

1 **Population dynamics of the primary malaria vector *Nyssorhynchus***
2 ***darlingi* in a high transmission setting dominated by fish farming in**
3 **western Amazonian Brazil**

4
5
6 Paulo Rufalco-Moutinho^{1,#a*}, Samir Moura Kadri², Diego Perez Alonso², Marta Moreno³,
7 Gabriel Carrasco-Escobar⁴, Catharine Prussing^{5,6}, Dionicia Gamboa^{7,8}, Joseph M.
8 Vinetz^{4,8,9}, Maria Anice Mureb Sallum¹⁰, Jan E. Conn^{5,6}, Paulo Eduardo Martins Ribolla^{1,2}

9
10
11 ¹ Departamento de Parasitologia, Instituto de Biociências de Botucatu, Universidade
12 Estadual Paulista, Botucatu, São Paulo, Brazil

13 ² Instituto de Biotecnologia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil

14 ³ Department of Infection Biology, London School of Hygiene & Tropical Medicine,
15 London, UK

16 ⁴ Laboratorio ICEMR-Amazonia, Laboratorios de Investigacion y Desarrollo, Facultad de
17 Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

18 ⁵ Department of Biomedical Sciences, School of Public Health, State University of New
19 York-Albany, NY, US

20 ⁶ Wadsworth Center, New York State Department of Health, Albany, NY, US

21 ⁷ Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía,
22 Universidad Peruana Cayetano Heredia, Lima, Peru

23 ⁸ Instituto de Medicinal Tropical “Alexander von Humboldt”, Universidad Peruana
24 Cayetano Heredia, Lima, Peru

25 ⁹ Section of Infectious Diseases, Department of Internal Medicine, Yale School of
26 Medicine, New Haven, CT, USA

27 ¹⁰ Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São
28 Paulo, São Paulo, Brazil

29 #a Current affiliation: Núcleo de Medicina Tropical, Universidade de Brasília, Distrito
30 Federal, Brazil

31

32

33 ***Correspondence:**

34 E-mail: paulorufalco@gmail.com (PRM)

35

36 **Abstract**

37 *Vale do Rio Juruá* in western Acre, Brazil, has reported highest malaria numbers since
38 2005, and is considered persistent transmission hotspot. Fish farming development was
39 encouraged to improve standard of living, resulting in productive breeding sites for
40 Amazonian malaria vector species, including *Nyssorhynchus darlingi* that, combined with
41 the high human density and mobility, adds to the local malaria burden. This study reports
42 entomological profile of immature and adult *Ny. darlingi* at three sites in Mâncio Lima,
43 Acre, during the rainy and dry season (February to September, 2017). From 63 fishponds,
44 10,859 larvae were collected, including 5,512 first-instar Anophelinae larvae and 4,927

45 second, third and fourth-instars, of which 8.5% (n = 420) were *Ny. darlingi*. This species
46 was most abundant in not-abandoned fishponds and in the presence of emerging aquatic
47 vegetation. Seasonal analysis of immatures in urban landscapes found no significant
48 difference in the numbers of *Ny. darlingi*, corresponding to equivalent population density
49 during the rainy to dry transition period. However, in the rural landscape, significantly
50 higher numbers of *Ny. darlingi* larvae were collected in August (IRR = 5.80, $p = 0.037$) and
51 September (IRR = 6.62, $p = 0.023$) (dry season), compared to February (rainy season),
52 suggesting important role of fishponds for vector population maintenance during the
53 seasonal transition in this landscape type. Adult sampling detected mainly *Ny. darlingi*
54 (~93%), with similar outdoor feeding behavior, but different abundance according to
55 landscape profile: urban site 1 showed higher peaks of human biting rate in May (46
56 bites/person/hour), than February (4) and September (15), while rural site 3 shows similar
57 HBR during the same sampling period (22, 24 and 21, respectively). This study contributes
58 to a better understanding of the larvae biology of the main malaria vector in the *Vale do Rio*
59 *Juruá* region and, ultimately will support vector control efforts.

60

61 **Introduction**

62 The link between anthropogenic environmental change and the emergence of
63 malaria is well-documented in the Amazon basin [1-3]. Increased human population and
64 land use/land cover change (LULC) influence the biological community, including
65 Anophelinae mosquitoes, particularly those with some degree of synanthropy and
66 competence to transmit *Plasmodium* sp. that circulate in the Amazon region [4]. This vast

67 region is responsible for 99.5% of human malaria in Brazil, mainly *Plasmodium vivax*
68 (>90% in 2019) [5]. Disease indicators vary according to the types of LULC and the socio-
69 environmental aspects of occupied environments, influencing spatiotemporal malaria
70 distribution trends [6]. Although from 2008-2016 Brazil reported annual reductions of the
71 disease, with 2016 having the lowest incidence in the past 35 years, in 2017 the incidence
72 increased by 50% compared with the previous year, decreasing only in 2019 [5]. This
73 resurgence emphasizes the need for routine and integrated surveillance, even when disease
74 rates are low, a characteristic feature of seasonal infectious diseases [7]. A key factor
75 involved in the successful eradication policy of mosquito-borne diseases with a broad
76 distribution and different focal transmission, such as malaria in the Amazon, is the
77 identification and characterization of vector sources, following evaluation of potential tools
78 for an integrated intervention framework [8].

79 Fish farming has been associated with malaria risk in the Amazon in Brazil [9], Peru
80 [10], Colombia [11,12], and in sub-Saharan Africa in Nigeria [13] and Cote d'Ivoire [14].
81 The Vale do Juruá, Mâncio Lima municipality, is a classic example of the potential hazards
82 of extensive fish farming in a periurban/urban setting. A local government program
83 provided resources to residents to construct fish farms, frequently located in their
84 backyards. The unwanted effect of this development program was the increased number of
85 suitable larval habitats of *Nyssorhynchus darlingi* and other local malaria vectors which
86 affect density and spatial distribution and threaten control strategies in the area [15-17].
87 Nowadays, the Vale do Juruá in western Acre is the region with the highest malaria
88 numbers in Brazil, for both *P. vivax* and *P. falciparum*. In a scenario where anthropogenic
89 fish farms have been demonstrated to be major contributors to vector abundance and

90 *Plasmodium* transmission, larval source management (LSM) can be a practical component
91 of integrated vector management (IVM) to reduce or eliminate immature stages of
92 mosquito vectors [18-20]. Further, the recognition that variation in larval habitats,
93 particularly in nutrient availability, strongly influences mosquito fitness, longevity, and
94 malaria transmission dynamics, has renewed interest in larval environments [21,22]. On the
95 other hand, LSM as part of a vector-borne disease control management plan has limitations
96 when dealing with natural aquatic habitats in rural and forest areas, especially when
97 breeding sites are extensive, inaccessible, and require frequent intervention such as clearing
98 aquatic vegetation [23, 24]. To address the application and effectiveness of any control
99 strategies on mosquito borne-disease transmission, local vector biology information is
100 essential, considering the diversity of *Ny. darlingi* in different environmental profiles of the
101 Amazon Basin, reflected in malaria epidemiology. Although entomological surveys
102 addressing Anopheline larvae and the main vector *Ny. darlingi* presence in fishponds have
103 been conducted in the Vale do Juruá [15-17], these studies did not focus on follow-up with
104 short intervals between observations (one/two months per collection), nor characterize
105 environmentally the fishponds associated with larvae sampled.

106 In the present study, an entomological survey of larvae and adult malaria vectors
107 was conducted to evaluate the presence of the main vector *Ny. darlingi* in fishponds and
108 neighboring households in Mâncio Lima, Acre. To address this, our study examined: (i)
109 aquatic habitat parameters associated with Anophelinae larval abundance; (ii) differences in
110 the abundance of *Ny. darlingi* during the rainy to dry seasonal transition; (iii) the
111 microgeographic effect of urban and rural landscapes on the population dynamics of *Ny.*

112 *darlingi*; and (iv) a comparison of human biting rates (HBR) and patterns of *Ny. darlingi*
113 biting times influenced by different landscape scenarios.

114

115 **Methods**

116 **Ethics Statement**

117 This study was approved by the World Health Organization Ethics Review
118 Committee (0002669). Verbal consent was obtained from residents for collections on their
119 properties, with the collaboration of the Mâncio Lima Endemics Diseases Coordination. A
120 monthly report of fishpond physiochemical conditions was provided to each resident. Adult
121 captures were conducted only by the authors, who used antimalarial prophylaxis as
122 recommended by the Brazilian Ministry of Health.

123

124 **Study Area**

125 The municipality of Mâncio Lima is located in western Acre state, Brazil (7° 36' 50"
126 S 72° 53' 45" W) along Highway BR 364 (Fig 1). An Anophelinae larval survey in artificial
127 and natural breeding-sites reported four times more immatures in fishponds [15] compared
128 with natural habitats. A time-series analysis (2003 to 2013), strongly suggested a
129 spatiotemporal association between fish farming and malaria incidence [16]. The estimated
130 population of Mâncio Lima is 17,545 [25], with the municipality registering for *P. vivax*:
131 6,632 infections in 2016 (API = 378 per 1000 habitants) and 7,049 infections in 2017

132 (API= 400); for *P. falciparum*: 1,172 in 2016 (API= 70) and 1,752 in 2017 (API = 99.8)
133 (<http://www2.datasus.gov.br/DATASUS>, 2018). Notifications for monthly malaria shows
134 significant linear correlation (>0.5) with rainfall: for *P. vivax* in 2016: $r = 0.75$, in 2017: $r =$
135 0.43 ; for *P. falciparum*: in 2016: $r = 0.51$, in 2017: $r = 0.47$ (S1 Fig). The most recent
136 livestock census (2016) registered a total of 5,392 cattle in Mâncio Lima, mainly in rural
137 areas (unpublished document, Institute of Agriculture and Forestry Defense of Acre, 2016).

138

139 **Fig 1. Satellite image of Mâncio Lima municipality, showing the three study**
140 **sites.** Site 1, urban, yellow, near Federal Highway BR 364 and Mâncio Lima town; Site 2,
141 urban, red, near Federal Highway BR 364 and more distant from Mâncio Lima; Site 3,
142 rural, green, distant from both BR 354 and Mâncio Lima. Each site shows the residence and
143 two perimeters: 0.5 and 1.0 km (source: ©2017 Google-Images ©2017 TerraMetrics, Nasa,
144 Cartographical data©2017 Maplink). The insert is a map of Brazil indicating the location of
145 Mancio Lima in Acre state.

146

147 **Study Design**

148 This research entailed an observational study of malaria vector ecology. For the
149 Anophelinae survey, independent geographical areas were delimited based on two sampling
150 criteria: the presence of a human residence occupied for at least the past 12 months for
151 adult mosquito collection, and nearby fishponds for larval collection, whether economically
152 active (used for pisciculture at the moment of the survey), or abandoned. Two perimeters

153 (500 m and 1000 m) were virtually attributed for each residence to delimit each study site,
154 and to support the localization of fishponds (Fig 1). These distances were chosen based on
155 the flight range of *Ny. darlingi* in a rural settlement in Rondonia state, between 500 and
156 1000 m [26]. To test the influence of an urban area on local transmission, two sites (Sites 1
157 and 2) were selected near Federal Highway BR 364; and one site (Site 3) that was more
158 distant from the highway (Fig 1). Highway BR 364 is important for socio-economic
159 landscape concepts in Acre state: usually, urban landscape profiles include paved streets
160 and have several residences and other human dwellings (schools, hospital, commercial
161 facilities), and this may be reflected in a higher number of families and houses, leading to
162 the establishment of more fishponds. On the other hand, rural landscape profiles consist of
163 a lower human presence, fewer dwellings, and primary or secondary forest cover, if the
164 landscape has not been exploited for logging, agriculture, or livestock [27]. The presence of
165 at least one fishpond near the house (within at least 500 m), positive previous larvae and
166 adult captures (in December 2016), ease of access to the property, and co-operation of the
167 residents were other considerations for the three residence selections and the respective
168 representative sites.

169

170 **Larval and Adult Capture**

171 Monthly larval collections were performed for six months in 2017 spanning rainy
172 and dry season (February, March, April, May, August, and September). Each fishpond was
173 sampled by 1) determining fixed sampling-points along fishpond margins (n=4, A-D); and
174 2) sampling by dipper at 10 sampling-points along each margin. The 10 dips were evenly

175 distributed according to the length of each margin. Dippers were standard: 10 cm in
176 diameter, with a volume of 350 ml and a 1.5 m long handle, and white in color for better
177 visibility of immatures [28]. Larval specimens were placed in 50 ml microtubes labeled
178 according to sampling date, site, fishpond number and sampling-point margin letter (A-D)
179 and number (1-10). All material was fixed in the field in 80% ethanol. Presence of aquatic
180 fauna collected in the dippers were also recorded (i.e., *Culex* sp., amphibians, fish).

181 Adult collections were performed at each of the three sites in February, May, and
182 September 2017. We used human landing catch (HLC), performed only by the
183 professionally trained authors (two people indoors and two peridomestic simultaneously,
184 rotating every two hours at each spot), using manual aspirators to capture mosquitoes, for
185 12 h /night (18:00-06:00). Collected mosquitoes were separated by date, location, and hour
186 of capture. In months and sites with low mosquito density, we sampled one additional night
187 (12h) and adjusted later for analysis. In February, there were two night collections at Site 1,
188 and one in Sites 2 and 3, respectively. In May and September, two collections were done at
189 Site 2, and one in Sites 1 and 3. Mosquitoes were stored in silica gel in microtubes (50 ml)
190 identified with a code that included: month, site, date, and hour of collection. On rainy
191 nights, adult captures were suspended and conducted on the following non-rainy night.

192 Field-collected specimens were identified at the Laboratory of Infectious Diseases of
193 the Federal University of Acre (UFAC - campus Cruzeiro do Sul, Acre state) and at the
194 School of Public Health of the University of São Paulo (USP - campus São Paulo, São
195 Paulo state). Adults and the larval stages L2–L4 were identified using a stereomicroscope
196 and entomological keys (Forattini, 2002). Because of the challenge to identify L1
197 morphologically [29], three larval groups were defined: Anophelinae L1 stage;

198 Anophelinae L2–L4 stages and *Ny. darlingi* L2–L4 stages: in this approach, Anophelinae
199 L2–L4 group included no *Ny. darlingi* species. After morphological identification, adults
200 and larvae were sent to the Biotechnological Institute of University of State of São Paulo
201 (UNESP - Campus Botucatu, São Paulo State) for further molecular analysis.

202

203 **Environmental Variables**

204 Fishponds were classified and measured according to environmental and physical-
205 chemical conditions. For the environment, categorical variables included periodicity
206 (permanent or temporary during the 6-month study period); abandoned fishpond- no
207 maintenance by the owner (yes or no); associated vegetation on the margins of fishpond (if
208 present: emerging, submerged, floating); the presence of *Culex* sp., amphibians, and fish.
209 For periodicity and abandoned by the owner, classification was at the fishpond level; for
210 vegetation and presence of other animals, classification was at the sampling-point level.
211 Physical-chemical variables included pH, temperature, and conductivity, measured using an
212 ExTECH multiparameter (extech.com/) probe that presented continuous values. However,
213 due to functionality limitations, data from this device were collected only in the first three
214 months (February, March, April). For the remaining three months (May, August,
215 September), pH, nitrates (mg/L), nitrites (mg/L), carbonate hardness (KH) and dissolved
216 chlorine (mg/L) were collected using a JBL ProScan kit (jbl.de/en), by immersion of a test
217 strip in the water and reading by smartphone app downloaded at Google Play Store
218 (play.google.com/store/apps/details?id=de.jbl.proscan). The data collected using the JBL
219 Proscan kit had a more limited range, i.e., categorical variables. Collections using the

220 ExTECH multiparameter probe were obtained at the sampling-point level; for the JBL
221 Proscan test kit, data were obtained at the fishpond level. Turbidity and shading were also
222 obtained only during the last three months of the survey (May, August, September). Water
223 turbidity was determined at the fishpond level using a LaMotte (lamotte.com) water column
224 test kit, with discrete values ranging between 0 - 200 JTU (where 0 represents translucent
225 water), at the fishpond level. Shading by canopy was collected at the sampling-point level
226 with a TerraGes spherical densitometer according to the manufacturer's specifications
227 (terrages.pt), with continuous values ranging between 0 – 24.96 1/4"-squares (where 0
228 represents shaded and 24.96 represents completely exposed), at the sampling-point level.
229 This information is summarized in S1 Table.

230 Monthly precipitation data were obtained from the CPTEC/INPE website
231 (clima1.cptec.inpe.br/). Adverse weather/air conditions (rain, mist, wind, smoke from
232 burning) were noted when they occurred during the adult night collections. Field
233 information was digitally stored through Open Data Kit (ODK). Data were compiled in
234 EXCEL (Microsoft). Visual resources (photographs) were also obtained from each
235 sampling-point, by ODK function. Georeferencing of the residences and fishponds was
236 conducted using GPS Garmin device and Google Earth Pro TM software.

237

238 **Data Setting and Statistical Analysis**

239 Statistical analyses were conducted to establish the association between larval
240 groups, environment, and physical-chemical variables, using multilevel regression models.

241 The seasonal pattern, according to rainfall trends of western Amazon Basin (Rainy Season:
242 Oct-Feb; Dry Season: Apr-Sept, see S1 Fig), was analyzed, considering the repeated
243 measures framework used for larval sampling: the month of the collection was assumed to
244 be a variable factor, with February being the chosen reference baseline according to rainfall
245 seasonality effect on Culicidae biology abundance [29]. Therefore we chose February to
246 represent a rainy month; September to represent a dry month; and the interval between
247 February and September as the rainy-dry transition (S1 Fig). Larval counts of three groups
248 (Anophelinae L1; Anophelinae L2 – L4; *Ny. darlingi* L2 - L4) were considered the
249 outcome variables.

250 Overdispersion was observed in data distribution resulting from large numbers of
251 zero values, thus a binomial negative regression analysis was used [30]. According to
252 assumptions of a negative binomial distribution, and the respective nature of dependent
253 variables, regression coefficients are presented as incidence rate ratios (IRRs), defined by
254 the number of events (Anophelinae counts) by fishpond (analysis unit) [31]. For all tests,
255 the statistical significance level assumed was 0.05. An initial univariate regression was
256 performed to verify any associations between single independent variables. Considering the
257 non-randomized approach, multivariate regression was performed to verify adjustments in
258 the coefficients. A cut-off value for p of less than 0.2 of univariate analysis was chosen, and
259 the order of insertion of the independent variable in the multivariate regression was from
260 the lowest to the highest p -value considering the univariate analysis [32]. Multicollinearity
261 was assessed for the following independent variables used in multivariate analysis, since
262 they were measured at the same sampling level: linear correlation for numerical variables

263 (continuous physical-chemical) and Spearman rank correlation coefficient for ordinal
264 variables (categorical physical-chemical).

265 Considering the hierarchical data structure (samplings-points nested within
266 fishponds), a mixed-effects model was conducted, mainly due to its flexibility in repeated
267 measures modeling of unbalanced data [33]. The dataset was structured in a long format,
268 with the *i*th row functioning as a time-point per specific sampling-point, and respective
269 fishpond (the subject of the analysis) [34]. Considering the biology of Anophelinae the
270 three study sites were not considered independent, the usual procedure for mixed models
271 that simulates repeated measures ANOVA, due to geographic proximity between sites
272 (mainly Sites 1 and 2) [35]. In addition to the overall regression, to distinguish effects
273 among sites, regressions were performed for each site. An unconditional model was built
274 first, followed by a model with a random component to indicate the subject of the repeated
275 term. A two-level model was chosen, combining samplings-points at the first level and
276 fishponds at the second level as the random component, according to the data structure
277 (samplings-points nested in fishponds). Due to some gaps in variables measured during the
278 monthly survey, a full dataset was the primary design (six-months), using the respective
279 independent environmental variables: periodicity, abandoned, associated vegetation,
280 presence of animals, and collection month. For physical-chemical variables, the turbidity of
281 water and shading, which were not possible to measure during the whole six-month survey,
282 three-month datasets were designed according to independent variables: pH, temperature
283 and conductivity (continuous data) in February, March, and April; shading, turbidity and
284 pH, nitrates, nitrites, carbonated hardness and dissolved chlorine (ordinal data), measured
285 in May, August, and September. For three-month physical-chemical datasets, individual

286 regressions were not performed for each site, due to reduced sampling effort. This
287 information is summarized in S1 Table.

288 For comparison of categorical variables between sites, as well as the hypothesized
289 adult abundance difference between indoor and outdoor, a Chi-square test was used.
290 Outliers and systematic errors were verified through box-plot graphs. All statistical
291 analyses were performed using Stata 14.2 (data analysis and statistical software - StataCorp
292 LP, College Station, TX, USA). A robust option for the variance component estimators
293 (VCE) was chosen according to the Stata configuration.

294

295 **RESULTS**

296 **Sampling sites**

297 Sixty-three fishponds in the three sites were identified and followed during the 2017
298 study period. Total numbers of fishponds monitored throughout the field survey was
299 variable because of seasonal precipitation or occasionally being emptied by owners, and
300 some fishponds could not be reached across flooded fields at some point during the
301 sampling period. Fig 2 shows satellite images for the three sites with each nearby residence
302 and the fishponds surveyed; number of fishponds sampled by period, along with dry
303 conditions and other characteristics is presented in S2 Table. S3 Table shows environment
304 variables by site: both urban Sites 1 (~66%, 92/139) and 2 (~88%, 126/143) show a higher
305 number of not abandoned fishponds compared with rural Site 3 (~22%, 12/64) ($p < 0.001$).
306 No significant difference in fishpond periodicity was identified between three sites

307 ($p=0.625$); all sites had high numbers of permanent fishponds: (Site 1: ~86%, 120/139; Site
308 2: 85% 122/143; Site 3: ~84%, 46/55).

309

310 **Fig 2: Satellite image of three study sites, with respective residence marked by**
311 **pushpin and fishponds by flags.** Note, using the virtual perimeters of each residence-
312 index, the different scales and fishpond distributions at each site. (Source: ©2017 Google-
313 Images ©2017 TerraMetrics, Nasa, Cartographical data©2017 Maplink).

314

315 Larval Collection

316 During the six-month sampling period of 2017, 10,859 larvae were collected:
317 $n=5,512$ corresponded to the group of Anophelinae L1 stage species; $n=4,927$ to the group
318 of Anophelinae L2–L4 stage species; and $n=420$ to the group of *Ny. darlingi* L2–L4 stages.
319 Urban Site 1 shows the highest number of larvae ($n=6,065$), followed by rural Site 3
320 ($n=3,017$) and urban Site 2 ($n=1,777$). Rural Site 3 had the highest density of larvae per
321 fishpond (54.85), followed by urban Sites 1 (43.63) and 2 (12.46). For the *Ny. darlingi* L2–
322 L4 group, urban Site 1 had the highest total number (249) and density per breeding site
323 (1.80); urban Site 2 had 97 and 0.68, respectively; rural Site 3 had 74 and 1.32,
324 respectively.

325 Anophelinae species and *Ny. darlingi* (both in L2–L4 stages) distributed by
326 fishpond, site, and period are shown in Fig 3. The higher proportion of non-*Ny. darlingi*
327 Anophelinae species compared with *Ny. darlingi* in practically all fishponds during the

328 rainy season from February through May (except for Fishpond number 07, Site 1, in
329 February) is noteworthy. The exclusive presence of *Ny. darlingi* was observed in some
330 fishponds at urban Site 2, however, these count values were minimal (1 or 2 specimens).
331 For the dry season (August and September), urban Site 1 had more fishponds that were
332 positive exclusively for *Ny. darlingi*: 01 in August; 01, 04, 08, 11 18, 25, and 26 in
333 September. Urban Site 2 also had some fishponds with *Ny. darlingi* exclusively, but also
334 with low counts. Rural Site 3 had *Ny. darlingi* in August and September in fishponds 54, 55
335 and 58 where this species was not observed during the rainy season months.

336

337 **Fig 3. Summary of larvae collected in the study. Anophelinae spp. and *Ny.***
338 ***darlingi* (both L2 to L4 stages), distributed by fishpond, site and period.**

339

340 **Statistical Analysis**

341 Table 1 shows the IRR coefficient results for the six-month dataset using univariate
342 analysis, by identified Anophelinae larval group. Table 2 shows the multivariate analysis
343 results, according to the selection criteria for independent variables. The Spearman rank
344 coefficient detected no correlation between independent variables of the six-month dataset,
345 with values lower than 0.1, except for the presence of *Culex* sp. and amphibians (0.34), and
346 submerged aquatic vegetation with floating vegetation (0.23).

347 **Table 1: Incidence Rate Ratio (IRR), 95% Confidence Interval and *p* values for**
 348 **Anophelinae identity-group, for six month survey univariate two level negative**
 349 **binomial regression:**

Anophelinae identity-group	Independent Variable		Univariate Two-level Negative Binomial							
			Overall		Site 1		Site 2		Site 3	
			IRR (95% C.I.)	<i>p</i>	IRR (95% C.I.)	<i>p</i>	IRR (95% C.I.)	<i>p</i>	IRR (95% C.I.)	<i>p</i>
Anophelinae species (L1)	Month Collection	February	1		1		1		1	
		March	1.23 (.73-1.92)	0.373	1.19(.64-2.23)	0.572	.87 (.23-3.26)	0.839	1.19(.61-2.30)	0.613
		April	1.29 (.73-2.25)	0.374	1.16(.58-2.33)	0.658	3.75(.90-15.60)	0.069	.39 (.18-.82)	0.013
		May	.94 (.55-1.61)	0.842	1.07(.56-2.06)	0.822	1.05(.28-3.89)	0.940	.35 (.13-.81)	0.016
		August	.56 (.24-1.28)	0.175	.13 (.04-.35)	0.000	4.21(1.27-13.99)	0.019	.64 (.19-2.20)	0.487
		September	.63 (.29-1.34)	0.256	.25 (.09-.64)	0.004	1.99 (.63 - 6.29)	0.239	1.21(.38-3.85)	0.742
	Periodicity	Temporary	1		1		1		1	
		Permanent	2.19 (.55-8.70)	0.262	1.32 (.45-3.87)	0.608	5.01 (.73-34.07)	0.100	2.12(1.20-3.75)	0.009
	Abandoned	Yes	1		1		1		1	
		No	.37 (.13-1.02)	0.056	1.63 (.70-3.77)	0.249	2.49 (.26-23.44)	0.423	1.08 (.32-3.59)	0.900
	Associated Vegetation	Emerging	1.86(1.07-3.25)	0.021	1.05 (.43-2.59)	0.906	3.98(1.25-12.68)	0.020	1.45 (.93-2.28)	0.097
		Submerged	.74 (.39-1.38)	0.343	.48 (.20-1.14)	0.101	.83 (.39-1.75)	0.629	.64 (.16-2.47)	0.521

		Floatin g	1.06 (.60- 1.89)	0.8 74	.73 (.31- 1.70)	0.4 73	1.83 (.84- 4.01)	0.1 29	.82 (.46- 1.47)	0.5 11	
	Presenc e	<i>Culex</i> sp.	1.73(1. 16- 2.57)	0.0 07	1.69(1. 04- 2.74)	0.0 32	1.79 (.64- 5.05)	0.2 64	1.84 (.9- 3.78)	0.0 94	
		Amphi bian	1.53 (.96- 2.44)	0.0 73	1.32 (.86- 2.03)	0.1 93	5.78 (.56- 59.4)	0.1 40	1.34 (.59- 3.01)	0.4 74	
		Fish	3.34(1. 58- 7.04)	0.0 02	5.55(2. 19- 14.09)	0.0 00	.45 (.072- 2.84)	0.3 98	2.09 (.72- 6.06)	0.1 75	
		Month Collecti on	Februar y	1	1	1	1	1	1		
Anopheli nae species (L2, L3, L4)		March	1.12 (.69- 1.79)	0.6 49	1.30(6 77- 2.51)	0.4 26	1.12 (.44- 2.86)	0.8 07	.77 (.38- 1.54)	0.4 57	
		April	1.17 (.69- 2.01)	0.5 50	1.20 (.66- 2.18)	0.5 43	4.65(1. 11- 19.46)	0.0 35	.30 (.17- .54)	0.0 00	
		May	1.07 (.63- 1.82)	0.7 96	1.25 (.64- 2.46)	0.5 12	1.93 (.72- 5.21)	0.1 93	.33(.15 -.73)	0.0 07	
		August	.85 (.38- 1.92)	0.7 09	.28 (.08- .87)	0.0 28	3.51(1. 22- 10.18)	0.0 20	1.09(.3 3- 3.67)	0.8 78	
		Septem ber	.87 (.41- 1.84)	0.7 17	.49 (.16- 1.56)	0.2 32	2.28 (.72- 7.25)	0.1 61	1.11 (.45- 2.95)	0.8 35	
		Periodi city	Tempo rary	1	1	1	1	1	1		
			Perman ent	2.88 (.54- 15.31)	0.2 14	1.24 (.21- 7.33)	0.8 05	13.9(1. 54- 125.35)	0.0 19	1.69 (.82- 3.5)	0.1 50
		Abando ned	Yes	1	1	1	1	1	1		
			No	.44 (.15- 1.26)	0.1 29	2.34 (.83- 6.64)	0.1 07	1.82 (.18- 18.42)	0.6 10	1.15 (.35- 3.77)	0.8 11
		Associa ted Vegetat ion	Emergi ng	1.82(1. 01- 3.31)	0.0 50	.95 (.43- 2.07)	0.9 02	3.71(1. 06- 13.04)	0.0 40	1.89 (.68- 5.19)	0.2 17
			Submer ged	.55 (.27- 1.12)	0.0 98	.29 (.13- .68)	0.0 04	.56 (.22- 1.45)	0.2 35	.98 (.21- 4.60)	0.9 85

		Floatin g	1.41 (.78- 2.53)	0.2 52	1.37 (.61- 3.10)	0.4 45	1.77 (.65- 4.86)	0.2 64	.73 (.64- .83)	0.0 00	
	Presenc e	<i>Culex</i> sp.	1.78(1. 16- 2.74)	0.0 09	1.86(1. 09- 3.19)	0.0 23	1.06 (.32- 3.57)	0.9 17	2.2 (1.1- 4.39)	0.0 25	
		Amphi bian	1.37 (.74- 2.52)	0.3 13	1.11 (.55- 2.23)	0.7 62	5.44 (.87- 33.83)	0.0 69	1.29 (.46- 3.65)	0.6 20	
		Fish	2.92(1. 23- 6.93)	0.0 15	2.84(1. 08- 7.51)	0.0 35	3.92 (.33- 46.71)	0.2 79	4.18 (.54- 32.11)	0.1 69	
			Februar y	1	1	1	1	1	1		
<i>Ny. darling</i> (L2, L3, L4)	Month Collecti on	March	.74 (.34- 1.60)	0.4 49	.55 (.18- 1.65)	0.2 94	.94 (.22- 3.93)	0.9 28	1.31 (.35- 4.93)	0.6 83	
		April	1.01(.4 8- 2.11)	0.9 84	.89 (.32- 2.45)	0.8 31	1.82(.2 7- 12.35)	0.5 38	.21 (.02- 2.26)	0.1 94	
		May	.81 (.41- 1.57)	0.5 32	.74 (.30- 1.81)	0.5 12	.56 (.11- 2.79)	0.4 80	1.03 (.14- 7.58)	0.9 71	
		August	1.39 (.57- 3.41)	0.4 65	.39 (.09- 1.58)	0.1 90	3.01 (.75- 11.94)	0.1 18	5.80(1. 08- 30.94)	0.0 39	
		Septem ber	1.55(.6 8- 3.54)	0.2 91	.91 (.28- 2.88)	0.8 79	1.61(.4 2- 6.09)	0.4 81	6.25(1. 19- 32.76)	0.0 30	
			Tempo rary	1	1	1	1	1	1	1	
		Perman ent	1.21 (.38- 3.77)	0.7 42	.86 (.22- 3.36)	0.8 35	5.89 (.46- 75.12)	0.1 72	.654 (.18- 2.27)	0.5 04	
		Abando ned	Yes	1	1	1	1	1	1		
	No		1.81 (.66- 4.95)	0.2 43	10.27(2.7- 39.07)	0.0 01	1.00 (.21- 4.87)	0.9 96	.91 (.19- 4.27)	0.9 06	
		Associa ted Vegetat ion	Emergi ng	2.08(1. 25- 3.46)	0.0 05	1.61 (.94- 2.76)	0.0 79	3.07 (.96- 9.78)	0.0 58	2.08 (.49- 8.72)	0.3 18
			Submer ged	1.45 (.68- 3.07)	0.3 34	1.27(.5 0- 3.22)	0.6 11	.79 (.26- 2.42)	0.6 92	2.77 (.43- 17.63)	0.2 79

		Floatin g	1.37 (.73- 2.57)	0.3 20	1.62 (.73- 3.60)	0.2 32	1.48 (.43- 5.0)	0.5 27	*	0.0 00
	Presenc e	<i>Culex</i> sp.	1.23 (.73- 2.07)	0.4 33	1.27 (.72- 2.25)	0.4 07	2.2 (.55- 8.78)	0.2 61	.57 (.21- 1.55)	0.2 74
		Amphi bian	1.32 (.63- 2.76)	0.4 55	1.05 (.53- 2.08)	0.8 88	6.53(2. 34- 18.21)	0.0 00	.88 (.065- 11.78)	0.9 24
		Fish	1.24 (.6- 2.57)	0.5 58	1.55 (.64- 3.73)	0.3 28	.71 (.11- 4.85)	0.7 23	1.48 (.22- 9.81)	0.6 83

350 Statistically significant values at the 0.05 level are highlighted

351 *IRR value omitted due low decimal number (10^{-10}).

352

353 **Table 2: Incidence Rate Ratio (IRR), 95% Confidence Interval and *p* values for**
 354 **Anophelinae species identity-group, for six-month survey multivariate two level**
 355 **negative binomial regression:**

Anophelin ae identity- group	Independent Variable		Multivariate Two-level Negative Binomial					
			Site 1		Site 2		Site 3	
			IRR (95% C.I)	<i>p</i>	IRR (95% C.I)	<i>p</i>	IRR (95% C.I)	<i>p</i>
Anophelina e species (L1)	Month Collectio n	February	1		1		1	
		March	1.14 (.56- 2.31)	0.72 1	.89 (.23- 3.50)	0.87 0	1.21(.62- 2.37)	0.57 9
		April	1.30 (.52- 3.24)	0.56 8	2.30(.57- 9.28)	0.24 2	.33 (.16- .69)	0.00 4
		May	1.31 (.56- 3.04)	0.53 4	1.07(.29- 3.92)	0.91 6	.25 (.13- .50)	0.00 0
		August	.21 (.07- .56)	0.00 3	3.70(1.0 5-13.03)	0.04 1	.54 (.16- 1.79)	0.31 7
		Septembe r	.31 (.10- .91)	0.03 3	1.19 (.33- 4.22)	0.78 9	1.19(.31- 4.55)	0.79 7

	Periodicity	Temporary			1		1		
		Permanent			2.98 (.53-16.77)	0.21 4	1.98 (.88-4.45)	0.09 6	
	Associated Vegetation	Emerging			5.27 (.91-30.46)	0.06 3	2.79(1.51-5.17)	0.00 1	
		Submerged	.67 (.38-1.20)	0.18 2					
		Floating			.62 (.14-2.73)	0.53 1			
	Presence	<i>Culex</i> sp.	1.50 (.99-2.27)	0.05 6			1.07 (.66-1.74)	0.77 3	
		Amphibian	.97 (.55-1.73)	0.93 2	6.10(1.13-32.94)	0.03 6			
		Fish	1.94 (.72-5.23)	0.19 2			2.03 (.76-5.39)	0.15 7	
Anophelinae species (L2, L3, L4)	Month Collection	February	1		1		1		
		March	1.43 (.76-2.67)	0.26 8	1.09 (.42-2.80)	0.86 4	.81 (.37-1.76)	0.59 3	
		April	2.00 (1.11-3.59)	0.02 0	2.56 (.73-8.99)	0.14 1	.46 (.17-1.25)	0.12 9	
		May	2.48 (1.29-4.76)	0.00 6	1.64 (.58-4.62)	0.35 1	.46 (.15-1.41)	0.17 5	
		August	.55 (.18-1.68)	0.29 3	2.74 (.95-7.88)	0.06 2	1.71 (.55-5.33)	0.35 3	
		September	.81 (.26-2.50)	0.72 1	1.46 (.31-6.77)	0.62 8	2.40 (.60-9.58)	0.21 4	
	Periodicity	Temporary				1		1	
		Permanent				9.01(1.36-59.77)	0.02 3	1.12 (.61-	0.72 1

							2.06)	
	Abandoned	Yes	1					
		No	1.65 (.76-3.56)	0.20 1				
	Associated Vegetation	Emerging			3.04(1.14-8.15)	0.02 7	66 (.22-1.96)	0.45 4
		Submerged	.34 (.19-.58)	0.00 0				
	Presence	<i>Culex</i> sp.	2.24 (1.36-3.69)	0.00 1			1.96 (.82-4.72)	0.13 1
		Amphibian			6.47(1.89-22.14)	0.00 3		
		Fish	1.12 (.43-2.86)	0.81 9			6.14 (.86-43.76)	0.07 0
<i>Ny. darlingi</i> (L2, L3, L4)	Month Collection	February	1		1		1	
		March	.65 (.21-1.98)	0.45 2	1.11 (.22-5.54)	0.89 7	1.31 (.35-4.86)	0.68 1
		April	1.01 (.36-2.81)	0.98 9	1.36 (.20-9.14)	0.75 3	.21 (.02-2.31)	0.20 4
		May	.66 (.26-1.64)	0.37 1	.83 (.16-4.36)	0.82 5	1.04 (.14-7.55)	0.96 7
		August	.48 (.11-2.00)	0.31 4	2.71 (.57-12.88)	0.20 9	5.80(1.11-30.41)	0.03 7
		September	1.11 (.34-3.65)	0.86 4	1.32 (.31-5.67)	0.70 5	6.62(1.29-33.89)	0.02 3
	Periodicity	Temporary			1			
	Permanent			3.84 (.55-26.57)	0.17 3			
	Abandoned	Yes	1					

	No	11.40 (3.06-42.52)	0.00 0				
Associated Vegetation	Emerging	2.12 (.83-5.45)	0.11 7	2.23 (.79-6.23)	0.12 6		
	Submerged					*	0.00 0
Presence	Amphibian			5.86(2.42-14.156)	0.00 0		

356 Statistically significant values at the 0.05 level are highlighted

357 *IRR value omitted due low decimal number (10^{-10}).

358

359

360 Considering the February baseline value and a statistical significance at 95% C.I.,
 361 seasonality differences were not detected in the overall regression for the three identified
 362 Anophelinae larval groups. For each site, Anophelinae L1 shows a particular pattern in the
 363 univariate regression: a decrease in counts for August and September in urban Site 1, and
 364 April and May in rural Site 3; and an increase in counts for urban Site 2 in August. The
 365 IRR values observed in the multivariate analysis were maintained relative to the univariate
 366 IRR values, indicating that these results were not influenced by possible confounding
 367 factors. The Anophelinae L2 - L4 group also shows a unique pattern for each site, however,
 368 in this case the IRR values were substantially different between the univariate and
 369 multivariate analysis. For the *Ny. darlingi* L2 - L4 group, there was a similar pattern in both
 370 urban sites (1 and 2), with no significant statistical difference in monthly larval numbers for
 371 the baseline value (February). In rural Site 3, an increase was observed for August [5.8
 372 (95% C.I.:1.11-30.41)] and for September [6.62 (95% C.I.:1.29-33.89)]. Multivariate and
 373 univariate regression showed comparable IRR values in the three sites. For periodicity and

374 abandoned characteristics, permanent condition was significant for Anophelinae L1 group
375 in rural Site 3, for univariate analysis only for the Anophelinae L2 - L4 group, whereas
376 urban Site 2 shows an increase in larval number for both univariate and multivariate
377 regression. The non-abandoned condition for *Ny. darlingi* shows an increase in larval
378 number in urban Site 1, for both univariate and multivariate regression. Emerging
379 associated vegetation shows an increase of larval number in the overall regression for all
380 three Anophelinae groups. Presence of *Culex* sp. and fish was significant for Anophelinae
381 L1 and L2, L3 and L4, in the overall regression. Urban Site 1 showed a similar association
382 for both groups, however only the Anophelinae L2 - L4 group maintains this value in
383 multivariate analysis. The presence of amphibians was positively associated with
384 Anophelinae L1 and L2- L4 groups in urban Site 2 only in the multivariate regression. The
385 *Ny. darlingi* group showed a positive association with amphibian presence only in urban
386 Site 2, for both univariate and multivariate regressions.

387 Table 3 shows IRR coefficient results for a three-month dataset using univariate
388 analysis, by identified Anophelinae larval group. Table 4 shows multivariate analysis,
389 according to the selection criteria for independent variables. The Spearman rank test shows
390 a high correlation between the categorical physical-chemical variables nitrates and nitrites
391 ($r=0.89$), and a low correlation between carbonate hardness and pH ($r=0.38$), and carbonate
392 hardness and dissolved chlorine ($r=0.4$). Physical-chemistry variables for continuous values
393 (pH, temperature, and conductivity) were not statistically associated at 95% C.I. with the
394 abundance of any of the three larval groups. Turbidity shows a significant negative
395 association for the Anophelinae L1 only in the univariate regression [0.98 (IC95%:.97-
396 .99)]. *Ny. darlingi* L2 - L4 shows a significant positive association with turbidity in

397 multivariate analysis only [1.01 (IC95%:1.00-1.01), $p=0.045$]. Shading reduction shows a
 398 significant negative association with the abundance of both Anophelinae L1, and
 399 Anophelinae L2 - L4 in both univariate and multivariate regressions, but for the *Ny.*
 400 *darlingi* group, the univariate was not significant at the 0.05 level, however, it was near the
 401 limit [0.96 (IC95%:.93-1.00) with $p=0.052$], whereas in the multivariate analysis shading
 402 was significant [0.95 (IC95%:.92-.99), $p=0.02$].

403

404 **Table 3: Incidence Rate Ratio (IRR), 95% Confidence Interval and p values for**
 405 **Anophelinae species identity-group, for three-month survey univariate two level**
 406 **negative binomial regression:**

Independent Variable		Univariate Two-level Negative Binomial					
		Anophelinae species (L1)		Anophelinae species (L2, L3, L4)		<i>Ny. darlingi</i> (L2, L3, L4)	
		IRR (95% C.I.)	<i>p</i>	IRR (95% C.I.)	<i>p</i>	IRR(95% C.I.)	<i>p</i>
Physical-Chemistry (continual values)	pH	1.02 (.78-1.33)	0.901	.91 (.69-1.21)	0.521	1.05(.72-1.54)	0.779
	Temperature	.92 (.81-1.06)	0.259	.95 (.82-1.1)	0.505	.87 (.71-1.06)	0.165
	Conductibility	1.00 (.99-1.01)	0.432	1.01 (.99-1.07)	0.297	1.02 (.99-1.009)	0.488
Turbidity (discrete value)		.98 (.97-.99)	0.050	.98 (.97-1.00)	0.120	1.01 (.99 - 1.02)	0.100
Shading (continual value)		.95 (.91-.99)	0.008	.96 (.94-	0.035	.96 (.93-1.00)	0.052

					.99)			
Physical- Chemistry (categorical values)	pH	>6	1		1		1	
		6.4	.14 (.05-.39)	0.000	.06 (.03-.12)	0.000	.87 (.43-1.76)	0.695
		6.6	.26 (.03-2.36)	0.231	.19 (.01-3.02)	0.237	1.97 (.61-6.5)	0.261
		6.8	.79 (.20-3.15)	0.747	.14 (.01-3.06)	0.210	.87 (.13-5.79)	0.884
		7	.31 (.04-2.20)	0.245	.10 (.02-.46)	0.003	*	0.000
		7.2	*	0.000	*	0.000	*	0.000
		7.6	*	0.000	*	0.000	*	0.000
	nitrates (mg/L)	0	1		1		1	
		10	1.53 (.51-4.64)	0.449	1.39 (.51-3.83)	0.519	.91 (.38-2.19)	0.839
		25	1.39 (.22-7.84)	0.705	1.36 (.23-7.95)	0.734	.72 (.15-3.59)	0.688
		40	.22 (.16-.32)	0.000	.06 (.04-.09)	0.000	*	0.000
	nitrites (mg/L)	0	1		1		1	
		0.25	1.05 (.44-2.49)	0.917	.84 (.40-1.77)	0.651	.96 (.42-2.21)	0.934
		0.5	1.54 (.16-14.64)	0.707	1.27 (.09-16.31)	0.854	.47 (.04-5.57)	0.547
	carbonated hardness (KH)	0	1		1		1	
		1.5	1.90 (.98-3.68)	0.056	2.05 (1.01-4.19)	0.049	.89 (.60-1.34)	0.604

		3	.71 (.25-1.99)	0.515	93 (.22-3.96)	0.921	.24 (.04-1.28)	0.094
		4.5	.17 (.06-.47)	0.001	*	0.000	.30 (.08-1.2)	0.089
		6	.10 (.04-.27)	0.000	*	0.000	.56 (.14-2.26)	0.415
		8	.13 (.06-.26)	0.000	.33 (.14-.79)	0.013	.82 (.19-3.49)	0.791
		15	3.49(1.69-7.17)	0.001	.23 (.12-.43)	0.000	2.08(.93-4.62)	0.073
	dissolved chlorine (mg/L)	0	1		1		1	
		0.8	.94 (.26-3.39)	0.922	.66 (.15-2.99)	0.588	.82 (.27-2.51)	0.730
		1.5	2.04 (.94-4.42)	0.070	.72 (.28-1.88)	0.506	2.21(1.17-4.19)	0.015

407 Statistically significant values at the 0.05 level are highlighted

408 *IRR value omitted due low decimal number (10^{-10}).

409

410

411 **Table 4. Incidence Rate Ratio (IRR), 95% Confidence Interval and p values for**

412 **Anophelinae species identify-group, for three-month survey multivariate two level**

413 **negative binomial regression:**

Independent Variable	Multivariate Two-level Negative Binomial					
	Anophelinae species (L1)		Anophelinae species (L2, L3, L4)		<i>Ny. darlingi</i> (L2, L3, L4)	
	IRR (95% C.I.)	p	IRR (95% C.I.)	p	IRR(95% C.I.)	p

Turbidity (discrete value)			.99 (.98-1.00)	0.087	.99 (.98-1.01)	0.216	1.01(1.00-1.01)	0.045
Shading (continual value)			.95 (.92-.98)	0.003	.97 (.94-.99)	0.041	.95 (.92-.99)	0.020
Physical- Chemistry (categorical values)	pH	>6	1		1		1	
		6.4	.18 (.03-1.06)	0.059	.08 (.025-.24)	0.000	.28 (.06-1.37)	0.118
		6.6	.71 (.06-8.19)	0.782	.68 (.04-10.58)	0.781	.84 (.12-5.62)	0.856
		6.8	6.95 (.35-136.97)	0.202	4.73 (.49-45.68)	0.179	.53 (.04-7.78)	0.645
		7	.34 (.02-6.38)	0.473	.27 (.03-2.73)	0.268	*	0.000
		7.2	*	0.000	*	0.000	*	0.000
		7.6	*	0.000	*	0.000	*	0.000
	nitrates (mg/L)	0	1		1		1	
		10	1.13 (.43-2.93)	0.804	1.12 (.46-2.72)	0.796	.74 (.27-2.07)	0.574
		25	.79 (.06-9.86)	0.861	.91 (.15-5.43)	0.915	.74 (.08-7.16)	0.794
		40	.68 (.18-2.62)	0.577	.30 (.09-1.04)	0.057	*	0.000
	carbonated hardness (KH)	0	1		1		1	
		1.5	1.39 (.75-2.58)	0.287	1.38 (.78-2.44)	0.272	.85 (.57-1.26)	0.423
		3	.33 (.14-.75)	0.008	.39 (.18-1.21)	0.103	.24 (.09-.63)	0.003
		4.5	.01 (.01-.52)	0.020	*	0.000	.13 (.01-1.88)	0.135

		6	.03 (.02-.38)	0.006	*	0.000	.37 (.08-1.71)	0.203
		8	.15 (.05-.46)	0.001	.25 (.07-.86)	0.027	3.87 (.99-15.17)	0.052
		15	.04 (.02-.51)	0.013	*	0.000	.34 (.03-4.23)	0.401
	dissolved chlorine (mg/L)	0	1				1	
		0.8	1.67 (.63-4.37)	0.299			1.34 (.64-2.78)	0.435
		1.5	4.23 (1.58-11.36)	0.004			3.41(1.51-7.68)	0.003

414 Statistically significant values at the 0.05 level are highlighted

415 *IRR value omitted due low decimal number (10^{-10}).

416

417 For ordinal physical-chemistry variables, increased pH values were associated with
 418 decreased in larval counts in all three groups, for both univariate and multivariate
 419 regression. Similarly, the highest nitrate level (40 mg/L) was associated with decreased
 420 larval counts for all three larval groups, and this was maintained in multivariate analysis for
 421 the *Ny. darlingi* group. Nitrites were not significantly associated with larval counts, and
 422 excluded from the multivariate regression analyses. For carbonated hardness, whereas both
 423 Anophelinae L1 and L2, L3 and L4 groups show a highly significant negative association
 424 (except Anophelinae L1 at 15 KH in univariate analysis, not kept in the multivariate
 425 regression), the *Ny. darlingi* group did not show statistical significance for any range,
 426 except a decrease in larval numbers observed at a range of 3 KH in the multivariate analysis
 427 [0.24 (IC95%:.09-.63)]. Dissolved chlorine showed a significant positive association at a
 428 range of 1.5 mg/L for the Anophelinae L1 in multivariate analysis [4.23 (IC95%:1.58-

429 11.36)], and the *Ny. darlingi* for both univariate [2.21 (IC95%:1.17-4.19)] and multivariate
430 analysis [3.41 (IC95%:1.51-7.68)].

431

432 **Adult Collection**

433 A total of 692 Anophelinae specimens was collected and identified as *Ny. darlingi*.
434 Fig 4 shows HBR for each site, adjusted for two night captures depending on site (Site 1 in
435 February; Site 2 in May and September). There was a significant difference in the
436 proportion of indoor vs. outdoor *Ny. darlingi* among the 3 sites ($X^2=19.833$, $p<0.001$), with
437 a higher abundance in the peridomestic area. The proportion indoors was higher in Site 3
438 (~25%) than in Site 1 (~12%) or Site 2 (~11%). In Site 1, May showed a higher number of
439 *Ny. darlingi* in all night captures (21/173 indoor/outdoor) than February (3/13
440 indoor/outdoor) or September (5/29 indoor/outdoor). Site 2 showed the lowest adult
441 collections: February (3/10 indoor/outdoor), May (0/8 indoor/outdoor), September (0/7
442 indoor/outdoor). In Site 3, mosquito numbers were consistently high for outdoor
443 collections, and increased for indoor captures in the last two months: February (11/104,
444 indoor/outdoor), May (51/101 indoor/outdoor), September (42/97, indoor/outdoor).

445 Regarding HBR per hour, Site 1 shows more activity in May between 19:00-20:00
446 (HBR= 46), while February presents low numbers between 19:00-20:00 (HBR=4), as does
447 September (18:00-19:00=13). Site 2 also presents low numbers (with peaks reaching at
448 maximum of four mosquitoes/hr). In contrast, *Ny. darlingi* from Site 3 showed higher
449 outdoors peaks during the first part of the night (February, 19:00-20:00=22; May, 18:00-
450 19:00=24; September, 20:00-21:00=21), also demonstrating, besides low values, some

451 indoor peaks that exceeded outdoors ones, in May (00:00-01:00, indoor=12, outdoor=7;
452 02:00-03:00, indoor=7, outdoor=0) and September (for 00:00-01:00, indoor=10,
453 outdoor=7).

454

455 **Fig 4. Blood-feeding pattern by Human Biting Rate (HBR: *Ny. darlingi* per**
456 **human captures per hour), by night-capture, site and period.**

457

458 **Discussion**

459 For effective control of Amazon malaria transmission it is essential to recognize the
460 diverse eco-epidemiologic profiles of the disease in local areas: municipalities, cities,
461 districts, subdistricts, along with “off the grid” areas: mining, rubber extraction (*seringal*),
462 rural settlements and indigenous populations. For such heterogeneity, the design and the
463 application of specific control methodologies according to each eco-epidemiologic profile
464 is needed [36]. The Brazilian Amazonian Basin has a total area of five million km²
465 (corresponding to an estimated 60% of the federal territory), but only 15% of the human
466 population, most in big cities and state capitals [25]. This heterogeneous distribution is
467 reflected in local characteristics of vector biology, thus malaria epidemiology, following
468 human dynamics that drive Amazonian occupation [37]. Interdisciplinary methods for
469 disease intervention are common but rarely tailored to specific local conditions [38, 39].
470 For effective eradication at a global scale, many aspects of public health need to be
471 included, such epidemiological and syndromic surveillance, early diagnosis, clinical

472 treatment, environmental sanitation, and improved methods for economical land use to
473 reduce inequity and poverty [40].

474 Ours is the first study to conduct a detailed microgeographic spatiotemporal analysis of
475 larvae and adult Anophelinae, with a focus on the major vector, *Ny. darlingi*, in Vale do
476 Jurua, western Acre, characterized by high malaria transmission associated with urban and
477 periurban fishponds. In this area, we determined that *Ny. darlingi* larval dynamics was not
478 affected by seasonality in urban landscapes, similar to findings in previous studies in the
479 area [15,16]. This feature may help to maintain the population density of *Ny. darlingi*
480 during the transition of rainy to dry seasons. We detected other fishpond characteristics
481 associated with *Ny. darlingi* abundance: active fishponds, emergent vegetation (normally
482 secondary growth that has emerged from deforested areas), and shade. A particular
483 fishpond characteristic verified by the present study was the presence of *Ny. darlingi* larvae
484 in water with dissolved chlorine, suggesting possible resilience for chemical pollution [41],
485 although the increase of pH and nitrates was observed as a limiting factor. Adult collections
486 were conducted for a single night per study site per month and therefore our conclusions
487 are preliminary. Most *Ny. darlingi* were collected outdoors, during the first part of the night
488 (18:00-00:00), a pattern reported for this species in other Amazonian occupied areas [42-
489 46], although we observed a greater abundance (not significant) and peaks of indoor
490 activity in the rural landscape.

491 In Amazonian malaria transmission, the most common type of breeding site, whether
492 natural or artificial (or both), contributes substantially to the dynamics and seasonality of
493 malaria [26,47,48]. Two main variables of natural aquatic habitats that affect larval survival
494 are water flow intensity during the rainy season (larval mortality rate); and low water

495 capacity during the dry season (loss of available aquatic niche) [24,48]. These conditions
496 are generally neutralized in artificial aquatic habitats such as dams, micro dams, cisterns,
497 fishponds, and other types of flow-limited water bodies [23], increasing the vectorial
498 capacity of primary vectors, such as *Ny. darlingi* [49]. A successful breeding site in a
499 malaria-endemic region should provide geographic and temporal coexistence for the
500 epidemiologic triad: vector, etiological agent, and human reservoir, according to ecological
501 strategies of Anopheline species [50,51] as well *Plasmodium* sp. [52], facilitating
502 adaptation to host behavior [53]. The presence of the primary malaria vector in human
503 residences and adjacent fishponds in Mancio Lima suggests that transmission may occur
504 both in and around houses, although our HBR data demonstrate that most biting occurs
505 outdoors.

506 A lower proportion of *Ny. darlingi* larval specimens was identified in the present study
507 (8.5%) compared to that found in the same municipality in earlier studies (16.1% [15];
508 22.5% [54]), and in other distinctive local Amazonian environments [24,48]. However, *Ny.*
509 *darlingi* L1 larvae were not morphologically identified herein and this stage represented
510 more than 50% of the total numbers of larvae surveyed. Furthermore, similar to other
511 entomological studies in malaria-endemic areas, our adult survey detected only *Ny. darling*
512 [43-46], although we recognize that HLC can generate a bias due to the mainly
513 anthropophilic behavior of this species, as well for *Nyssorhynchus* sp. in general.
514 Biodiversity of Anophelinae can be an indicator of environmental disruption, a putative
515 signal of future outbreaks [55]. There is both a notably increased abundance and/or the
516 emergence of *Nyssorhynchus* species in human-colonized Amazonian areas [44,56], and
517 low natural abundance of this genus in primary Amazon forest [57,58]. *Nyssorhynchus*

518 *darlingi* is not always the dominant species in the *Nyssorhynchus* larval community that
519 emerges with anthropogenic change: for example, in Mâncio Lima, Acre state, it is *Ny.*
520 *albitarsis s.l.* [15]; in Labrea, Amazon state, *Ny. triannulatus* [24]; and in Pôrto Velho,
521 Rondônia state, *Ny. braziliensis* [59]. However, *Ny. darlingi* may be the species that best
522 adapts to human behavior in the Amazon region relative to vectorial capacity [49,60-62].

523 Our study was noteworthy for the micro-geographical analysis of larvae sampled,
524 measuring different characteristics of vector ecology at sampling-point and fishpond levels.
525 Anophelinae species L1 showed different behavior among the three sites: urban Site 1 had a
526 decrease in August and September; however there was an increase in August in urban Site
527 2; whereas for rural Site 3, there was a decrease in larval counts in April and May. These
528 results did not change in the multivariate analysis, in contrast with Anophelinae L2 - L4,
529 which present an inverse association in urban Site 1 after adjustment, indicating some
530 influential cofactor that was not measured by this study.

531 Nevertheless, the primary vector *Ny. darlingi* L2 - L4 group - identified to species level-
532 shows a singular pattern: no difference of larval numbers in fishponds detected in urban
533 Sites 1 and 2 during the rainy to dry season transition, in both univariate and multivariate
534 analysis. There was also no seasonal difference for *Ny. darlingi* in early study [15],
535 however, they incorporated a larger time frame (2 years) with larger intervals between
536 larval sampling efforts (5-6 months). Interestingly, in our Site 3 (rural), there was a
537 significant increase in larval numbers between the February baseline and both August and
538 September, months that correspond to the dry season. Possibly, fishponds play a more
539 important role in the maintenance of *Ny. darlingi* during the transition from rainy to dry

540 season in rural landscapes than our study demonstrates. Similar results were found in rural
541 settlements with the presence of artificial breeding-sites [10,47].

542 Seasonal malaria is common in the Amazon region, associated with *Ny. darlingi*
543 population density and rainfall patterns. In urban and suburban areas in Rondônia state
544 [45], malaria increased at the end of the dry season and the beginning of the next rainy
545 season in landscapes with mainly natural breeding-sites (riverside malaria); in contrast, in
546 landscape dominated by artificial breeding-sites (so-called dryland malaria), both malaria
547 and *Ny. darlingi* remain high throughout the year. Here, a simple linear correlation between
548 monthly precipitation and *P. vivax* notifications showed a positive association for rainfall
549 seasonality and malaria cases, mainly in 2016 (2016 $r = 0.75$; 2017 = 0.43), indicating
550 some seasonal effect on malaria numbers (S1 Fig). However, these monthly notifications
551 could have been more informative had they been adjusted for the appropriate landscape
552 profile (urban/rural). For *Ny. darlingi* larvae, a major sampling effort with more sites in
553 each landscape type in a multi-year survey is needed to confirm this seasonal pattern.

554 In urban Site 1, the increase of *Ny. darlingi* larvae in active fishponds, not detected for
555 Anophelinae L1 and L2 - L4 groups, supports the earlier study [15], demonstrating that
556 economically active fishponds are important larval habitats for primary vectors. Emerging
557 aquatic vegetation was strongly associated with all three Anophelinae groups in the overall
558 regression, reinforcing the recommendation by WHO [63], that cleaning the margins can be
559 an effective environmental control for Amazon *Nyssorhynchus* sp. The presence of *Culex*
560 sp. species and egg rafts was constant in the survey, suggesting they share the same
561 ecological niche as the Anophelinae L2 - L4 group. Most *Culex* sp. were identified as
562 subgenus *Melanoconion*, a group that contains species that are regional arbovirus vectors

563 [64]. Thus, fish farming may open larval habitats for other Culicidae species of
564 epidemiological importance. The presence of fish was common in the fishponds surveyed
565 (even abandoned ones), showing that Anophelinae larval species readily coexist with the
566 local fish community, or amphibians according to a microecological food web of aquatic
567 habitat [65]. Prospects for putative biological control seem unclear in this case unless exotic
568 larvivorous fish species were to be utilized, but they represent other risks for the local
569 environment and are not a feasible option [66].

570 Water turbidity was slightly associated with Anophelinae numbers, with *Ny. darlingi*
571 being found previously in turbid water [24]. We report a significant association with shaded
572 or low light environments for the three Anophelinae groups, a feature associated previously
573 with *Ny. darlingi* ecology [48]. High values of pH (>7) and nitrates (40 mg/L) appear to be
574 limiting factors for the Anophelinae aquatic habitat. Although carbonated hardness (an
575 alkalinity indicator), shows a similar pattern in the decrease of Anophelinae L1 and L2 - L4
576 groups, for *Ny. darlingi* there was no significant association. More surprisingly, the
577 increase in *Ny. darlingi* larvae in waters with dissolved chlorine suggested possible
578 tolerance of immatures to polluted aquatic habitats. This was also detected for the
579 Anophelinae L1, representing an important feature of opportunistic species that invade new
580 aquatic niches in human occupation without environmental sanitation, and may be linked to
581 phenotypic plasticity of ion regulation of Amazon mosquito Culicidae larvae under
582 different physical-chemical conditions [67].

583 Aside from the non-identification of L1 larvae, mentioned above, a second limitation of
584 this study was that we planned to measure the perimeter of each fishpond to test for an
585 association with larval abundance [10]. We initially measured each fishpond but, due the

586 high number of ponds (n=63), it was not realistic to accurately measure change in water
587 level in each one for each of the six months. Thirdly, there were some technical problems
588 with measuring instruments, resulting in gaps in some of the independent variables of the
589 survey, reducing sampling effort. Fourthly, we intensively sampled two urban sites but only
590 one rural one, mainly due to complex logistical issues. Finally, there is an important
591 relationship between households with malaria incidence and distance to breeding sites for
592 *Ny. darlingi* [48] and *P. vivax* infection [68] measurement of which was beyond the scope
593 of our study.

594 Nevertheless, our study does provide important information about temporal variation
595 and environment features of larvae of the primary vector *Ny. darlingi* at micro-spatial levels
596 (sampling points of fishponds), as well as *Ny. darlingi* adult profiles in nearby households.
597 Tailored LSM strategies accounting for this heterogeneity, such the use of biological
598 larvicides [69], need to be routinely incorporated in malaria integrated control to reduce
599 transmission in Mâncio Lima, and in other cities of Vale do Juruá region.

600

601 **Acknowledgements**

602 We thank Marcelo Urbano Ferreira and Pablo Secato Fontoura (Instituto de
603 Ciências Biomédicas - Universidade do São Paulo); Francis de Melo Santos (Gerente de
604 Endemias de Mâncio Lima); Rodrigo Medeiros de Souza (Laboratório de Doenças
605 Infeciosas na Amazônia Ocidental - Universidade Federal do Acre); Vera Lucia Carvalho
606 da Silva (Ministério da Saúde do Brasil); Reginaldo Grenzi da Silva (Biblioteca, FSP-
607 USP); and the residents of Mâncio Lima and Cruzeiro do Sul (AC) for their hospitality.

608

609 **References**

610

- 611 1. Ilacqua RC, Chaves LSM, Bergo ES, Conn JE, Sallum MAM, Laporta GZ. A
612 method for estimating the deforestation timeline in rural settlements in a
613 scenario of malaria transmission in frontier expansion in the Amazon Region.
614 Mem Inst Oswaldo Cruz. 2018; 113(9):e170522.
- 615 2. Tucker Lima JM, Vittor A, Rifai S, Valle D. Does deforestation promote or
616 inhibit malaria transmission in the Amazon? A systematic literature review and
617 critical appraisal of current evidence. 2017 Philos Trans R Soc Lond B Biol Sci.
618 2017; 5(372):172.
- 619 3. Hahn MB, Gangnon RE, Barcellos C, Asner GP, Patz JA. Influence of
620 deforestation, logging, and fire on malaria in the Brazilian Amazon. PLoS One.
621 2014; 9(1): e85725.
- 622 4. Pimenta PF, Orfano AS, Bahia AC, Duarte A., Ríos-Velásquez CM, Melo FF,
623 Pessoa FA, Oliveira GA, Campos KM, Villegas LM, Rodrigues NB, Nacif-
624 Pimenta R, Simões RC, Monteiro WM, Amino R, Traub-Cseko YM, Lima JB,
625 Barbosa MG, Lacerda MV. An overview of malaria transmission from the
626 perspective of Amazon *Anopheles* vectors. Mem Inst Oswaldo Cruz. 2015; 110
627 (1): 23–47.
- 628 5. Ministry of Health of Brazil, 2020, available at
629 [https://antigo.saude.gov.br/images/pdf/2019/novembro/20/Boletim-
630 epidemiologico-SVS-35.pdf](https://antigo.saude.gov.br/images/pdf/2019/novembro/20/Boletim-epidemiologico-SVS-35.pdf)
- 631 6. Oliveira-Ferreira J, Lacerda MVG, Brasil P, Ladislau JLB, Tauil PL. Daniel-
632 Ribeiro CT. Malaria in Brazil: an overview. Malar J. 2010; 9: 115.
- 633 7. Lederberg J. Infectious disease as an evolutionary paradigm. Emerg Infect Dis.
634 1997; 3(4): Special Issue.
- 635 8. Zhang S, Zhang L, Feng J, Yin J, Feng X, Xia X, Frutos R, Manguin S, Zhou S.
636 Malaria Elimination in the People’s Republic of China: Current Progress,
637 Challenges, and Prospects. Towards Malaria Elimination: A Leap Forward.
638 Chapter 10. 2018
- 639 9. Costa KM, de Almeida WA, Magalhaes IB, Montoya R, Moura MS, de Lacerda
640 MV, et al. Malaria in Cruzeiro do Sul (Western Brazilian Amazon): analysis of
641 the historical series from 1998 to 2008. Rev Panam Salud Publica. 2010; 28:
642 353–360
- 643 10. Vittor AY, Pan W, Gilman RH, Tielsch J, Glass G, Shields T, Sánchez-
644 Lozanom W, Pinedo VV, Salas-Cobos E, Flores S, Patz JA. Linking
645 deforestation to malaria in the Amazon: characterization of the breeding habitat

- 646 of the principal malaria vector, *Anopheles darlingi*. Am J Trop Med Hyg. 2009;
647 81 (1): 5–12.
- 648 11. Conde M, Pareja PX, Orjuela LI, et al. Larval habitat characteristics of the main
649 malaria vectors in the most endemic regions of Colombia: potential implications
650 for larval control. Malar J. 2015; 14:476. doi:10.1186/s12936-015-1002-y.
- 651 12. Buitrago LS, Brochero HL, McKeon SN, Lainhart W, Conn JE. First published
652 record of urban malaria in Puerto Gaitán, Meta, Colombia. Mem Inst Oswaldo
653 Cruz. 2013;108(8):1045-1050. doi:10.1590/0074-0276130217.
- 654 13. Oladepo O, Tona GO, Oshiname FO, Titiloye MA. Malaria knowledge and
655 agricultural practices that promote mosquito breeding in two rural farming
656 communities in Oyo State, Nigeria. Malar J. 2010;9:91. doi:10.1186/1475-2875-
657 9-91.
- 658 14. Diakit  NR, Guindo-Coulibaly N, Adja AM, et al. Spatial and temporal
659 variation of malaria entomological parameters at the onset of a hydro-
660 agricultural development in central C te d’Ivoire. Malar J. 2015a; 14:340.
661 doi:10.1186/s12936-015-0871-4.
- 662 15. Reis IC, Codeço CT, Degener CM, Keppeler EC, Muniz MM, de Oliveira FG,
663 Cort s JJ, de Freitas Monteiro A, de Souza CA, Rodrigues FC, Maia GR,
664 Hon rio NA. Contribution of fish farming ponds to the production of immature
665 *Anopheles* spp. in a malaria-endemic Amazonian town. Malar. J. 2015; 14: 452.
- 666 16. Reis IC, Hon rio NA, Barros FS, Barcellos C, Kitron U, Camara DC, Pereira
667 GR, Keppeler EC, Silva-Nunes M, Codeço CT. Epidemic and endemic malaria
668 transmission related to fish farming ponds in the Amazon frontier. PLoS One.
669 2015; 10: e0137521.
- 670 17. Reis IC, Codeço, C.T., C mara, D.C.P. et al. Diversity of *Anopheles* spp.
671 (Diptera: Culicidae) in an Amazonian Urban Area. Neotrop Entomol. 2018; 47,
672 412–417. <https://doi.org/10.1007/s13744-018-0595-6>
- 673 18. Maheu-Giroux M, Castro MC. Impact of Community-Based Larviciding on the
674 Prevalence of Malaria Infection in Dar es Salaam, Tanzania. PLoS One. 2013;
675 8:e71638.
- 676 19. Maheu-Giroux M, Castro MC. Cost-effectiveness of larviciding for urban
677 malaria control in Tanzania. Malar J. 2014; 13:477.
- 678 20. Galardo AK, Zimmerman R, Galardo CD. Larval control of *Anopheles*
679 (*Nyssorhynchus*) *darlingi* using granular formulation of *Bacillus sphaericus* in
680 abandoned gold-miners excavation pools in the Brazilian Amazon rainforest.
681 Rev Soc Bras Med Trop. 2013; 46(2):172-7.
- 682 21. Moller-Jacobs LL, Murdock CC, Thomas MB, 2014. Capacity of mosquitoes to
683 transmit malaria depends on larval environment. Parasit Vectors. 2014; 7: 593.
- 684 22. Brady OJ, Godfray HC, Tatem AJ, Gething PW, Cohen JM, McKenzie FE,
685 Perkins TA, Reiner RJ, Tusting LS, Sinka ME, Moyes CL, Eckhoff PA, Scott
686 TW, Lindsay SW, Hay SI, Smith DL. Vectorial capacity and vector control:

- 687 reconsidering sensitivity to parameters for malaria elimination. *Trans R Soc*
688 *Trop Med Hyg.* 2016; 110 (2): 107–117.
- 689 23. Conn JE, Ribolla PE. Ecology of *Anopheles darlingi*, the primary malaria vector
690 in the Americas and current nongenetic methods of vector control. 2015 In:
691 Adelman, Z.N. (Ed.), *Genetic Control of Malaria and Dengue*. Oxford:
692 Academic Press, pp. 81–102.
- 693 24. Rufalco-Moutinho P, Schweigmann N, Bergamaschi DP, Mureb Sallum MA.
694 Larval habitats of *Anopheles* species in a rural settlement on the malaria frontier
695 of southwest Amazon, Brazil. *Acta Trop.* 2016; 164:243-258.
- 696 25. Instituto Brasileiro de Geografia e Estatística (IBGE). Available at
697 <https://ww2.ibge.gov.br/home/geociencias/geografia/amazonialegal.shtm?c=2>.
- 698 26. Barros FSM, Honório NA. Man biting rate seasonal variation of malaria vectors
699 in Roraima, Brazil. *Mem Inst Oswaldo Cruz.* 2007; 102(3): 299-302.
- 700 27. Lana RM, Riback TIS, Lima TFM, et al. Socioeconomic and demographic
701 characterization of an endemic malaria region in Brazil by multiple
702 correspondence analysis. *Malar J.* 2017; 16:397. doi:10.1186/s12936-017-2045-
703 z.
- 704 28. SVS-MS, Secretaria de Vigilância em Saúde, Ministério da Saúde,
705 2011. Padronização dos métodos utilizados em pesquisa larvária de *Anopheles*
706 *narotina* dos laboratórios de entomologia. Nota Técnica no.012-
707 CGPNM/DIGES/SVS/MS. Registro Número: 25000-088097/2007-80,
708 Brasília.
- 709 29. Forattini O.P., 2002. *Culicidologia Médica*. Edusp, 2 v. pp: 250–252, 325, 337,
710 São Paulo, Brasil.
- 711 30. Martin TG, Wintle BA, Rhodes JR, Kuhnert PM, Field SA, Low-Choy J, Tyre
712 AJ, Possingham HP. Zero tolerance ecology: improving ecological inference by
713 modelling the source of zero observations. *Ecol Lett.* 2005; 8,1235–1246.
- 714 31. Kenea O., Balkew, M., Tekie, H. et al. Human-biting activities of *Anopheles*
715 species in south-central Ethiopia. *Parasit Vectors* 2016; 9, 527.
716 <https://doi.org/10.1186/s13071-016-1813-x>
- 717 32. Zar J.H., 2010. *Biostatistical Analysis*, 5th edition. Pearson Education, pp. 434–
718 437.
- 719 33. Quené H, van den Bergh H. 2004. On multi-level modeling of data from
720 repeated measures designs: a tutorial. *Speech Communication.* 2004; 43 (1–2):
721 103-121.
- 722 34. Rabe-Hesketh S and Skrondal A, 2012. *Multilevel and Longitudinal Modeling*
723 *Using Stata*. Third Edition. Vol I. Stata Press, Texas, US.
- 724 35. Hurlbert SH. Pseudoreplication and the Design of Ecological Field Experiments.
725 *Ecological Monographs* 1984; 54(2), pp. 187-2.

- 726 36. Hagenlocher M., Castro, M.C. Mapping malaria risk and vulnerability in the
727 United Republic of Tanzania: a spatial explicit model. *Popul Health Metrics*
728 2015; 13, 2. <https://doi.org/10.1186/s12963-015-0036-2>
- 729 37. Smith DL, Perkins TA, Reiner RC, Barker CM, Niu T, Chaves LF, Ellis AM,
730 George DB, Le Menach A, Pulliam JR, Bisanzio D, Buckee C, Chiyaka
731 C, Cummings DA, Garcia AJ, Gatton ML, Gething PW, Hartley DM, Johnston
732 G, Klein EY, Michael E, Lloyd AL, Pigott DM, Reisen WK, Ruktanonchai N,
733 Singh BK, Stoller J, Tatem AJ, Kitron U, Godfray HC, Cohen JM, Hay SI, Scott
734 TW. Recasting the theory of mosquito-borne pathogen transmission dynamics
735 and control. *Trans R Soc Trop Med Hyg.* 2014; 108 (4): 185–187.
- 736 38. Corrêa A.P.S.A., Galardo, A.K.R., Lima, L.A. et al. Efficacy of insecticides
737 used in indoor residual spraying for malaria control: an experimental trial on
738 various surfaces in a “test house”. *Malar J.* 2019; 18, 345.
739 <https://doi.org/10.1186/s12936-019-2969-6>.
- 740 39. Nascimento J, Sampaio VS, Karl S, Kuehn A, Almeida A, Vitor-Silva S, de
741 Melo GC, Baia da Silva DC, Lopes SCP, Fé NF, Lima JBP, Guerra MGB,
742 Pimenta PFP, Bassat Q, Mueller I, Lacerda MVG, Monteiro WM. Use of
743 anthropophilic culicid-based xenosurveillance as a proxy for *Plasmodium vivax*
744 malaria burden and transmission hotspots identification. *PLoS Negl Trop Dis.*
745 2018; 12;12(11):e0006909. doi: 10.1371/journal.pntd.0006909. PMID:
746 30418971; PMCID: PMC6258424.
- 747 40. Houweling TA, Karim-Kos HE, Kulik MC, Stolk WA, Haagsma JA, Lenk EJ,
748 Richardus JH, de Vlas SJ. Socioeconomic Inequalities in Neglected Tropical
749 Diseases: A Systematic Review. *PLoS Negl Trop Dis.* 2016;
750 12;10(5):e0004546. doi: 10.1371/journal.pntd.0004546. PMID: 27171166;
751 PMCID: PMC4865383.
- 752 41. Azrag RS, Mohammed BH. *Malar J.* *Anopheles arabiensis* in Sudan: a
753 noticeable tolerance to urban polluted larval habitats associated with resistance
754 to Temephos. *Malar J.* 2018; 17(1):204. doi: 10.1186/s12936-018-2350-1.
- 755 42. Saavedra M.P., Conn, J.E., Alava, F. et al. Higher risk of malaria transmission
756 outdoors than indoors by *Nyssorhynchus darlingi* in riverine communities in the
757 Peruvian Amazon. *Parasit Vectors.* 2019; 12, 374.
- 758 43. Prussing C., Moreno, M., Saavedra, M.P. et al. Decreasing proportion of
759 *Anopheles darlingi* biting outdoors between long-lasting insecticidal net
760 distributions in peri-Iquitos, Amazonian Peru. *Malar J.* 2018; 17, 86.
761 <https://doi.org/10.1186/s12936-018-2234-4>
- 762 44. Barbosa LMC, Souto RNP, Dos Anjos Ferreira RM, Scarpassa VM. Behavioral
763 patterns, parity rate and natural infection analysis in anopheline species involved
764 in the transmission of malaria in the northeastern Brazilian Amazon region. *Acta*
765 *Tropica.* 2016; 164: 216–225.

- 766 45. Gil LHS, Rodrigues MS, Lima AA, Katsuragawa TH. Seasonal distribution of
767 malaria vectors (Diptera: Culicidae) in rural localities of Porto Velho, Rondônia,
768 Brazilian Amazon. *Rev Inst Med Trop*. 2015; 57(3): 263–267.
- 769 46. Moutinho PR, Cruz RMB, Gil LHS, Ribolla PEM. Population dynamics,
770 structure and behavior of *Anopheles darlingi* in a rural settlement in the Amazon
771 rainforest of Acre. *Malar J*. 2011; 10: 174.
- 772 47. Barros FSM, Honório NA. Deforestation and malaria on the Amazon frontier:
773 larval clustering of *Anopheles darlingi* (Diptera: Culicidae) determines focal
774 distribution of malaria. *Am J Trop Med Hyg*. 2015; 93 (5): 939–953.
- 775 48. Barros FSM, Arruda ME, Gurgel HC, Honório NA. Spatial clustering and
776 longitudinal variation of *Anopheles darlingi* (Diptera: Culicidae) larvae in a
777 river of the Amazon: the importance of the forest fringe and of obstructions to
778 flow in frontier malaria, *Bull Entomol Res*. 2011; 101 (6): 643–658.
- 779 49. Sallum MAM, Conn JE, Bergo ES, Laporta GZ, Chaves LSM, Bickersmith SA,
780 de Oliveira TMP, Figueira EAG, Moresco G, Olivêr L, Struchiner CJ, Yakob L,
781 Massad E. Vector competence, vectorial capacity of *Nyssorhynchus darlingi* and
782 the basic reproduction number of *Plasmodium vivax* in agricultural settlements
783 in the Amazonian Region of Brazil. *Malar J*. 2019; 4;18(1):117. doi:
784 10.1186/s12936-019-2753-7. PMID: 30947726; PMCID: PMC6449965.
- 785 50. Scott TW, Takken W. Feeding strategies of anthropophilic mosquitoes result in
786 increased risk of pathogen transmission. *Trends Parasitol*. 2012; 28(3): 114-121.
- 787 51. Cohuet A, Harris C, Robert V, Fontenille D. Evolutionary forces on *Anopheles*:
788 what makes a malaria vector? *Trends Parasitol*. 2010; 26 (3): 130-136.
- 789 52. Reece SE, Ramiro RS, Nussey DH. Plastic parasites: sophisticated strategies for
790 survival and reproduction? *Evol Appl*. 2009; 2(1):11-23.
- 791 53. Packard RM. The making of a tropical disease: A short history of malaria.
792 *Emerg Infect Dis*. 2008;14(10):1679.
- 793 54. Monnerat R, Magalhães I, Daniel S, Ramos F, Sujii E, Praça L, Martins E,
794 Soares CM. Inventory of breeding-sites and species of Anopheline mosquitoes
795 in the Juruá valley, *Inter J Mosq Res*. 2014; 1(3): 1-3.
- 796 55. Laporta GZ, Prado PIKL, Kraenkel RA, Coutinho RM, Sallum MAM.
797 Biodiversity can help prevent malaria outbreaks in tropical forests, *PLoS Negl*
798 *Trop Dis* 2013; 7 (3): e2139.
- 799 56. Martin LMO, David MR, Maciel-de-Freitas R, Silva-do-Nascimento TF.
800 Diversity of *Anopheles* mosquitoes from four landscapes in the highest endemic
801 region of malaria transmission in Brazil. *J Vector Ecol*. 2018; 43(2):235-244.
802 doi: 10.1111/jvec.12307. PMID: 30408291
- 803 57. Hutchings RSG, Hutchings RW, Sallum MAM. Culicidae (Diptera:
804 Culicomorpha) from the central Brazilian Amazon: Nhamundá and Abacaxis
805 rivers, *Zoologia*. 2013; 30 (1): 1–14.

- 806 58. Hutchings RSG, Hutchings RW, Menezes IS, Motta MA, Sallum MAM.
807 Mosquitoes (Diptera: Culicidae) from the northwestern Brazilian Amazon:
808 Padauari river, J Med Entomol. 2016; 53(6):1330-1347.
- 809 59. Morais SA, Urbinatti PR, Sallum MAM, Kuniy AAM, Gilberto G, Fernandes A,
810 Nagaki SS, Natal D. Brazilian mosquito (Diptera: Culicidae) fauna: I.
811 Anopheles species from Porto Velho, Rondônia state, western Amazon, Brazil.
812 Rev Inst Med Trop São Paulo 2012; 54(6), 331-335.
- 813 60. Bourke B.P., Conn, J.E., de Oliveira, T.M.P. et al. Exploring malaria vector
814 diversity on the Amazon Frontier. Malar J. 2018; 17, 342.
815 <https://doi.org/10.1186/s12936-018-2483-2>
- 816 61. Conn JE, Grillet ME, Corre M, Sallum MAM. Malaria Transmission in South
817 America—Present Status and Prospects for Elimination. (July 18th 2018).
818 Malaria Transmission in South America—Present Status and Prospects for
819 Elimination, Towards Malaria Elimination Sylvie Manguin and Vas Dev, Intech
820 Open, DOI: 10.5772/intechopen.76964.
- 821 62. Hiwat H, Bretas G. Ecology of Anopheles darling root with respect to vector
822 importance: a review, Parasit Vectors. 2011; 4: 177.
- 823 63. WHO Larval source management – a supplementary measure for malaria vector
824 control. An operational manual July 2013.
- 825 64. Turell MJ, Sardelis MR, Jones JW, Watts DM, Fernandez R, Carbajal F, Pecor
826 JE, Klein TA. Seasonal distribution, biology, and human attraction patterns of
827 mosquitoes (Diptera: culicidae) in a rural village and adjacent forested site near
828 Iquitos, Peru, J Med Entomol. 2008; 45 (6): 1165–1172
- 829 65. Rejmankova E, Grieco J, Achee N, Roberts DR 2013. Ecology of larval habitats.
830 In S Manguin (ed.), Anopheles mosquitoes - New insights into malaria vectors,
831 9th ed., InTech, Rijeka, p. 397-446.
- 832 66. Walshe DP, Garner P, Adeel AA, Pyke GH, Burkot TR. Larvivorous fish for
833 preventing malaria transmission, Cochrane Database of Systematic Reviews.
834 2017; 12:CD008090.
- 835 67. Patrick ML, Gonzalez RJ, Wood CM, Wilson RW, Bradley TJ, Val AL. The
836 characterization of ion regulation in Amazonian mosquito larvae: evidence of
837 phenotypic plasticity, population-based disparity, and novel mechanisms of ion
838 uptake. Physiol Biochem Zool. 2002; 75(3):223-36.
- 839 68. Nissen A, Cook J, Loha E, Lindtjørn B. Proximity to vector breeding site and
840 risk of *Plasmodium vivax* infection: a prospective cohort study in rural Ethiopia.
841 Malar J. 2017; 16(1):380. doi: 10.1186/s12936-017-2031-5.
- 842 69. Fontoura PS, Costa AS, Ribeiro FS, Ferreira MS, Castro MC, Ferreira MU.
843 Field Efficacy of VectoMax FG and VectoLex CG Biological Larvicides for
844 Malaria Vector Control in Northwestern Brazil. J Med Entomol. 2020;
845 4;57(3):942-946. doi: 10.1093/jme/tjz220.

846

847 **Support Information**

848

849 **S1 Fig. Monthly distribution of reported malaria cases (*P. vivax* and *P. falciparum*)**

850 **and accumulated monthly precipitation. Mancio Lima municipality 2016 and 2017.**

851 Pearson correlations: in 2016 for *P. vivax* and precipitation $r = 0.75$; for *P. falciparum* and

852 precipitation $r = 0.51$; in 2017 for *P. vivax* and precipitation $r = 0.43$; for *P. falciparum* and

853 precipitation $r = 0.47$. (Source: Malaria: <http://www2.datasus.gov.br/DATASUS>;

854 Precipitation: clima1.cptec.inpe.br).

855

856 **S1 Table. Independent variable and response options (by classification or instrument**

857 **measure), level of subject analysis (sampling-point or fishpond), and sampling effort**

858 **during 2017 according to the sampling schedule (6-month for Feb, Mar, Apr, May,**

859 **Aug, Sept; 3-month for Feb, Mar, Apr; 3-month for May, Aug, Sept).**

860

861 **S2 Table. Fishpond numbers by site and collection month in Mancio Lima, Acre,**

862 **Brazil 2017.** Fishpond column: numbers of fishponds identified per respective month;

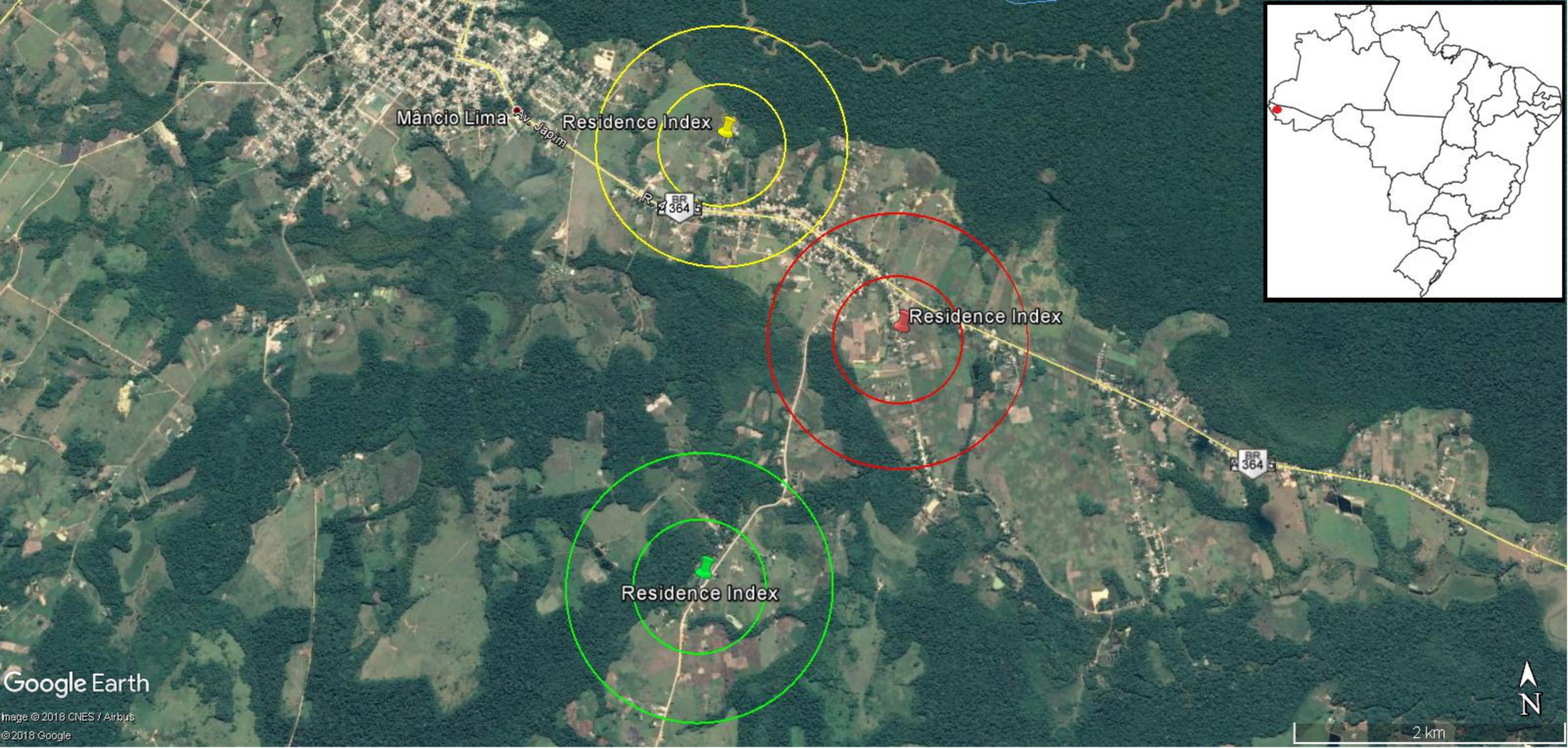
863 values in brackets are total fishponds surveyed for the respective month. Dry column:

864 fishponds with absence of water (by seasonality or owner management). Not surveyed

865 column: fishponds unsampled (due to flooded fields or no possible access to property).

866

867 **Supplementary Table 3: Environment independent variables by site in Mancio Lima,**
868 **Acre, Brazil 2017.** *fishpond level. **sampling-point level.



Mâncio Lima

Av. Japim

Residence Index

BR 364

Residence Index

Residence Index

BR 364

Google Earth

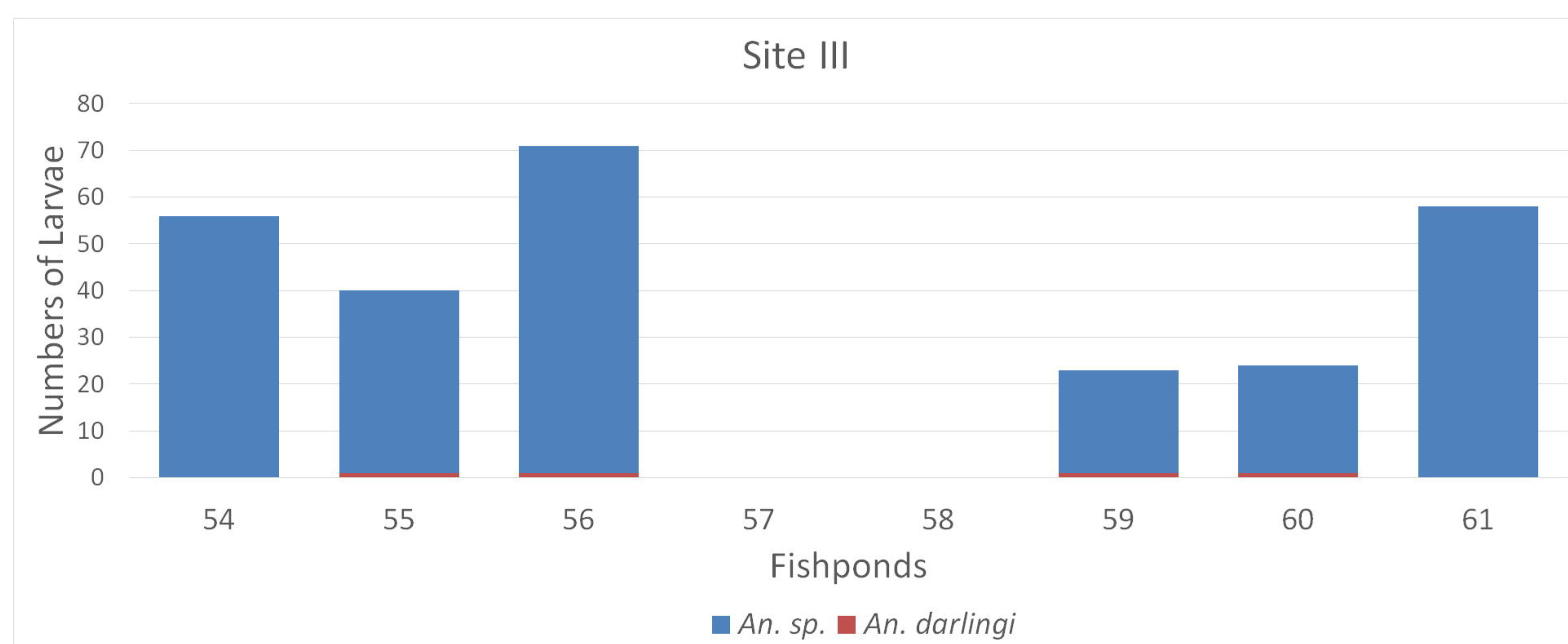
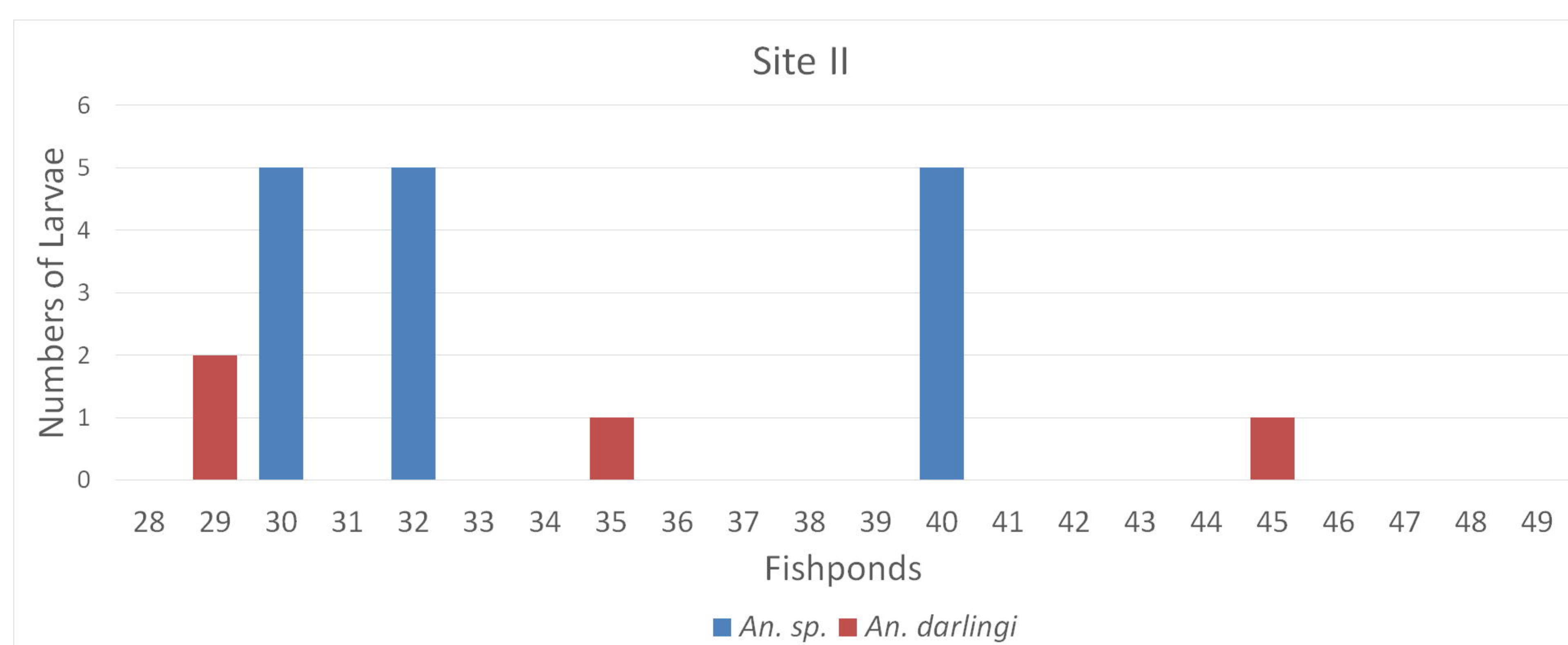
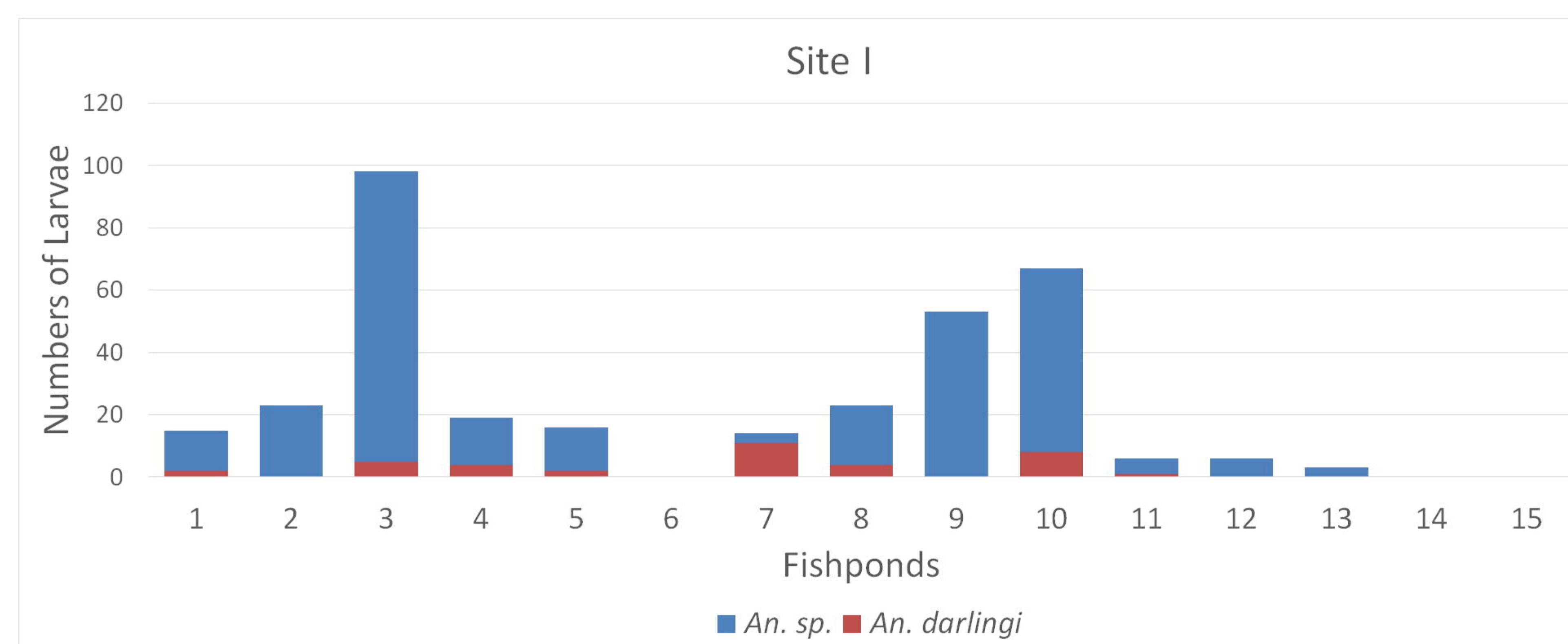
Image © 2018 CNES / Airbus
© 2018 Google

2 km

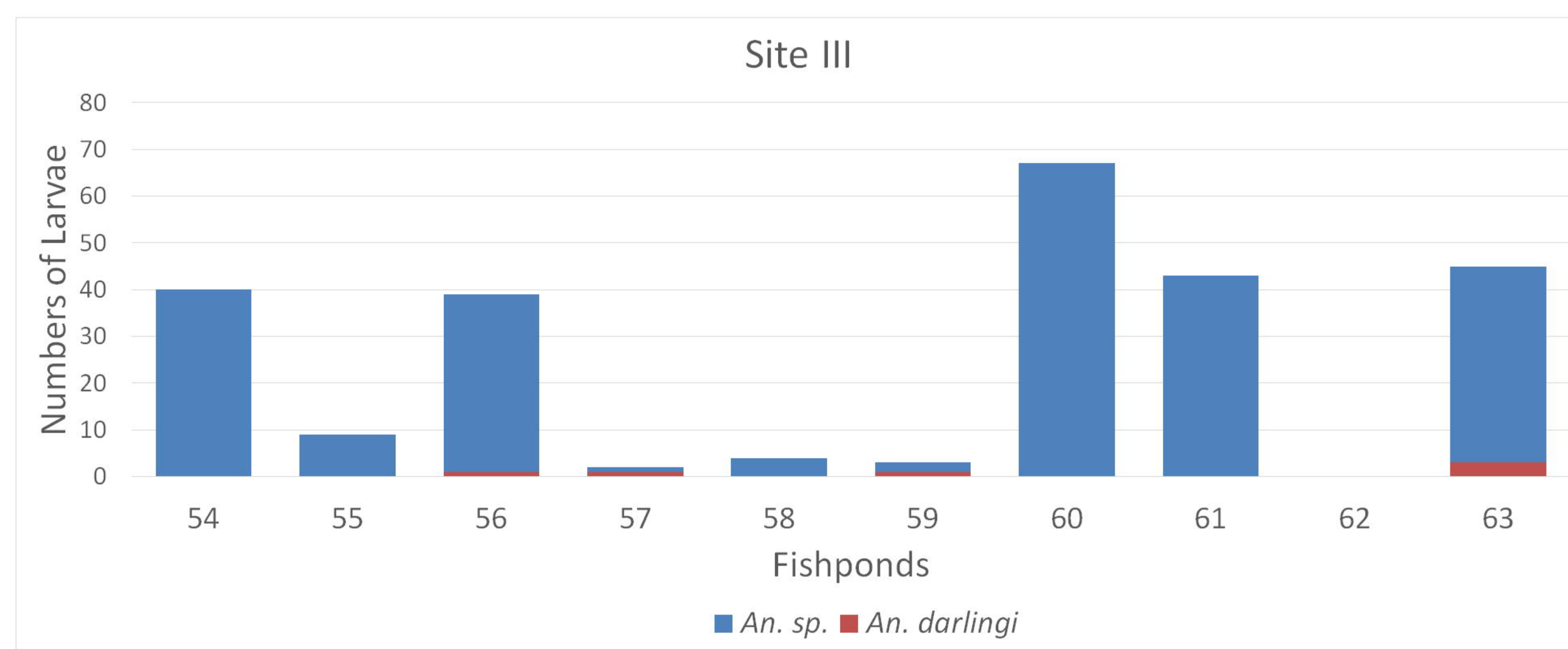
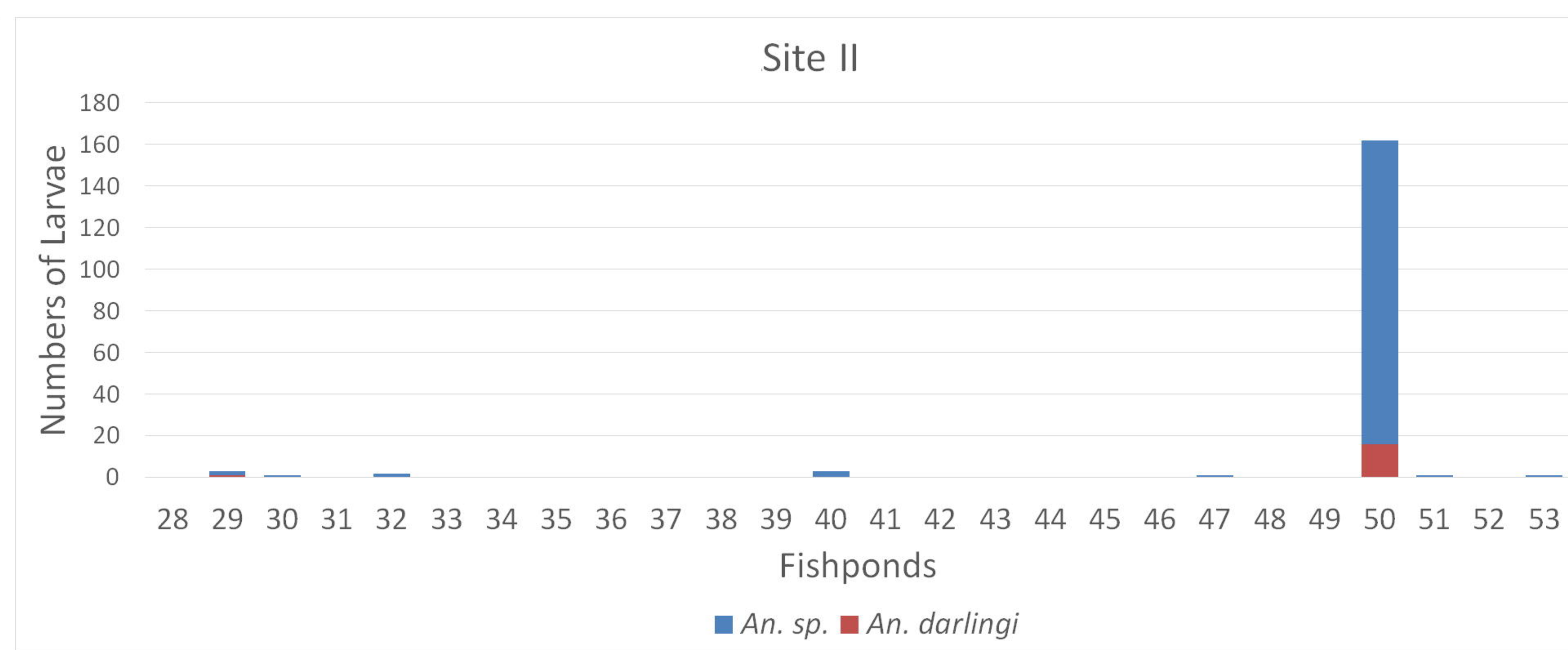
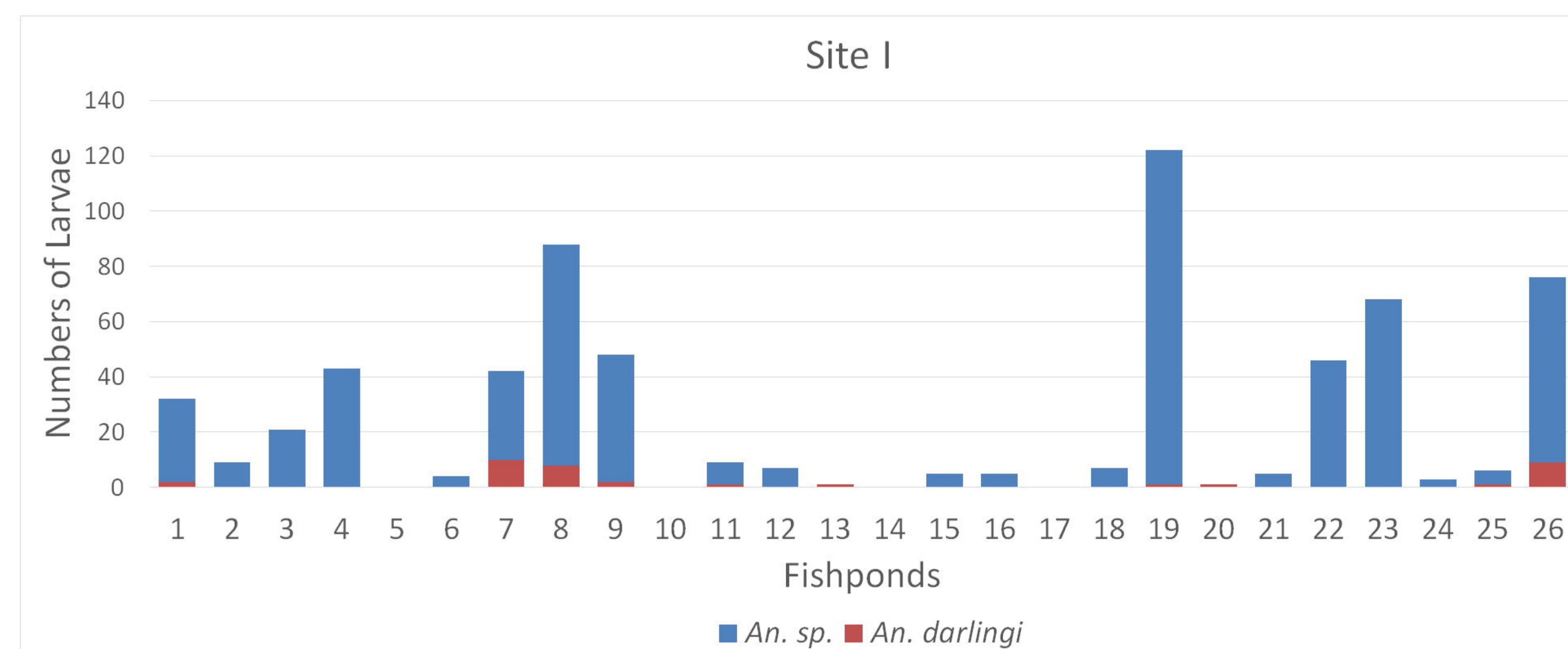




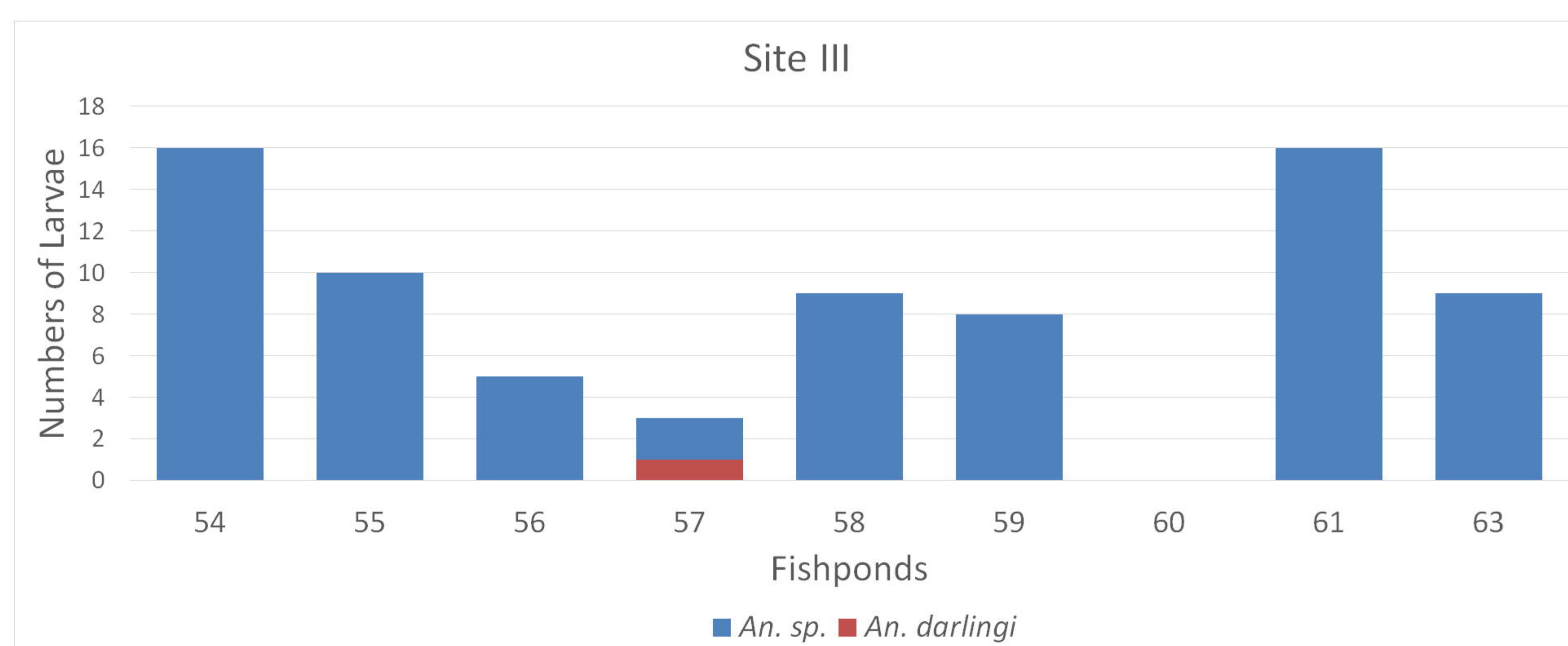
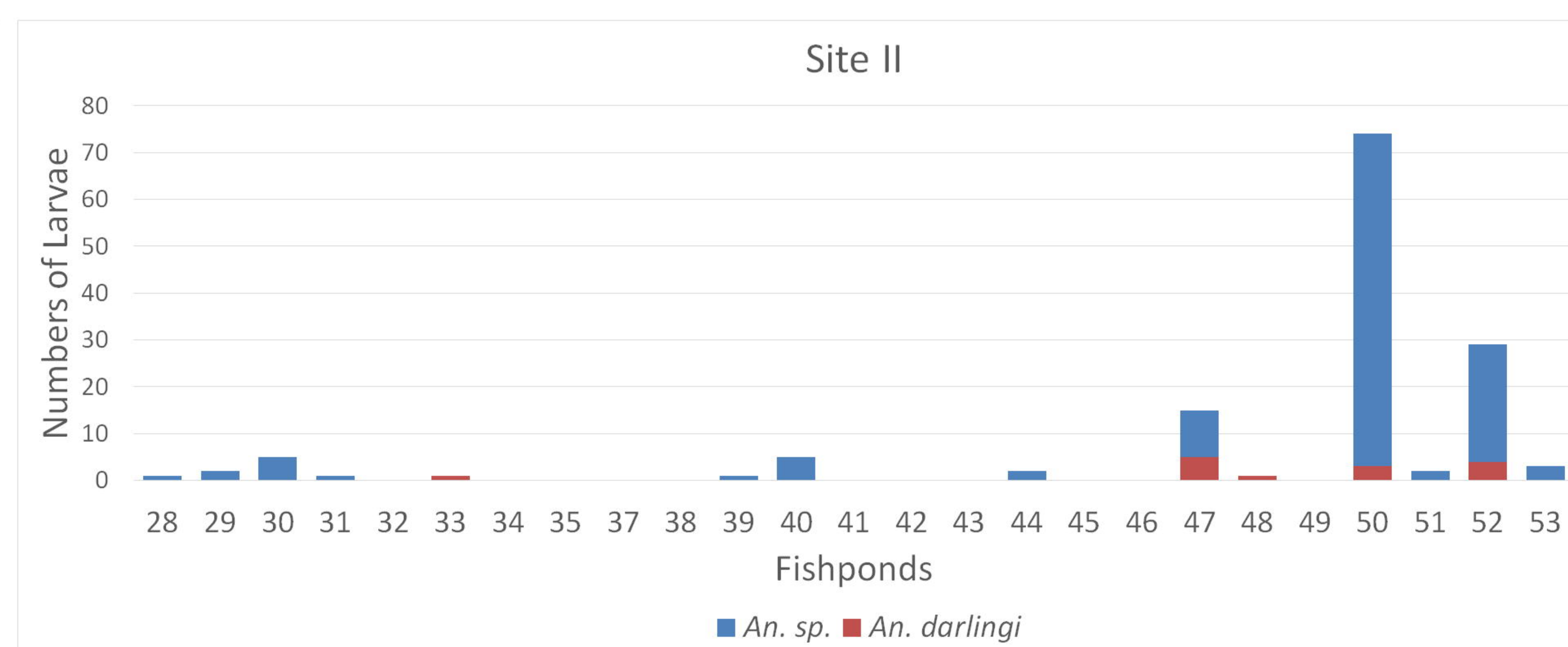
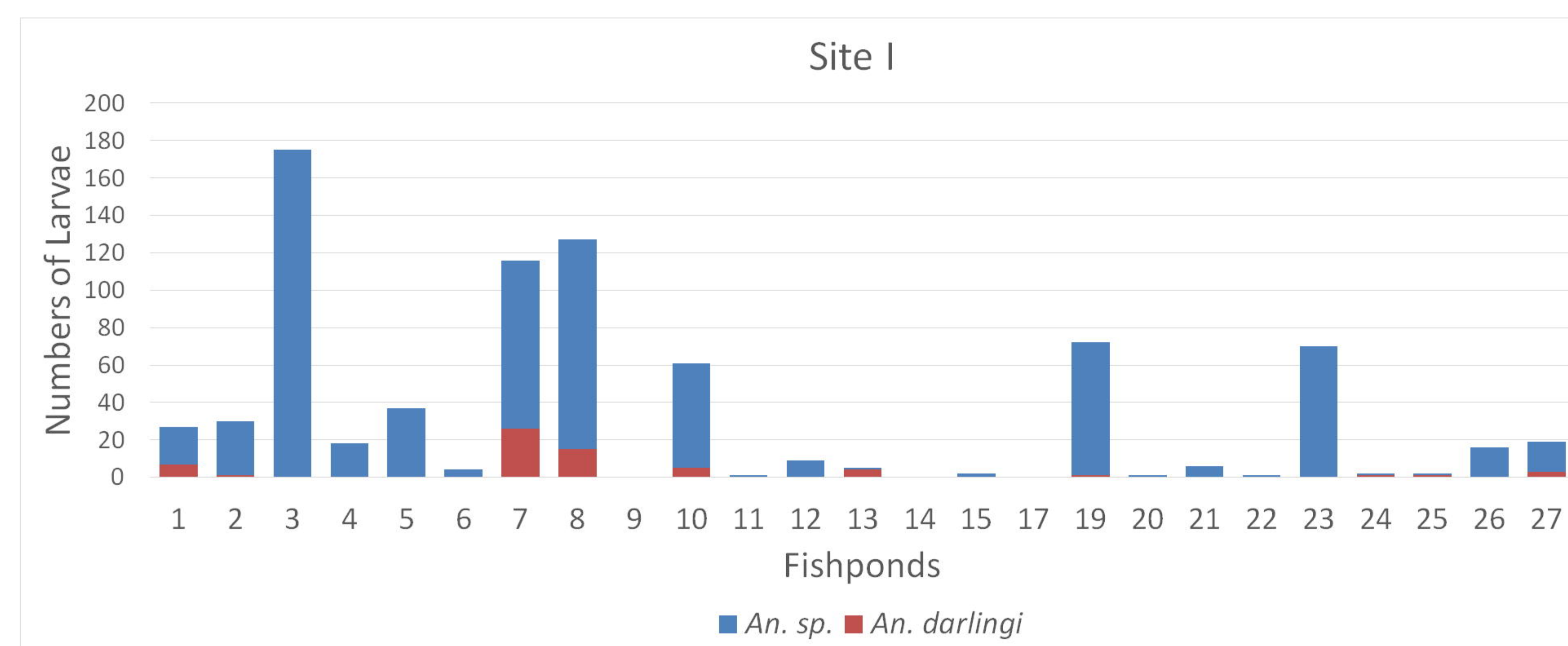
February



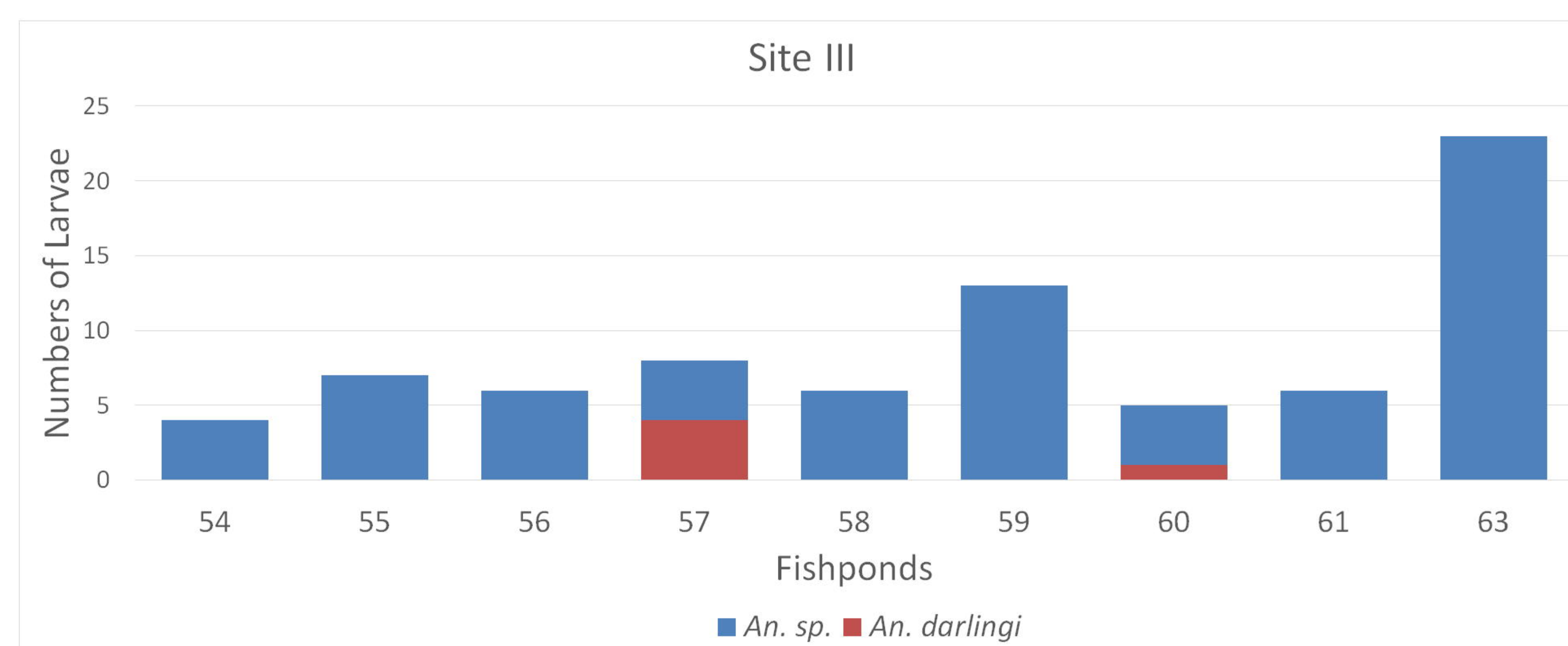
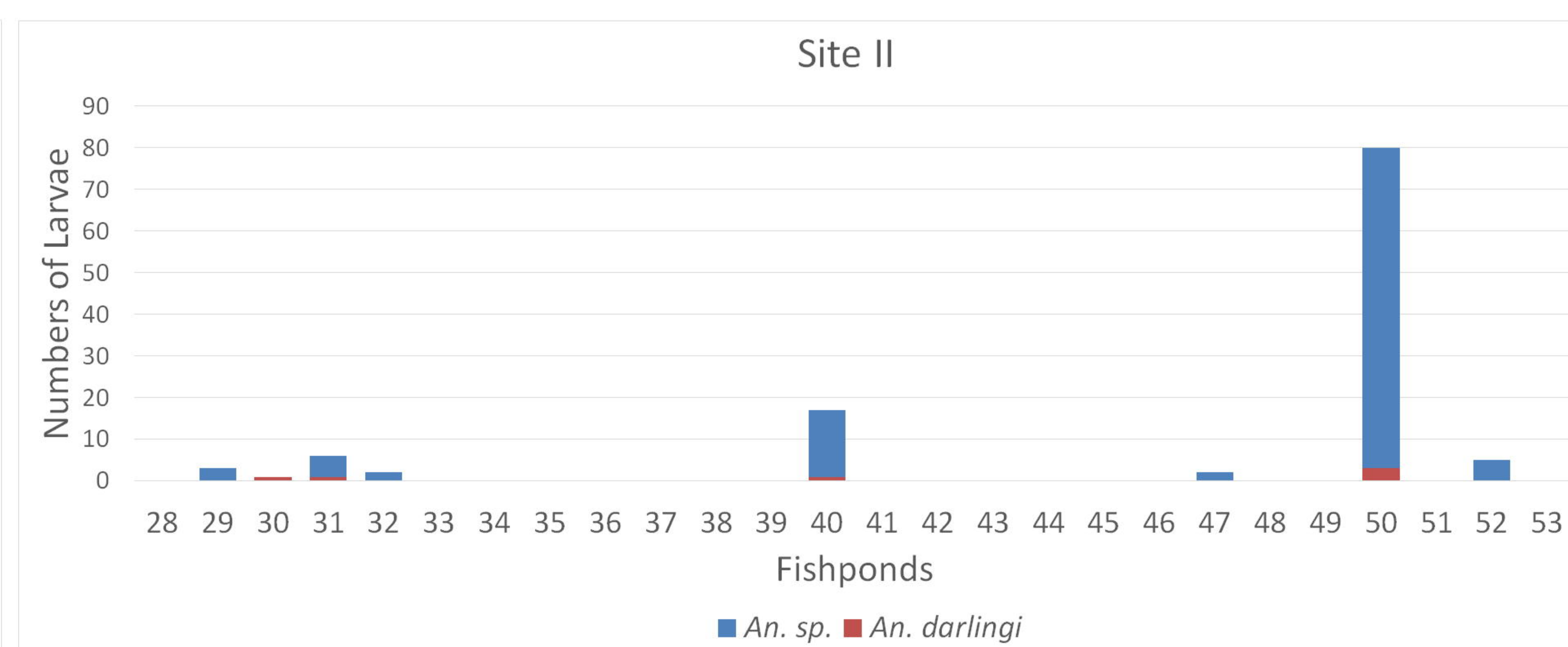
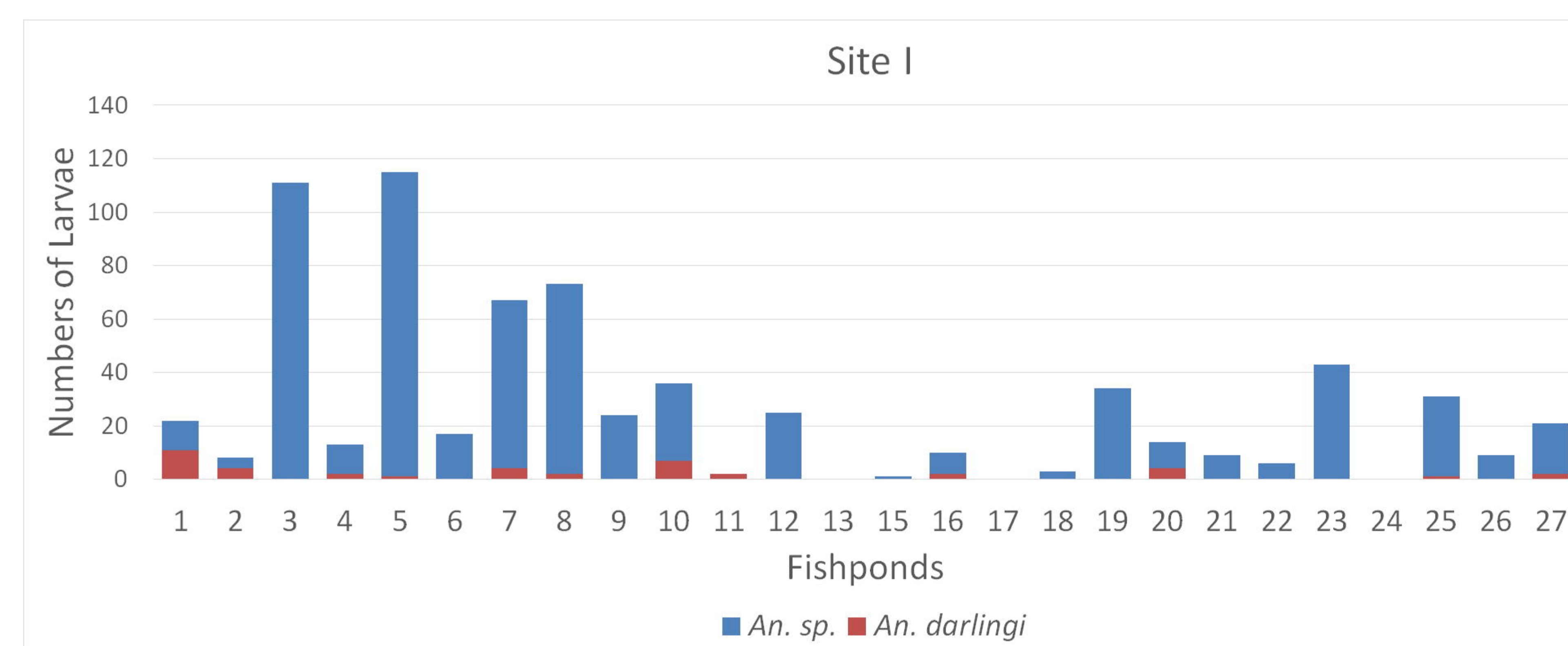
March



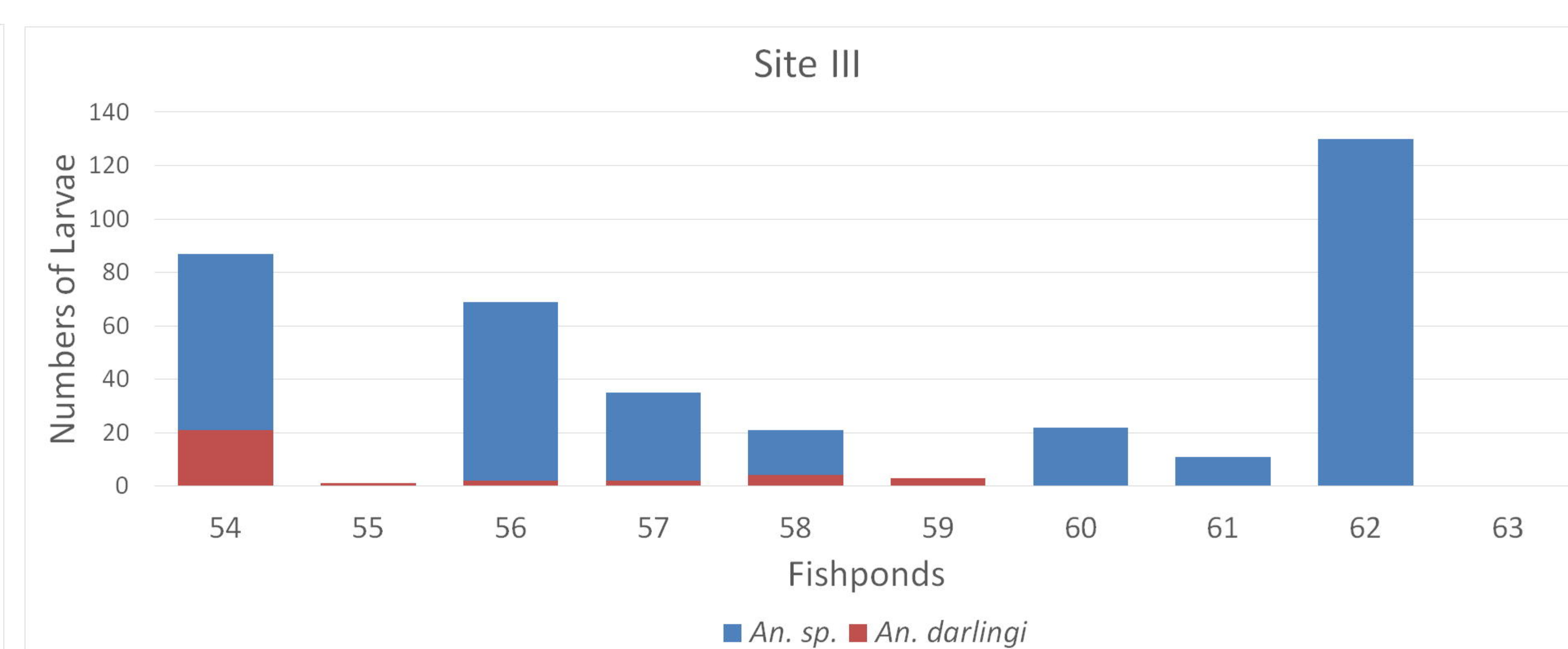
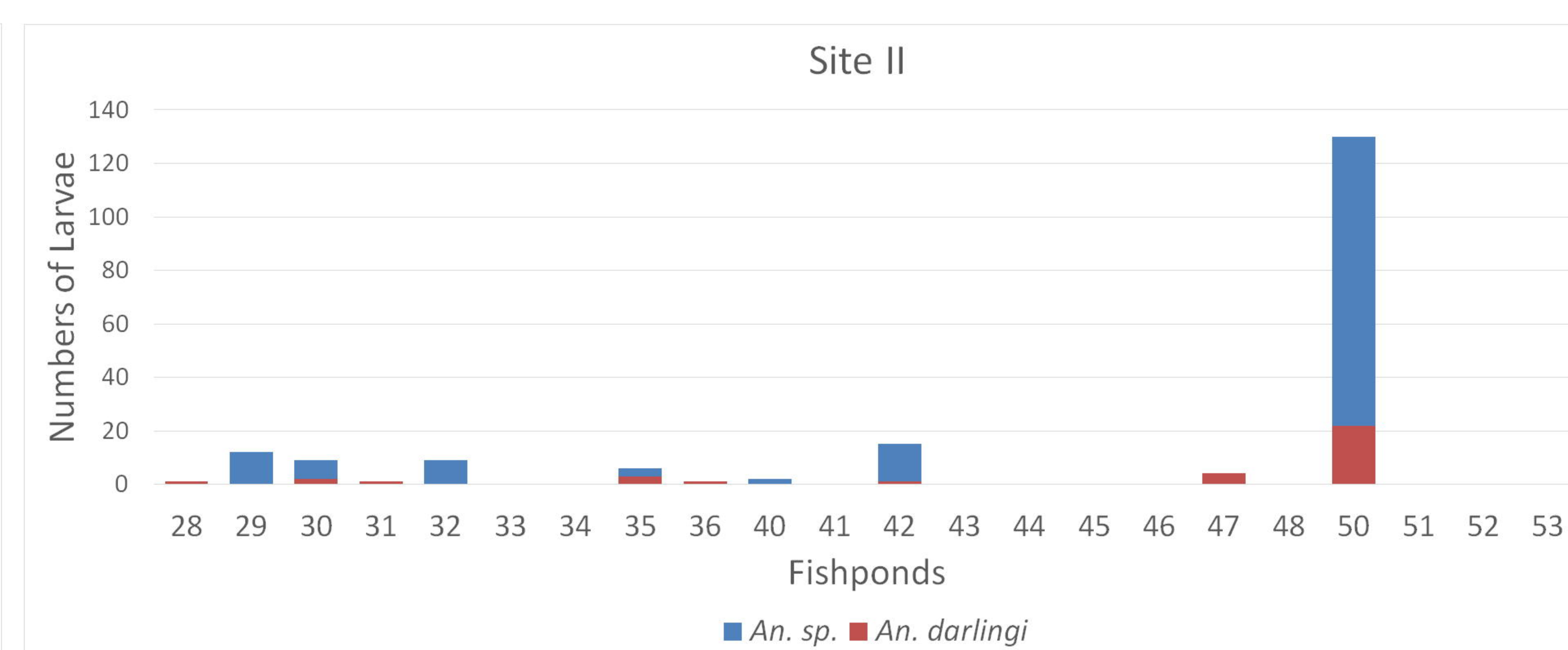
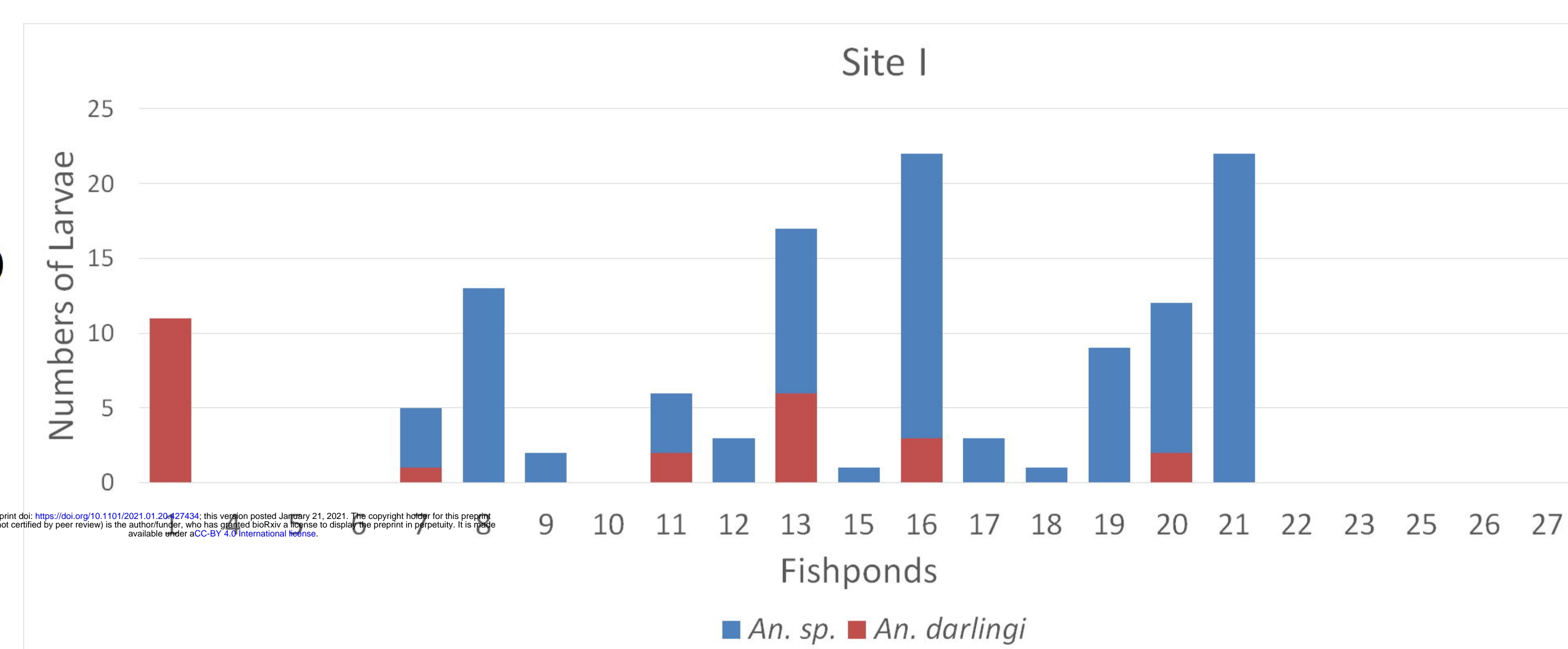
April



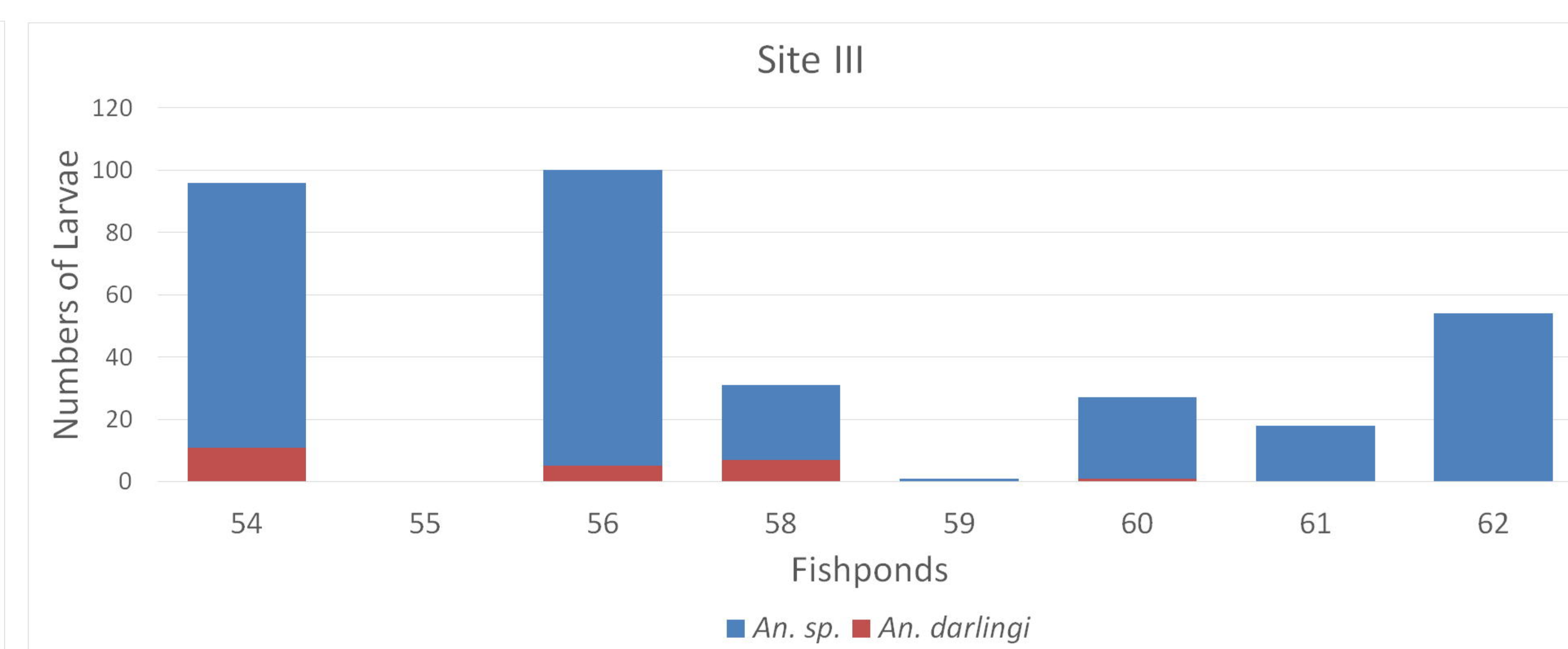
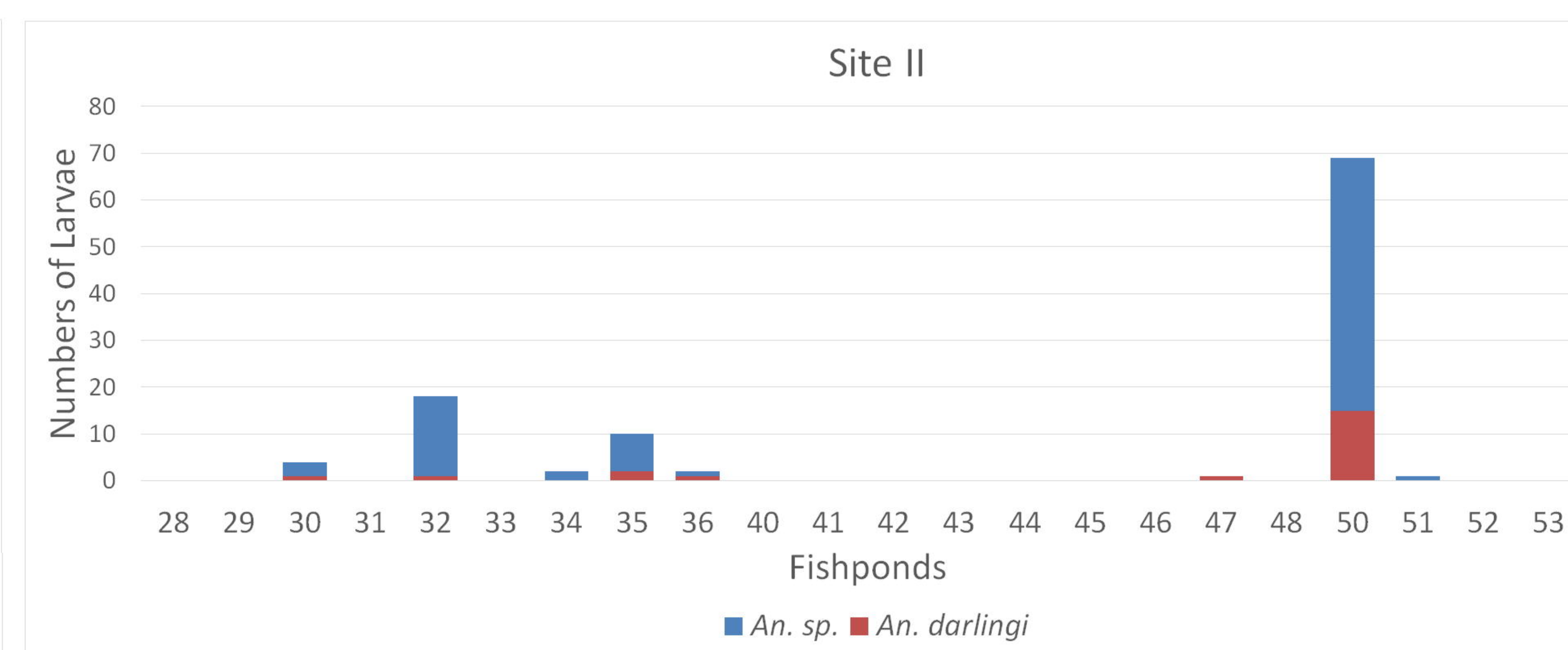
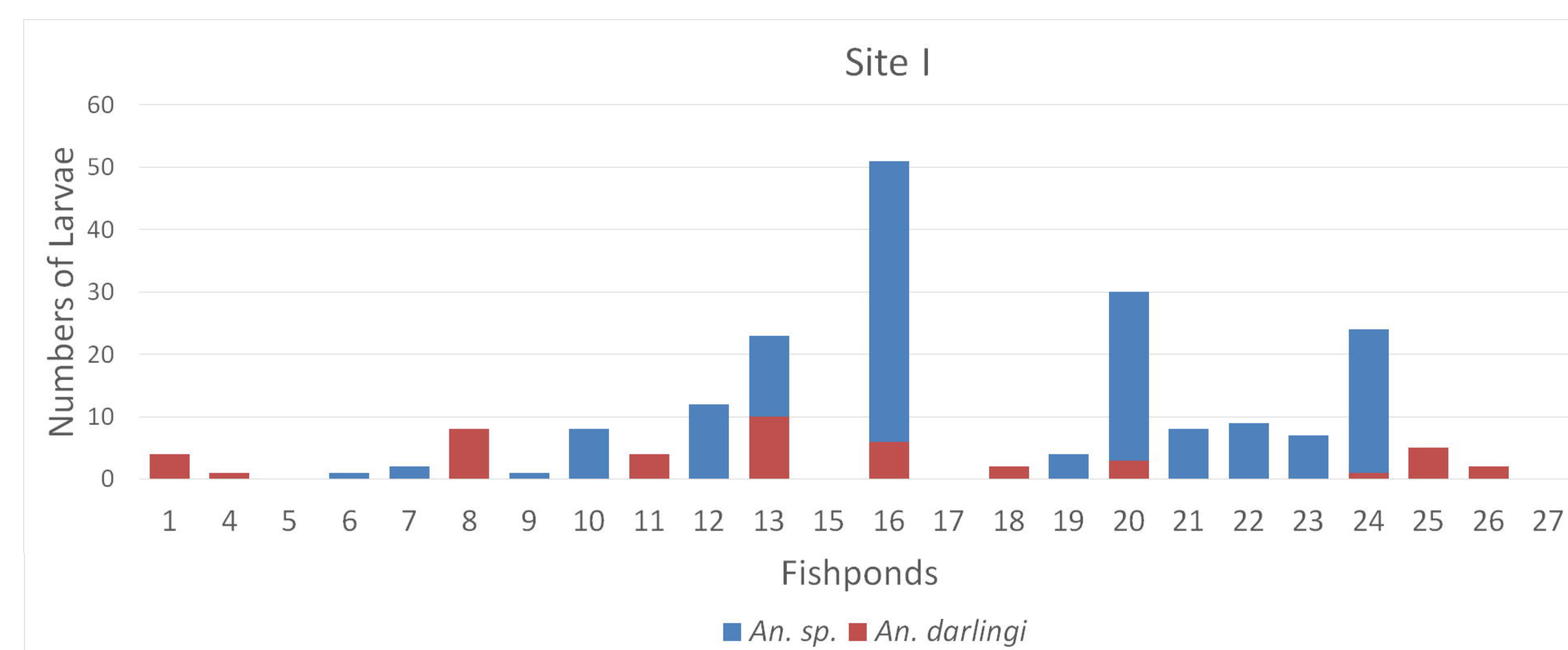
May



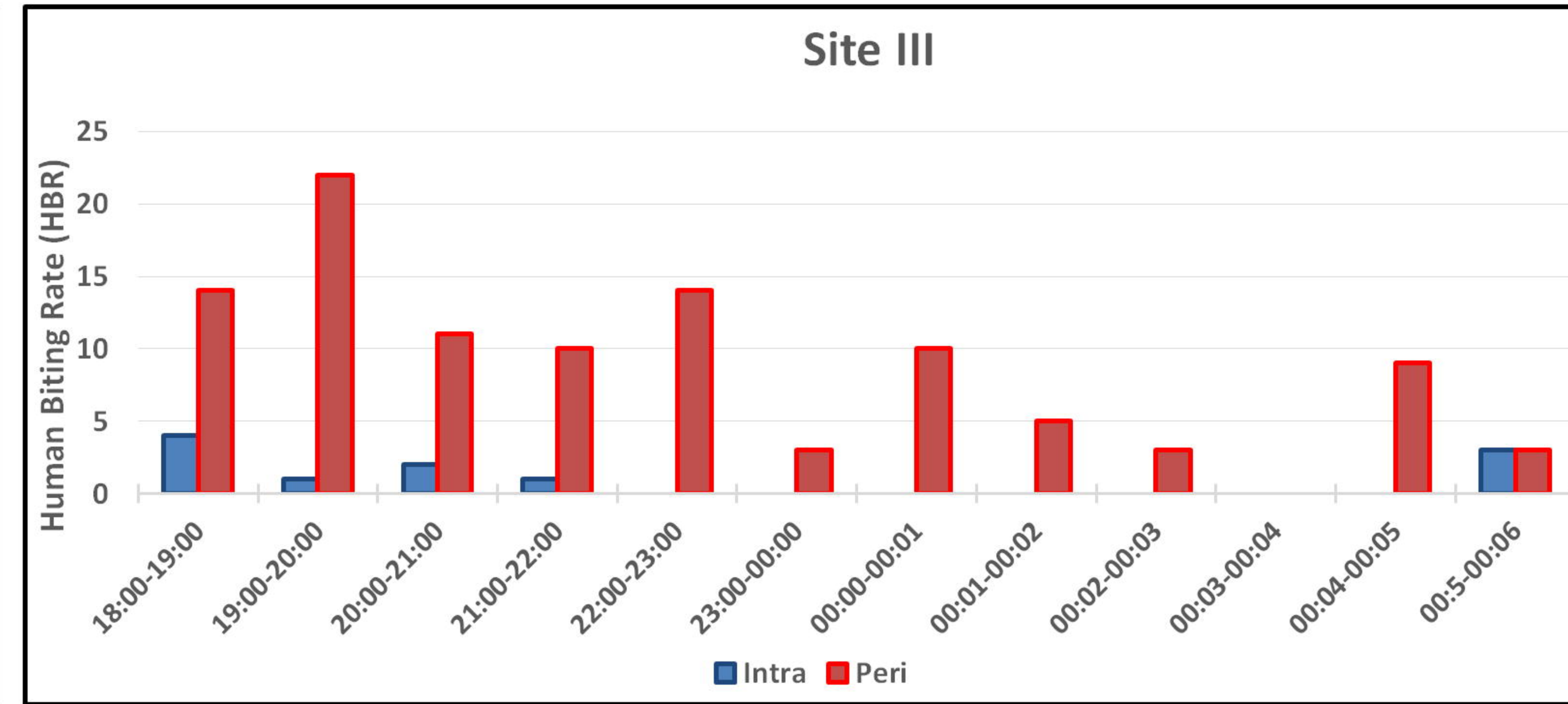
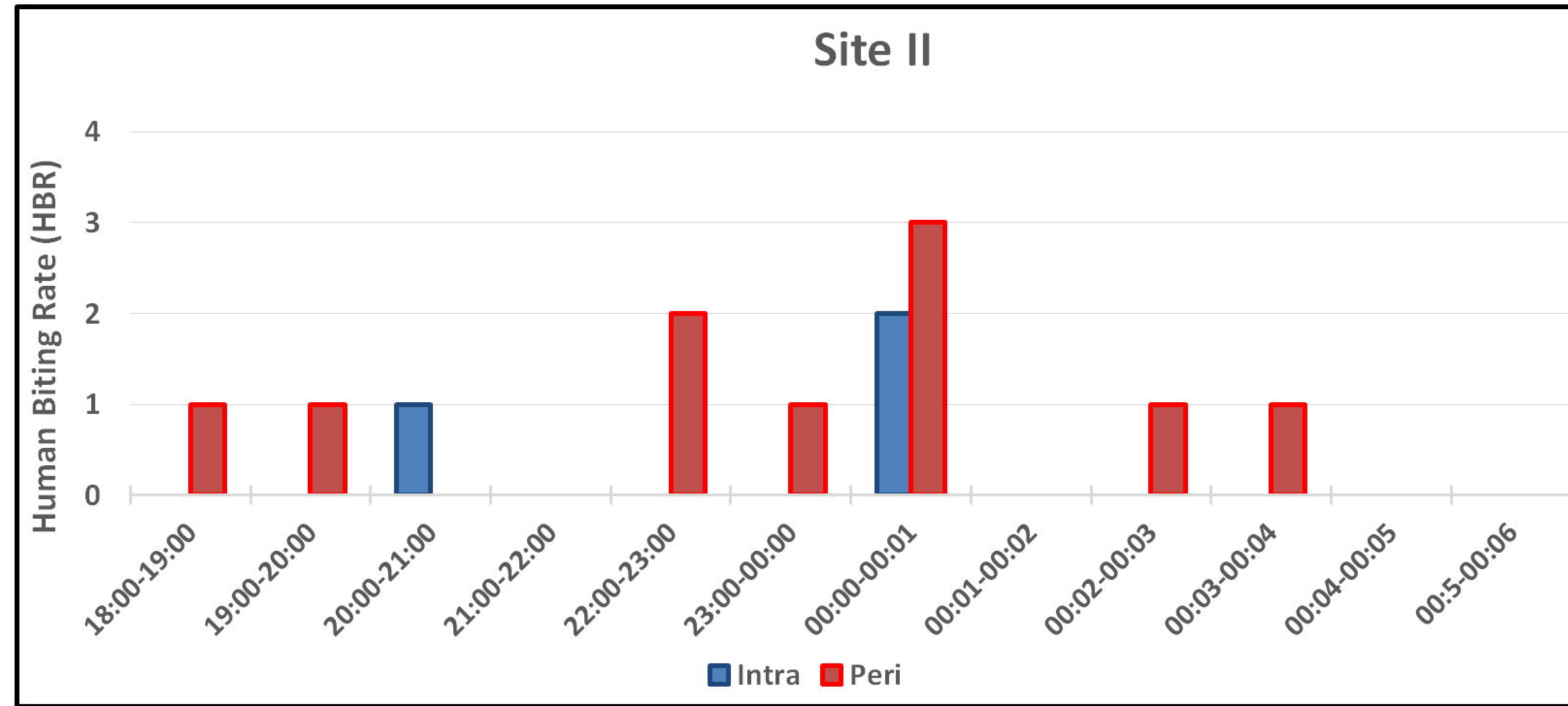
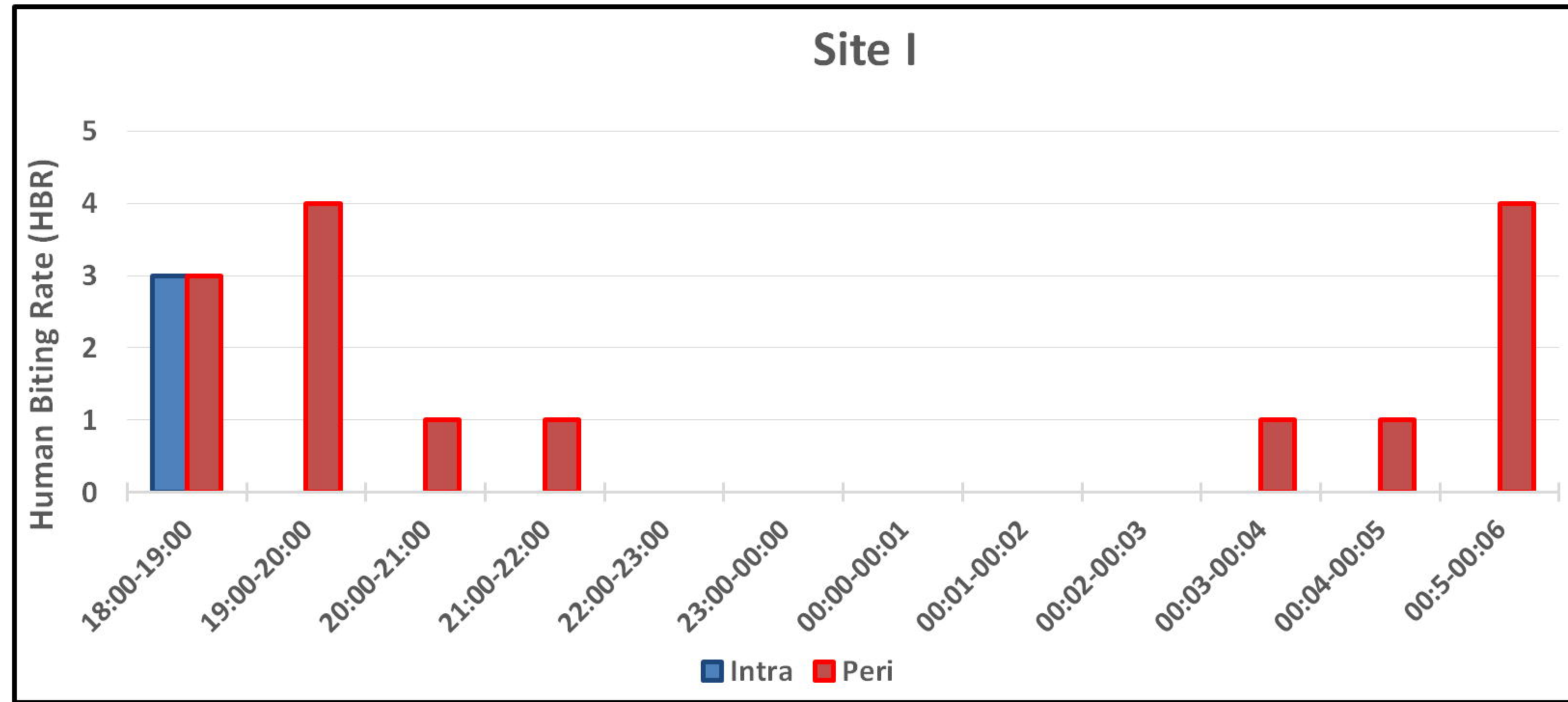
August



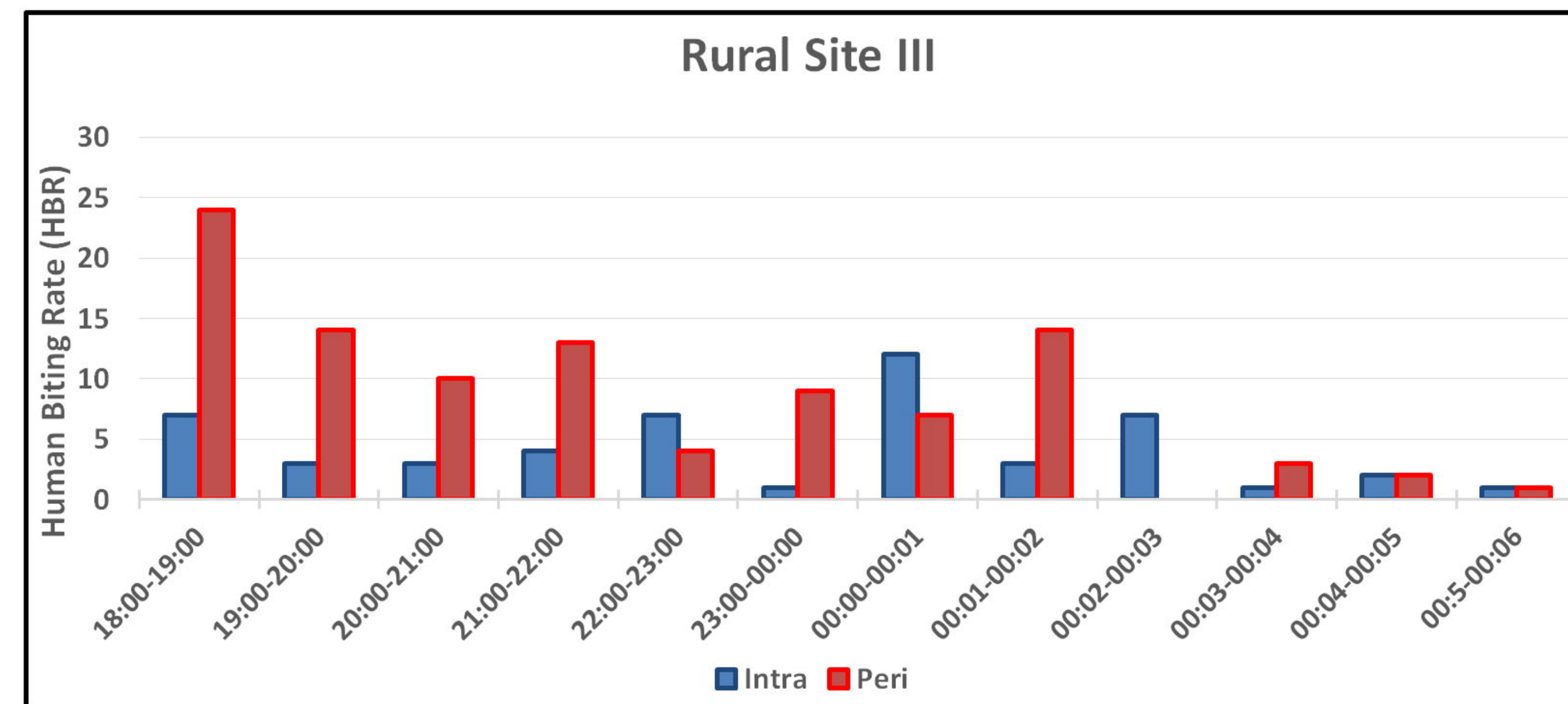
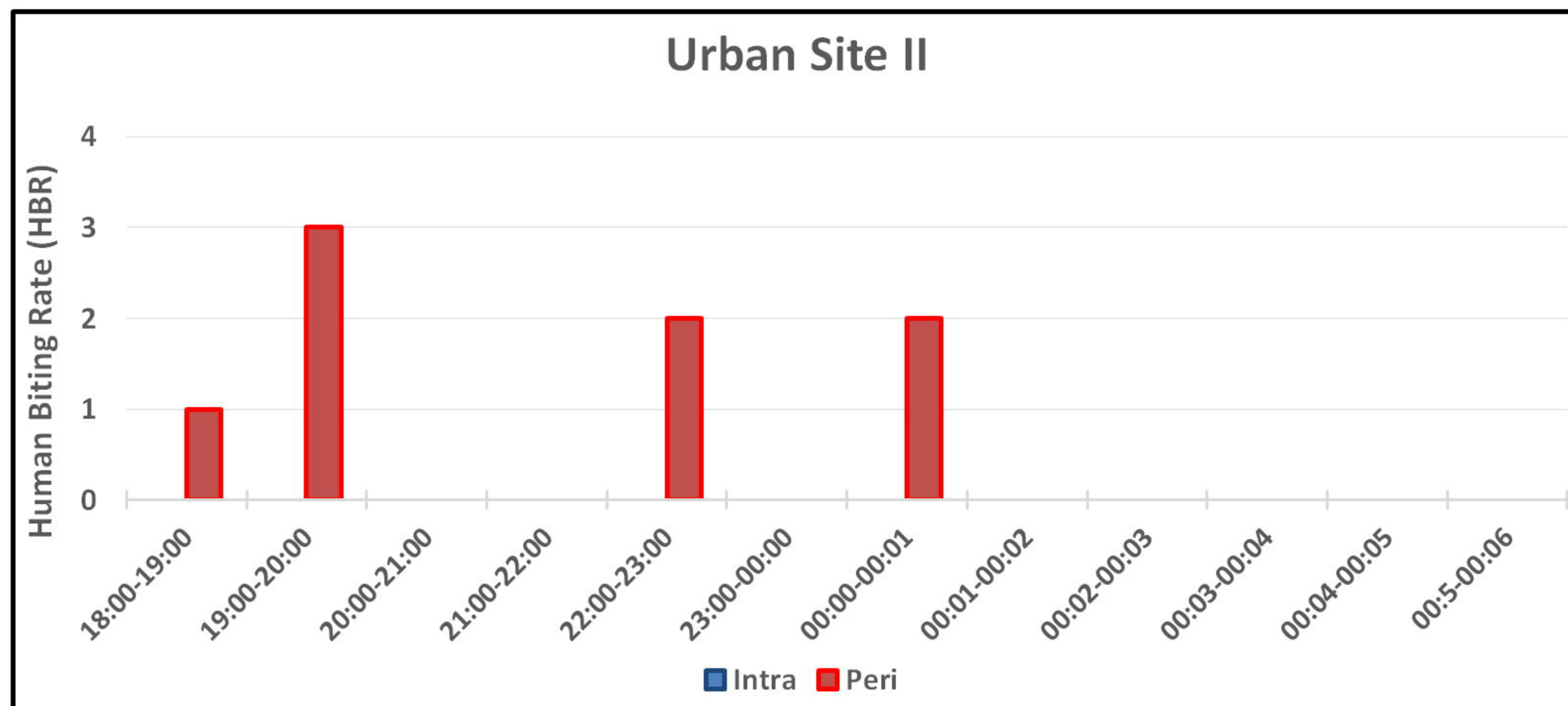
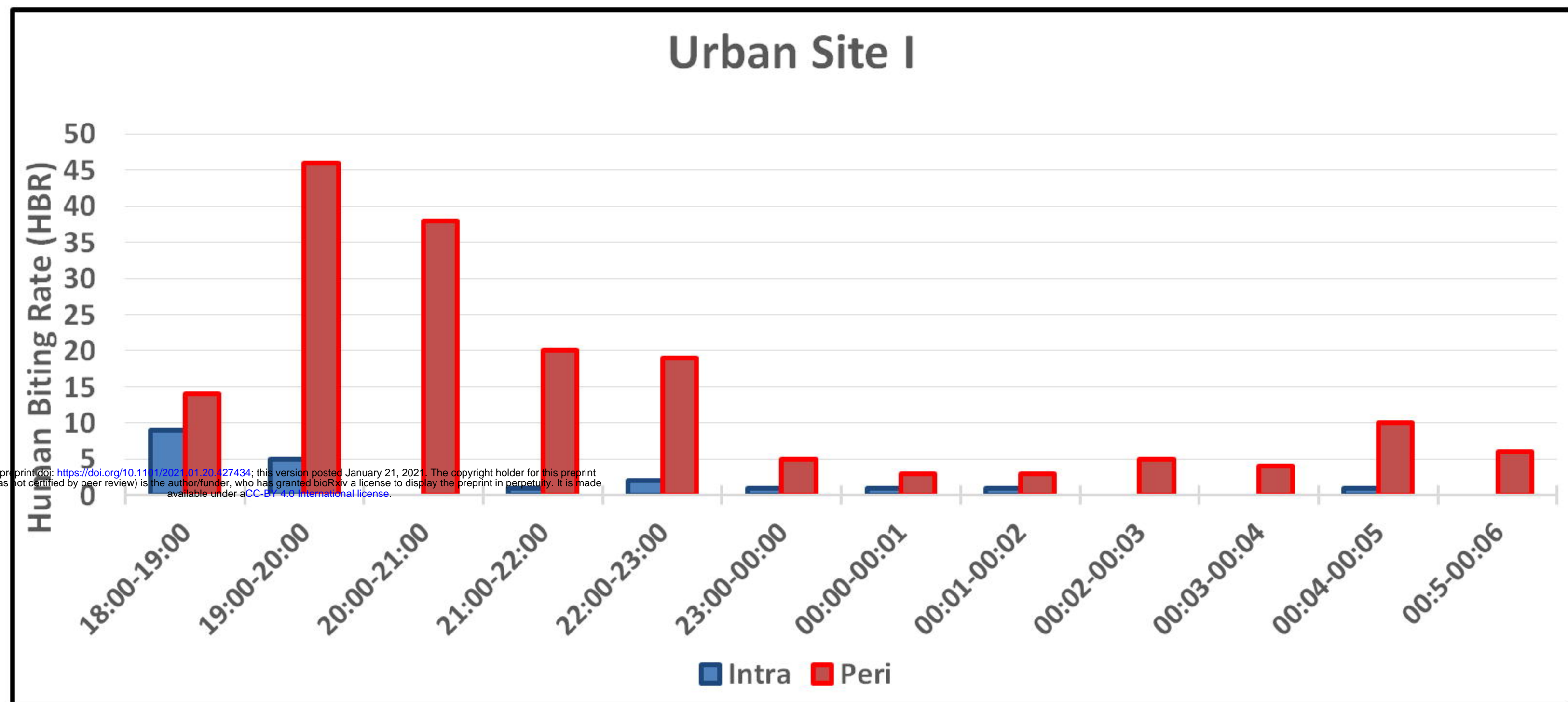
September



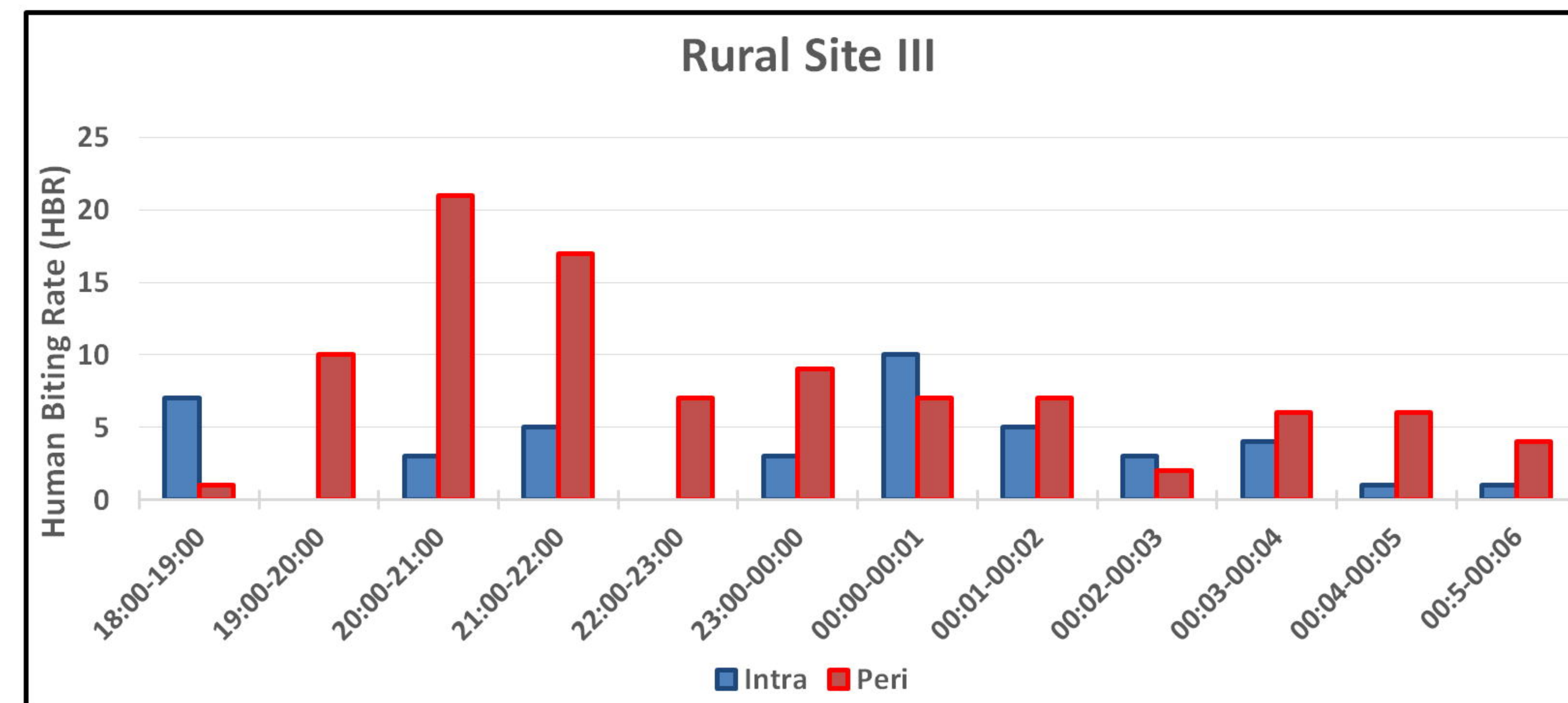
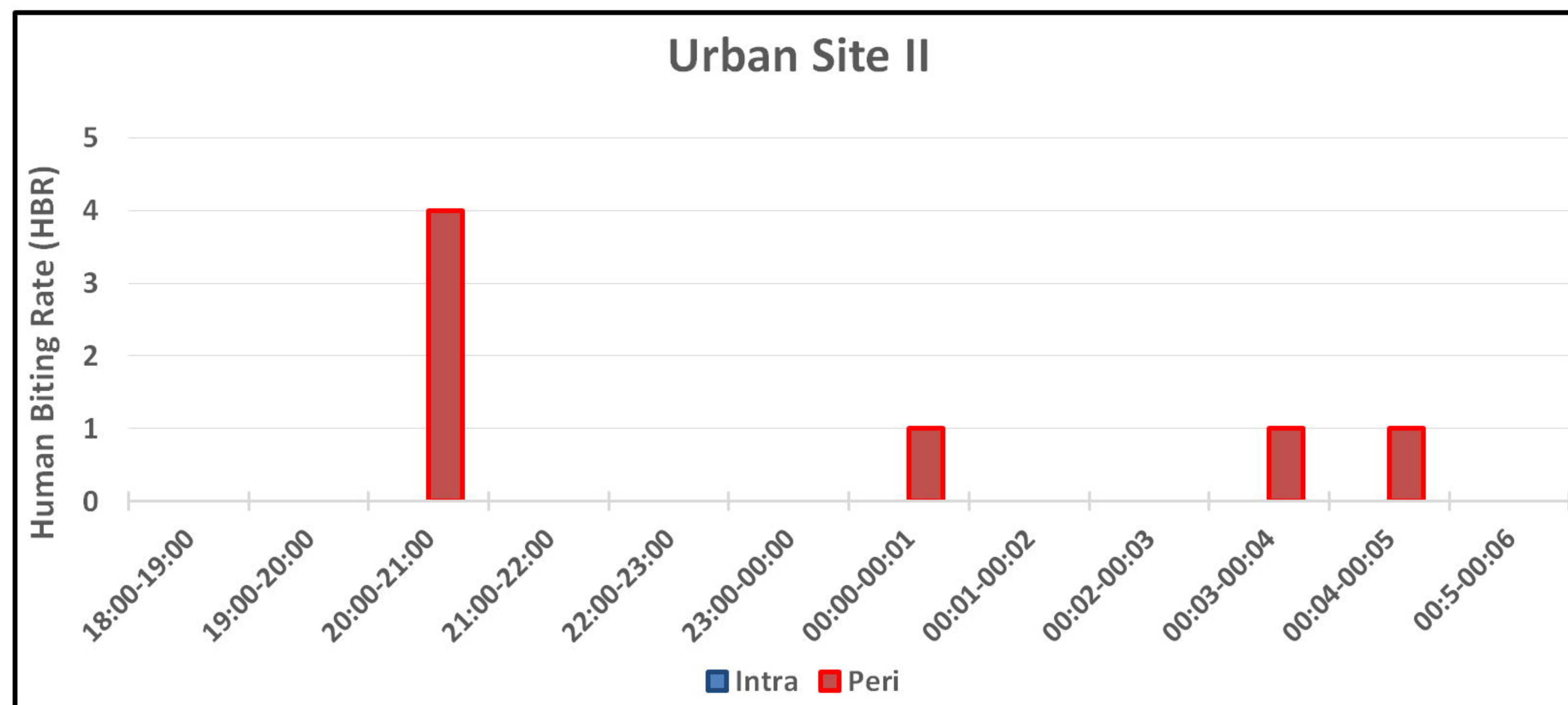
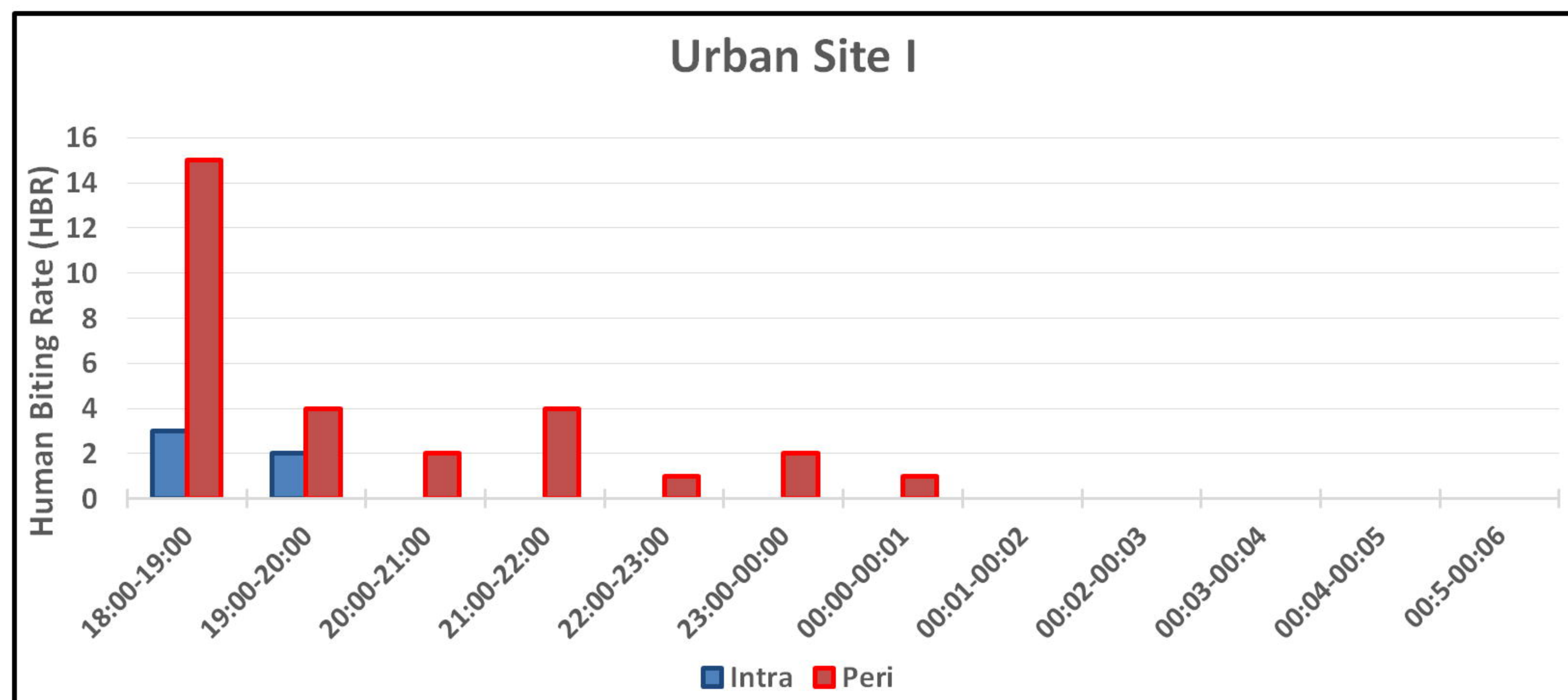
February



May



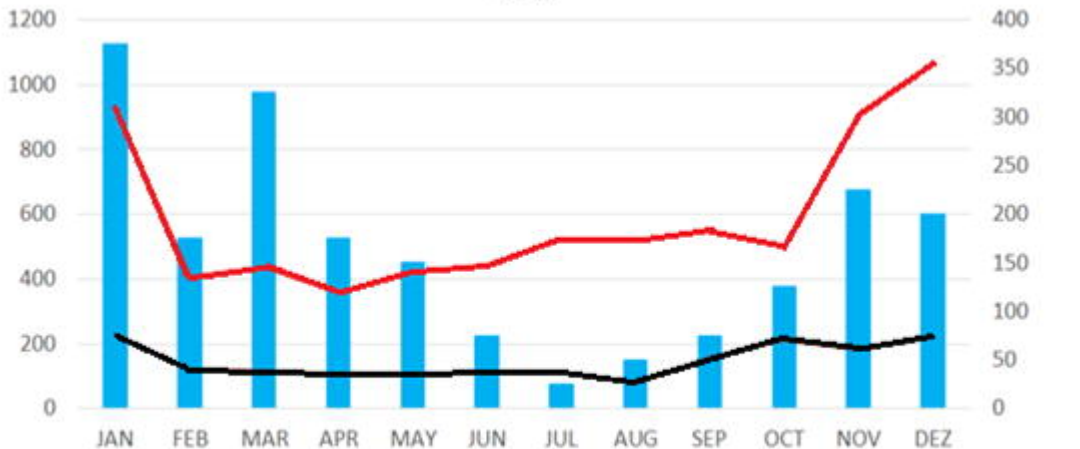
September



2016



2017



Precipitation

Vivax malaria

Falciparum malaria