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Permanent colonization of creek sediments, creek water and limnic water plants by four *Listeria* species in low population densities

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Abstract: During a 1-year longitudinal study, water, sediment and water plants from two creeks and one pond were sampled monthly and analyzed for the presence of *Listeria* species. A total of 90 % of 30 sediment samples, 84 % of 31 water plant samples and 67 % of 36 water samples were tested positive. Generally, most probable number counts ranged between 1 and 40 g^{-1}, only occasionally >110 cfu g^{-1} were detected. Species differentiation based on FT-IR spectroscopy and multiplex PCR of a total of 1220 isolates revealed *L. innocua* (46 %), *L. seeligeri* (27 %), *L. monocytogenes* (25 %) and *L. ivanovii* (2 %). Titers and species compositions were similar during all seasons. While the species distributions in sediments and associated *Ranunculus fluitans* plants appeared to be similar in both creeks, RAPD typing did not provide conclusive evidence that the populations of these environments were connected. It is concluded that (i) the fresh-water sediments and water plants are year-round populated by *Listeria*, (ii) no clear preference for growth in habitats as different as sediments and water plants was found and (iii) the RAPD-based intraspecific biodiversity is high compared to the low population density.

Keywords: biodiversity; fresh water; *Listeria monocytogenes*; *Listeria* spp.; sediment; water plants.

Dedication: This work is dedicated to the memory of Professor Peter Böger. His continuous support and thoughtful guidance over many years encouraged the senior author to aim at an academic career in microbial ecology and evolutionary biology.

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1 Introduction

The genus *Listeria* consists of the species *Listeria monocytogenes, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri, L. grayi* [1], *L. marthii* [2], *L. rocourtiae* [3], *L. fleischmannii* [4] and *L. weihenstephanensis* [5]. The species *L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis* [6], *L. booriae* and *L. newyorkensis* [7] were validly described recently. *L. monocytogenes* is a zoonotic facultative pathogen responsible for severe infections with high mortality rates in susceptible population groups such as infants, pregnant women, immune compromised and elderly persons [8–12]. Owing to its ability to overcome food preservation barriers such as low temperatures, low pH and high salt concentrations [13], the pathogen poses a potential risk to human health. Especially the cold-tolerant nature of *L. monocytogenes* is an important parameter for infection by contaminated food since low initial numbers of the bacterium can grow to infective doses even in the refrigerator of consumers.

One source of *L. monocytogenes* are farm animals such as cows. A relationship between listeriosis in domestic animals and the feeding of low quality, improperly fermented silages with pH values >4.0 has been reported [14, 15]. *L. monocytogenes* is carried asymptomatically in silage-fed livestock and is dropped in feces [16]. Shedding of the pathogen by a variety of domestic animals like sheep and cattle has been reported [16–20]. A possible route of infection into the human food chain, for instance, is through the ingestion of uncooked food plants grown on soils irrigated with contaminated water or via the consumption of vegetables cultivated on manure treated fields [21–24]. Also, ready-to-eat food or dairy products that have not been pasteurized, such as raw milk cheese or smoked salmon pose significant risks to consumers. Due to its importance as a food-borne pathogen, numerous studies on this genus deal with the occurrence and tracing of *L. monocytogenes* in food processing plants and in farm environments [17, 25–30].

In early publications, a ubiquitous distribution and saprophytic nature of the genus *Listeria* has been

suggested [31, 32]. *L. monocytogenes* has been isolated from different natural surroundings including soil [31, 33–38], vegetation [24, 36, 37], surface water [38–44] and wild animals [45–48]. In general, systematic studies concerning the occurrence of *L. monocytogenes* in soil and fresh water are rare. Even less is known on the occurrence of the other species of *Listeria* in habitats different from farm environments and food processing plants (for a review, see Sauders and Wiedmann [49]). However, studying the ecology of *Listeria* is important because knowledge on environmental niches will help to understand contamination routes. Therefore, the objective of this work was to systematically evaluate the occurrence, intraspecific biodiversity, population density and temporal fluctuation of four *Listeria* species in the limnic habitats fresh water, water plants and sediment.

2 Materials and methods

2.1 Sampling sites and sampling procedures

Samples were taken from (i) the creek Moosach in Freising, which discharges eastward into the river Isar, (ii) the creek Wolnzach in the city Wolnzach, a branch of the river Ilm, which discharges into the river Abens, which is a tributary of the river Danube, and (iii) the pond Postweiher in the city Wolnzach. The two creeks are separated by a water divide. The surrounding of the sampling site at Moosach was rural with meadows that had not been used as grazing land for more than 15 years. Manure from livestock operations had not been applied for many years. Thus, this site was not proximately influenced by cattle farming. In contrast, the sampling site at the creek Wolnzach was directly impacted by cattle grazing in the summer months since domestic animals were kept on an adjacent pasture. The pond Postweiher at Wolnzach is located within playing fields, streets and houses, but no cattle grazing areas were located nearby. The pond is fed by two subsurface wells and discharges into the creek Wolnzach upstream of the Wolnzach sampling site. The water levels of this pond were extremely variable over the sampling period. Due to high water levels from February to August, sampling of sediment was not feasible. In contrast to the creeks, water of this site was frozen in January and February.

Samples were collected monthly over a 1-year period. The sampled matrices were sediment, water plants and water. Also, the water temperature was recorded at every sampling. pH values of water and sediment samples were determined once according to the conventional pH-meter method VDLUFA I A 5.1.1 proposed by the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA Verlag, Darmstadt, Germany).

From the creeks Moosach and Wolnzach, the water plant investigated was *Ranunculus fluitans*. At the pond Postweiher, *R. fluitans* was not growing and the water plant flora varied over the sampling period. Therefore, water plants examined from Postweiher consisted of various genera: *Lemna trisulca* and *Ceratophyllum* spec. (October and July), *Lemna trisulca* (April, June, August and September), *Mentha* × *piperita* (February and March), *Carex* spec (January) and a representative of the *Nymphaceae* (May).

In total, 36 water samples, 30 sediment and 31 water plant samples were analyzed. Using sterile gloves, sediment samples were collected up to a depth of approximately 5 cm and transferred into sterile bags. Plants were gently rinsed to separate the roots from sediment traces before placing them into sterile bags. The root mass was below 1 % of the total mass of the plant sample. Plants were also investigated without roots. Water samples were collected using sterile glass flasks. All samples were transported at ambient temperature and analyzed within 2 h after sampling.

2.2 Determination of *Listeria* cell counts by most probable number (MPN)

For the enumeration of *Listeria* spp. from sediment and water plant matrices, two different selective enrichment media were compared in a preliminary test. The media tested were ½ Fraser broth (Sifin, Berlin, Germany) and UVM II broth (University of Vermont Medium according to the US Department of Agriculture, Oxoid, Wesel, Germany). Selective enrichments were performed according to § 64 LFGB DIN EN ISO 11290-2 applying the following modifications: 100 g of sample was added to 400 ml of ½ Fraser basis broth or UVM II broth, leaving out the selective media components. The water plant samples were minced using an Ultraturax machine (Janke Kunkel, Staufen, Germany). Samples were incubated 1 h at room temperature to permit resuscitation of stressed cells. Then, aliquots of the sample preparations were transferred to ½ Fraser broth or UVM II broth supplemented with corresponding selective agents producing 10-fold dilutions containing 10, 1 and 0.1 g of sample matrix. All dilution steps were prepared five-fold as specified in DIN EN ISO 7218:2005. Inoculated broth were stirred thoroughly and incubated 24 h at 30 °C. Of each enriched dilution sample,

50 µl was streaked onto PALCAM agar (Sifin, Berlin, Germany) and incubated at 30 °C for 24–48 h.

2.3 Confirmation of presumptive *Listeria* spp. and differentiation to the species level

From each PALCAM plate, well-detached presumptive *Listeria* colonies were picked and streaked onto tryptone soy agar (Oxoid, Wesel, Germany). After incubation for 24–48 h at 30 °C, confirmation was performed by examination under obliquely transmitted light. Atypical isolates were examined microscopically and by plating on LAOA agar [50]. Cell counts were determined according to DIN EN ISO 7218:2005 using MPN tables.

Differentiation to the species level was performed using FT-IR spectroscopy [51] and multiplex PCR [52]. Template DNA for the latter method was prepared as follows: cells were grown on tryptone soya agar for 48 h at 30 °C, half a loop (2 mm in diameter) of cells were collected and suspended in 200 µl sterile water containing 10–15 mg of zirconia/silica beads (0.1 mm in diameter; Roth, Karlsruhe, Germany). The suspension was treated twice for 45 s at 6.5 m/s in a ribolyser (PastPrep, Eschwege, Germany) at ambient temperature and subsequently heated for 5 min at 95 °C. After heating, a centrifugation step at 18,000× *g* for 10 min was performed. The supernatant was used for further analysis. Species determination of ambiguous isolates was investigated either by employing a commercial biochemical differentiation kit (API *Listeria*; bioMérieux, La Balme les Grottes, France) [53] or by 16S rDNA sequencing as described elsewhere [54].

2.4 RAPD typing of *Listeria* spp.

Subtyping of *Listeria* isolates was performed by randomly amplified polymorphic DNA analysis. The use of a combination of oligonucleotide primers in a single PCR reaction is suggested to provide more reproducible patterns [55, 56]. Therefore, three oligomer primers which had been successfully applied in other studies were used simultaneously. The primers were M13 (5′-GAG GGT GGC GGT TCT-3′) [57], Eric2 (5′-AAG TAA GTG ACT GGG GTG AGC G-3′) [58] and inlA.F (5′-CAG GCA GCT ACA ATT ACA CA-3′) [25]. These primers address different genomic regions: M13 is directed to sections containing micro- or mini-satellites, Eric.2 focuses repeated regions and inlA.F targets internalin genes [25]. RAPD PCR reactions were performed in a final volume of 25 µl containing 50–100 ng of template DNA, 3 µl of 10× PCR Buffer IV (Thermo Fisher Scientific, Dreieich, Germany), 1 mM dNTP Mix (Thermo Fisher Scientific), 3.5 mM $MgCl_2$, 6.25 pmol/µl of each primer and 0.5 U of Thermoprime Plus Taq Polymerase (Thermo Fisher Scientific). Reaction conditions were as follows: 94 °C for 2 min, 35 cycles, each at 94 °C for 1 min, 35 °C for 1 min and 72 °C for 2 min. The final step was done 5 min at 72 °C. The PCR products were resolved by agarose gel electrophoresis (1.6 % w/v rotiagarose; Roth) in 0.5× TBE buffer (50 mM Tris, 45 mM boric acid, 0.5 mM EDTA, Roth) at 300 V for 1 h. After separation, DNA was visualized by UV light after staining with ethidium bromide (1 µg/ml). Image acquisition was carried out using an Image Station 440CF (Kodak, Rochester, NY, USA). To test reproducibility, from every isolate RAPD testing was performed twice applying cell lysate from two independent cultivations. One *L. monocytogenes* and one *L. innocua* strain with known RAPD band pattern served as positive controls for validation of PCR runs.

For normalization of gels and band detection, the TL120 software was used (biostep, Jahnsdorf, Germany). Parameters for band detection are listed in the Supplementary Table 2. For band pattern analysis, a database was established using biostep TL120DM software. Applying the mentioned parameters for band pattern analysis, UPGMA clusters of the control strains exhibited similarities ≥95 %. Pattern reproducibly differing by at least one band were defined as different RAPD types.

2.5 Statistical treatment of data

For the total numbers of *Listeria* isolates (Table 3 and Figure 1), mean values and standard deviations have been calculated (Figure 2). To consider outliers, median values are also given in Table 3. Generally, a very high variance was found in all data sets which is typical for longitudinal ecological studies. A potential relatedness of sediment and *R. fluitans* populations based on the RAPD patterns of the isolates was evaluated by estimating the P values using the exact Fisher test (data not shown).

3 Results and discussion

3.1 Comparison of enrichment media

For enumeration, direct plating methods are suitable if *Listeria* populations >100 cfu/g are present in the sample

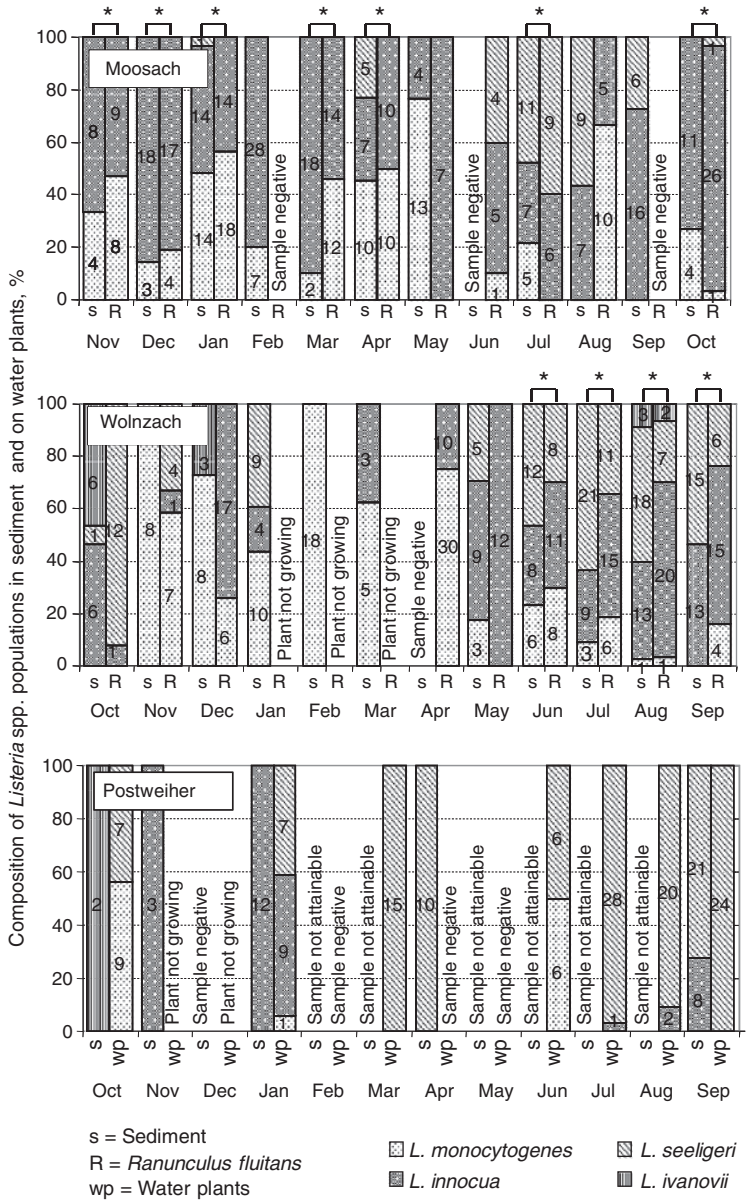

Figure 1: Species composition of the *Listeria* populations in creek sediment and on water plants of three sampling sites determined in a longitudinal study. Numbers within the bars indicate the absolute numbers of isolates analyzed. s, sediment; R, *R. fluitans*; wp, water plants. Asterisks mark samples of sediment with corresponding *R. fluitans* specimen with apparently similar species compositions.

matrix. For quantification of populations <100 cfu/g, an enrichment procedure followed by an MPN analysis is necessary. Although MPN methods are commonly used to quantify *Listeria* in food samples, reports on the use of MPN techniques in samples from natural environments are rare. Due to little information on the quantification of *Listeria* in natural habitats, MPN cell counts were first determined using the two enrichment media ½ Fraser broth and UVM II broth. Generally, both broths consisted of corresponding concentrations of components except for lithium chloride, which was absent from UVM II broth. The selective agents in ½ Fraser broth were acriflavine hydrochloric acid, ammonium ferric III sulfat and nalidixic acid, while selectivity in UVM II broth was achieved by cycloheximide, acriflavine hydrochloric acid and nalidixic acid. The ability of ½ Fraser and UVM II broth to promote growth of *Listeria* spp. was investigated by the examination of five sediment and five *R. fluitans* samples applying five-fold MPN approaches. Recovery was slightly higher using ½ Fraser broth for both tested sample types, although differences were not significant (data not shown). From each PALCAM plate with presumptive *Listeria* colonies, up to five colonies were picked and identified. The same *Listeria* species

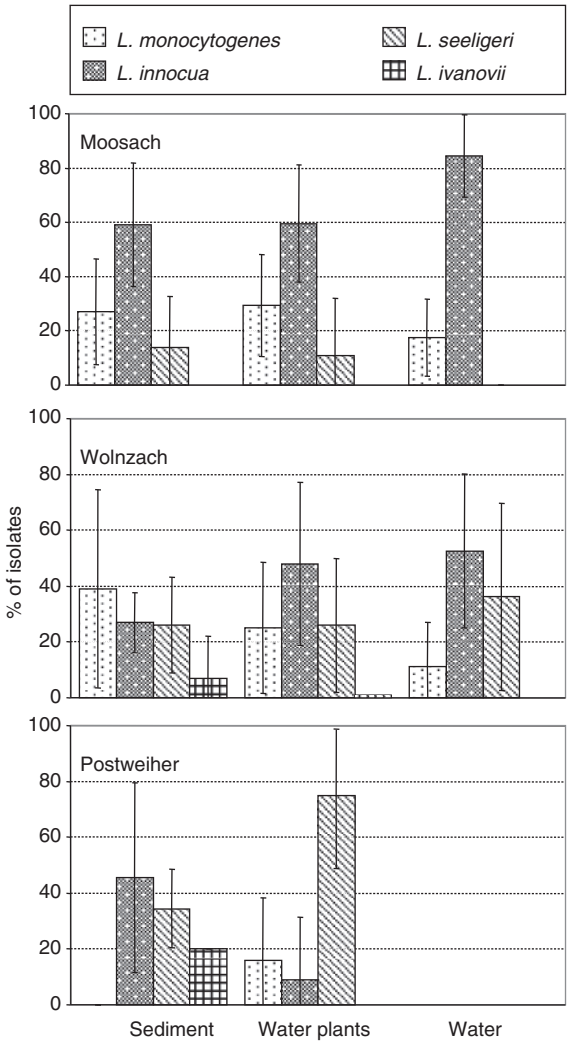

Figure 2: Average species composition of *Listeria* populations in three habitats of three sampling sites. Values expressed in % are given, covering a 1-year sampling period of fresh-water sediments, water plants and water. The species composition of water is based on five samples taken from Moosach and five samples taken from Wolnzach. Mean values and standard deviation are indicated. Species determination of water isolates from Postweiher was not performed. Note that considerable scattering is observed in the data which limits potential conclusions on significant differences in the habitats.

were isolated by the use of the tested enrichment media (data not shown). Based on these results, ½ Fraser broth and a three-fold MPN approach were used in the 1-year longitudinal study.

However, a note of caution has to be added since it is unknown whether any *Listeria* species may grow better than other species in the same sample during enrichment of sediment or plant samples. It has been reported that, during selective enrichment of food samples, growth of *L. innocua* is preferred over *L. monocytogenes* [59, 60]. However, it is unknown, whether this effect plays a role when natural samples are used for enrichment.

3.2 Year-round occurrence of the genus *Listeria*

Over the 12-month sampling period, water temperatures ranged between 6 °C in winter and 14 °C in summer at Moosach and Wolnzach, while surface water at Postweiher was largely frozen in winter and reached up to 18 °C in summer (Supplementary Figure 1). The pH values of water (pH 7.80) and sediment (pH 7.50) were similar in both Moosach and Wolnzach. In total, 30 sediment samples, 31 water plant samples and 36 water samples were analyzed for the presence of *Listeria* spp. After a one-step ½ Fraser broth selective enrichment procedure, 27 sediment samples (90 %), 26 water plant samples (84 %) and 24 water samples (67 %) were tested positive (Table 1). Compared to the high occurrence of *Listeria* spp. in water samples from Moosach and Wolnzach (100 and 83 %, respectively), the occurrence in water from Postweiher was low (25 %). The observed year-round occurrence is in accordance with the psychrotolerant nature of *Listeria* species.

In other studies on the occurrence of *Listeria* spp. in natural habitats based on rather sporadic samplings, 6–90 % of the samples were tested positive (Table 2), but only few limnic environments have been investigated. The strikingly frequent occurrence of *Listeria* spp. in the three limnic habitats observed in this study confirms an early report of Welshimer and Donker-Voet [32] stating that fresh-water environments frequently harbor *Listeria* spp.

It has been reported that the isolation frequency of *Listeria* from surface water was increased after heavy rainfalls, suggesting runoffs and washouts from surrounding surfaces to have a significant influence on the detection of *Listeria* in water [36, 38, 64]. Moreover, slope gradients have been reported to impact the detection of *Listeria* in water samples [35, 37, 67]. We found no indication that pelagic cell counts of *Listeria* are higher than cell counts on water plants and in sediment at all areas sampled. Run-off from adjacent surfaces due to rainy periods did probably not play an important role in these habitats, at least during the sampling performed in this study. This was in agreement with an occasional examination of adjacent soil and plant samples which tested only very rarely positive for *Listeria* spp. (data not shown). Our data, therefore, are in agreement with the hypothesis that *Listeria* spp. in sediments and on water plants are not allochthonous but form part of an autochthonous microbiota.

Table 1: Occurrence of *Listeria* spp. in sediments, on water plants and in water of two creeks (Moosach and Wolnzach) and one pond (Postweiher).

Sampling site	Sediment		Water plants		Water	
	No. of samples	*Listeria* positive	No. of samples	*Listeria* positive	No. of samples	*Listeria* positive
Moosach	12	11	12	10	12	11
Wolnzach	12	11	9	9	12	10
Postweiher	6	5	10	7	12	3
Total	30	27	31	26	36	24

Specimens were sampled monthly over a 1-year period. The total numbers of samples examined and the numbers of samples tested positive for the presence of *Listeria* are listed.

Table 2: Occurrence of *Listeria* spp. in natural habitats.

Species	Habitat	Occurrence (%)	No. of samples	Sampling	References
Listeria spp.	Irrigation water	58	33	Sample collection over a 6 week period	[24]
	Terrestrial samples (arable land)	44	80		
Listeria spp.	Irrigation water	90	52	Systematical up to 192 h after an irrigation or rain event	[36]
	Soil (arable land)	12	1092		
	Spinach leaves	6	334		
Listeria spp.	Soil (humus, sand and clay specimen)	30	467	Occasional sampling over a 2-year period	[38]
	Fresh water	26.5	n.s.		
Listeria spp.	Soil of natural environments	19	303	Sporadic sampling over a 2-year period	[61]
	Vegetation (decaying logs, grass, moss)	34	302		
	Fresh water	16	294		
L. monocytogenes	Natural environment (n.s.)	10.7	56	Sporadic sampling over a 5-month period	[62]
	Vegetation	27.3	11		
Listeria spp.	Sea water	20.5	200	5 Samplings over a 1-year period	[63]
Listeria spp.	Inshore sea bottom soil from a fish farm	40.6	32	Sporadic sampling within a 2-year survey	[64]
L. monocytogenes	Marine sediment	6.9	160	Sample collection during a 2-year period	[65]
	Sea water	8.1	161		
Listeria spp.	Fresh water or low salinity water	81	37	Sampling during a winter period	[66]
	Sediment	30.4	46		
L. monocytogenes	Mud samples from creeks, rivers and ponds	31.5	38	n.s.	[31]
Listeria spp.	Creek water	67	36	Sample collection monthly over 13 months	This study
	R. fluitans	90	21		
	Other water plants	70	10		
	Creek sediment	90	30		

n.s., not specified.

3.3 Cell counts of *Listeria* species

Listeria cell counts are listed in Table 3. Occasionally, *Listeria* cell counts above 110 MPN/g were found in sediment and on plant samples, but never in water. Due to these rare high cell counts, and comparatively low sample numbers, standard deviations were exceedingly high in this data set, which makes it difficult to tell whether the occurrence on water plants is higher than in sediments. Generally, *Listeria* titers did not change in an obvious pattern over the sampling period, indicating that in the habitats examined no clear seasonal trends occur. To our knowledge, data on the population density of *Listeria* spp. in a longitudinal study in sediment and water plant samples have not yet been reported. Other studies focus on *L. monocytogenes* in waste water and sludge [68], fresh surface water and untreated sewage [69], sewage, sewage sludge and river water specimen [70]. These studies report

Table 3: Cell counts of *Listeria* spp. (MPN/g) in sediments, on water plants and in water of two creeks (Moosach M, Wolnzach W) and one pond (Postweiher P).

Habitat	Sampling site	MPN/g															
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	MD	m	sd
Sediment	M	n.d.	15	24	21	4.3	4.3	2.3	9.3	<0.3	46	4.3	15	0.7	9.3	13.3	±1.04
	W	0.7	2.1	0.9	15	0.7	0.4	<0.3	0.9	7.5	>110	46	110	n.d.	1.5	18.4	±35.1
	P	0.4	>110	<0.3	>110	n.d.	n.d.	4.3	n.d.	n.d.	n.d.	n.d.	46	n.d.	4.3	16.9	±25.3
R. fluitans	M	n.d.	24	2.3	15	<0.3	2.3	21	3.6	0.9	4.3	2.1	<0.3	110	4.0	18.6	±33.2
	W	46	>110	9.3	n.d.	n.d.	n.d.	21	15	93	>110	>110	46	n.d.	33.5	38.4	±31.0
Water plants	P	>110	n.d.	n.d.	4.3	<0.3	15	<0.3	<0.3	2.0	24	15	24	n.d.	15	14.1	±9.4
Water	M	n.d.	46	4.3	2.3	<0.3	9.3	0.4	0.9	0.4	1.5	0.3	0.7	46	1.5	10.2	±17.9
	W	0.9	9.3	<0.3	24	0.4	<0.3	0.4	0.9	0.4	9.3	4.3	2.3	n.d.	1.6	5.2	±7.5
	P	<0.3	4.3	<0.3	4.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	2.3	<0.3	n.d.	4.3	3.6	±1.2

Analyzed samples were taken over a 1-year period. n.d., not determined; MD, median; m, mean value; sd, standard deviation. By calculating the median and mean values, MPN counts of <0.3 and >110 MPN could not be included since these were outside the range of the MPN procedure.

on occasional samplings only. The numbers of *Listeria* cells reported in such studies, however, are comparable to the population densities determined in this work.

3.4 Biodiversity of *Listeria* at the species level

From all samples tested positive, on average, 20 presumptive *Listeria* colonies were randomly picked and analyzed. A total of 508 isolates from sediments, 560 isolates from water plants and 152 isolates from water were differentiated to the species level (Supplementary Table 1). The monthly occurrence of all *Listeria* spp. found in the habitats investigated is shown in Figure 1. With the exception of *L. seeligeri*, which was predominant at Moosach in the summer months, no clear seasonal trend in population size and species composition was observed. This finding suggests that *Listeria* populations were rather constant during the 1-year sampling period. Other investigations concerning the seasonal occurrence of *Listeria* populations in natural environments are inconsistent. While a higher occurrence in fall, winter and spring periods was observed [40, 42, 43, 71], contrary findings with a higher occurrence of *Listeria* spp. in the summer months have also been reported [37, 72].

The overall species compositions of *Listeria* populations of water, water plants and sediments are shown in Figure 2. *Listeria ivanovii* was only rarely found at Wolnzach and at Postweiher, but never at Moosach. Moshtaghi et al. detected *L. ivanovii* only in soil taken from an animal-inhabited area [33]. In another study, at sites in proximity to cattle grazing, the occurrence of *L. monocytogenes* was significantly higher [42]. Lyautey et al. [44] reported on a link between the occurrence of *L. monocytogenes* in surface water, an upstream dairy farm and the presence of cropped adjacent surfaces, while, to the contrary, Sauders et al. found *L. monocytogenes* and *L. innocua* to be more common in urban environments [72]. In our study, the creek Moosach was not influenced by cattle farming while adjacent pastures were located at the creek Wolnzach. However, no major difference in the frequency and size of *L. monocytogenes* populations was found in these two creeks (Figures 1 and 2).

The overall species distribution of *Listeria* appeared to be similar in sediment and water plants of the two creeks, especially in Moosach (Figures 1 and 2). This was somewhat surprising since *R. fluitans* and sediment are vastly different habitats with respect to oxygen and nutrient availability, substrates, predators and species interaction [35]. However, it is known that *L. monocytogenes* and *L. innocua* harbor an unusually large regulatory gene repertoire in their genomes, including more than 200 transcriptional regulators (ca. 7 % of the genome) and, in addition, 331 genes (ca. 11.6 % of the genome) responsible for carbohydrate transport [12, 73]. This is clear evidence indicating a high ecological flexibility which may enable these species to colonize a broad range of habitats such as water plants and sediments.

3.5 Substantial microbiodiversity of *Listeria* below the species level

The intraspecific biodiversity (microbiodiversity) of the *Listeria* species detected in these habitat's was assessed

by RAPD typing of strains isolated from water plants, sediment and water. RAPD is a common typing method characterised by a high discriminatory power [58, 74]. An example of RAPD pattern is shown in the Supplementary Figure 2. All RAPD patterns obtained in our study were found to be species specific (data not shown).

A total of 309 *Listeria* isolates originating from creek samplings, each comprising the habitats sediment and *R. fluitans* of the same location probed at the same time were RADP typed. Additionally, 64 isolates from the corresponding water samples were typed (Table 4). The microbiodiversity found is substantial. For instance, among 245 isolates from sediment and *R. fluitans*, 84 different RAPD types were detected. No evidence was found that the microbiodiversity is different in the three habitats investigated or within the three species isolated in higher numbers (data not shown). However, it is not feasible to calculate biodiversity indices based on the data available for two reasons. First, a limited number of isolates was sampled from each habitat per time point. Second, and more important, the sampling method applied involves an enrichment step. As long as, owing to its low abundance, *Listeria* clones cannot be detected directly in environmental samples, all conclusions drawn from such data, which refer to population structure, critically depend on the unknown potential of different *Listeria* clones to survive and compete in a selective enrichment procedure [59, 60, 75, 76].

Table 4: Total numbers of typed isolates and total numbers of RAPD types detected in the habitat's sediment, *R. fluitans* and water.

Habitat	*Listeria* spp.	No. of isolates examined	No. of RAPD types detected	RAPD types/no. of isolates
Sediment	*L. monocytogenes*	65	23	0.354
	L. innocua	82	24	0.293
	L. seeligeri	53	21	0.396
R. fluitans	*L. monocytogenes*	38	9	0.237
	L. innocua	61	22	0.361
	L. seeligeri	18	10	0.555
	*L. monocytogenes**	41	5	0.122
	*L. innocua**	41	6	0.146
	*L. seeligeri**	3	2	0.666
Water	*L. monocytogenes*	17	3	0.176
	L. innocua	44	11	0.25
	L. seeligeri	3	2	0.666

Species marked by an asterisk indicate isolates from *R. fluitans* analyzed without roots.

3.6 Are the *Listeria* populations in the three habitats sediment, *R. fluitans* and water related?

A visual analysis of the relative fractions of *Listeria* spp. on water plants and in corresponding sediments of creek locations and sampling times showed somewhat similar distributions in 11 out of 17 cases, when data from both habitats collected at the same time point were available (marked by an asterisk in Figure 1). This led us to hypothesize that the *Listeria* populations in both habitats may not be independent. One may speculate that the *Listeria* cells attached to the surface of the water plants partly originated from the creek sediments and colonized the plant surface from the sediment. To test this hypothesis, RAPD patterns of *Listeria* originating from the three habitats were compared. Table 5 shows the overall result, summarizing all *Listeria* species. Between 14 and 16 % of the RAPD types were isolated both from sediment and *R. fluitans*. However, identical RAPD types were also detected in sediment and water (2 %) as well as on *R. fluitans* and water (5 %). In addition, on rare occasions, the same RAPD type was found in both Wolnzach and Moosach. The limited fraction of identical RAPD-types obtained from both habitats did not allow the attainment of statistically significant evaluations (exact Fisher test, data not

Table 5: Occurrence of RAPD types of *L. monocytogenes, L. innocua*, and *L. seeligeri* isolated from sediment, *R. fluitans* and water of the creeks Moosach and Wolnzach.

Habitats compared	No. of sample pairings analyzed	No. of isolates examined	No. of RAPD types differentiated	RAPD types found in both habitats (%)
Sediment/*R. fluitans*[a]	10	245	84	14
Sediment/*R. fluitans*[b]	3	157	25	16
Sediment/water	5	152	50	2
R. fluitans[a]/water	5	139	43	5

Samples of the habitats compared (sample pairings) were taken at the same time. The habitats compared were (i) sediment to *R. fluitans*, (ii) sediment to water and (iii) *R. fluitans* to water, respectively. [a]*R. fluitans* samples including roots, [b]*R. fluitans* samples without roots.

shown). The data available so far are therefore inconclusive as to whether the *Listeria* populations in sediment and on *R. fluitans* are related.

4 Conclusion

To our knowledge, this study reports for the first time a monthly longitudinal study of the occurrence of *Listeria* spp. in three associated aquatic habitats. Also, the occurrence of *Listeria* spp. on *R. fluitans* has not been reported before. Creek sediment and water plants were continuously populated by several *Listeria* species, but, interestingly, no clear species specific habitat preference was found. Additionally, despite low cell counts of *Listeria* in the habitats investigated, a rather high intraspecific biodiversity was observed.

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