated during the study.

Technique	Dimensions & Details	Area Sampled
Full-width Belt Transect (FWBT)	300 m X 10 m (length X width) transect covered while walking at the speed of 25 m per two minutes.	3,000 m^2 in 24 minutes
Half-width Belt Transect (HWBT)	300 m X 5 m (length X width) transect covered while walking at the speed of 25 m per two minutes. The width of the transect was restricted to the area facing the riverside.	1,500 m^2 in 24 minutes
Full-circle Point Count (FCPC)	Point counts with a radius of 5 m (full circle) placed at an interval of 25 m (such as 0 m, 25 m, 50 m, and so on) across a 300 m straight line.	78.54 m^2 per point X 13 stations = 1,021.02 m^2 surveyed in 26 minutes
Half-circle Point Count (HCPC)	Point counts with a radius of 5 m (semi-circle) placed at an interval of 25 m (such as 0 m, 25 m, 50 m, and so on) across a 300 m straight line. The semi-circle was restricted to cover the area facing riverside.	39.27 m^2 per point X 13 stations = 510.51 m^2 surveyed in 26 minutes

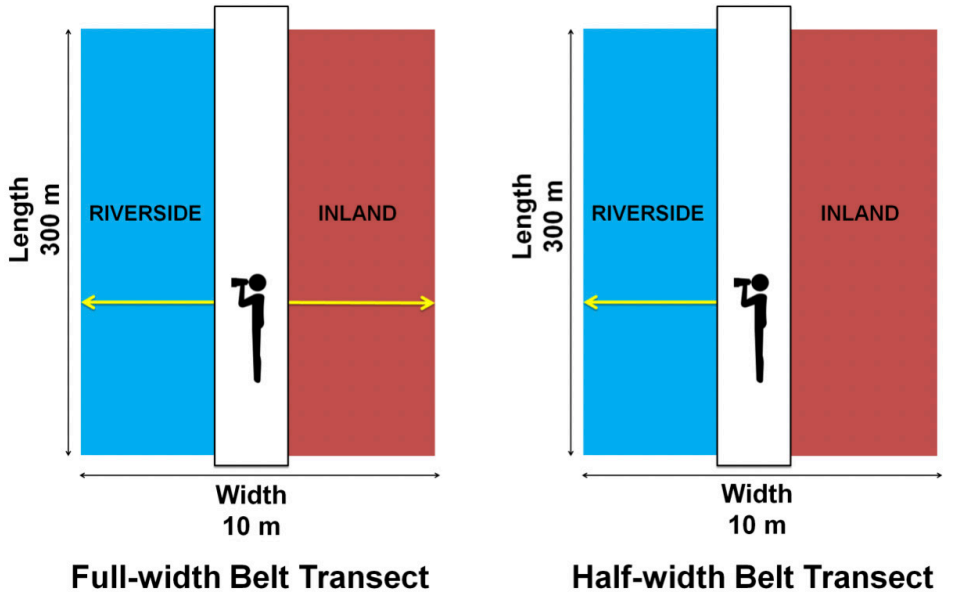

Figure 1. Graphic explaining Full-width Belt Transect (FWBT) and Half-width Belt Transect (HWBT) techniques. The details of the dimensions are provided in Table 1.

to add a variant in method, where we maximized the sampling effort at the riverside. Finally, we used two main sampling methods (belt transect and point count) with a variant in each (Table 1, Figure 1, Figure 2). We used two variants of the belt transect method: 1. Full-width belt transect, where we counted the adult individuals of the species present on the banks inland and at the riverside covering the 300 m belt transect (Table 1, Figure 1). 2. Half-width belt transect, where we counted the adult individuals of the species present only at the riverside covering the 300 m belt transect (Table 1, Figure 1). 3. Full-circle point count, where we counted the adult individuals of the species present in a circle of 5 m radius across a 300 m straight line at each 25 m interval (Table 1, Figure 2). 4. Half-circle point count, where we counted the adult individuals of the species present in a half circle facing the riverside across a 300 m straight line at each 25 m interval (Table 1, Figure 2).

Data Analysis

We performed the analysis in the statistical software PAST (v.3.) (Hammer et al. 2008) and R (R core team 2014). We compared the cumulative number of species detected per unit area, cumulative number of species detected per unit time, new species added per unit area, and new species added per unit time. We used nested analysis of variance (ANOVA) to compare the four techniques: FWBT, HWBT, FCPC and HCPC. Additionally, we used box-plots to visualize the differences in the capture of parameters across the techniques. We calculated species estimates CHAO1 (Chao 1984; Colwell & Coddington 1994), CHAO2 (Chao 1987; Colwell &

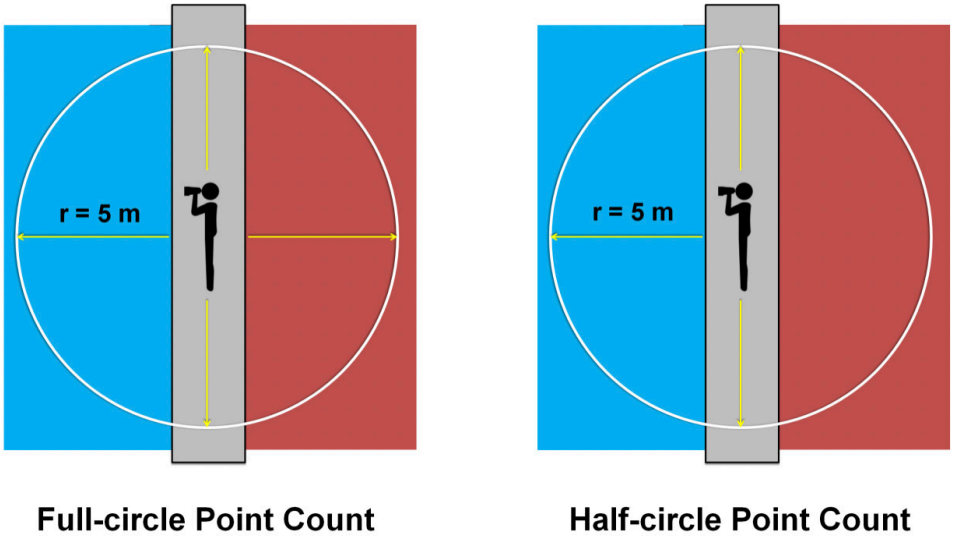

Figure 2. Graphic explaining Half-circle Point Count (HCPC) and Full-circle Point Count (FCPC). Both the methods were carried out along 300 m straight distance. The details of the dimensions are provided in Table 1.

Coddington 1994), Jack1 and Jack2 using Biodiversity Pro v2.0 (McAleece et al. 1997) for each technique and compared it with the cumulative number of species observed per technique. We kept a separate list of off-sampling observations to compare with our data and species estimates.

RESULTS

In total, we recorded 19 odonate species at the site; a complete list of species detected is given in Supplementary Table 1. We obtained statistically significant results for comparisons among techniques (Supplementary Table 2 & 3) for species detected per unit area ($F = 28.79$, $P << 0.0001$) (Figure 3A) and new species added per unit area ($F = 5.15$, $P = 0.0012$) (Figure 3B), through nested ANOVA (Supplementary Table 3).

The proportion of species detected and new species added per square meter were highest for HCPC (Figure 3A, 3B). There was no significant difference across techniques for species detected and new species added per minute ($P > 0.3$) (Figure 3C, 3D). We found through species estimate analysis all techniques except FWBT produced conservative estimates (Table 2).

DISCUSSION

Given equal effort in terms of replicates, HWBT, FCPC, and HCPC methods produced comparable ranges of species estimates, whereas the FWBT method had the maximal coverage (89.5% of 19) of the total number of species observed at the site (Table 2). Overall, belt transects had higher coverage of total species richness (80–90 %) than point counts (63–74 %), indicating that belt transects are highly time-efficient techniques, suggesting the reason for their popularity amongst ecologists. Our comparison of sampling techniques revealed that through the HCPC technique, we recorded significantly more species per unit area (Figure 3A & 3B, Supplementary Table 1 & 2). A reason for this is probably the more intensive search (two minutes) in a smaller area (39.25 m^2) per point count that can be achieved through HCPC compared with other techniques used. The HCPC method perhaps is the best method for intensive sampling but is not time-efficient.

This was a short-term study, but it provided some future research directions. We carried out standardization of adult odonate sampling technique only at one field site, however, taking multiple temporal replicates helped in eliminating sampling errors. Our analysis provided statistically significant results, but we think this procedure needs to be replicated at several sites for an extended time period to test if our results are consistent. In addition, these techniques need to be evaluated at other types of wetlands such as ponds, lakes, and streams, to come up with standard methods for assessing adult odonates at various habitats. For all the techniques used in the present study, double-counting seems to be a potential flaw. Individuals aggregating at a location may also introduce overestimation error,

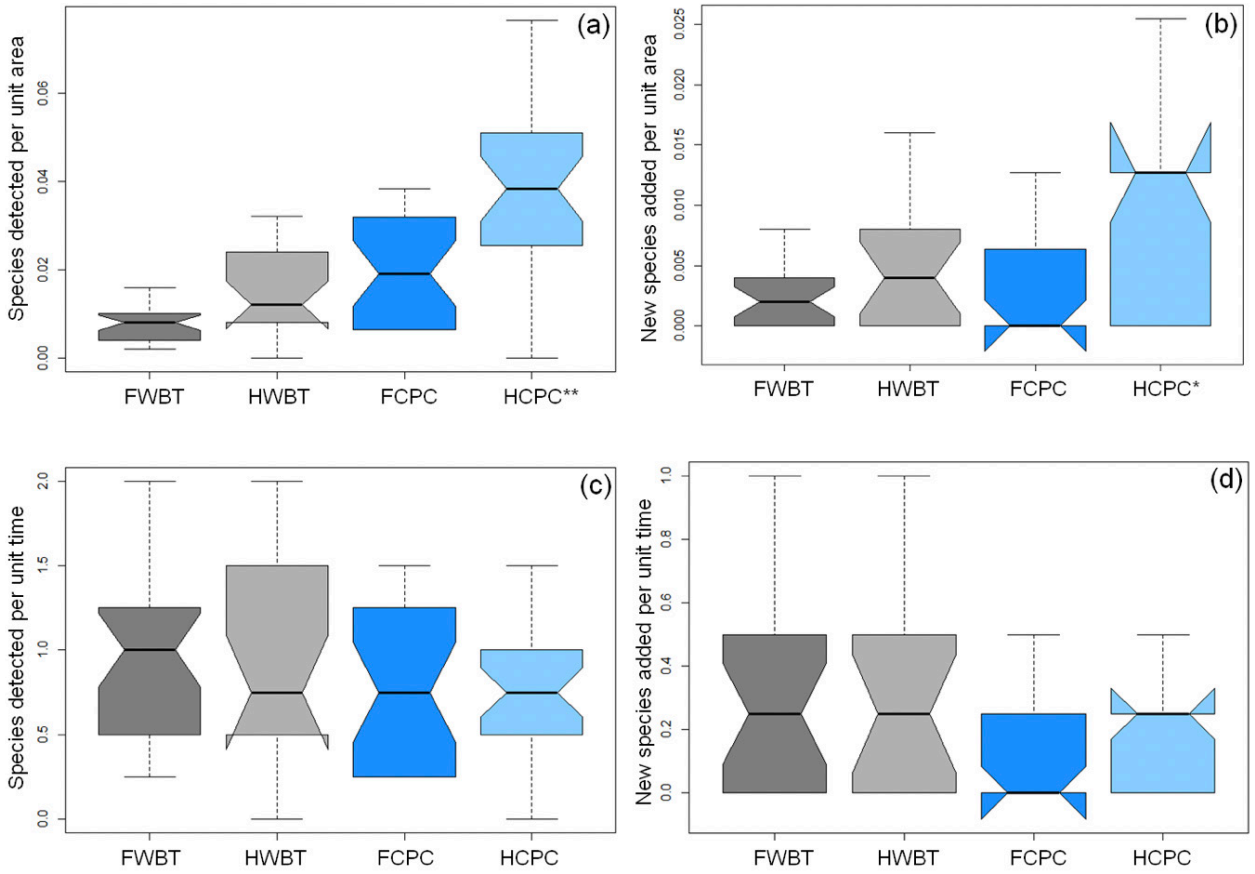

Figure 3. Comparison among sampling techniques. FWBT—Full-width Belt Transect | HWBT—Half-width Belt Transect | FCPC—Full-circle Point Count | HCPC—Half-circle Point Count | *—P <0.05 | **—P <<0.01.

Table 2. Species estimates across various adult odonate counting methods. The cumulative number of species observed across all techniques was 19.

Estimate	FWBT (n = 5)	HWBT (n = 4)	FCPC (n = 5)	HCPC (n = 5)
CHAO 1	18	15	12	15
CHAO 2	26.7	18.13	20	17.6
Jack 1	23.6	18.75	15.2	18.2
Jack 2	26.45	19.92	17.15	19.25
Observed	17	15	12	14
% coverage of all the species observed	89.5	80	63.2	73.7

n—number of temporal replicates | FWBT—Full-width Belt Transect | HWBT—Half-width Belt Transect | FCPC—Full-circle Point Count | HCPC—Half-circle Point Count.

especially if such sites fall on point count stations. These potential flaws can be fixed only with capturing and marking individuals or alternatively, by adding several sampling replicates to reduce the error. Since dragonflies and damselflies have different flight abilities and habits, it is necessary to adjust strategies and techniques to sample them (Koparde 2016). A one size fits all strategy is not suitable to sample both the suborders or habitats.

We draw attention to the desirability of standardized sampling protocols in species diversity sampling. Our preliminary analysis indicates that HCPC might be a suitable method to sample adult odonates at the riverbanks when time is not a limiting factor, and FWBT when it is.

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Supplementary Table 1. List of species recorded during the study.

	Species Name	FWBT (n = 17)	HWBT (n = 15)	FCPC (n = 12)	HCPC (n = 14)
ANISOPTERA (n = 12)					
Family:					
Aeshnidae (n = 1)	*Anax guttatus* (Burmeister, 1839)	X	X		
Gomphidae (n = 1)	*Ictinogomphus rapax* (Rambur, 1842)	X	X	X	X
Libellulidae (n = 10)	*Acisoma panorpoides* (Rambur, 1842)	X			
	Brachythemis contaminata (Fabricius, 1793)	X	X	X	X
	Crocothemis servilia (Drury, 1770)	X	X	X	X
	Orthetrum pruinosum (Burmeister, 1839)		X		X
	Orthetrum sabina (Drury, 1770)	X	X	X	X
	Pantala falvescence (Fabricius, 1798)	X		X	X
	Rhyothemis variegata (Linnaeus, 1763)		X		X
	Tramea basilaris (Palisot de Beauvois, 1805)	X	X	X	
	Trithemis aurora (Burmeister, 1839)	X	X	X	X
	Trithemis palidinervis (Kirby, 1889)	X	X	X	X
ZYGOPTERA (n = 7)					
Family					
Coenagrionidae (n = 6)	*Agriocnemis pygmaea* (Rambur, 1842)	X	X	X	X
	Ceriagrion coromandelianum (Fabricius, 1798)	X	X		X
	Ischnura aurora (Brauer, 1865)	X			
	Ischnura senegalensis (Rambur, 1842)	X	X	X	X
	Pseudagrion decorum (Rambur, 1842)	X	X	X	X
	Pseudagrion hypermelas (Selys, 1876)	X	X	X	X
Platycnemididae (n = 1)	*Disparoneura quadrimaculata* (Rambur, 1842)	X			

N—Number of species | FWBT—Full-width Belt Transect | HWBT—Half-width Belt Transect | FCPC—Full-circle Point Count | HCPC—Half-circle Point Count | X—Presence.

Supplementary Table 2. Point count standardization at six point count stations. Each PC column represents the cumulative number of species observed and numbers in brackets represent the cumulative number of individuals observed.

Minutes	PC 1	PC2	PC3	PC4	PC5	PC6
1st	1 (1)	3 (3)	1 (1)	1 (1)	1 (1)	1 (1)
2nd	3 (3)	4 (6)	1 (1)	1 (1)	1 (1)	1 (1)
3rd	3 (4)	5 (8)	1 (1)	1 (1)	2 (2)	1 (1)
4th	3 (5)	5 (9)	2 (2)	1 (1)	2 (2)	1 (1)
5th	4 (6)	5 (9)	2 (2)	1 (1)	2 (2)	1 (1)

Supplementary Table 3. Nested ANOVA across techniques reveal significant differences in species detected per unit area ($F = 28.79$, $P << 0.0001$) and new species added per unit area ($F = 5.15$, $P = 0.012$) across techniques.

	Sum of squares	Degrees of freedom	Mean square	Fs	*P*	Variance component (percentage)
Species detected per unit area						
among groups	0.0146	3	0.0048	28.79	0.0000	50.51
subgroups within groups	0.0025	15	0.0001	1.016	0.4457	0.14
within subgroups	0.0153	92	0.0001			49.35
total	0.0325	110				100
Species detected per unit time						
among groups	1.1142	3	0.3714	1.0956	0.3834	0.5010
subgroups within groups	5.0768	14.92	0.3389	1.6005	0.0887	9.2833
within subgroups	19.4552	92	0.2114			90.215
total	25.6464	110				100
New species added per unit area						
among groups	0.0010	3	0.0003	5.1571	0.0119	19.345
subgroups within groups	0.0011	15.93	0.0000	1.4270	0.1542	6.9312
within subgroups	0.0034	71	0.0000			73.723
total	0.0055	90				100
New species added per unit time						
among groups	0.3424	3	0.1141	1.1728	0.3530	0.9945
subgroups within groups	1.5558	15.93	0.0973	1.4417	0.1477	8.7746
within subgroups	4.7885	71	0.0674			90.23073
total	6.6868	90				100

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