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2 Towards precision ecology: Relationships of multiple sampling
3 methods quantifying abundance for comparisons among studies

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16 **Short title** Relationships of sampling methods

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21 invasive,

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34 ABSTRACT

35 Because different sampling techniques will provide different abundance values, it is
36 currently difficult to compare results among many studies to form holistic
37 understandings of how abundance influences ant ecology. Using three sampling
38 methods in the same location we found pitfall traps best confirmed *A. gracilipes*
39 presence recording the fewest zero values (9.1%), card counts were the least reliable
40 (67.1%), and tuna lures were intermediate (30.1%). The abundance of *A. gracilipes*
41 from card counts ranged from 0 to 20, in pitfall traps from 0 to 325, and the full range
42 of tuna lure abundance scores (0-7) were sampled. We then determined the
43 relationships between these three standard ant sampling techniques for the abundance
44 of yellow crazy ant *Anoplolepis gracilipes*. Irrespective of the data transformation
45 method, the strongest relationship was between pitfall traps and tuna lures, and the
46 least strong was between pitfall traps and card counts. We then demonstrate the utility
47 of this knowledge by analysing *A. gracilipes* abundance reported within published
48 literature to show where the populations in those studies sit on an abundance
49 spectrum. We also comment on insights into the relative utility of the three methods
50 we used to determine *A. gracilipes* abundance among populations of varying
51 abundance. Pitfall traps was the most reliable method to determine if the species was
52 present at the sample level. Tuna lures were predominantly reliable for quantifying
53 the presence of workers, but were limited by the number of workers that can gather
54 around a spoonful of tuna. Card counts were the quickest method, but were seemingly
55 only useful when *A. gracilipes* abundance is not low. Finally we discuss how
56 environmental and biological variation needs to be accounted for in future studies to
57 better standardise sampling protocols to help progress ecology as a precision science.

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61 INTRODUCTION

62 There is a myriad of ways to sample ant abundance [1,2]. For clarity, all use of the
63 word abundance in this paper refers solely to “momentary above-ground worker
64 abundance” from 30 second counts to 24 hour counts, not overall abundance which
65 would incorporate non-foraging workers. Although there will likely be a positive
66 relationship between forager abundance with overall population levels and worker
67 density [3], these relationships and other potential metrics are not considered further.

68

69 To quantify abundance, the sampling method of choice for any study will depend
70 upon many factors including effort, desired sampling completeness, the science
71 questions, the foraging biology of the target species, and the data requirements of
72 proposed analyses. But because different sampling techniques will provide different
73 abundance values, it is currently difficult to compare results among many studies to
74 form holistic understandings of how abundance influences ant ecology, and largely
75 impossible for quantified comparisons to give predictive knowledge. This issue is
76 most prominent when there is little complementarity between both sampling
77 technique and the environments (e.g. card counts in a rainforest vs pitfall traps in an
78 open environment).

79

80 Most research investigating comparability of ant sampling methods focuses on
81 sampling entire faunas (species richness) and has investigated variations of the same
82 type of method such as comparing different pitfall trap diameters [4] or preservatives
83 [5,6], as well as comparing data collected using different sampling methods
84 [8,9,10,11]. Assessments focusing on individual species appear to be largely restricted
85 to matrix preferences of invasive species for toxic bait attractancy [e.g. 12,13]. For all
86 such work, as far as we are aware, there has never been a comparative study that has
87 produced quantitative relationships of the outcomes of using different methods that
88 can be used to compare dissimilar data among other studies.

89

90 Resolving the issue of non-complementarity of data among studies will be one of the
91 most productive ways to accelerate knowledge and address key science questions.
92 One of the best examples of the need to address this data issue is the attempts to gain
93 a predictive understanding the impacts of invasive species. Impacts of biological
94 invasions are density dependent [14,15,16], so having data that can be precisely
95 compared among studies sampling the target invader is critical if we are to obtain
96 truly predictable and quantifiable understandings of the effects of invasive species. To
97 some extent we can make comparisons and broad generalisations when sampling and
98 environments are similar, or even when sampling is consistent among very different
99 environments, but even this output remains far from quantifiable.

100

101 Here, we attempt to partly resolve this issue by determining the relationships between
102 three standard ant sampling techniques for the abundance of the invasive yellow crazy
103 ant *Anoplolepis gracilipes*. We then demonstrate the utility of this knowledge by
104 analysing *A. gracilipes* abundance reported within published literature to show where
105 the populations in those studies sit on an abundance spectrum. We also comment on
106 insights into the relative utility of the three methods we used to determine *A.*
107 *gracilipes* abundance among populations of varying abundance.

108

109 MATERIAL AND METHODS

110

111 **Study area**

112 The study was conducted within northeast Arnhem Land (12°11'S, 136°46'E) in
113 Australia's Northern Territory. The regional climate is tropical monsoonal with high
114 temperatures (17-33C) throughout the year and an annual rainfall of approximately
115 1200 mm falling predominantly during the summer wet season. The landscape is
116 predominantly savanna woodland dominated by *Eucalyptus tetrodonta* (height and
117 canopy cover approximately 15 m and 20% respectively), with an understorey to three
118 meters predominantly of Acacias and grasses [17]. The weather was consistent
119 throughout the sample period, being dry and sunny (temperature range 21-32C) and
120 all sites were sampled simultaneously, therefore we do not consider that weather
121 variation influenced the results.

122

123 **Sampling**

124 We quantified *A. gracilipes* abundance at three sites, being three spatially discrete *A.*
125 *gracilipes* populations with visually different active ant abundance. This species
126 occurs throughout the region in discrete populations [18], and all populations were
127 familiar to the authors as they had been recently surveyed in preparation for
128 eradication [19]. Sampling was conducted along transects, with 11 sampling locations
129 along each transect, and both transects and sampling locations spaced 10 m apart.
130 There were 13 transects at two sites with visually low and medium-level *A. gracilipes*
131 abundance, and 15 transects at the third site with visually high abundance. Within a
132 site a single person conducted sampling on each transect and a different person was
133 used for each transect. Sampling was conducted using three methods at all three sites,
134 being card counts, tuna lures and pitfall traps. A single sample of each method was

135 used at each sample location in three separate sampling occasions. Card sampling was
136 conducted first at two sites in the late afternoon (after 4 pm) of 18 September 2009.
137 The following day tuna lures were used at the same two sites at exactly the same time
138 of day. Pitfall traps were set the following day and operated for 24 hours. The same
139 sampling order and timing was conducted at the third site commencing 21 September
140 2009.

141

142 Cards were 10 cm x 10 cm laminated paper. At each sampling location a card was
143 placed on the ground with the edges in contact with substrate as far as possible to
144 allow easy access for the ants to walk onto the card. The card was observed for 30
145 seconds with the number of *A. gracilipes* workers that walked over the square
146 recorded.

147

148 Tuna lures were a teaspoon amount of canned tuna (Home brand Seafood Basket cat
149 food containing tuna). At each sampling location a lure was placed directly onto the
150 ground and *A. gracilipes* abundance at and within 1cm of each lure was scored after
151 20 minutes according to the following scale that has been applied to many
152 publications containing ant abundance datasets: 0 = no ants; 1 = 1 ant; 2 = 2-5 ants; 3
153 = 6-10 ants; 4 = 11-20 ants; 5 = 21-50 ants; 6 = 50-100 ants; and 7 = >100 ants. This
154 scale is used because of the impracticality of accurately counting large numbers of
155 ants at a lure.

156

157 Pitfall traps were plastic containers with an internal diameter of 65 mm, one third
158 filled with ethylene glycol as a preservative. A single pitfall trap was set at each
159 sample location, operated for 24 hours, collected, and *A. gracilipes* abundance was
160 counted back in a laboratory. Because of logistics constraints only 13 transects were
161 sampled using pitfall traps, with these transects chosen to cover the full abundance
162 variation found prior by card counts and tuna lures. Six, four and three transects were
163 sampled at the three sites respectively (Figure 1).

164

165 **Analysis**

166 Four of the transects in the low-abundance site obtained no *A. gracilipes* in both card
167 counts and tuna lures, and were also not subsequently sampled using pitfall traps, so
168 these transects were excluded from analyses, predominantly because we could not

169 confirm if *A. gracilipes* were definitely present or not when the data were analysed.
170 First, the scaled data of the tuna lures was transformed to give an actual abundance
171 value, being the value or mid-range value of each abundance category, being: 0 = 0; 1
172 = 1; 2 = 3; 3 = 8; 4 = 15.5; 5 = 35.5; 6 = 75.5; and 7 = 125. Next, the abundance data
173 for each sampling location were transformed to reduce the spatial scatter of the data,
174 and was done so two ways to compare the two most extreme mathematical
175 possibilities. First was a $\log(x+1)$ transformation on data first summed at the transect-
176 level (data of 11 samples first combined and then transformed) and second was
177 sample-level data first transformed and then summed to transect-level. The data were
178 then compared first pairwise in 2D scatterplots fitted with linear Pearson correlations,
179 then together in 3D scatterplots.

180

181 To demonstrate the utility of these correlations we then compared ant abundances
182 among published studies. First, we identified 12 publications that sampled yellow
183 crazy ants using one of these methods and also provided data. Although most studies
184 contained numerous sites with varying levels of crazy ant abundance, we only used
185 the data from the sampling unit with the highest abundance. Where possible we
186 obtained sample-level data, but in most instances all that was available was an
187 average per sample or a total of summed samples (e.g. 5 pitfall traps summed as a
188 plot), in which case we averaged the data to obtain an abundance value per sample,
189 and if needed standardised the data to the methods used here (e.g. halving the data of
190 the 1-minute card count in [20] to match the 30 second card count used here).

191 Because traps were used the most (5 out of 11 studies), we used the correlation
192 equations to calculate abundance in traps for the other six studies. To ensure sample
193 number was the same among studies all calculations were made using 11 samples as
194 the sample number to make data comparable with this study. This was done for both
195 data transformation methods. These trap data were then used to calculate all
196 remaining data of the other two sample types for each study using the same
197 methodologies, but by re-arranging the $y=ax+b$ equations to $x=(y-b)/a$. The data were
198 then visually compared graphically.

199

200 RESULTS

201 At the sample level, where all three sampling methods were used, pitfall traps best
202 confirmed *A. gracilipes* presence recording the fewest zero values (13; 9.1%), card

203 counts were the least reliable (96; 67.1%), and tuna lures were intermediate (43;
204 30.1%). In the 13 instances where *A. gracilipes* workers were not present in a trap, no
205 *A. gracilipes* were also recorded by both other methods in 12 instances, and a single
206 worker was recorded at the tuna lure in the other instance, confirming that *A.*
207 *gracilipes* was largely absent from these few locations. The abundance of *A.*
208 *gracilipes* from card counts ranged from 0 to 20, in pitfall traps from 0 to 325, and the
209 full range of tuna lure abundance scores (0-7) were sampled.

210

211 The raw sample-level data showed great variation (Figures 2a-c), but naturally this
212 variation was greatly reduced after combinations of log transformation and pooling
213 (Figures 2d-i). Irrespective of the data transformation method, the strongest
214 relationship was between pitfall traps and tuna lures, and the least strong was between
215 pitfall traps and card counts. The two transformation and pooling methodologies gave
216 similar but distinctly different results. However, the 3D compilation of sample-level
217 data that had undergone transformation prior to pooling provided a greater data spread
218 (Figure 3). Notably two transects with intermediate *A. gracilipes* abundances that also
219 had zero values on card counts look anomalous in the figures.

220

221 The standardisation calculations of pitfall trap abundance from published data using
222 the two transformation and pooling methodologies (Figure 4) ordered the publications
223 very similarly. But the two methods gave greatly different results for the two studies
224 with greatest crazy ant abundance (studies 11 and 12) whose original data were based
225 on card counts, with the method that used log transformation of already pooled data
226 (Figure 4b) providing a seemingly artificially low abundance count. The 3D
227 compilation of sample-level data that had undergone transformation prior to pooling
228 largely maintained the order of the studies as determined by pitfall trap data, but with
229 some clear nuances of lower abundances relative to the general pattern, predominantly
230 (2 of 3 studies) associated with studies that whose data were based on card counts
231 (studies 2 and 11) (Figure 5).

232

233 DISCUSSION

234 Despite great differences in sampling methodology, the abundance data obtained from
235 the three sampling techniques attained reasonably strong relationships, but only after
236 transformation and pooling. This outcome provided a strong basis by which

237 abundance among studies using different sampling techniques could be calculated and
238 compared. Here we used two types of transformations and found similar but distinct
239 results, and ultimately of these two we recommend the use of the data that transforms
240 samples before pooling. However, this does not preclude the use of other
241 transformations that may prove to be better. Indeed, we have provided our raw data as
242 Supplementary Material to encourage other researchers to explore other mathematical
243 relationships, and perhaps other analytical techniques, that may prove to be superior
244 to what we have found here.

245

246 Even with the relatively small gradient of *A. gracilipes* abundance assessed within
247 this study, coupled with universal implications for their use, the three techniques had
248 clearly different capacity and practicality for use. Pitfall traps had the lowest
249 occurrence of absence data, so was the most reliable method to determine if the
250 species was present at the sample level. The downside of this method though is that it
251 is very time consuming and laborious and does not give results in real-time because
252 the ants need to be counted back in a laboratory. Pitfall traps are also prone to prone
253 to inflated abundance data if a trap is positioned close to a nest. Tuna lures were
254 predominantly reliable for quantifying the presence of workers, but were limited by
255 the number of workers that can gather around a spoonful of tuna, being not too many
256 more than 100 for *A. gracilipes*, which can also be influenced by user-induced
257 variation in lure size and shape. Therefore lures (at least of this size) would be less
258 useful in areas with high and very high *A. gracilipes* abundance. Card counts were the
259 quickest method, but were seemingly only useful when *A. gracilipes* abundance is not
260 low. In the most extreme case, a zero count was obtained by a card, but the associated
261 pitfall trap collected 201 workers. Notably cards are not limited by ant numbers like
262 tuna lures are, and also potentially pitfall traps, and so are great to use when ant
263 abundance is very high.

264

265 What remains unclear is how variation with the ant's diurnal foraging cycle [21],
266 prevailing environmental conditions, and annual population cycle would affect results
267 and ultimately the mathematical relationships. Because pitfall traps are operated
268 normally for 24 to 48 hours, sometimes up to a week, this method has the advantage
269 that it is not so subject to short-term variations in environmental conditions, and
270 captures forager abundance throughout full diurnal cycles. But ant abundance at lures,

271 and especially card counts due to their “snapshot” assessment times” will be
272 completely subject to momentary abundance at that point on the diurnal cycle coupled
273 with effects of prevailing environmental conditions. So for both methods, different
274 abundance values would likely be attained at different stages of the diurnal cycle and
275 between when environmental conditions are ideal vs less ideal. Similarly, attendance
276 at lures may also be influenced by the presence of other resources and dietary needs
277 of the colony at the time relative to the composition of the lure. Indeed even just lure
278 placement in sunny vs shaded locations is known to affect ant presence [22]. As a
279 prediction for all epigeic ant species, we suspect that such variation issues will be
280 greater in open environments more than closed environments due to the effects of
281 solar insolation, and relatively greater in other locations where there are big changes
282 in daily temperature such as on mainland systems relative to lowlands of island
283 systems. Finally, all three methods would also give differing results between peak and
284 low times of the ant’s annual population cycle.

285

286 The combined analysis of published work we presented here does not account for any
287 of the variation issues detailed above, and so therefore should be interpreted with
288 some caution and not seen as being definitive. Indeed even just the differences in the
289 results between the two data transformations used demonstrates the lack of definitive
290 nature of the analysis. Regardless, the calculations appear to confirm the claim that
291 yellow crazy ant abundance quantified on Christmas island within [3] are the highest
292 of all reported for yellow crazy ant, and probably remains the highest quantified for
293 any ant species in the world.

294

295 Our study has advanced the potential for meta-analyses of *A. gracilipes* studies, by
296 providing a mathematical way to calculate abundance that is standardised among
297 studies that have quantified abundance using either pitfall traps, lures or cards to
298 determine an abundance spectrum. But we have not yet been able to advance the
299 utility of many studies that use other methods to quantify *A. gracilipes* abundance
300 using methods like insecticide knockdown, standardised hand collections, foliage
301 beating, sugar-soaked pads [23,24,25,26,27,28) and of course when abundance data
302 are not used or reported (e.g. 29). It remains unclear how universal the relationships
303 would be for other ant species. We suspect that these relationships would not be
304 universal, but may be similar enough for ecologically similar species that have similar

305 abundance, similar locomotion speeds, and similar foraging strategies. Ultimately,
306 understanding and accounting for momentary, daily and seasonal variations in ant
307 abundance, even avoiding these issues altogether within future studies perhaps
308 through the use of standardised sampling protocols, is a great science challenge that
309 overcoming will help progress ecology as a precision science.

310

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315

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- 426
- 427
- 428

430 Figure Legends

431

432 Figure 1. Transect-level combined counts of tuna lures and card counts showing
433 transects that were (white circles) and weren't (black circles) sampled with pitfall
434 traps.

435

436 Figure 2. Pairwise relationships of the three sampling methods of card counts, pitfall
437 trapping and tuna lures with raw sample-level data (a-c), transect-level data compiled
438 from first pooling sample-level data then applying a $\log(x+1)$ transformation (d-f) and
439 transect-level data compiled by pooling sample-level data that had first undergone a
440 $\log(x+1)$ transformation (g-i).

441

442 Figure 3. 3D scatterplots of the relationship between *A. gracilipes* abundance
443 determined by the three sampling methods of card counts, pitfall trapping and tuna
444 lures using transect-level data compiled from first pooling sample-level data then
445 applying a $\log(x+1)$ transformation (a) and transect-level data compiled by pooling
446 sample-level data that had first undergone a $\log(x+1)$ transformation (b).

447

448 Figure 4. Standardised highest yellow crazy ant abundance reported within fourteen
449 studies as calculated by the relationships between the three sampling methods of card
450 counts, pitfall trapping and tuna lures using a) summed $\log(x+1)$ -transformed sample
451 data, and b) $\log(x+1)$ -transformed pooled data. Studies are: 1) [30]; 2) [31] New
452 Caledonia site; 3) [31] Australia site; 4) [20]; 5) [32]; 6) this study; 7) [33]; 8) [34] 9)
453 [18]; 10) [35]; 11) [36]; 12) [37]; 13) [38]; and 14) [3].

454

455 Figure 5. 3D scatterplot of standardised highest yellow crazy ant abundance among
456 fourteen studies as calculated by the relationships between the three sampling
457 methods of card counts, pitfall trapping and tuna lures using sample-based $\log(x+1)$ -
458 transformed pooled data. Studies are: 1) [30]; 2) [31] New Caledonia site; 3) [31]
459 Australia site; 4) [20]; 5) [32]; 6) this study; 7) [33]; 8) [34] 9) [18]; 10) [35]; 11)
460 [36]; 12) [37]; 13) [38]; and 14) [3].

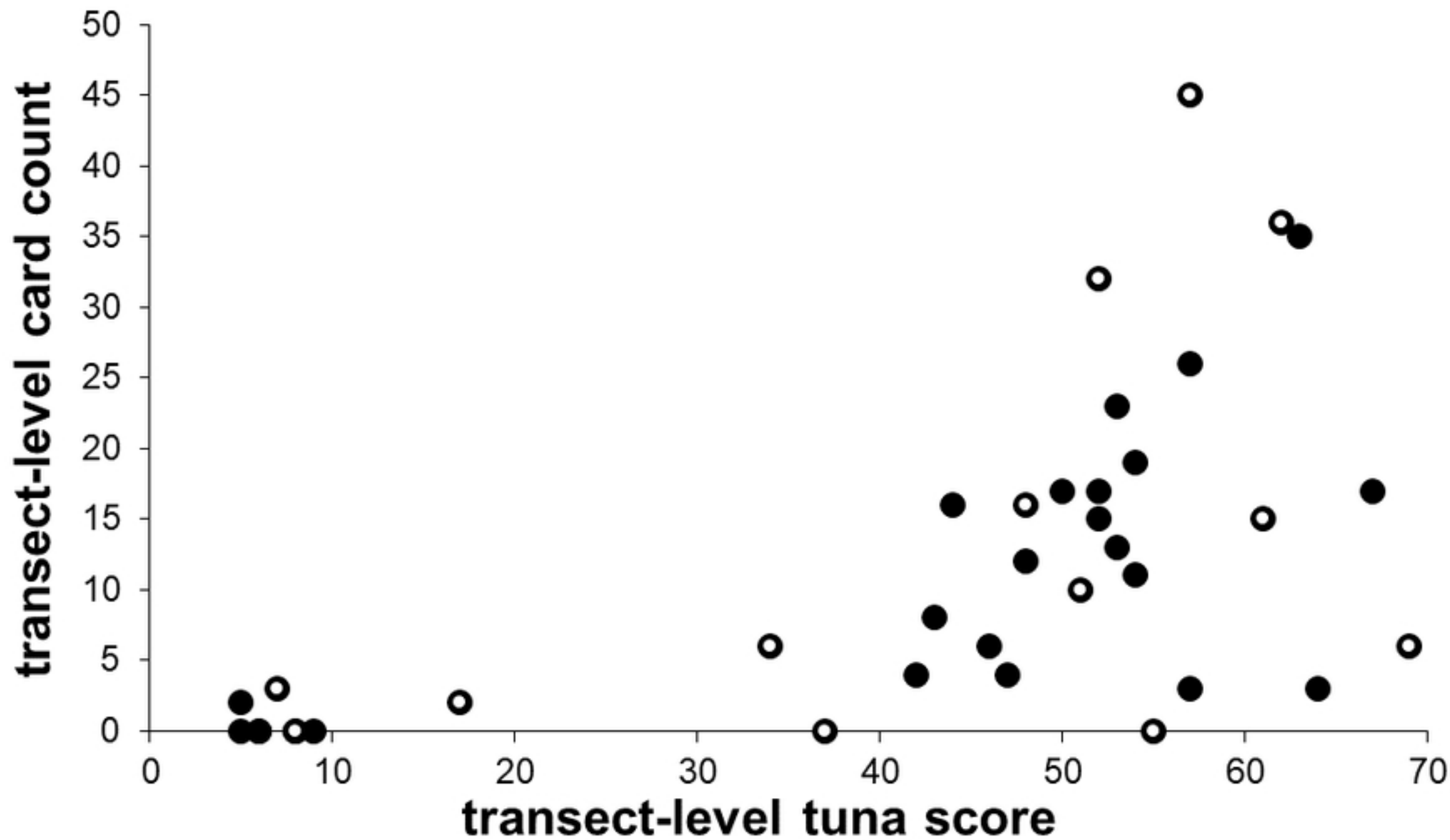


Figure 1

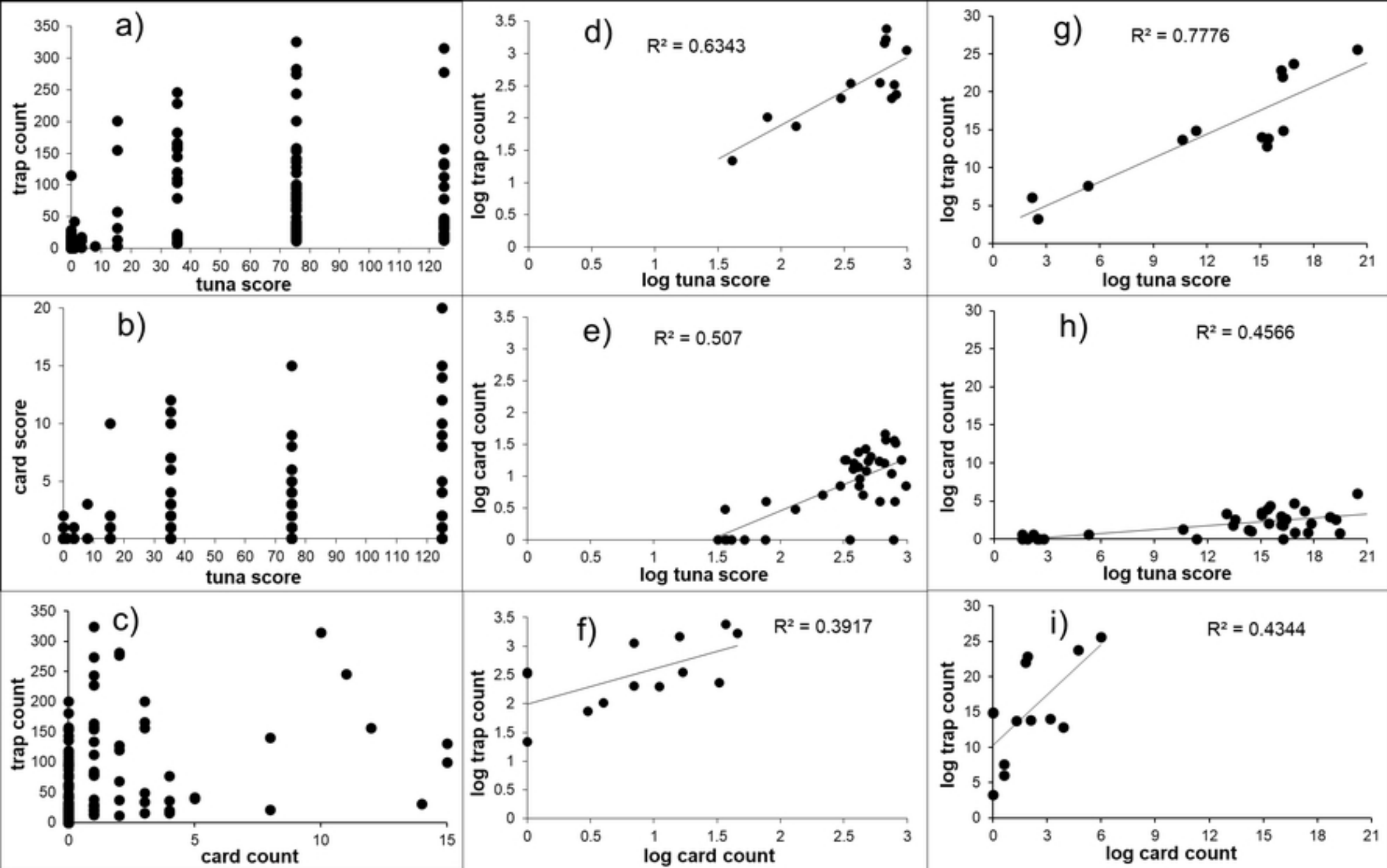


Figure 2

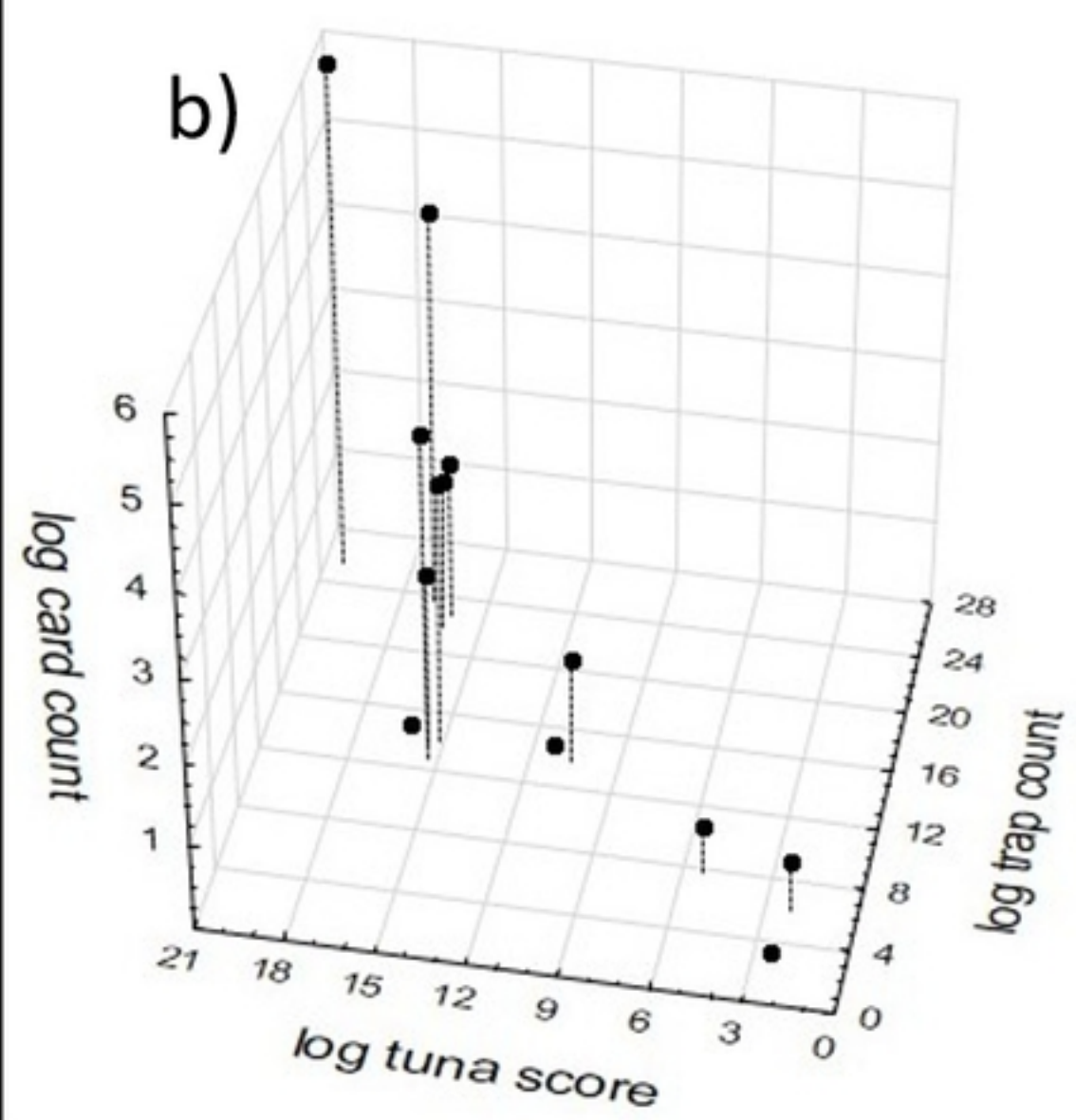
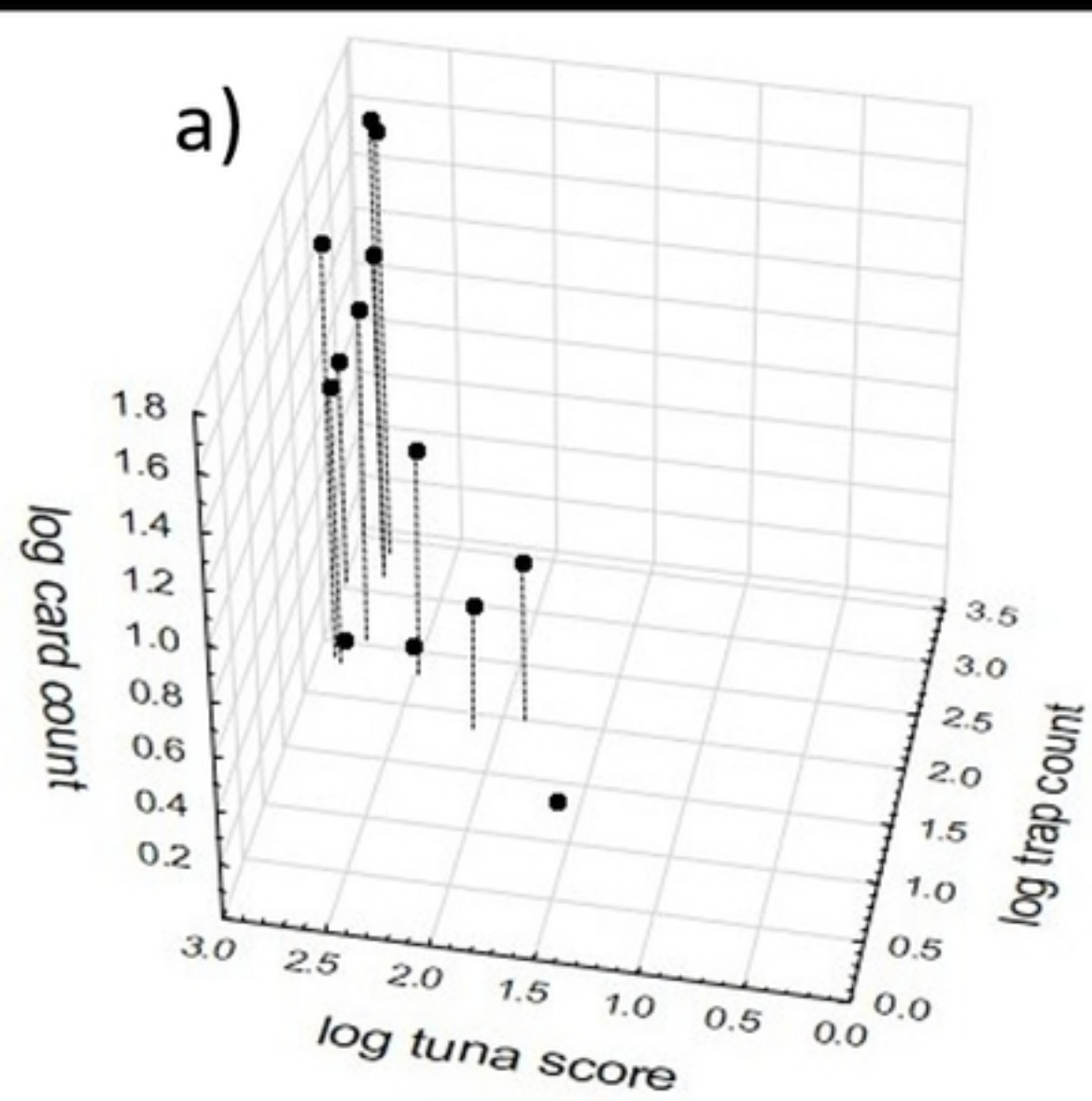


Figure 3

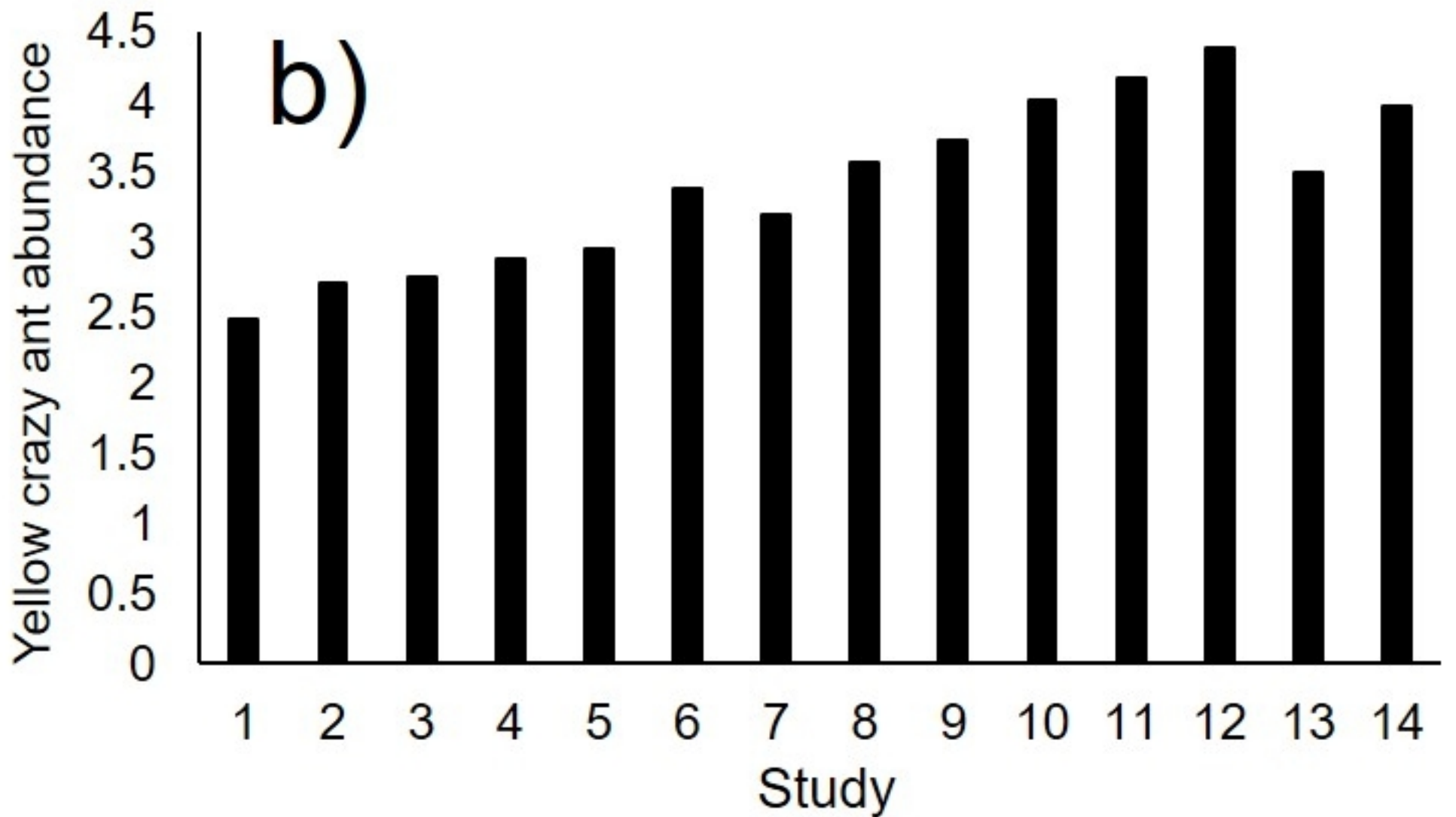
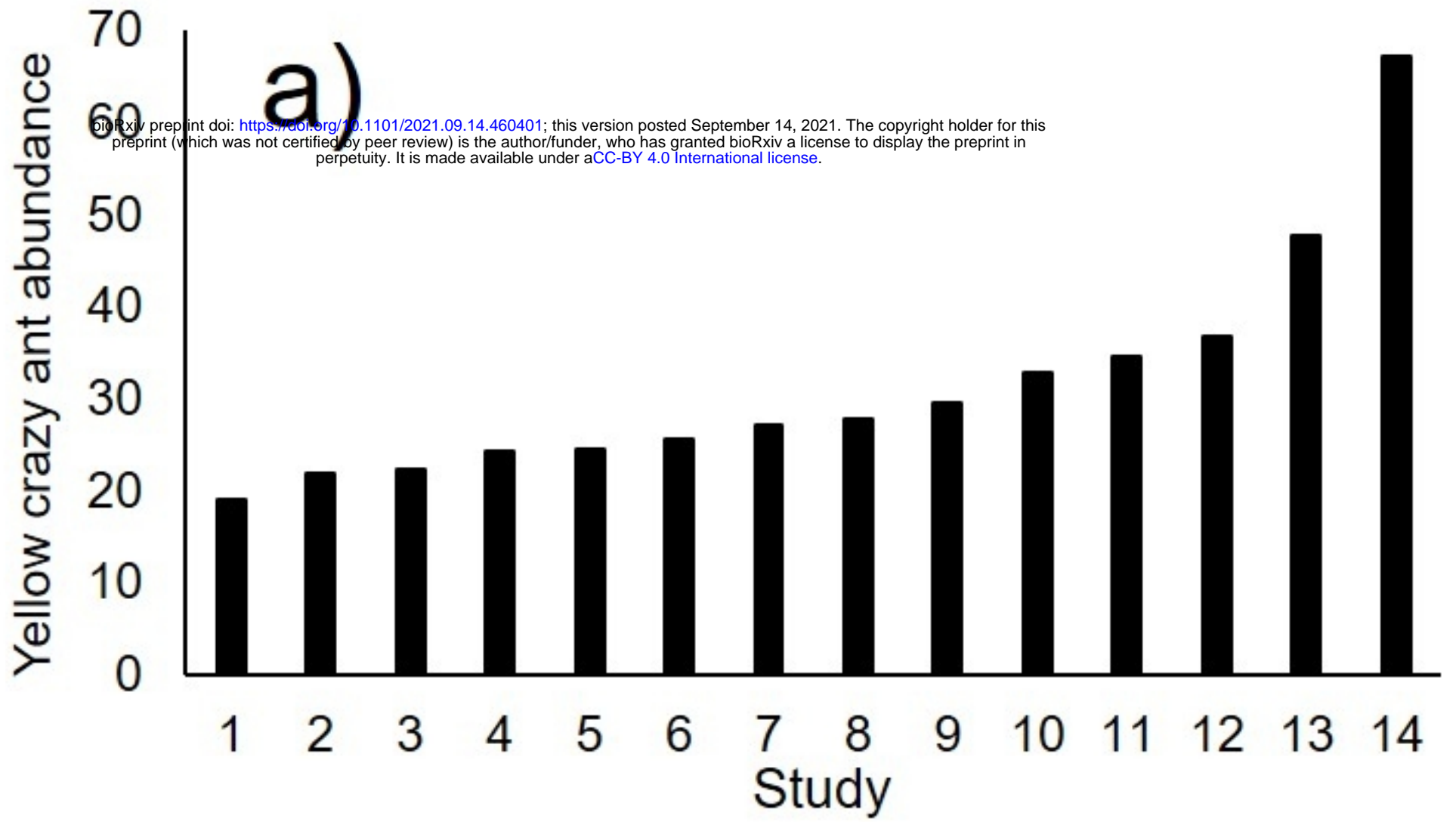


Figure 4

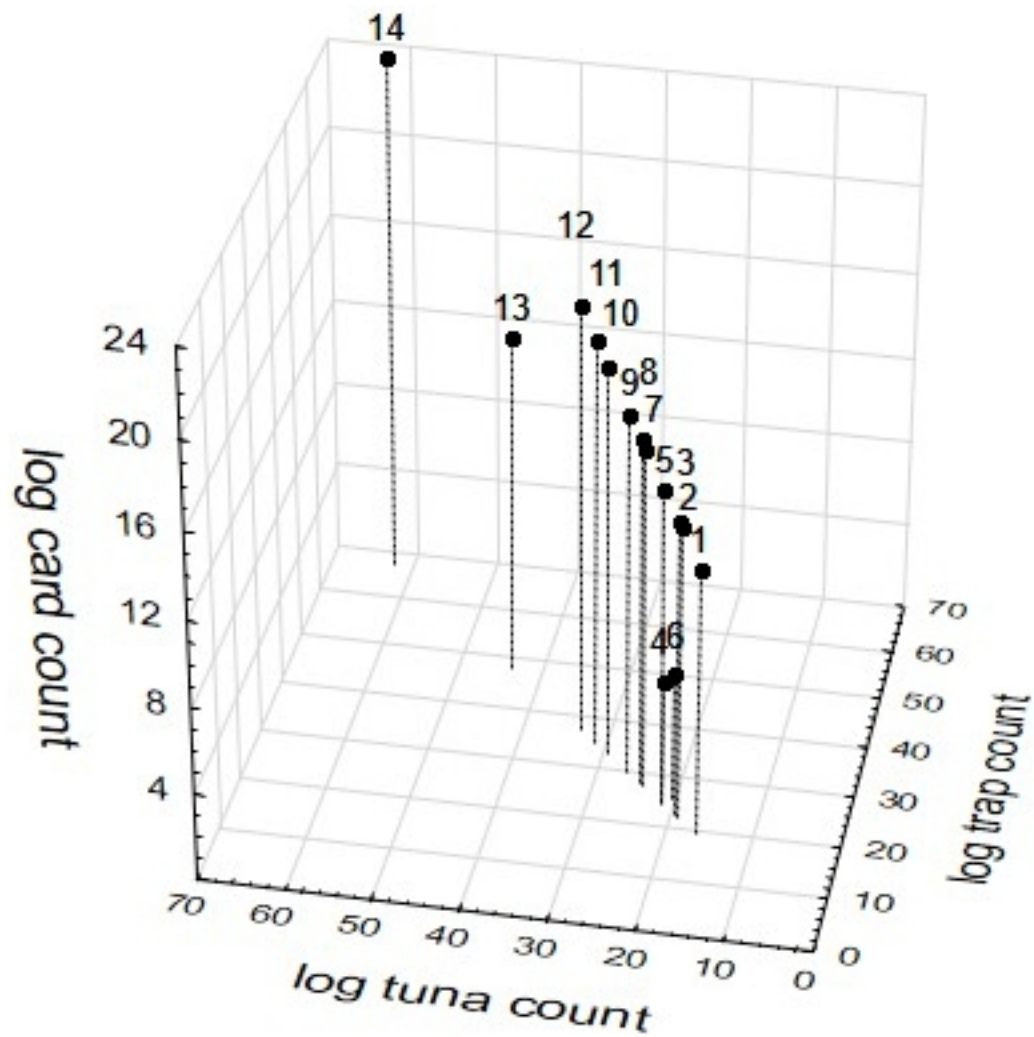


Figure 5