Settlement preferences of leeches (Clitellata: Hirudinida) for different artificial substrates

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Abstract We examined the settlement preferences of leeches by analysing colonization patterns on artificial substrates. The intention was to check whether the type of material, texture and size of artificial substrates would influence the abundance of leeches. To test substrate type preferences, we used the following nine artificial substrates: polyester, polycarbonate, aluminium, glass, rubber, steel, polyethylene, ceramics and polypropylene. In addition, substrates of different textures (structural vs. smooth) and sizes (900, 400, 100 cm²) were used. We found that rubber, polyethylene and aluminium were most frequently chosen by the leeches. Surface structure and size of the objects were other factors which determined substrate selection by these organisms. Leeches more readily inhabited rough surfaces and the surfaces of smaller-sized objects. The species composition of leech assemblages on analysed artificial

substrates was dominated by predatory taxa of the genus *Erpobdella* and *Helobdella*.

Keywords Artificial materials · Substrate preferences · Settling behaviour · Substrate roughness and size

Introduction

Leeches are among the most numerous inhabitants of the littoral zone in aquatic ecosystems and they are important components of the food webs of different types of water bodies (Koperski, 2003; Strzelec et al., 2010). These predators and bloodsuckers attack various groups of hydrobionts and are themselves prey to many carnivores (Chernaya & Koval'chuk, 2009; Siciński, 2009). Previous studies have shown that one of the most important environmental factors which determine the number and diversity of this taxon is the presence of a solid substrate (Pawłowski, 1936; Sawyer, 1986; Krodziewska, 2003; Koperski, 2010). Most leeches inhabit stones, pieces of wood and other hard underwater substrates (Bielecki et al., 2008; Beracko & Košel, 2011; Utevsky et al., 2012). Many studies have also highlighted the role of macrophytes as agents increasing the abundance and biological diversity of Hirudinida (Sawyer, 1986; Bielecki et al., 2004). Leeches clearly avoid soft and unstable bottoms, including sand and silt, which do not allow

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them to attach their suckers and, as a result, significantly reduce their ability to move freely, feed or escape from predators (Agapow, 1980; Brönmark, 1992).

Anthropogenic pressure on the environment has resulted in an increased amount of solid waste introduced to aquatic ecosystems. The introduction of solid waste can replace natural habitats and cause fragmentation (e.g. changing the nature of the substrate, creating barriers), resulting in the disappearance of certain taxa and a decline in local biodiversity (Beaven et al., 2000; Brauns et al., 2007). However, most often they create new habitats and increase the space available for many plants and invertebrates (Winston, 1982; Chapman & Clynick, 2006; Jatulewicz, 2007; Czarnecka et al., 2009). Matysiak (1985), Bertman & Bielecki (1997), Czerniawska-Kusza (2004) have shown that leeches find favourable conditions for life on anthropogenic wastes and often prefer them, especially when the bottom is not suitable, e.g. sandy bottom (Czarnecka et al., 2009). Artificial substrates may also play a prominent role in the reproduction of leeches. Most species of the family Erpobdellidae and Piscicolidae deposit their cocoons on a hard surface (Sawyer, 1986; Young, 1988; Dimitriu & Shain, 2004; Mason et al., 2005). Waste as a sustainable and stable element of the environment seems to form a favourable place for leeches to leave cocoons. Results by Koperski (2003) have shown that the density of cocoons of the family Erpobdellidae laid on the artificial surface was several times higher when compared to those laid on other biotic and abiotic elements of the environment.

Factors influencing the settlement of leeches on different habitats are still poorly known. Most attention has been devoted to biological and chemical agents (Mazzoni et al., 2005; Garcia-Perez et al., 2007; Bisson & Torre, 2011; Bisson et al., 2012) and the role of light (Pawłowski, 1936; Elliott, 1973; Sawyer, 1986; Bielecki, 1999; Bielecki et al., 2011a). However, there is a lack of information concerning physical properties of substrates, particularly those of anthropogenic origin, which often represent the more frequent substrate type for settlement by these invertebrates.

The purpose of this study was to investigate leech settlement on several substrates, which could be potentially introduced into aquatic ecosystems as a result of human activity. We hypothesized that leech

numbers would depend on material composition of artificial substrate (experiment 1), texture (experiment 2) and size of the tested objects (experiment 3).

Materials and methods

Study site

The study was carried out in Lake Ukiel, the deepest and largest water body (maximum depth 43 m, mean depth 10.6 and surface area 412 ha) in Olsztyn city (Mazurian Lake District, northeastern Poland). Lake Ukiel is characterized by high species diversity and abundance of leeches (Żółtowski, 1965; Bielecki & Dzika, 2000; Bielecki et al., 2011b). Sampling was conducted in the northwestern part of the lake called Gutkowskie (54°62'N; 20°66'E) (Fig. 1). The selected study site possessed a substrate consisting of relatively uniform portions of sand, gravel and small stones. The emerged phytolittoral comprises sparse patches of *Phragmites australis* (Cav.) Trin. Ex Steudel, with no submerged macrophytes. During the study, fluctuation in various physical and chemical characteristics and water level were not noted (Table 1).

Experimental design

Settlement plates were exposed in the near shore area of Lake Ukiel from September to October in 2013. Leeches were collected from the plate's surface during five separate sampling events, occurring every twelve days starting September 13 and concluding October 31. The settlement unit composed of plates attached directly to the bottom, arranged horizontally, in three rows, parallel to the shore of the lake. The first row of plates was anchored to the bottom of the lake at a depth of 30 cm. The plates in the rows were spaced about 5 cm from each other. Schemes of the arrangement of plates in each variant of experiments are shown in Figs. 2, 3 and 4. After each collection, the plates were cleaned of contaminants and organisms that deposited on them by brush. Specimens collected from the three plates made of the same type of analysed substrate were combined into one composite sample. There were 5 replicates for each substrate type, representative of the 5 collection dates spanning the 12 sampling day intervals per collection period.

Fig. 1 Map of Lake Ukiel in northeastern Poland, with location of study site (S1)

Table 1 Physical and chemical parameters of the water during experiment period

Parameter	$\bar{x}$	SD
pH	9.0	0.0084
Dissolved oxygen (mg/l)	10.6	1.0840
Conductivity ($\mu\text{S}/\text{cm}$)	240	1.4832
Total suspended solids (mg/l)	158	0.9771
Temperature ($^{\circ}\text{C}$)	14.8	3.4928

$\bar{x}$ mean, SD standard deviation, $n = 5$

In the first experiment, to test the preference of material, the following substrates were used: 1-poly-carbonate (hard, rigid and transparent), 2-polyester (soft and flexible), 3-aluminium (hard and rigid), 4-glass (hard, rigid and transparent), 5-rubber (hard and flexible), 6-steel (hard and rigid), 7-polyethylene (hard and rigid), 8-ceramics (hard and rigid) and 9-polypropylene

(hard and rigid)—Fig. 2. The size of one plate was $300 \times 300 \times 2$ mm with exception to ceramic plates which had a different thickness than the rest of the elements ($300 \times 300 \times 5$ mm). We could not prepare or purchase a proper thickness of this type of plates. To analyse the effect of texture on settling preferences, in the second experiment, three sets of settlement units were constructed that comprised plates of (1) smooth and frosted glass, (2) smooth and rough aluminium and (3) smooth and riffled rubber (Fig. 3). The riffle height on the plates was 1 mm, whereas the distance between two riffles was 3 mm. The third experiment tested how plate size affected the density of leeches. Polyethylene, polypropylene, aluminium and glass settlement units of three sizes were deployed at the bottom ($300 \times 300 \times 2$ mm, $200 \times 200 \times 2$ mm, $100 \times 100 \times 2$ mm)—Fig. 4. Leeches collected from the plates were moved into the laboratory where they were relaxed in 10% ethyl alcohol (10 min) and preserved in 70% ethyl alcohol. Collected individuals were classified to the species level based on Pawłowski (1936).

Fig. 2 Experimental design and position of plates used to test for leech settlement preferences based on substrate type; *PC* polycarbonate, *PES* polyester, *AL* aluminium, *GL* glass, *RU* rubber, *ST* steel, *PE* polyethylene, *CER* ceramics, *PP* polypropylene

Fig. 3 Experimental design and position of plates used to test for leech settlement preferences based on substrate texture; *GL* glass, *AL* aluminium, *RU* rubber, *SM* smooth, *RO* rough, *RI* riffled

Statistical analysis

Due to the heterogeneity of variance (Levene test, $P = 0.0004$), a Kruskal–Wallis test was used for assessing the significant differences in the abundance of leech assemblages on the particular materials (experiment 1). In the case of significant results, we applied a post hoc multiple comparison of mean ranks

for all groups. Differences between the number of individuals collected from a particular material with a smooth and structural surface (experiment 2) and differences between the leech number and density on plates of different sizes (experiment 3) were compared using two-way analyses of variance (ANOVA) (factors: material and texture in experiment 2, material and size in experiment 3). To avoid violations of ANOVA assumptions of normality and homoscedasticity (checked with Levene test), we log-transformed the data for both analyses. When an overall ANOVA produced significant results, a Turkey's post hoc test was used to find the difference between the particular groups. The results were regarded as statistically significant at $P < 0.05$. Statistical analyses and figures were made using STATISTICA 10 PL, (StatSoft, Inc. (2011), STATISTICA (data analysis software system), version 10. www.statsoft.com).

Results

In the first experiment, a total of 512 leeches were collected. These individuals were represented by five

Fig. 4 Experimental design and position of plates used to test for leech settlement preferences based on substrates size: *PE* polyethylene, *PP* polypropylene, *AL* aluminium, *GL* glass, *A* plate size 300 × 300 mm, *B* plate size 200 × 200 mm, *C* plate size 100 × 100 mm

families and twelve leech species. In the analysed assemblages, the most numerous species collected were *Erpobdella octoculata*, *E. nigracollis* and *Helobdella stagnalis* accounting for 88.67% of total collected individuals. The polyester substrates differed significantly in the number of leeches from polyethylene, aluminium and rubber (Kruskal–Wallis test: $H = 24.47$; $P = 0.0019$). Polyester was significantly less settled with leeches than other materials mentioned above (Fig. 5).

The maximum number of species, nine and eight, was found on aluminium, polyethylene, rubber and ceramics. The minimal number, two and three, was found on polyester and steel (Table 2). *E. octoculata* and *E. nigracollis* were dominant taxa found across all substrate types (Table 2). However, the percentage of *E. octoculata* and *E. nigracollis* in the leech assemblages that inhabited various substrates was different, ranging from 20 to 68.18% and 10.91 to 80%, respectively (Table 2). In addition to these two species, *H. stagnalis* also had a high percent of occurrence, ranging from 6.45 to 19.57%. However, this taxon was not present in all the tested substrates (Table 2).

Deliberations about which substrate the species prefer were conducted based on the four species accounting for the maximum percentage number and

frequency of occurrence (Table 2, 3). *E. octoculata* was found in the highest proportions on the polyethylene and rubber substrates were these accounted respectively for 21.4 and 22.57% of total assemblage (Table 3). Other materials were settled less by the individuals of *E. octoculata*, where the species never exceeded 15% of the total assemblage. *E. nigracollis* were found in the highest proportions in rubber (25.00%), aluminium (20.31%) and polyethylene (17.19%), and never exceeded 15% in all other substrates (Table 3).

The maximum percentage of all the collected specimens of *H. stagnalis* was recorded, similar to *E. octoculata*, on polyethylene, aluminium and rubber, 31.88, 26.09 and 18.84%, respectively. There were not any individuals of *H. stagnalis* on polyester and steel (Table 3). The percentage number of *H. stagnalis* individuals on the other substrates did not exceed 9% of the total colonizing assemblage. The maximum number of *E. monostriata* inhabited rubber, polyethylene and polypropylene (Table 3). On these substrates, leeches of this species ranged from 20.69 to 24.14%. The occurrence of *E. monostriata* was not noted on polyester.

A significant interaction between the material and plate texture was found (ANOVA: $F_{2, 18} = 9.35$;

Fig. 5 Average number of leeches on different artificial substrates: *PC* polycarbonate, *PES* polyester, *AL* aluminium, *GL* glass, *RU* rubber, *ST* steel, *PE* polyethylene, *CER* ceramics, *PP* polypropylene; statistically significant differences are indicated by different letters

Table 2 Species composition (%), number of individuals in the leech assemblages (*N*) and frequency of species on different materials (F)

	PC <i>N</i> = 27	PES <i>N</i> = 5	AL <i>N</i> = 92	GL <i>N</i> = 22	RU <i>N</i> = 115	ST <i>N</i> = 21	PE <i>N</i> = 113	CER <i>N</i> = 55	PP <i>N</i> = 62
<i>E. octoculata</i>	40.74	20.00	41.30	68.18	50.43	57.14	48.67	61.82	53.23
<i>E. nigricollis</i>	37.04	80.00	28.26	13.64	27.83	33.33	19.47	10.91	29.03
<i>H. marginata</i>	3.07	–	–	–	–	–	–	1.82	–
<i>E. monostrata</i>	3.07	–	2.17	4.55	6.09	9.52	6.19	5.45	9.68
<i>H. stagnalis</i>	14.81	–	19.57	9.09	11.30	–	19.47	10.91	6.45
<i>G. concolor</i>	–	–	1.09	4.55	0.87	–	0.88	–	–
<i>E. testacea</i>	–	–	–	–	0.87	–	2.65	5.45	–
<i>G. complanata</i>	–	–	2.17	–	1.74	–	1.77	1.82	1.61
<i>A. striata</i>	–	–	1.09	–	–	–	0.88	1.82	–
<i>A. heteroclita</i>	–	–	1.09	–	–	–	–	–	–
<i>A. hyalina</i>	–	–	3.26	–	–	–	–	–	–
<i>A. papillosa</i>	–	–	–	–	0.87	–	–	–	–

PC polycarbonate, PES polyester, AL aluminium, GL glass, RU rubber, ST steel, PE polyethylene, CER ceramics, PP polypropylene

$P = 0.0016$). With regards to particular materials, a significantly higher number of leeches have been found on a structural surface than on a smooth surface of the substrates made of aluminium—Fig. 6. The average number of collected leeches on the plates of various sizes was similar (ANOVA: $F_{2, 96} = 2.73$; $P = 0.0699$) ranging from 9 on the surface of 100 cm² to 12 on those of 400 and 900 cm². The average density of leeches on plates of various sizes ranged from 49 (glass substrate of 900 cm²) to 880 individuals per m² (aluminium substrate with an area of 100 cm²)—Fig. 7. A significant interaction between the substrate material and its size was recorded (ANOVA: $F_{6, 96} = 4.67$; $P = 0.0003$). A higher density of leeches was found on 100 cm² plates made of aluminium, polypropylene and glass in relation to those 900 cm², on 100 cm² plates made of polypropylene and glass compared to those 400 cm² and on 400 cm² glass surfaces with respect to those of 900 cm². In the case of polyethylene substrates of different sizes, there were no significant differences (Fig. 7).

Discussion

Studies concerning the influence of physical parameters of the environment and types of substrates,

including artificial ones, are quite common for macrozoobenthos (Bonzini et al., 2008; Jae-Hoon et al., 2013; Spagnolo et al., 2014). Much has been written about their use as a tool in the assessment of aquatic environment quality (De Pauw et al., 1994; Czerniawska-Kusza, 2004; MacDonald et al., 2012; Loke et al., 2014) or biomass production of phyto and zoobenthos (Moura et al., 2007; Plá & Alveal, 2012). In relation to inland waters, research has mainly focused on their habitat-creation role and concerns entire groups of benthic fauna (De Pauw et al., 1986; De Pauw et al., 1994; Czerniawska-Kusza, 2004; Poznańska et al., 2009; Czarnecka et al., 2009). Studies about the preferences of particular species are still rare and tend to focus on sessile organisms (Czarneński et al., 2004; Kobak, 2004; Kobak & Januszewska, 2006; Porter & Marsden, 2008; Richards & Beger, 2011; Braccia et al., 2014; Spagnolo et al., 2014). With regard to free-living taxa, most studies have focused on representatives of Gammaridae, especially non-native species in European waters (Devin et al., 2004; Czarnecka et al., 2010; Kobak et al., 2013), but there is still little information about leech settlement on human-made substrates.

Indirectly, we can obtain this information from research devoted to entire groups of benthic fauna, which includes representatives of Hirudinida.

Table 3 Species preferences with respect to different types of materials

		PC	PES	AL	GL	RU	ST	PE	CER	PP
<i>E. octoculata</i>	<i>N</i>	11	1	38	15	58	12	55	34	33
	%	4.28	0.39	14.79	5.84	22.57	4.67	21.40	13.23	12.84
<i>E. nigricollis</i>	<i>N</i>	10	4	26	3	32	7	22	6	18
	%	7.81	3.13	20.31	2.34	25.00	5.47	17.19	4.69	14.06
<i>H. marginata</i>	<i>N</i>	1	–	–	–	–	–	–	1	–
	%	50	–	–	–	–	–	–	50	–
<i>E. monostrata</i>	<i>N</i>	1	–	2	1	7	2	7	3	6
	%	3.45	–	6.90	3.45	24.14	6.90	24.14	10.34	20.69
<i>H. stagnalis</i>	<i>N</i>	4	–	18	2	13	–	22	6	4
	%	5.80	–	26.09	2.90	18.84	–	31.88	8.70	5.80
<i>G. concolor</i>	<i>N</i>	–	–	1	1	1	–	1	–	–
	%	–	–	25.00	25.00	25.00	–	25.00	–	–
<i>H. marginata</i>	<i>N</i>	–	–	–	–	–	–	–	1	–
	%	–	–	–	–	–	–	–	100	–
<i>E. testacea</i>	<i>N</i>	–	–	–	–	1	–	3	3	–
	%	–	–	–	–	14.29	–	42.86	42.86	–
<i>G. complanata</i>	<i>N</i>	–	–	2	–	2	–	2	1	1
	%	–	–	25.00	–	25.00	–	25.00	12.50	12.50
<i>A. striata</i>	<i>N</i>	–	–	1	–	–	–	1	1	–
	%	–	–	33.33	–	–	–	33.33	33.33	–
<i>A. heteroclita</i>	<i>N</i>	–	–	1	–	–	–	–	–	–
	%	–	–	100	–	–	–	–	–	–
<i>A. hyalina</i>	<i>N</i>	–	–	3	–	–	–	–	–	–
	%	–	–	100	–	–	–	–	–	–
<i>A. papillosa</i>	<i>N</i>	–	–	–	–	1	–	–	–	–
	%	–	–	–	–	100.00	–	–	–	–

N number of individuals, % percent of total colonizing assemblage, *PC* polycarbonate, *PES* polyester, *AL* aluminium, *GL* glass, *RU* rubber, *ST* steel, *PE* polyethylene, *CER* ceramics, *PP* polypropylene

Nevertheless, there is a lack of data about leech preferences to artificial substrates. To the authors' knowledge, this is the first time such an analysis has been conducted on this group of organisms. According to the literature, some taxa are more numerous on artificial substrates in relation to the natural bottom (Czarnecka et al., 2009); this particularly applies to thigmotactic organisms, which include leeches (Czer-niawska-Kusza, 2004). In general, they inhabit tight-fitting places to achieve maximum body contact (Bisson & Torre, 2011).

This study indicates that leech assemblages on artificial substrates are dominated by predatory taxa. Although the collected material was characterized by high species diversity and rare species of leeches were found (*Alboglossiphonia hyalina*, *A. striata* and *A. papillosa*), the only representative of bloodsucking taxa was *Hemiclepsis marginata*. However, several

previous studies have already identified other species in this reservoir belonging to this particular trophic guild (Żółtowski, 1965; Bielecki & Dzika, 2000; Bielecki et al., 2011b). Difference in the species composition, described by us in comparison with previous research, may result from the fact that above-mentioned authors used several different methods of collection of leeches including sampling directly from the fish hosts and manually from submerged elements of the environment. Koperski (2003) explained the absence of bloodsuckers on artificial substrates by unequal and concentrated distribution, but in our opinion, their physiological state is equally important. When hungry, bloodsucker species emerge from their hiding places beneath submerged substrates and move to habitats where contact with a host is more probable (Mann, 1962). More often they occur on the external surfaces of solid elements of the environment, near the

Fig. 6 Average number of leeches on substrates of different textures: *SM* smooth, *RO* rough/riffled, *RU* rubber, *GL* glass, *AL* aluminium; statistically significant differences are indicated by different letters; error bars indicate confidence levels of 0.95

Fig. 7 Average density of leeches on substrates of various sizes made of different materials: *AL* aluminium, *PE* polyethylene, *PP* polypropylene, *GL* glass; statistically significant differences are indicated by different letters; error bars indicate confidence levels of 0.95

water surface or reservoir banks, where they wait for their host (Pawłowski, 1936; Bielecki, 1999; Bielecki et al., 2011a). Therefore, we would conclude that

Piscicola leeches were not found in our samples as they are likely attached to their hosts, which are consistent with their life cycles. The other species may

not stay near the bottom zone and, as a result, they were beyond the range of the experiment.

In our experiment, all collected species on artificial substrates represent two morphological body forms: “leaf” (genus *Alboglossiphonia*, *Glossiphonia*, *Hemiclepsis*, *Helobdella*) and “tape” (*Erpobdella*) (division in accordance with Bielecki, 1997). Possibly, due to the strong dorsoventrally flattened body, these two forms show a greater affinity for the hard substrate than other shapes found in leeches and are more dependent on it, which is associated with their frequent settlement on artificial substrates. The cylindrical form was not recorded during the study.

An important factor which could influence the density and the composition of the leech community in artificial habitats is the presence of periphyton. The results of Koperski (2003) have shown that a significantly lower density of molluscivorous species is reported on the artificial substrates with poorly developed biofilm. The presence of biofouling algae entailed an increase in the individuals of snails and thus of leeches of the family Glossiphoniidae, which feed on their body fluids. In turn, a thick layer of periphyton hinders capturing success and feeding rates of omnivorous leeches of the genus *Erpobdella* and *Helobdella*, resulting in their greater number on substrates with a less-developed layer of biofouling algae (Koperski, 2003), which is confirmed in our study. In our experiments, periphyton may develop only in small degrees on settlement units exposed to the environment for about 2 weeks, which makes them unattractive for gastropods and thus for molluscivores of the Glossiphoniidae family. This is probably why during the experiment a dominance of omnivorous species such as *E. octoculata*, *E. nigricollis*, *E. monostriata* and *H. stagnalis* was noted.

Our research has shown that leeches inhabit structural substrates in greater numbers than smooth ones. Similar to many other benthic species (Marsden & Lansky, 2000; Czarnołęski et al., 2004; Saliu & Ovuorie, 2006), leeches prefer this texture type because of the quick and intensive development of the biofouling alga layer. Despite the low development of periphyton during the experiment, it could spread more on a rough surface. Bergey (1999) showed that small crevices on rough surfaces are used by the diatoms as refuge against water waves and grazer pressure, resulting in more intensive development of periphyton on less smooth surfaces. Hart (1978),

Clifford et al. (1989) and Bergey (1999) noted that small aquatic organisms and detrital particles accumulate in depressions on rough surfaces which result in greater diversity and abundance of macroinvertebrates. Thus, an increase in food resources can be a key factor in more numerous occurrences of both predator and parasite leeches.

In our study, the most densely inhabited substrates were rubber, polyethylene and aluminium, and the least frequently colonized was polyester. This tendency is consistent with observations of other authors (Pawłowski, 1936; Mann, 1962; Sawyer, 1986; Czarnecka et al., 2009; Koperski, 2010) and confirms preferences for the hard surfaces. Leeches less frequently colonize a soft substrate, subjected to the movements of water masses. This finding was consistent with the results of Czarnecka et al. (2009), who showed that there were no leeches on soft materials such as cotton, nylon or linen. Unstable substrates decrease their ability to move, escape or obtain food (Agapow, 1980). This is especially important for predatory leeches of the genus *Helobdella* and *Erpobdella* (Adamiak-Brud et al., 2014). Notwithstanding, substrates made of glass and polycarbonate are hard and guarantee sufficient stability, Hirudinida settled on them in a relatively small number may be because of the greater amount of solar radiation. All of the above-mentioned materials were transparent, which prevents settlement for negative phototactic leeches (Pawłowski, 1936; Mann, 1962; Elliott, 1973; Sawyer, 1986).

The results of field experiments on leech density on substrates of different sizes have shown that smaller plates have significantly higher densities of individuals. This may be due to several factors, primarily leeches tend to inhabit the edges of various submerged elements, which were observed in our study (Garcia-Perez et al., 2007; Bisson & Torre, 2011). The substrate is even more attractive for them, when circumference to the surface area ratio is higher, which ensures a greater availability of attractive places for leeches to inhabit. Perhaps there is a minimum size of objects of a substrate element beyond which leeches are unable to occupy; this may be conditioned by body size, reduction of movement capacity, etc.

Leech density also depends on the presence of conspecifics (Lang, 1974; Garcia-Perez et al., 2007; Bisson & Torre, 2011; Bisson et al., 2012). When choosing a place to colonize, leeches actively penetrate

micro-habitats (Garcia-Perez et al., 2007). If this exploratory phase is accompanied by sufficient physical contact with conspecifics, leeches form a group and inhabit such regions (Bisson & Torre, 2011; Bisson et al., 2012). In order to settle they must exceed a certain threshold of meetings. Too few contacts result in further exploration and selection of another place (Bisson & Torre, 2011). The smaller plates have a higher leech density, and since they are more crowded, it is easier to have direct contact between individuals. It should be emphasized that a group enhances their stability and lifetime in a particular micro-habitat by adding successive specimens (Bisson et al., 2012). This mechanism is connected with the secretion of neuromodulators, serotonin 5-HT and to a lesser extent dopamine into the bloodstream of individuals during physical contact with members of the same taxon (Bisson & Torre, 2011; Bisson et al., 2012). The benefits of group formation by leeches are not understood, but Bisson et al. (2012) have observed a seasonal variation in the creation of social agglomeration. In late spring and summer, leeches are more active and prone to social behaviour, while in the winter their mobility and willingness to form groups slightly decrease. This may indicate that gregariousness plays a role in finding a sexual partner. However, there is still a lack of information whether encounters with individuals of other species of Hirudinida initiate the agglomeration and affect the choice of location for settlement.

In conclusion, all our hypotheses have been corroborated. We confirm that leech abundance depends on the type of material, from which the artificial substrates are made, and their texture. There is a high probability that leeches numerously inhabit hard anthropogenic substrates such as rubber, polyethylene and aluminium with a rough surface. The density of these organisms is also affected by the size of artificial objects. Indeed, leeches prefer smaller elements. The above considerations provide information on artificial substrates from the point of view of their attractiveness for leeches. They can become a theoretical base that supports the preparation of anthropogenic substrates for collecting representatives of Hirudinida and analysing water quality with biotic indices. Improvement of sampling methods is important because until now the universal method of quantitative leech collection has not yet been developed and current methods have many limitations.

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