

31 **Abbreviations:** BDMF–biodiversity maintenance function; CONN–Connectance Index; CONTAG–
32 Contagion Index; DIV–Landscape Division Index; ENN–Euclidean Nearest-Neighbor Distance; FRAC–
33 Fractal Dimension Index; GYR–Radius of Gyration; LPI–Large Patch Index; LSI–Landscape Shape
34 Index; PRD–Patch Richness Density; SHDI–Shannon's Diversity Index; SHEI–Shannon's Evenness
35 Index; StN–Syrph The Net database.

36

Introduction

37 Global biodiversity is constantly being eroded as a consequence of human-induced pressures (Pimm
38 1995). One such pressure is landscape change (Foley et al. 2005, Tscharrntke et al. 2005). Besides biotic
39 and abiotic parameters, human influence has been determined as one of the main factors shaping
40 landscape patterns (Rackham 1998, Moser et al. 2002). Disturbance of those patterns influences multiple
41 ecological processes, thereby affecting both ecosystem functions and species within ecosystems (With
42 1997). In order to alleviate the negative consequences of landscape disturbances and to preserve imperiled
43 species and areas, varying conservation measures have to be applied. However, due to limited resources
44 for conservation action, proper estimation of conservation priorities is needed (Faith 1992). Therefore, it is
45 crucial to identify bioindicator taxa that can reflect broad-scale impacts and exhibit measurable responses
46 to different changes in the environment. Although species level is the most often considered taxonomic
47 resolution, genus-level indicators could have significant values. Due to the specific larval food type of
48 phytophagous genera, one can assume that the whole genera could be sensitive to changes in the
49 environment and would have timely and measurable responses to these changes.

50 Landscape structure is a key element of our understanding of species diversity (Walz 2011) and it has
51 been proven to significantly influence insect communities (Didham et al. 1996). Different landscape
52 features (such as isolation of habitat fragments, patch area, patch quality, ratio of habitat edge to interior,
53 etc.) affect insect richness and abundance in space. Thus, it is clear that insects can be used to assess
54 changes in landscapes across time (Hunter 2002).

55 In our study, we focused on the Syrphidae; a Dipteran insect family. Around 6000 hoverfly species have
56 been described worldwide to date (Pape et al. 2011). They mainly feed on pollen and nectar and are

57 considered the second-most significant group of pollinators after bees (Petanidou et al. 2011). In
58 contribution to significance of these species tells the fact that areas significant for their survival (PHA-
59 Prime Hoverfly Area) are defined in Serbia (Vujić et al. 2016), while Miličić et al. (2017) conducted area
60 prioritization for Southeast Europe based on distribution and vulnerability of hoverflies. Their role as a
61 bioindicator has been particularly recognized through the development of the Syrph The Net (StN)
62 database, representing an expert system for analyzing and evaluating hoverfly communities. The
63 “biodiversity maintenance function” (BDMF) can be used as an estimate of site quality and is calculated
64 by comparing the expected biodiversity within a habitat type on a site with its observed biodiversity.
65 BDMF is the main output of StN and represents the ratio between observed number of species to the total
66 number predicted by StN (Speight 2008). Lists of predicted species can be generated by considering
67 regional lists of species and matching the habitat preferences of each species to the habitats available at a
68 given site (Speight and Castella 2001). Numerous studies have successfully used this database for habitat
69 evaluations, confirming the potential of hoverflies as bioindicators (Speight and Castella 2001, Sarthou et
70 al. 2005, Velli et al. 2010, Sommaggio and Burgio, 2014). However, unlike the previous studies assessing
71 the bioindicator role of syrphids based only on present information, in this study we examine the changes
72 over time both in landscape structure and in species richness. Specifically, we targeted the two largest
73 European hoverfly genera, *Cheilosia* Meigen, 1822 and *Merodon* Meigen, 1822. These genera have been
74 the focus of numerous field surveys in Serbia over the last 35years, so their distributions and habitat
75 preferences are well known (Vujić, pers. comm.). Additionally, species of these two genera can be
76 considered specialists, having larvae that are phytophagous and often linked to a specific plant genus or
77 species (Rotheray and Gilbert 2011). It is widely acknowledged that specialized species are more sensitive
78 to environmental change than generalists (O’Grady et al. 2004, Isaac et al. 2009), implying that these
79 species will exhibit rapid and measurable responses to landscape changes.

80
81 Jovičić et al. (2017) showed that landscape structure and land use patterns affect both *Cheilosia* and
82 *Merodon* species. Here, we investigate (i) the effects of landscape structural change on *Merodon* and

83 *Cheilosia* species richness at both spatial and temporal scales, and (ii) the bioindicator potential of these
84 species using BDMF calculated for data spanning 25 years. To fulfill our objectives, we assess whether
85 there have been shifts in the communities of these two hoverfly genera and, if so, we test whether these
86 shifts are associated with changes in landscape structure.

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Material and methods

89 Data on hoverfly species richness

90 Hoverfly species distributions throughout Serbia have been investigated regularly over the last 35 years.
91 The Faculty of Science of the University of Novi Sad, Serbia, hosts an internal database comprising a
92 large amount of geo-referenced data on hoverfly species presence. For the purposes of this study, we
93 selected 10 sites from the database (Table 1), which were recently surveyed by the authors over a 4-year
94 period (2011–2014). Sites were chosen by experts based on knowledge about the ecological preferences of
95 species from the genera *Merodon* and *Cheilosia*. A detailed description of the sites and all of their
96 macrohabitats can be found in Jovičić et al. (2017).

97 Specimens were counted during peak flight periods, from April to the end of August, using entomological
98 netting. The StN database consists of information on adult hoverfly species collected using Malaise traps.
99 However, a major limitation of using Malaise traps for sampling hoverflies is that they are often
100 vandalized or damaged by grazing animals (Speight et al. 2000). We chose to use entomological netting as
101 a sampling method for our study instead of Malaise traps for two reasons. First, for a large number of our
102 sites, we could not adequately protect Malaise traps. Secondly, data in our internal database for the period
103 1990-2010 was collected using entomological nets. Thus, in order to compare our findings among years,
104 we decided to use the same sampling method. Additionally, entomological netting is considered to be
105 more efficient than Malaise traps (Marcos-García et al. 2012).

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108 **Data on landscape structural change**

109 Landscape structural change was evaluated using GIS tools and relevant ecological software. We based
110 our analysis on CORINE land cover maps in vector format from 1990, 2006 and 2012, using the ArcGIS
111 software package (ArcGIS10, ESRI). We established circular zones with radii of 2 km and 5 km around
112 each site. The Fragstat 4.2 software (McGarigal et al. 2002) was used to calculate landscape metrics based
113 on prepared maps that had previously been converted into ERDAS raster format (15m/pixel). In total, we
114 selected 11 landscape metrics aimed at describing landscape structure and change over 25years, three of
115 which were based on previous research on the influence of landscape structure on *Merodon* and *Cheilosia*
116 communities (Jovičić et al. 2017) and an additional eight metrics were added (Table 2; indicated with
117 asterisks) because we assumed that over longer time periods they would influence species richness of the
118 two investigated genera.

119 **Data analysis**

120 **Syrph The Net analysis**

121 A detailed description of the process of calculating BDMF can be found in Speight et al. (2000). We
122 calculated BDMF for each of the 10 analyzed sites. We adopted a threshold of 50% to indicate sites of
123 good conservation status. Thus, if less than 50% of expected species were recorded for a given site
124 (BDMF < 50%), it may be considered degraded (Speight et al. 2000), whereas BDMF > 50 % indicates
125 sites with good habitat quality.

126 **Correlations among ecological and landscape parameters**

127 Our dataset was comprised of ecological (*Merodon* and *Cheilosia* species richness and BDMF) and
128 landscape parameters [Radius of Gyration (GYR), Large Patch Index (LPI), Fractal Dimension Index
129 (FRAC), Contagion Index (CONTAG), Landscape Shape Index (LSI), Landscape Division Index (DIV),

130 Patch Richness Density (PRD), Shannon's Evenness Index (SHEI), Shannon's Diversity Index (SHDI),
131 Euclidean Nearest-Neighbor Distance (ENN), Connectance Index (CONN)]. We had two data points for
132 the ecological parameters, i.e. for periods 1990-2006 and 2006-2014, and three data points for landscape
133 parameters, i.e. for individual years 1990, 2006 and 2014. In order to bring two sets of parameters to the
134 common time-frame, we calculated the landscape parameters for the periods for which we had the
135 measurements of ecological parameters (1990-2006 and 2006-2014). We did this by calculating the
136 average value for each period:

137 1) $(p_{1990} + p_{2006}) / 2$;

138 2) $(p_{2006} + p_{2012}) / 2$

139 where p stands for parameter value.

140 To test whether there was a relationship between changing landscape parameters over the 25-year time-
141 frame and the three ecological parameters, we calculated the correlation between the corresponding
142 columns from the first and the second matrix. Kolmogorov-Smirnov test showed that there was a large and
143 significant distance between the normal distribution and empirical distribution function of the three
144 ecological parameters (all $p < .001$). This means that we can assume with a high certainty that the samples
145 are not normally distributed. Hence, the use of Pearson correlation is not appropriate and Spearman's rank
146 correlation was used instead. The resulting correlations, calculated in MATLAB, are given in Table
147 3, where all statistically significant results are indicated by asterisks.

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Results

153 **Landscape structural change over 25 years**

154 We found interesting trends regarding landscape structural change for the first time period (1990-2006).
155 Within the 2km buffer, an increase in the LPI and LSI indices indicated a simplification of landscape
156 patches (Appendix 1). The larger and more symmetric patches, together with the higher complexity of
157 patch perimeter shapes (decreased FRAC index), confirm that over this period patches became more
158 regular in shape. Moreover, within the 5 km buffer areas, the CONTAG and CONN metrics exhibited
159 negative trends, signifying that similar patches became less connected. The different CONN values
160 between the 2 and 5 km buffers indicate different landscape patterns at these two scales; the 2 km buffers
161 manifest higher connectivity (a mean of approximately 70%), whereas connectivity was approximately
162 40% for the 5 km buffers. Our data also revealed an increase in the LSI index for 1990-2006, with an
163 average value of +6.4% indicating an increase in the regularity of landscape patterning in this period.
164 However, this trend was reversed for the following years (an average value of -7.5% for 2006-2014), with
165 the lowest value at site 5 where urbanization is more pronounced. We found the same trend for the DIV
166 index. One of the most widely used landscape metrics in landscape ecology, Shannon's Diversity Index,
167 indicated a decrease in dispersion of patches across the investigated landscapes.

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169 **Analysis of changes in species richness and site quality (BDMF) over 25 years**

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171 In percentage terms, the greatest decrease in species richness in both genera for the period 1990-2014 was
172 observed at sites 1, 2, and 5 (Fig. 1, Appendix 2). The greatest decrease in *Merodon* species richness was
173 recorded at site 8, whereas the genus *Cheilosia* suffered the greatest decrease in species richness at site 2.
174 The only site where a change in species richness was not observed was site 4.

175 Mean BDMF for the first period (BDMF1; 1990-2006) was 50.7%; the highest mean value was observed
176 for site 7 (77.8%), whereas the lowest mean value was found for site 9 (29%). All BDMF1 and BDMF2

177 values (2006-2012) are presented in Fig. 2. According to the BDMF classification, currently six sites can
178 be considered as degraded habitats, with BDMF values $< 50\%$ (sites 1, 2, 4, 5, 9 and 10), whereas three
179 sites can be classed as "good quality" habitats (3, 6 and 8) with BDMF values ranging between 50 and
180 74%. Only one site (7) presented a value $> 75\%$, indicating the highest habitat quality.

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182 **Correlations among ecological and landscape parameters**

183 Our results showed differences in correlation patterns between changes in landscape parameters and
184 species richness of the two genera (Table 3 and 4). Although there was no correlation between *Merodon*
185 species richness and landscape changes, *Cheilosia* species richness proved to be significantly positively
186 correlated to LSI ($r=0.683$, $p<0.05$), and CONN ($r=0.689$, $p<0.05$). Additionally, BDMF was strongly and
187 positively correlated to CONN ($r=0.726$, $p<0.05$), and negatively correlated to PRD ($r=-0.707$, $p<0.05$). It
188 is also worth noting that spatial scale influenced the response of all investigated ecological parameters
189 since statistical significance was only observed at the smaller spatial scale (2km), while on 5km scale
190 parameters did not show statistically significant correlations.

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192 **Discussion**

193 **Influence of landscape parameters on hoverflies over 25years**

194 Our analysis revealed quantitative changes in landscape structure over a 25-year period, as well as
195 significant hoverfly species richness loss during this time frame. Landscape changes can be driven by
196 quite distinct sets of factors (Koomen et al. 2007). SHDI, one of the most widely-used metrics in
197 landscape pattern analysis, characterizes landscape composition in terms of diversity at the landscape
198 level. Values of this metric for the 2 and 5 km buffer zones, together with CONTAG values, revealed an
199 overall decrease in dispersion of the investigated sites, probably due to reduced fragmentation. Two

200 components contribute to calculations of SHDI: richness (defined as the number of different patch types)
201 and evenness in the distribution of areas among patch types (Eiden et al. 2000). Previous studies have
202 documented the potential of SHDI to explain contemporary hoverfly species richness (Földesi et al. 2015,
203 Jovičić et al. 2017). However, the results of the present study showed no significant relationship between
204 this landscape parameter and species richness, nor between SHDI and BDMF over the 25-year study time
205 frame. Heterogeneous land cover types can increase hoverfly species richness (Büchs 2003), but if
206 increased landscape heterogeneity involves an increase in the number of habitats that are not suitable for
207 hoverflies, heterogeneity in itself will not support hoverfly macro-habitat requirements. Another measure
208 of landscape diversity used in our analysis was PRD. The negative correlation between PRD and *BDMF*
209 confirms that an understanding of biology and ecology of bioindicators is of utmost importance in
210 landscape analyses, and that the selection of landscape parameters and their interpretation almost always
211 depends on species preferences. The influence of landscape diversity on hoverfly species richness has
212 rarely been studied through the lens of historical ecology, so additional research is needed to better
213 understand its effects.

214 LSI is a landscape shape index, values of which increase with increasing shape irregularity and
215 disaggregated areas within the landscape. This index was positively related to *Cheilosia* species richness,
216 but did not significantly influence the response of the genus *Merodon* nor BDMF over the 25-year period.
217 Our correlation analysis revealed a strong relationship between BDMF and the CONN parameter during
218 the time frame we considered. The strong positive correlation most likely indicates that loss of
219 connectivity in the landscape is the main cause of habitat quality degradation, ultimately leading to loss of
220 species. However, this outcome primarily relates to the genus *Cheilosia*, since a statistically significant
221 positive correlation was found between *Cheilosia* species richness and CONN, but not between *Merodon*
222 species richness and CONN. The effects of landscape structure on different insect pollinator groups vary
223 according to species mobility and foraging behavior (Steffan - Dewenter et al. 2002), clearly highlighting
224 the response as being taxon - specific (Jovičić et al. 2017). Given the fact that connectivity is a key
225 concept relating to the ecological effects of environmental change, future research should include more

226 detailed methods for quantifying the network connectivity of landscapes mosaics, i.e. the Harary index
227 (Ricotta et al. 2006).

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230 **Bioindicator role of hoverflies**

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232 Due to its inherent complexity, biodiversity cannot be easily measured so appropriate descriptors
233 (surrogates, indicators) need to be selected (Schindler et al. 2012). Here, we tested the bioindicator role of
234 two phytophagous hoverfly genera by utilizing the StN database and BDMF values to assess habitat
235 quality. A decrease of 9.25% for the mean value of BDMF across all sites over the last 25 years indicates
236 decreased site quality. We found that sites belonging to both the "degraded" and "good quality" categories
237 exhibited quality degradation. For example, two sites (1 and 5) were downgraded from being good quality
238 to degraded sites. These sites have been affected by agricultural activities, which could contribute to
239 habitat disturbance and, consequently, impact species richness (loss). In particular, expansion of
240 agricultural fields at the expense of forests has had a negative impact on species of *Cheilosia*. Moreover,
241 site 5 has undergone urbanization, which can strongly influence its capacity to support hoverfly
242 assemblages. Our StN analysis of these two taxonomic groups provides insights into the relationship
243 between the species richness of these two genera and landscape structural change. We conclude that due to
244 its sensitivity, the genus *Cheilosia* could be used as an effective indicator of landscape change over
245 longtime periods. Moreover, a recent study by Radenković et al. (2017) confirms a higher sensitivity of
246 the genus *Cheilosia* to environmental changes; the genus *Cheilosia* would be more negatively affected by
247 future climate change than *Merodon* on the Balkan Peninsula. Meyer et al. (2009) found that land - use
248 change differentially affects hoverfly species depending on their specific larval feeding habits as well as
249 their microhabitats. Modified ecosystems can support better *Merodon* species due to the availability of
250 their larval host plants (Jovičić et al. 2017). On the contrary, *Cheilosia* species are sensitive to

251 environmental disturbance, especially of forests. Undisturbed forest habitats enable them to have
252 continuity of the microclimate they prefer. If the microclimate changes, species may become endangered.

253 It is important to underline that hoverflies are a diverse taxon, constituted by genera with different
254 ecological requirements (Sommaggio, 1999, Rotheray and Gilbert, 2011). Extending this type of analysis
255 to all genera in the Family Syrphidae could be useful, especially if taxa with trophic characteristics other
256 than phytophagy are considered. The use of functional traits rather than numbers of species seems to be
257 more useful for assessing the conservation of habitats (Moretti et al. 2009, Vandewalle et al. 2010). Our
258 research confirms StN as a useful tool for detecting differences between sites, including capturing the
259 effect of changes in landscape complexity over a long period of time.

260 Our study confirms that spatio-temporal patterns of landscape change need to be considered when
261 planning for conservation management activities (Senapathi et al. 2015). We conclude that shifts in
262 hoverfly assemblages occur in those landscapes that have experienced the greatest change in various
263 landscape characteristics, such as aggregation, isolation / connectivity and diversity. Consequently, we
264 have confirmed the bioindicator role of hoverflies through the patterns our data has revealed. Thus, we
265 recommend that the landscape metrics that best describe these patterns, together with the StN database, be
266 used as management tools in conservation management strategies to ensure the sustainable conservation of
267 hoverfly diversity.

268
269 **Acknowledgements:** We kindly thank John O'Brien for English proofreading and Dr Giovanni Burgio for
270 contributions while developing the original idea of this study. This work was supported by the Ministry of
271 Education, Science and Technological Development, Republic of Serbia, under Grant No. 173002 and
272 Grant No. 43002, the Provincial Secretariat for Science and Technological Development under Grant No.
273 114-457-2173/2011-01, and H2020 project ANTARES under Grant No. 664387.

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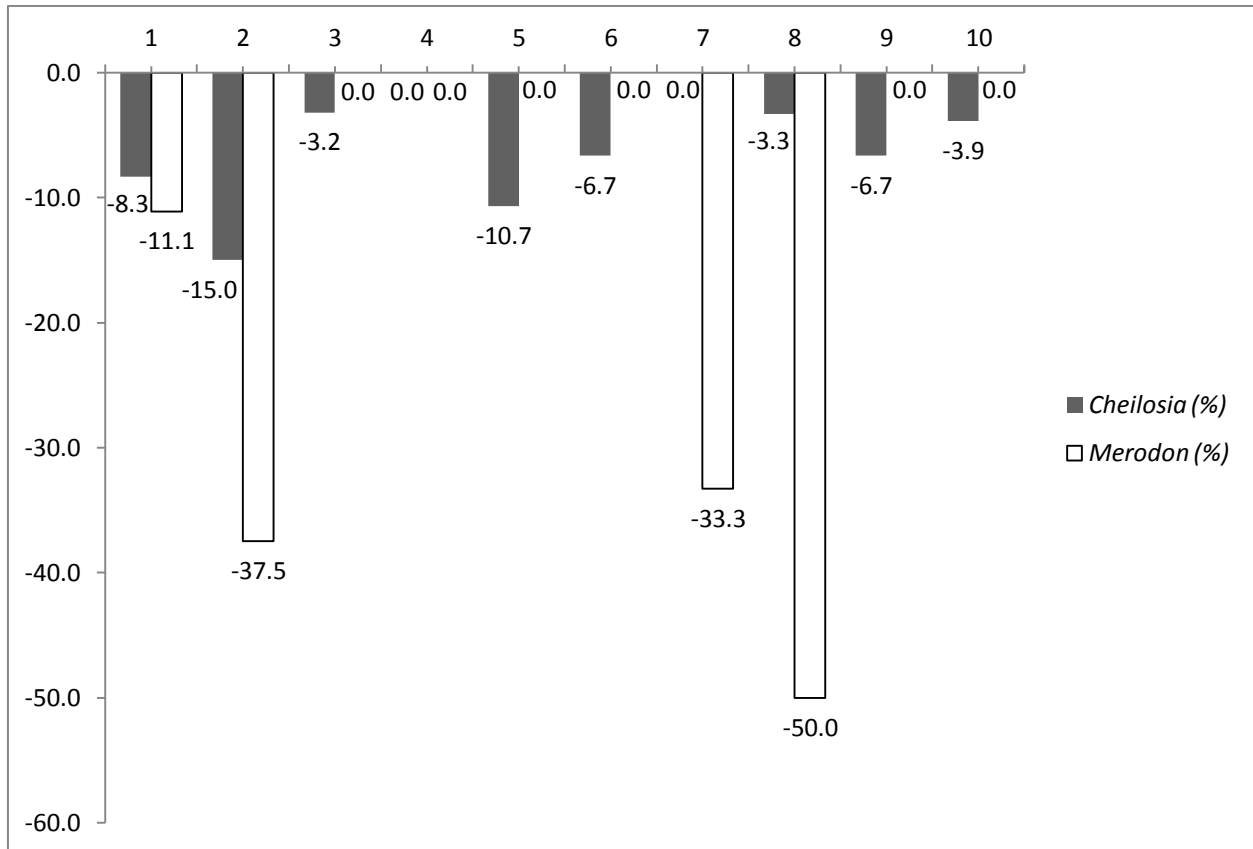
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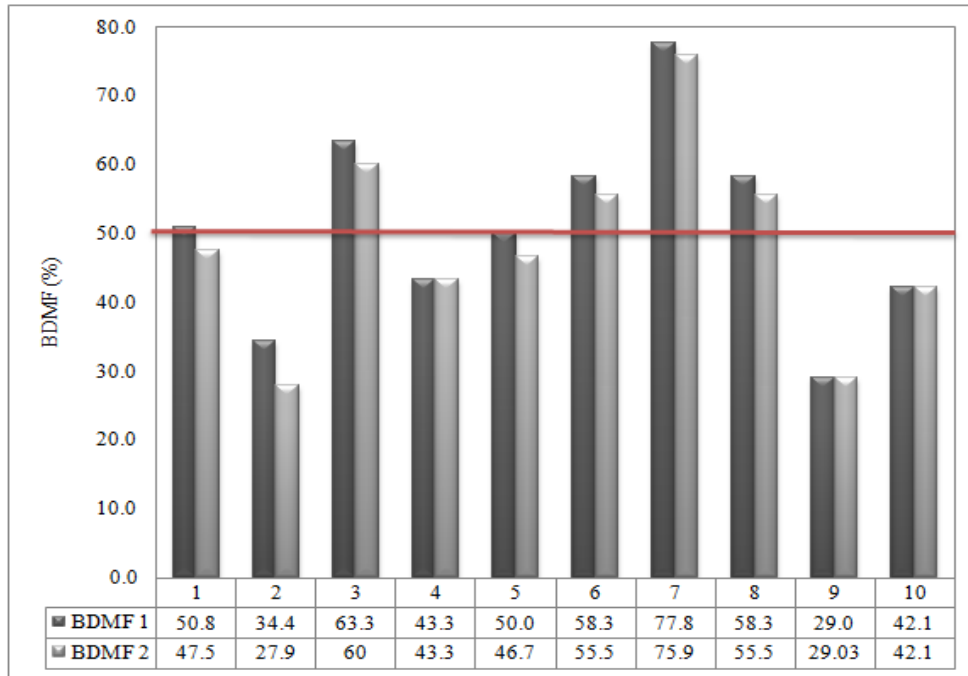
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Figure 1. Observed percentage change in *Merodon* and *Cheilosia* species richness during the period 1990-2014 for ten study sites (1-10).



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393 Figure 2. Comparison of BDMF values for 10 study sites (1-10) for two time - periods: BDMF1 (1990-

394 2006) and BDMF2 (2006-2014). The red line represents the threshold (50%) for good quality habitats.

395 BDMF= biodiversity maintenance function; the ratio between observed and predicted species.

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398 Table 1. Research study sites: GPS coordinates and summary of landscape characteristics.
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Sites	Coordinates	Landscape matrix	Type of Landscape
1	N44°0'55.48 E21°52'54.77	Broadleaf forest (Quercus & Fagus)	Low mountain
2	N44°0'47.12 E21°55'32.81	Broadleaf forest (Quercus & Fagus)	Low mountain
3	N44°1'43.59 E21°57'29.33	Broadleaf forest (Quercus)	Low mountain
4	N44°1'1.22 E21°57'35.77	Broadleaf forest (Quercus)	Low mountain
5	N45°10'44.22E 19°51'55.54	Broadleaf forest (Quercus)	Low mountain
6	N43°16'39.11 E20°46'32.24	Conifer forest (Picea)	High mountain
7	N43°21'15.38 E20°44'40.33	Conifer forest (Picea) & Broadleaf forest (Fagus)	High mountain
8	N43°19'22.80 E20°44'57.84	Conifer forest (Picea)	High mountain
9	N43°19'0.64 E22°48'5.98	Conifer forest (Picea)	High mountain
10	N43°14'1.79" E22°46'53.35	Broadleaf forest (Fagus) & Conifer forest (Picea)	High mountain

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408 Table 2. Landscape metrics used to quantify landscape structure and to assess landscape structural change;
409 calculated in Fragstat.

GroupType	Landscape metrics	Description
Area & edge	Radius of Gyration (GYR)*	Measure of patch extent; it describes how far across the landscape a patch extends its reach.
Shape	Large Patch Index (LPI)*	Index of dominance that equals the percentage of landscape comprised by the largest patch
	Fractal Dimension Index (FRAC)	Describes the complexity of a patch's perimeter.
Aggregation	Contagion Index (CONTAG)*	Index measuring the degree of clumping of attributes on raster maps.
	Landscape Shape Index (LSI)*	Describes the regularity of landscape patches in the considered landscape
Subdivision	Landscape Division Index (DIV)*	Describes how much the landscape is subdivided into patches.
Diversity	Patch Richness Density (PRD)*	Measure of landscape diversity.
	Shannon's Evenness Index (SHEI)*	Describes the proportion of the landscape occupied by a certain class.
	Shannon's Diversity Index (SHDI)	Describes how many patches of the same type are dispersed in the landscape.
Isolation	Euclidean Nearest-Neighbor Distance (ENN)*	Quantifies patch isolation.
	Connectance Index (CONN)	Describes connectivity between patches of the same class.

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413 Table 3. Correlations among ecological (*Cheilosia* and *Merodon* species richness and BDMF) and
 414 landscape parameters [Radius of Gyration (GYR), Large Patch Index (LPI), Fractal Dimension Index
 415 (FRAC), Contagion Index (CONTAG), Landscape Shape Index (LSI), Landscape Division Index (DIV),
 416 Patch Richness Density (PRD), Shannon's Evenness Index (SHEI), Shannon's Diversity Index (SHDI),
 417 Euclidean Nearest-Neighbor Distance (ENN), Connectance Index (CONN)].

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	LPI	LSI	GYR	FRAC	ENN	CONTAG	CONN	DIV	PRD	SHI	SHEI
<i>Cheilosia</i>	-0.470	0.683*	0.128	0.329	0.195	0.067	0.689*	0.433	-0.604	0.098	-0.018
<i>Merodon</i>	-0.464	0.212	0.369	0.505	-0.055	0.225	0.615	0.553	-0.137	0.355	-0.225
BDMF	-0.390	0.232	0.591	0.567	0.183	0.067	0.726*	0.396	-0.707*	0.159	0.006

419 *p<.05

420

421 Table 3. P-values of correlations among ecological (*Cheilosia* and *Merodon* species richness and BDMF)
422 and landscape parameters [Radius of Gyration (GYR), Large Patch Index (LPI), Fractal Dimension Index
423 (FRAC), Contagion Index (CONTAG), Landscape Shape Index (LSI), Landscape Division Index (DIV),
424 Patch Richness Density (PRD), Shannon's Evenness Index (SHEI), Shannon's Diversity Index (SHDI),
425 Euclidean Nearest-Neighbor Distance (ENN), Connectance Index (CONN)].

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	LPI	LSI	GYR	FRAC	ENN	CONTAG	CONN	DIV	PRD	SHI	SHEI
<i>Cheilosia</i>	0.171	0.030	0.724	0.353	0.589	0.854	0.028	0.211	0.065	0.789	0.960
<i>Merodon</i>	0.176	0.557	0.294	0.136	0.881	0.531	0.059	0.097	0.707	0.314	0.531
BDMF	0.265	0.519	0.072	0.087	0.613	0.854	0.018	0.257	0.022	0.662	0.987

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