Mosquito species composition and phenology (Diptera, Culicidae) in two German zoological gardens imply different risks of mosquito-borne pathogen transmission

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ABSTRACT: Due to their large diversity of potential blood hosts, breeding habitats, and resting sites, zoological gardens represent highly interesting places to study mosquito ecology. In order to better assess the risk of mosquito-borne disease-agent transmission in zoos, potential vector species must be known, as well as the communities in which they occur. For this reason, species composition and dynamics were examined in 2016 in two zoological gardens in Germany. Using different methods for mosquito sampling, a total of 2,257 specimens belonging to 20 taxa were collected. Species spectra depended on the collection method but generally differed between the two zoos, while species compositions and relative abundances varied seasonally in both of them. As both sampled zoos were located in the same climatic region and potential breeding sites within the zoos were similar, the differences in mosquito compositions are attributed to immigration of specimens from surrounding landscapes, although the different sizes of the zoos and the different blood host populations available probably also have an impact. Based on the differences in species composition and the various biological characteristics of the species, the risk of certain pathogens to be transmitted must also be expected to differ between the zoos. ***Journal of Vector Ecology* 43 (1): 80-88. 2018.**

Keyword Index: Culicidae, biodiversity, urban, mosquito seasonality, vector, sampling methods.

INTRODUCTION

Zoological gardens represent unique ecosystems where exotic and native animal species coexist. These heterogeneous human-made environments are mostly located in urban areas and provide ideal living conditions for mosquito larvae and adults, with numerous opportunities for taking blood meals, mating, resting, and oviposition (Tuten 2011). Zoos also offer attractive habitats for wild native animals, such as squirrels, raccoons, and birds, both through the availability of food sources and the lack of natural predators (Nelder et al. 2009). If a potential mosquito vector population existed within the zoo area, the accidental entry of mosquito-associated pathogens could quickly lead to a disease outbreak among the zoo animals but might possibly also affect zoo visitors and workers.

The probability of disease-agent transmission, however, depends on many factors, such as the availability of vector-competent mosquito species, their population density, the presence of reservoir hosts, the proportion of infectious mosquitoes, and the rate at which people/animals are bitten, all of them contributing to the vector capacity (Smith et al. 2004). In addition to abiotic factors, like temperature and precipitation, the probability of disease transmission is strongly affected by biodiversity in the ecosystem. A study from the U.S.A. has shown that mosquitoes in zoos use captive animals as well as humans and free-roaming native animals as blood hosts, making a transmission of pathogens between the various groups possible (Tuten et al. 2012). A high diversity of the blood host population, which is typical for zoos, is assumed to reduce the risk of vector-borne disease, also known as the dilution effect (Ezenwa et al. 2006). The dilution effect, however, only takes effect if the mosquito is not host-specific and if the various possible blood host species are poor reservoir hosts. If the particular host species of the mosquito population contains competent reservoirs, high species diversity could lead to an increase in disease prevalence (Ostfeld and Keesing 2000, Ezenwa et al. 2006). By contrast, a high biodiversity of the mosquito vector population may reduce the risk of pathogen transmission, as mosquito species-rich communities usually have a lower density as compared to species-poor communities (Chaves et al. 2011).

Mosquito abundance can vary considerably within small geographic areas, for example between two neighborhoods of a city (LaDeau et al. 2013). This can be attributed to dispersal barriers, such as streets or the availability of larval habitats, although mosquito ecology in urban environments is still poorly understood (LaDeau et al. 2015). In Wellington, New Zealand, it was found that the density of adult mosquitoes was substantially higher in the urban zoo area than in a natural forest only a few kilometers away (Derraik et al. 2003).

Due to the vulnerability of zoo animals in a non-natural and non-native environment where pathogens that they are not adapted to may circulate, infections often have life-threatening consequences. Cases of mosquito-borne diseases in zoological gardens have repeatedly been reported. During the 1999 West Nile outbreak in New York, numerous specimens of the Bronx Zoo/Wildlife Conservation Park bird collection died after infection with the virus (Ludwig et al. 2002). Cases of avian malaria, eastern equine encephalomyelitis, and dirofilariosis are also documented for zoos (Adler et al. 2011).

Species affected by mosquito-borne diseases in zoological gardens are mainly penguins and owls, which are susceptible to West Nile virus (WNV), Usutu virus (USUV), and avian malaria parasites (Ludwig et al. 2002, Huijben et al. 2007, Steinmetz et al. 2011). The detection of St. Louis encephalitis virus and WNV in orcas particularly emphasizes the vulnerability of captive animals to mosquito-borne pathogens as compared to wild specimens of the same species which do not come into contact with mosquitoes in their natural habitat (Jett and Ventre 2012). In Germany, USUV was isolated from two perished captive great grey owls in the Zoological Garden Berlin in 2015 (Ziegler et al. 2016). Since such specific infections are often discovered in zoos sooner than in their natural surroundings, zoological gardens gain importance as epidemiological sentinel stations (McNamara 2007).

In order to better understand transmission cycles of mosquito-associated pathogens, to identify infection sources and to be able to successfully control the vectors, accurate investigations of vector communities and seasonal population dynamics are crucial. Detailed knowledge about mosquito species composition, however, is scarce in Germany, with hardly any data about mosquito communities in zoological gardens.

In order to assess differences between the mosquito faunas occurring in different kinds of zoological gardens, this study included a large zoo in a densely populated urban area and a smaller one that is surrounded by forest. Species composition and seasonal variation of mosquito populations in the two zoos were examined for a better understanding of mosquito community patterns and to identify potential vectors of mosquito-borne disease agents. To test if the collection method has an impact on the observed diversity, different methods for sampling were also compared.

MATERIALS AND METHODS

Study locations

Mosquitoes were collected in the Tierpark Berlin (Berlin, Germany, N52°49.8406', E13°53.0210') and the Zoological Garden Eberswalde (Brandenburg, Germany, N52°82.2664', E13°78.3025'). The distance between the two zoos is approximately 40 km (Figure 1).

The Tierpark Berlin is one of two zoological gardens in the city of Berlin. It covers 160 ha and is the largest so-called 'landscape' zoological garden in Europe. The zoo area is characterized by deciduous and coniferous trees, with artificial ponds and ditches traversing the park. The surroundings of the Tierpark are shaped by urban settlements, with the Spree River and the Treptower Park located at a linear distance of 4.2 km. The animal collection consists of a total of about 7,500 animals belonging to approximately 900 native and exotic species.

The Zoological Garden Eberswalde covers an area of 15 ha and is located in the southern outskirts of Eberswalde, federal state of Brandenburg. Small artificial ponds and ditches are present in the zoo area, but in contrast to the Tierpark Berlin, the Zoological Garden Eberswalde is adjacent to a nature reserve in the southeast, which is characterized by an alluvial forest, and to a forest in the northwest, which is dominated by beech and pine trees. The zoo is home to some 1,500 animals of approximately 150 native and exotic species.

Mosquito sampling

Mosquito sampling took place from May to September, 2016, in a four-week rhythm in each zoological garden. At both locations, eight EVS-traps (BioQuip Products, CA, U.S.A.; Rohe and Fall 1979) were evenly distributed at comparable locations and heights between 160 cm and 200 cm, with a minimum of 50 m distance to ensure independence. As access to animal enclosures was restricted, traps could be located at freely accessible sites only. They were equipped with dry ice producing CO_2 as an attractant and were operated for 24 h. At the Zoological Garden Eberswalde, EVS-trapping could not be done in September.

In addition to trapping, mosquitoes were collected one day per month from selected potential resting sites using a battery-powered Improved Prokopack Aspirator (model 1419; John W. Hock, FL, U.S.A.). In each zoo, 15 natural and artificial sites were sampled. Aspirator collections were performed for five min per resting site. Natural resting sites were represented mainly by understory vegetation, artificial resting sites by eaves, and wooden or stone constructions with shaded hiding places. Mosquitoes approaching and trying to bite the collector during the fieldwork were also captured and later referred to as 'hand catches.' All collected adult mosquitoes were preserved on dry ice and stored frozen (-80° C) until further processing.

Larval stages were collected with a dipper in breeding sites like puddles, ponds, and ditches. Smaller artificial breeding sites, such as plastic containers, tree-holes or tires, were sampled with a tea strainer. Each potential breeding site was dipped ten times, or until the water of the breeding site was exhausted before ten samples could be taken. Since the number of possible breeding sites varied over the season, the number of sampled sites was not standardized between the two zoos. In the Tierpark Berlin, a maximum of 15 breeding sites, and in the Zoological Garden Eberswalde, a maximum of 19 sites was sampled per visit.

Mosquito identification

Immature mosquito stages collected were raised to adults in the laboratory for easier morphological identification. Adults were morphologically determined to species using the identification keys by Schaffner et al. (2001) and Becker et al. (2010). Identification of

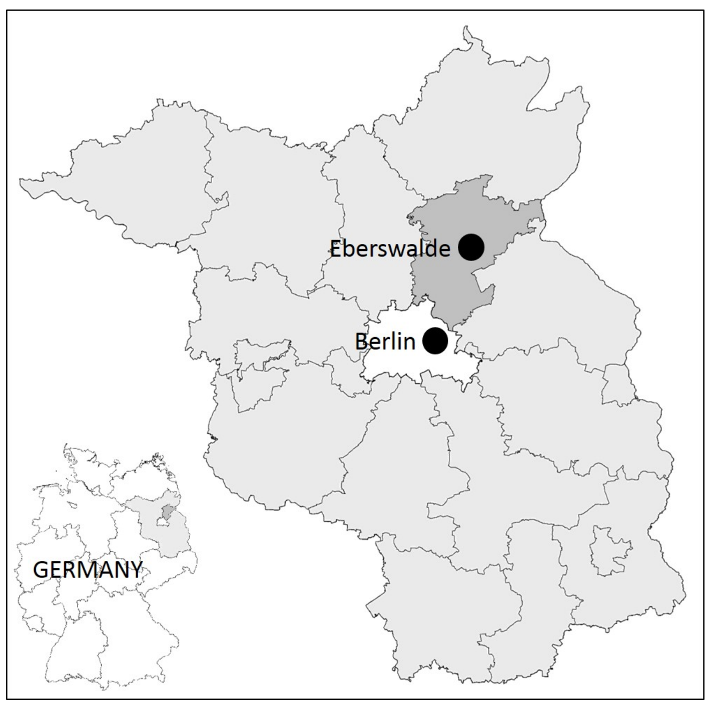

Figure 1. Location of the study areas in Germany.

Anopheles maculipennis complex specimens was done genetically by species-specific ITS2-PCR (Proft et al. 1999, Kronefeld et al. 2014), whereas identification of *Culex pipiens* complex specimens was conducted by a multiplex real-time PCR assay (Rudolf et al. 2013). Mosquitoes not belonging to these complexes but for some reason not morphologically identifiable (e.g., because of damage) were subjected to CO1 (cytochrome oxidase gene subunit 1) barcoding (Folmer et al. 1994, Hébert et al. 2003).

Females belonging to the *Aedes cinereus* and *Ae. annulipes* groups could neither be determined to species level morphologically nor by CO1 barcording and were evaluated as groups. Also, *Culiseta morsitans* and *Cs. fumipennis* females were evaluated jointly, as no reliable morphological or molecular differentiation was possible.

Data analysis

Statistical analyses were conducted in R Version 3.2.0. Non-identified isomorphic specimens belonging to the *Cx. pipiens* or *An. maculipennis* complexes were not included in the statistical analysis. For comparing the biodiversity of the studied zoos, the Shannon-Wiener index ([H′=Σpi×ln pi], where pi is the proportion of the i-th species in the studied zoo) and the Shannon evenness ([E'=H'/H'max], where H' is the Shannon-Wiener index and H'max is the maximum possible value of H') were calculated. Only adult mosquito samples were analyzed, as larval collections may be influenced by aggregate distributions of individuals, which could occur when large numbers of larvae of a single mosquito species are found in one breeding site (Medeiros-Sousa et al. 2015).

Because the observed number of species is often lower than the number of species actually present, sample adequacy was evaluated by a species accumulation curve, and total richness was estimated by the first-order jackknife method for each zoological garden (Burnham and Overton 1979, Medeiros-Sousa et al. 2015). For the analysis, 1,000 randomizations were implemented without replacement, and the 95% confidence intervals were estimated by the vegan package in R (Oksanen et al. 2016). This analysis was performed for both immature and adult forms according to Medeiros-Sousa et al. (2015), as it was assumed that species presence in the larval collections basically corresponded to their presence in the adult form.

To illustrate seasonal changes in the species composition, the five most common species of each zoo, as based on adult mosquito catches, were plotted in a radar chart according to month. Direct visual comparison was facilitated by $\log_{10}$ transformation of the data.

Finally, non-metric multidimensional scaling (NMDS) was performed with the vegan package in R (Oksanen et al. 2016) to compare the species communities between the two zoological gardens with regard to the used adult collection methods, EVS-trapping and aspirating. Because in Eberswalde EVS-collections had not been conducted in September, trap data for both locations were analyzed only from May to August. Species which had only been captured once during the study period were not considered for NMDS. A stress test was performed to check whether the data were suitable for the analysis. The distance measure used was the Bray-Curtis index; the maximum number of random starts in search of a stable solution was 100.

RESULTS

Mosquito species diversity

In total, 2,257 specimens belonging to 20 mosquito species were collected, represented by 16 taxa in each zoo (Table 1). *Aedes punctor, Ae. rusticus, An. daciae,* and *Cs. morsitans/fumipennis* were only collected in Eberswalde, while *Ae. cataphylla*, *Ae. geniculatus, Cx. modestus,* and *Cx. pipiens* biotype *molestus* were only sampled in Berlin. Ten mosquito species were collected as immature forms. *Aedes geniculatus* was only detected in the larval form, but all other species collected as larvae were also found as adults. The majority of the 'hand-catches' consisted of *Ae. annulipes* group species in the Zoological Garden Eberswalde and of *Ae. vexans* in the Tierpark Berlin (Table 1).

Regarding adult mosquitoes, species diversity obtained by EVS-traps was higher than by aspirator collections. As the Shannon evenness values were 0.63 (H′=1.82) for the Zoological Garden Eberswalde and 0.65 (H′=1.76) for the Tierpark Berlin, respectively, the abundance of different species was more even in Berlin than in Eberswalde. This can be explained by the much higher number of *Ae. annulipes* group mosquitoes (n=425) collected with EVS-traps in the Zoological Garden Eberswalde as compared to all other mosquitoes (second most frequently collected species: 129 *Cs. annulata*; Table 1).

The species accumulation curve of the Zoological Garden Eberswalde reaches an asymptote after 40 collections, demonstrating a good sample adequacy (Figure 2a). First-order jackknife total richness estimation indicates approximately 18 species (standard error (SE) = 0.99) for the Zoological Garden Eberswalde, which is close to the observed 16 species. For the Tierpark Berlin, the curve (Figure 2b) is still not stable at the end of the collection period, suggesting that more samples are needed to cover the whole biodiversity of this location. It also indicates that in the Tierpark Berlin more common and less rare mosquito species occur. The jackknife total richness estimator gives a higher probability for 21 species to occur than for the observed 16 taxa, although a high standard error (SE = 1.98) was measured.

Seasonal variation of mosquito species

Figure 3 visualizes the seasonal variation of mosquito taxa at the two locations. In Eberswalde, high numbers of *Ae. annulipes* group mosquitoes were observed in May. Altogether, specimens of this group accounted for 45.2% of all captured adult mosquitoes at this location. The second most frequently collected species in Eberswalde was *Cs. annulata* (17.2% of all adult collections), dominating from June until August. Starting in June, high numbers of *Cq. richiardii* (6.0% of all adult collections) were recorded, but this species was not present in the September collection. In September, *Cx. p.* biotype *pipiens* (4.2% of all adult collections in Eberswalde) predominated over *Cs. annulata*. High numbers of specimens were constantly collected for *An. messeae* (4.8% of all adults in Eberswalde).

The species pattern was different in Berlin, as the numbers of specimens of the dominant species remained relatively constant, confirming the results of the species accumulation curves and the higher evenness value (Figure 3). Here, *Cs. annulata*, *An. maculipennis* s.s., and *Cx. p.* biotype *pipiens* were collected with relatively constant high numbers over the whole season. *Culiseta*

Table 1. Species composition in the sampled zoos according to collection method.

	Tierpark Berlin				Zoological Garden Eberswalde			
Species	Aspirator	EVS-trap	Hand catches	Larval collection	Aspirator	EVS-trap	Hand catches	Larval collection
Ae. annulipes group[1]	-	3	3	-	11	425	19	2
Ae. cataphylla	-	1	-	-	-	-	-	-
Ae. cinereus group[1]	-	2	-	-	-	16	1	-
Ae. geniculatus	-	-	-	2	-	-	-	-
Ae. punctor	-	-	-	-	-	6	1	-
Ae. rusticus	-	-	-	-	-	1	-	-
Ae. vexans	2	194	16	-	1	5	-	-
An. claviger	-	2	-	-	28	19	1	1
An. maculipennis complex[2]	173	2	-	15	9	3	1	13
An. daciae	-	-	-	-	8	11	-	-
An. maculipennis s.s.	96	8	-	20	15	2	-	10
An. messeae	4	2	-	-	21	27	-	2
An. plumbeus	33	1	-	-	4	2	-	1
Cq. richiardii	1	-	-	-	1	58	1	-
Cs. annulata	124	16	-	13	48	129	-	-
Cs. morsitans/fumipennis[1]	-	-	-	-	14	-	-	-
Cx. pipiens complex[2]	44	30	-	73	30	6	-	119
Cx. p. biotype *molestus*	-	1	-	-	-	-	-	-
Cx. p. biotype *pipiens*	28	79	-	13	40	3	6	4
Cx. torrentium	5	18	-	6	10	7	-	31
Cx. modestus	-	-	1	-	-	-	-	-
Cx. territans	3	1	-	12	16	-	-	20
Total number of species[3]	9	13	3	6	13	14	6	8
Total collected	**513**	**361**	**20**	**154**	**256**	**720**	**30**	**203**

[1] reliable morphological or genetic differentiation not possible.
[2] not identified to species.
[3] *An. maculipennis* and *Cx. pipiens* complex specimens only considered if species differentiation was done.

annulata accounted for 15.7%, *Cx. p.* biotype *pipiens* for 12.0%, and *An. maculipennis* s.s. for 11.3% of all adult mosquitoes. In contrast to Eberswalde, high numbers of *Ae. vexans* were recorded in Berlin in July. Altogether, *Ae. vexans* specimens made up for 23.7% of adult mosquitoes collected in the Tierpark Berlin.

Differences in species composition between the zoos

Different community patterns at both locations and differences in species composition depending on aspirator or EVS-collections are visualized in the NMDS plot in Figure 4. Mosquito collections in the Tierpark Berlin were more similar to each other, while collections made in the Zoological Garden Eberswalde were more variable (Figure 4). Aspirator and EVS-collections in Eberswalde from May are characterized by a completely different species composition, attributed both to a high proportion of *Ae. annulipes* group mosquitoes in EVS-collections and high numbers of aspirator-collected *Cs. morsitans/fumipennis*. Monthly aspirator and EVS-collections from June to August from Eberswalde are more similar to each other than the May collections. From June to August, *An. messeae* and *Cq. richiardii* had a great influence on the observed species composition. Eberswalde aspirator collections from September are more similar to Tierpark Berlin EVS-collections from June. At both locations, *Cx. torrentium* was caught in high numbers. Monthly aspirator collections from Berlin were quite similar among each other, with a predominance of *An. maculipennis* s.s., *Cx. p.* biotype *pipiens*, *Cs. annulata*, and *An. plumbeus*. Berlin EVS-collections from June and July were dominated by *Ae. vexans* and from August by *Cx. territans*. Tierpark Berlin EVS-collections from May were dissimilar to the other collections, as *Ae. annulipes* group and *An. claviger* mosquitoes significantly influenced the mosquito composition but were not found in later samples.

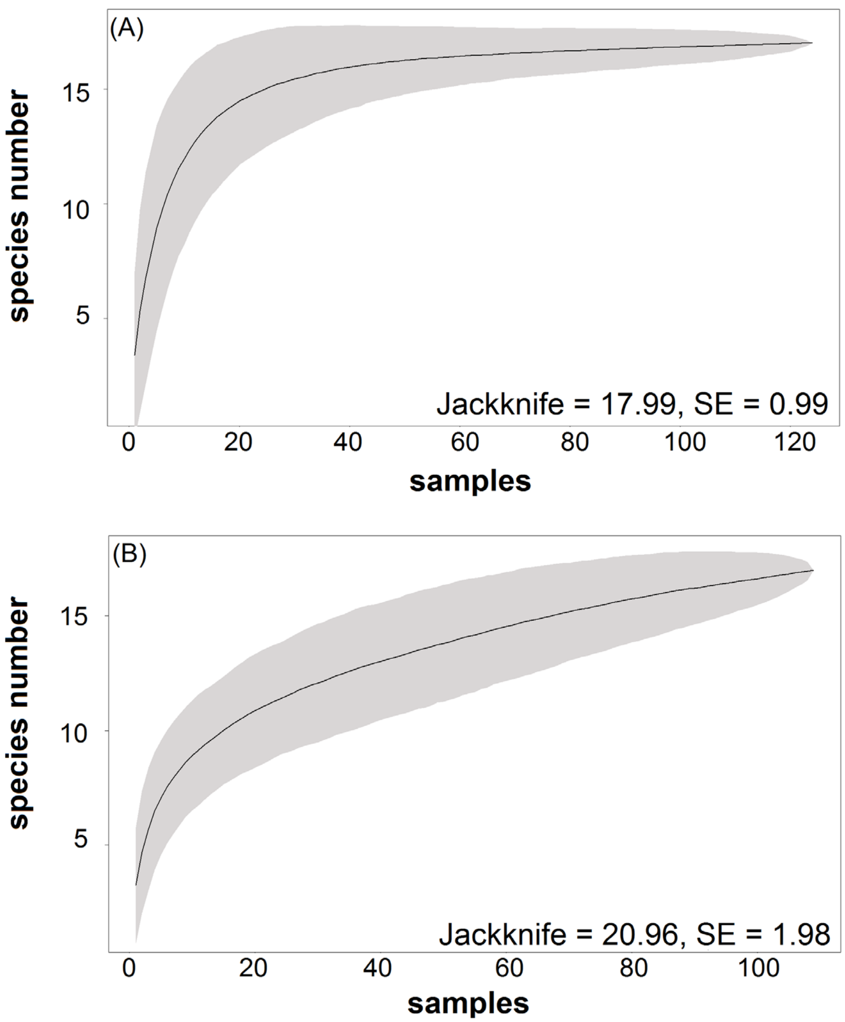

Figure 2. Species accumulation curves (SAC) for the Zoological Garden Eberswalde (A) and the Tierpark Berlin (B).

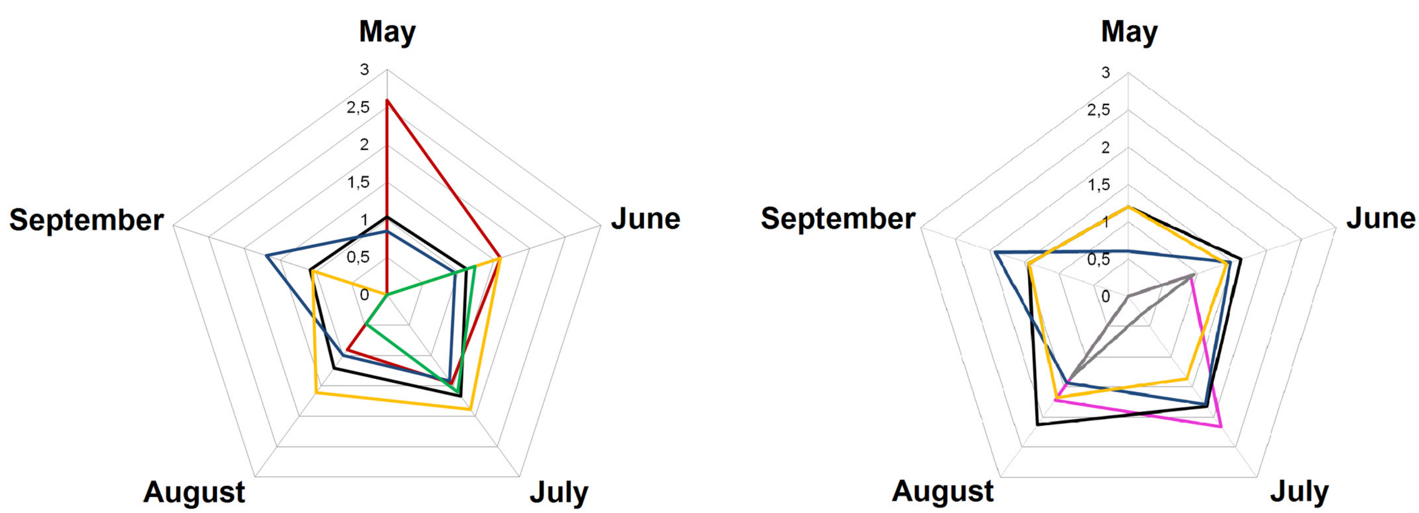

Figure 3. Seasonal variation of the five most frequently collected species of the Zoological Garden Eberswalde (left) and the Tierpark Berlin (right). Red: *Ae. annulipes* group, black: *An. messeae*, blue: *Cx. pipiens* biotype *pipiens*, yellow: *Cs. annulata*, green: *Cq. richiardii*, pink: *Ae. vexans*, grey: *An. maculipennis* s.s., brown: *An. plumbeus*.

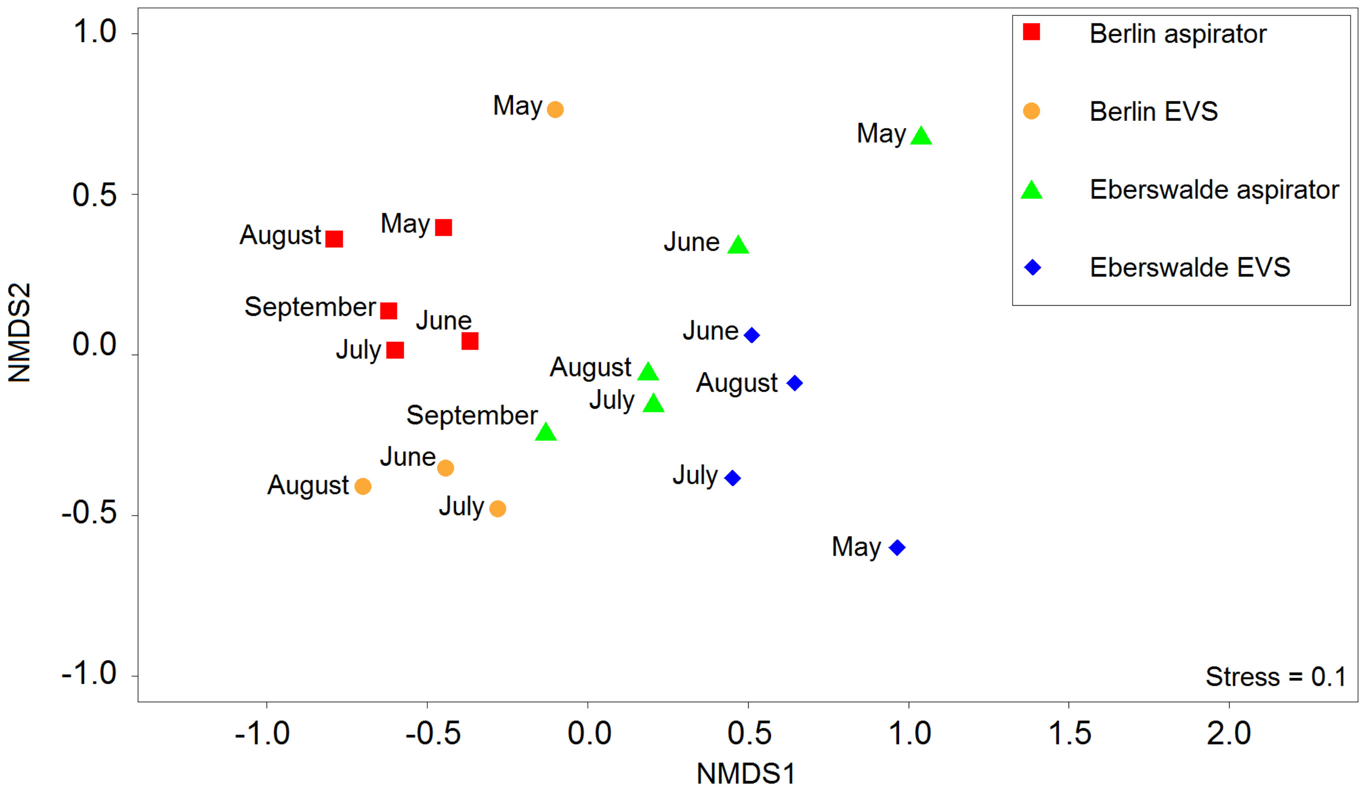

Figure 4. Non-metric multidimensional scaling (NMDS) plot showing mosquito species composition similarity between collections by different methods and at different locations. Each of the 18 dots corresponds to all samples collected at the same time with the same method.

DISCUSSION

Although the Tierpark Berlin and the Zoological Garden Eberswalde are located in the same climatic region, mosquito species compositions were different. These results agree with a study from the U.S.A. from 2013, where the composition of species varied substantially between two sites located in the same ecological region with the same regional pool of species (LaDeau et al. 2013). In the present study, the surveyed zoos differed in size and in surrounding landscapes but also in the spectrum of available blood hosts. Heterogeneous environments across space and time affect the demography and composition of mosquito populations and thus may influence the risk of mosquito-borne diseases (Smith et al. 2004). Consequently, disease risks in zoos embedded in different landscape structures have to be estimated according to the specific situation, as different species may have different significance as potential vectors of different pathogens. Factors that could make a mosquito species a relevant vector in zoos, in addition to its innate vector competence, include a wide spatial distribution at high population densities, host preferences for captive animals and humans, and an extended seasonal occurrence (Smith et al. 2004; Meyer Steiger et al. 2016). Of the 50 mosquito species native to Germany, 27 have been found associated with pathogens, although not necessarily with vector competence (Kampen and Werner 2015). Five of them were detected in the present study in one or both zoos with at least hundred captured specimens: *Ae. annulipes* group, *Ae. vexans*, *Cs. annulata*, *An. maculipennis* s.s., and *Cx. p.* biotype *pipiens*. Of note, the proportion and influence of isomorphic *An. maculipennis* and *Cx. pipiens* complex species could be underestimated, since not all individuals were identified to species level.

The *Ae. annulipes* group was not only the most frequently captured taxon in the Zoological Garden Eberswalde but is also widespread in Germany in general (Mohrig 1969). One member of the group, *Ae. cantans*, is suspected to be involved in the transmission of WNV, Ťahyňa virus (TAHV), Usutu virus (USUV), and of the filarial nematodes *Dirofilaria repens* and *Setaria tundra* (Czjaka et al. 2012; Kampen and Walther 2018). Preferred hosts of *Ae. cantans* are mammals and birds (Medlock et al. 2007), but blood-feeding on exotic animals has not been documented. In the present study, most of the hand-caught mosquitoes belonged to the *Ae. annulipes* group, confirming an association with human hosts. Controlling *Ae. annulipes* group mosquitoes in the zoo area could be difficult, as the preferred breeding sites, shaded ponds, and swampy waters which may develop after the snow melt or heavy rainfalls (Becker et al. 2010), do not only exist on the premises of the zoological garden but also in the surrounding forest.

As opposed to the Zoological Garden Eberswalde, spring species were less abundant in the Tierpark Berlin. Instead, high numbers of the floodwater species *Ae. vexans* were recorded in July. These had mainly been captured by EVS-traps, not excluding the possibility of attraction from outside the study area. A study by Burkett-Cadena et al. (2008) has shown that *Ae. vexans* prefers understory vegetation for rest, where mosquitoes have a higher probability of escaping from being caught than at shelter-type resting sites. The species is also known from urban environments

(Lebl et al. 2014), and is one of few mosquito species actively dispersing over longer distances (Verdonschot and Besse-Lototskaya 2014).

As a typical floodwater mosquito species, *Ae. vexans* could play an important role in the epidemiology of viral mosquito-borne diseases, as immature stages may develop in tremendous numbers in floodplains, the resting sites of resident and migratory birds which can be reservoirs of the causative viruses (Kampen et al. 2012). The species has been shown to be vector-competent for Rift Valley fever virus, various equine encephalitis viruses, TAHV, WNV, and Zika virus (ZIKV) and has been incremented as a potential vector of Batai virus (BATV), CHIKV, USUV, and filarial worms (Kampen and Walther 2017). Avian malaria parasites like *P. circumflexum* and *P. elongatum,* which frequently infect anseriform and galliform birds but also bird species in zoological gardens (Bennett et al. 1993), were also isolated from *Ae. vexans* (Huff 1965). *Aedes vexans* is a primarily ornithophilic mosquito species, but blood meals are occasionally also taken from mammals and exotic zoo animals (Becker et al. 2010; Schoenenberger et al. 2015). Additionally, this species seems to accept human hosts, as demonstrated by the attacks and biting attempts on the mosquito collector during the fieldwork of this study. *Aedes vexans* is not active throughout the complete vegetation period but mainly during the summer months when it was observed in the Tierpark Berlin. Since humans prefer to visit the zoos in summer and most zoo animals are only outdoors during the warmer periods of the year, both are more exposed to mosquitoes in summer. *Aedes vexans* therefore would have a higher probability to transmit pathogens than typical spring species. In addition, the extrinsic incubation period is shorter at summer temperatures.

Although *Cs. annulata* was caught regularly at both survey sites as adult and immature forms, its abundance was higher in the Zoological Garden Eberswalde. The species prefers the proximity to human settlements and hibernates as adult females in houses or cellars (Peus 1929). Preferred blood hosts are birds and mammals, including exotic zoo animals (Medlock et al. 2005; Schoenenberger et al. 2015). Field-collected *Cs. annulata* specimens were found infected with BATV, USUV, and *D. repens*, and transmission was shown for TAHV (Becker et al. 2014; Kampen and Walther 2017). Additionally, avian malaria parasites have been isolated from this species (Huff 1965). All of these findings give rise to the assumption that *Cs. annulata* might be a vector in both zoos.

Studies on the vector competence of isomorphic species belonging to species complexes are rare. Therefore, species of the *An. maculipennis* and the *Cx. pipiens* complexes are discussed on a complex level. Both species complexes were regularly found at the two study sites as adult and immature forms. Species of the *An. maculipennis* complex were one of the most frequently caught taxa in the Tierpark Berlin. Vector competence has been demonstrated for malaria parasites, BATV, Sindbis virus (SINV), and WNV, and *An. maculipennis* complex species are probably also involved in the transmission of dirofilariae (Kampen and Walther 2017). *Dirofilaria repens* was isolated from an *An. daciae* specimen captured in Germany in 2012 (Kronefeld et al. 2014), a mosquito species included in the collections from the Zoological Garden Eberswalde. *Anopheles maculipennis* complex species are described to be opportunistic feeders and have been shown to take blood meals on both mammals and birds (Danabalan et al. 2014). Also, feeding on exotic species in zoos has been described (Schoenenberger et al. 2015).

Various viruses have been detected in members of the *Cx. pipiens* complex, for example BATV, SINV, WNV, TAHV, and USUV (Kampen and Walther 2017). Avian malaria parasites, mainly *P. relictum*, were isolated from *Cx. pipiens* complex mosquitoes (Huff 1965). Also, *D. immitis* was demonstrated in *species of this complex* in Germany in 2012 (Kronefeld et al. 2014). Despite a preference for birds, *Cx. p.* biotype *pipiens* mosquitoes may take advantage of human hosts and thus increase transmission risk to humans (Kilpatrick et al. 2006). Furthermore, *Cx. pipiens* complex species accept exotic zoo vertebrates as hosts (Schoenenberger et al. 2015).

According to Smith (2004), mosquito biting rates are highest near breeding sites where adult emergence takes place. By contrast, vector infection rates are highest far from the breeding sites, where older mosquitoes can be found. Thus, the distance of adults to the breeding place reflects the age structure of mosquito populations. According to this hypothesis, species whose larvae develop within the zoo area would be more likely to become a mere nuisance, while species developing outside the zoo area have a higher probability to be infectious when collected within the zoo. For this reason, breeding site management in the zoo could reduce the nuisance situation but not necessarily the transmission risk of mosquito-associated pathogens. These aspects require further research, as the infection risk is also influenced by mosquito densities and biting rates, landscape, and, above all, blood and reservoir host densities (Tsuda et al. 2016), which are probably higher in the zoo than outside.

The results also demonstrate that the obtained mosquito spectra depend on the collection method applied. Therefore, the sampling methodology may have an impact on which species is perceived as a relevant vector as, for example, attractant EVS-traps collect higher numbers of mosquitoes and species compared to aspirator collections. On the other hand, species like *Cx. territans*, which is not or poorly attracted to commonly used lure traps, are more likely to be collected by aspiration or larval sampling (Küpper et al. 2006, Burkett-Cadena et al. 2008). Furthermore, different mosquito species prefer different types of resting sites (Burkett-Cadena et al. 2008), causing a bias in the species collected by the selection of sampled sites. Finally, the sampling season is crucial as the biodiversity is subject to change with progressive seasons.

According to the vector potential of the most commonly collected species in the Zoological Garden Eberswalde, TAHV, USUV, WNV, filariae, and avian malaria parasites seem to have the highest risk of being transmitted to zoo animals or humans. Given the vector potential of *Ae. vexans*, a theoretical risk for the transmission of a much broader spectrum of pathogens can be deduced for the Tierpark Berlin. Regarding transmission to zoo animals, Disease agents like BATV, SINV, TAHV, USUV, and filarial worms are of major importance, as these have recently been shown to circulate in Germany. Additionally, avian malaria parasites present a considerable risk to susceptible exotic bird species.

Although potential mosquito vectors occur in both zoos surveyed, in neither of them are mosquitoes actively controlled, although prophylactic treatment for the prevention of avian malaria is administered. Should control be implemented, it is important to consider mosquito breeding sites both within and outside the zoo areas.

As the vector potential of native mosquito populations is hardly known, detailed risk analyses are difficult, the more so as the transmission of mosquito-borne pathogens depends on many additional abiotic and biotic factors. The surveillance of mosquito species is only a first step for further research. The study demonstrates that in addition to the special structure of the zoo, the environment in which it is embedded needs to be considered when it comes to risk analysis, mosquito control, and mosquito-borne disease management. While the present study focused on the mosquito fauna within zoos, the special situation in the area surrounding the zoo should be included in future analyses.

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