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Species inventory, ecology and seasonal distribution patterns of Culicidae (Insecta: Diptera) in the National Park Donau-Auen

Die Stechmückenfauna (Culicidae) des Augebietes bei Orth an der Donau wurde 2011 erhoben um die ökologischen Ansprüche der Stechmücken an ihre Bruthabitate zu untersuchen und die Lebenszyklen der abundanten Arten zu rekonstruieren.

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nationalpark donauauen
KOSTBARE NATUR FÜR GENERATIONEN

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1. Introduction

More than 3,500 mosquito species have been recorded worldwide and more than 40 species are known to be endemic in central Europe (Becker et al. 2010). In Austria, the Culicidae inventory consists of 39 published species belonging to 6 genera (*Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Culiseta* and *Uranotaenia*) (Mohrig & Car 2002); since then three further species (*Ochlerotatus (Ochlerotatus) nigrinus* (Eckstein 1918), *Aedes (Stegomyia) albopictus* (Skuse 1894) and *Anopheles (Anopheles) hyrcanus* (Pallas 1771)) were reported by Seidel 2012) and Walter (2011).

Culicidae are endopterygotic insects belonging to insect order Diptera. They are divided into two subfamilies: *Anophelinae* and *Culicinae* (Fauna Europaea, 2011). Culicidae are mainly known as vectors for many medically important pathogens and parasites like viruses, bacteria, protozoans and nematodes and as transmitters of diseases like malaria, chikungunya, dengue and west nile fever and filariaris, among others (Eldridge & Edman 2000; Clements 2012; Becker et al. 2010). After the disappearance of endemic malaria in Austria in 1950 (Wernsdorfer 2002), there was a lack of interest in mosquito research, both in medical and natural sciences, although potentially culicid malaria vectors like *Anopheles* (*Anopheles*) *maculipennis* and *Anopheles* (*Anopheles*) *claviger* (Wernsdorfer 2002) are still present in Austria. Mosquito species distribution patterns and their ecology gain importance again, because global change favours the arrival of invasive mosquito species, followed by new emerging vector-borne diseases (European centre for disease prevention and control 2012). This is shown by the distribution of two invasive mosquitoes, *Aedes albopictus* and *Aedes (Stegomyia) aegypti* (Linnaeus 1762) (Zell 2008). *Ae. albopictus*, currently considered as the most invasive mosquito in the world (Benedict et al. 2007), originated from Southeast Asia (Scholte 2007; Paupy et al. 2009, Medlock et al. 2012) and has nowadays been recorded in 20 European countries since its first record in Albania in 1979 (Medlock 2012). In contrast, *Ae. aegypti* originated from Africa and had been reported for the first time in Europe in the Netherlands in 2010 but has not established in Europe (Scholte et al. 2010, Medlock 2012). Populations of another invasive mosquito species, *Ochlerotatus (Finlaya) japonicus* (Theobald 1901) is widely spread in northern Switzerland (Schaffner et al. 2003, 2009) and southwest Germany (Werner et al. 2012). Currently lesser-known invasive mosquito species like *Ochlerotatus (Ochlerotatus) atropalpus* (Coquillett 1902) (Medlock et al. 2012) and *Aedes (Finlaya) koreicus* (Edwards 1917), which is a potentially transmitter of *Dirofilaria*

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immitis and the Japanese encephalitis virus, have been reported from Europe recently (Capelli et al 2011). In the last decades, the Ecology of Culicidae has been poorly neglected except few investigations on population and community ecology of invasive mosquito species. Interspecific competition between *Ae. albopictus* and *Ae. aegypti* has been investigated in artificial containers in Brazil, where both species successfully established (Braks et al. 2003). A main effect of mosquito research was pest control; i.e. reduction of culicid populations by using methods like *Bacillus thuringiensis* Subsp. *israelensis* (*Bti*) to protect people from mosquito-borne diseases. Worldwide, 200 tonnes of *Bti* are used annually for mosquito control (Becker 1998). Since 1990 the effects of Bti on target (mosquitoes and black fly larvae) and non-target organisms were investigated (Boisvert & Boisvert 2000). The results showed that nematoceran species, such as Ceratopogonidae (Garcia et al. 1980), Chironomidae (Garcia et al. 1980; Miura et al. 1980; Kondo et al. 1995), Blephariceridae (Back et al. 1985), Dixidae (Becker & Margalit 1993) and Tipulidae (Garcia et al. 1980) are susceptible to *Bti*. Meanwhile it is known that the usage of *Bti* has negative effects on breeding success of birds, which is positively correlated with the intake of Culicidae and other Nematocera (Poulin et al. 2010). *Bti* treated and control sites were monitored for 3 years in the Camargue in southern France, where the clutch size and fledging survival was lower at the treated sites (Poulin et al. 2010). Ecological long-term effects of methropene and *Bti* on non-target organisms (zooplankton, insects and birds) were investigated in a 3 year study in wetlands of central Minnesota (Niemi et al. 1999). No negative trophic effects could be attributed to the change in the insect community, as the nest loss due to predation seems to be a greater limiting factor than the lack of mosquitoes (Niemi et al. 1999). These studies underline the need for long-term investigations to fully predict the consequences of mosquito control on effects of wetland communities. Despite increasing interest in mosquitoes there is a lack of information on species inventories, autecology and distribution patterns in Austria and the bordering countries (European Centre for Disease Prevention and Control 2012). The National Park Donau-Auen was chosen for this study, because it provides many different habitats for the larval development of mosquitoes. As one of the last remaining large wetlands in Central Europe, it serves both as a nature conservation zone and a recreational area which often leads to a conflict between human interests and species conservation. The goal of my study was to update the information on the species inventory, the ecology and the spatial distribution of mosquitoes

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in the area of the National Park Donau-Auen, which serves as a basis for further investigations in this region.

2. Material and Methods

2.1 Study area

The study area is situated in Orth an der Donau in the National Park Donau-Auen, Lower Austria. It is mainly influenced by the Pannonian climate with a precipitation of 500 to 700 mm per year. The precipitation maximum is in summer and the minima in spring and autumn, resulting in low summer water levels of Danube floodplain waters (Fink and Moog 1996). The sampling sites are situated in the Vienna basin (Pannonian plain and hills according to Illies 1987) and belong to the ecoregion Hungarian lowlands (Moog et al. 2001), and bioregion Eastern Regions and Lowlands (Moog et al. 2001). The latter extends from the cliff zone in the west to the March catchment in the east and from the Thaya catchment in the north to the Danube in the south. The bedrock consists of marine sedimentary rocks of the late tertiary (Fink et al. 2000).

Major element of the National Park Donau-Auen is the Danube River. It is a ninth-order river and originates eastwards from Donaueschingen in Germany, where Brigach joins Breg (Schiemer et al. 1999). It is the second largest river of Europe with a length of 2 845 km and a catchment area of about 801 463 km^2. At the Nationalpark the Danube's catchment area amounts to more than 104000 km^2, with an average annual discharge of 1 950 m^3s^{-1} , ranging from 900 to 5250 m^3s^{-1} (Tockner et al. 1998). In Austria the Danube is approximately 350 km long with a slope of approximately 0.45 ‰ and a flow velocity of approximately 2 ms^{-1} (Tockner et al. 1998; Schiemer et al. 1999). The flow regime of the Danube is characterized by summer floods; however, floods can occur throughout the year (Fink et al. 2000).

The National Park Donau-Auen is situated in the east of Austria between Vienna and Bratislava and preserves one of the last major wetland environments in Central Europe. It has a total area of more than 9 320 hectares (Lazowski 1997) with a length of 38 km and a maximum width of 4 km. Approximately 65% of the National Park area are riparian forest, 15% meadow, and more than 20 % is covered by water (Donau-Auen GmbH). In 1978 the Lobau was awarded the status of a nature protection zone, with the Lower Lobau being declared as UNESCO Biosphere Reserve. The Danube-March-Thaya wetlands were declared a

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nature preserve in 1979, and the Danube-March wetlands and the Lower Lobau as Wetlands of International Importance under the Ramsar Convention in 1983. Not before, 1996 the Austrian Danube wetlands were given the status of a National Park and exists in its current form, as a Riverine Wetlands National Park, Category II, recognized by the IUCN since 1997 (Donau-Auen GmbH). The National Park provides an enormous variety of different aquatic habitats, ranging from oxbow lakes, temporary side arms and sloughs to small water-filled tree holes (phytotelmata). Hence this area was chosen for the investigation of indigenous mosquito species and their ecological adaptions to different breeding habitats.

2.2 Sampling sites

20 sampling sites (Figure 1) were chosen in the National Park Donau-Auen in the area of Orth an der Donau at the northern shore of the Danube (Figure 1). 4 sample sites were located in the natural retention area for floodwaters and 16 sites were disconnected from the irregular flood regime of the Danube due to a bank. From March to September 2011 8 sampling sites, located along side arms, Fadenbach (F1-F3), Große Binn (GB1), Kleine Binn (KB1, KB2) and the Wachtelgraben (S1, S2), 5 temporary stagnant water bodies (T1-T2) as well as 6 water filled tree holes, Phytotelmata (PT1-PT2) were examined for 245 days.

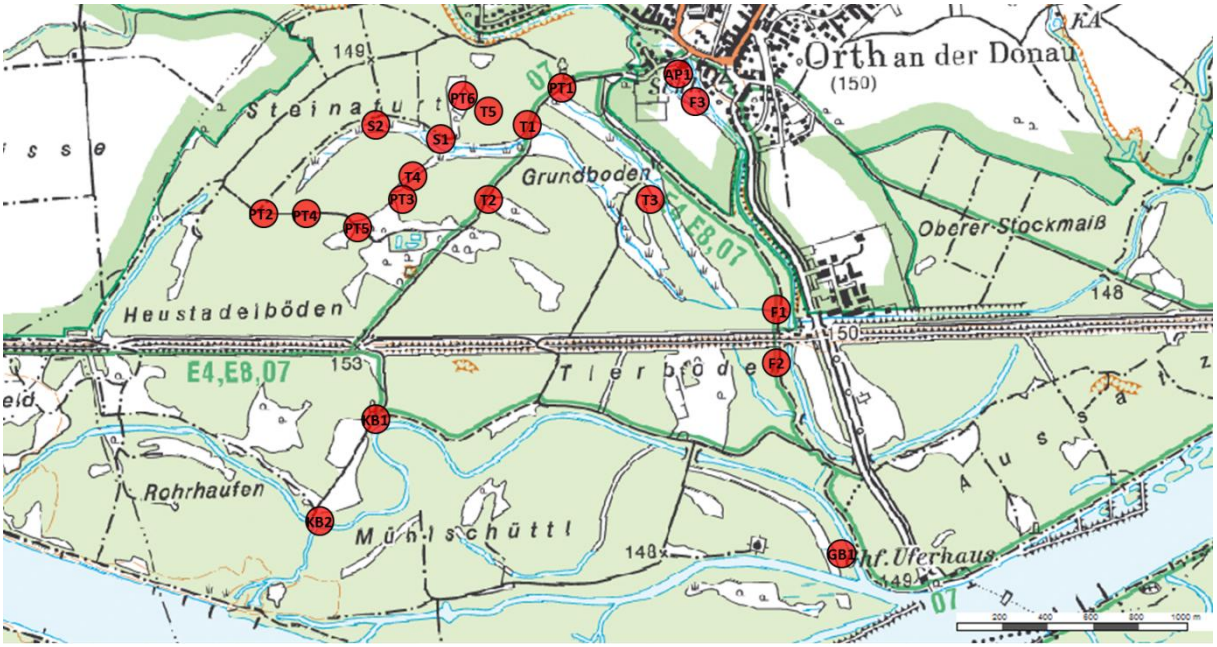

Figure 1: Distribution of sampling sites located in the National Park Donau-Auen, Lower Austria (www.austrian-map.at).

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2.2.1. Fadenbach (F1, F2 and F3)

The Fadenbach (Figure 2) is a former subsidiary branch of the Danube, 30 kilometres long which originates in Mühlleiten. This slow flowing water body is connected to the Danube by the Wachtelgraben. At Orth an der Donau, 3 different sampling sites (F1, F2 and F3) were chosen for further investigations. From May to September the Fadenbach was mainly covered by *Lemna minor* L. (1753). Bank vegetation at sampling sites consisted of *Lythrum salicaria* L. (1753), *Lysimachia nummularia* (Linnaeus 1753) and *Phalaris arundinacea* L. (1753). The water body was rarely shaded by *Tilia cordata* Mill. (1768) and *Carpinus betulus* L. (1753). At all three sampling sites *Lemna minor* L. (1753) was most abundant in July with *Ceratophyllum demersum* L. (1753) being abundant. Mean oxygen concentration was 5.39 mgL^{-1} and pH ranged from 7.58 to 7.43 at all sampling sites at the Fadenbach (Table 1).

From May to June *Notonecta glauca* (Linnaeus 1758) and larvae of *Chaoborus crystallinus* (De Geer 1776) were the dominating invertebrates at site F1. From July to September *Acilius sulcatus* (Linnaeus 1758) (larvae and adults) as well as *Nepa cinerea* (Linnaeus 1758) and larvae of *Hyphydrus ovatus* (Linnaeus 1761) were dominating (Figure 2). At F1, a mean conductivity of 813 μScm^{-1} and a turbidity of 23.94 NTU were measured (Table 1).

The second sampling site (F2; Figure 2) is slightly shaded when compared to F1. Larvae of *Hyphydrus ovatus* (Linnaeus 1761) were the dominating invertebrates in July, *Nepa cinerea* (Linnaeus 1758) and *Notonecta glauca* (Linnaeus 1758) from May to June and in lower abundance than F1. A comparatively low conductivity (mean = 523 μScm^{1}) and a mean oxygen concentration of 7.12 mgL^{-1} were measured at this site.

At site F3, larvae of *Hyphydrus ovatus* (Linnaeus 1761) occurred in early April and were abundant in July. Larvae of *Acilius sulcatus* (Linnaeus 1758) were present from April to early May, and larvae of *Chaoborus crystallinus* (De Geer 1776) were detected throughout the sampling period. This sampling site is characterized by a mean conductivity of only 524 μScm^{-1} and relatively high nutrient values due to high evaporation rates (Table 1).

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Table 1: Limnochemical parameters (arithmetic means with range) of sampling sites at the Fadenbach (F1-F3).

Parameter	F1		F2		F3	
Water level (cm)	58	(24-110)	592	(7.20-95)	53	(18.90-85)
Water persistence (d)	245		245		245	
Temperature (°C)	15.73	(10-21)	15.9	(10-22.6)	15.96	(10.4-23.1)
Oxygen saturation (%)	62.39	(0.9-106)	77.2	(19-110)	79.26	(13-116)
Oxygen concentration (mgl^{-1})	5.39	(9-10)	7.12	(1.60-10.5)	7.73	(0.90-10.8)
Turbidity (NTU)	23.94	(8.84-54.03)	19.6	(9.18-142)	33.35	(12.05-119.9)
pH	7.6	(7.4-7.8)	7.7	(7.4-7.9)	7.5	(6.5-7.8)
Conductivity (μScm^{-1})	812	(453-1160)	523	(344-1080)	959	(469-1186)
NH_4 (mgl^{-1})	0.15	(0-1.11)	0.08	(0-0.74)	0.65	(0-2.54)
Cl (mgl^{-1})	51.25	(16.73-80.3)	29.2	(14.41-75.2)	65.36	(26.20-80.51)
NO_3 (mgl^{-1})	7.78	(0.06-17.35)	1.09	(0.04-3.0)	15.95	(0.05-61.93)
PO_4 (mgl^{-1})	0.03	(0-0.12)	0.04	(0-0.12)	0.86	(0-26.2)
SO_4 (mgl^{-1})	110.8	(13-196.8)	50.6	(10.60-181.8)	130.8	(26.20-202.68)
Water hardness ($mmoll^{-1}$)	2.41	(2.34-2.7)	1.78	(1.62-1.98)	3.39	(2.70-5.58)
Carbonate hardness ($mmoll^{-1}$)	2.17	(1.98-2.52)	1.52	(1.44-1.80)	3.51	(2.52-4.86)

Figure 2: Fadenbach sampling sites F1 (A; northern view), F2 (B; southern view) und F3 (C; western view); pictures were taken on 18 August, 2011.

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2.2.2 Große Binn and Kleine Binn (GB1, KB1 and KB2)

Große (Figure 3A) and Kleine Binn (Figure 3B, C) are subsidiary branches of the Danube with the former connected to the Fadenbach by the Wachtelgraben. Riparian vegetation consisted of *Phragmites australis* (Cav.)Trin. ex Steud. (1841) and *Phalaris arundinacea* L. (1753). Mean conductivity at the sampling sites was 531 μScm^{-1}, mean oxygen concentration and nutrients were comparatively low (Table 2). Throughout the year, the Kleine Binn is a stagnant water body except in summer when higher current speeds were observed due to a higher discharge of the Danube. The water level of site KB1 (Figure 3B) fluctuated between 45 and 99 cm and of KB2 (Figure 3C) between 20 and 107 cm (Table 2). Both sampling sites at the Kleine Binn were mainly covered by *Phragmites australis* (CAV.) Trin. ex Steud (1841) and *Phalaris arundinacea* (L. 1753). Mean values of conductivity at KB1 (Figure 3B) were 602 μScm^{-1}, of oxygen concentration 9.2 mgL^{-1} and of pH 7.6, respectively. Turbidity was slightly increased (11.06 NTU) and nutrient concentration was low (Table 2). High abundances of *Gerris* sp. (Fabricius 1794) and *Hydrometra stagnorum* (Linnaeus 1758) were detected at the sampling sites of the Große and the Kleine Binn during the entire investigation period.

Table 2: Limnochemical parameters (arithmetic means with range) of sampling sites at the Große (GB1) and Kleine Binn (KB1, KB2).

Parameter	KB1		KB2		GB1	
Water level (cm)	66	(45-89.9)	46	(20-107)	39	(25.50-51.50)
Water persistence (d)	245		245		245	
Temperature (°C)	17.6	(13.1-23.4)	14.65	(13-22.1)	17	(12.10-21.70)
Oxygen saturation (%)	101.7	(73-122.1)	99.31	(66-143)	96.7	(70.6-114)
Oxygen concetration (mgl^{-1})	9.21	(7-11.4)	8.74	(5.8-14.5)	8.82	(6.51-10.10)
Turbidity (NTU)	11.06	(2-32.8)	8.89	(2-40.36)	12.6	(2-30.4)
pH	7.6	(6.5-8.3)	8.7	(7.5-9.2)	8.1	(7.6-9.6)
Conductivity (μScm^{-1})	601	(359-800)	495	(359-701)	531	(355-762)
NH_4 (mgl^{-1})	0.02	(0.0-0.34)	0	(0.0-0.0)	0	(0.0-0.0)
Cl (mgl^{-1})	17.12	(13.8-19.62)	18.03	(13.82-23.22)	16.9	(13.6-22.7)
NO_3 (mgl^{-1})	3.63	(0.15-7.55)	2.74	(0.01-7.87)	4.63	(0.01-8.55)
PO_4 (mgl^{-1})	0.02	(0.0-0.1)	0.02	(0.0-0.15)	0.02	(0.0-0.1)
SO_4 (mgl^{-1})	47.48	(20.8-69.13)	38.67	(20.1-60.3)	39.1	(21.22-61.52)
Water hardness ($mmoll^{-1}$)	2.92	(2.16-3.78)	2.86	(1.98-3.06)	2.65	(2.52-2.88)
Carbonate hardness ($mmoll^{-1}$)	2.55	(1.8-3.24)	2.1	(1.80-2.16)	2.08	(1.80-2.34)

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Figure 3: Sampling sites at the Große Binn GB1 (A; north-western view on 23 March, 2011) and at the Kleine Binn KB1 (B; south-western view on 23 March, 2011) and KB2 (C; south-western view on 27 March, 2011).

2.2.3. Wachtelgraben

Two sampling sites were investigated at the Wachtelgraben, a smaller cove-like water body. The connectivity between these two sampling sites was interrupted from 26 to 29 March, from 7 to 13 April and on 1 May, 2011, when sampling site S2 fell dry. Sampling site S1 (Figure 4) was subject to fluctuating water level (51-116 cm). The water body was slightly shaded by *Tilia cordata* (Mill. 1768) and *Corylus avellana* (L. 1753) and was mainly bordered by *Phragmites australis* (Cav.)Trin. ex Steud. (1841) and some isolated stocks of *Phalaris arundinacea* (L. 1753), Typha *latifolia (*L. 1753), *Typha angustifolia* L. (1753)*, Lythrum salicaria* L. 1753) and *Carex elata* ALL. (1785). *Lemna trisulca* (L. 1753), *Stratiotes aloides* (L. 1753) and *Ceratuphyllum demersum* (L. 1753) were detected in the water body. The most abundant invertebrates were *Dytiscus marginalis* (Linnaeus 1758) larvae, *Nepa cinerea* (Linnaeus 1758)*, Ranatra linearis* (Linnaeus 1758), *Ilyocoris cimicoides* Linnaeus 1758) and

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larvae of *Chaoborus crystallinus* (De Geer 1776). Mean conductivity was 822 µScm^{-1}, mean oxygen concentration 5.78 mgL^{-1} and pH was 7.7 (Table 3).

Sampling site S2 (Figure 5) was overgrown with *Phragmites australis* (Cav.)Trin. ex Steud. (1841), with some isolated stocks of *Lythrum salicaria* L. (1753) and *Rubus caesius* (L. 1753). Larvae of *Dytiscus marginalis* (Linnaeus 1758) and *Chaoborus crystallinus* (De Geer 1776) were detected in low abundances. Average water level was 6 cm from March to May, mean conductivity was 1176 µScm^{-1}, and oxygen concentration was very low (mean = 2.87 mgL^{-1}). However, nutrient concentrations were high due to high evaporation rates (Table 3).

Figure 4: Wachtelgraben sampling site S1 (A, 23 March, 2011; B, 18 September, 2011; southern view).

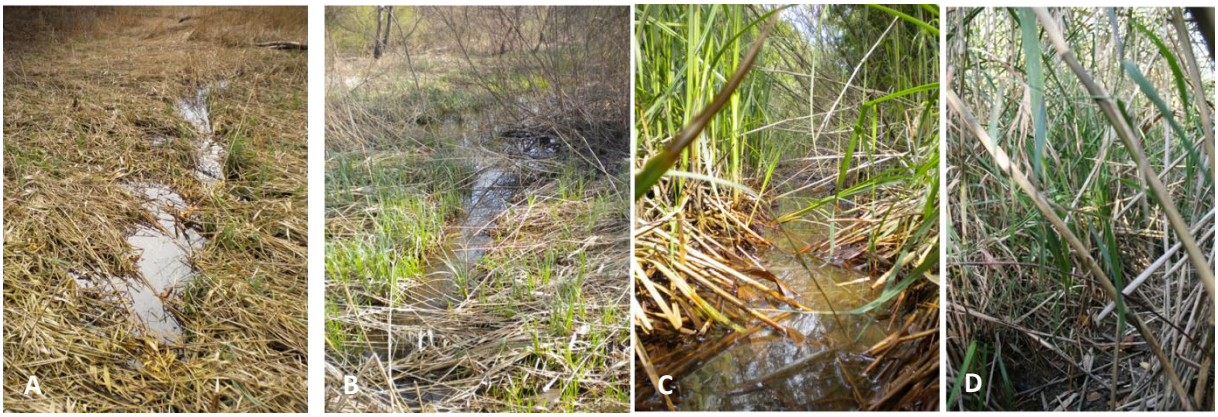

Figure 5: Wachtelgraben sampling site S2 (A, 23 March; B, 08 April; C, 04 May and D, 18 September, 2011; north-eastern view).

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Table 3: Limnochemical parameters (arithmetic means with range) of the sampling sites at the Wachtelgraben (S1, S2).

Parameter	S1		S2	
Water level (cm)	98	(51.3-116)	6	(0-7)
Water persistence (d)	245		73	
Temperature (°C)	19.5	(15.4-26.6)	10	(9.2-11.3)
Oxygen saturation (%)	63.7	(22-99.2)	31.5	(7.9-48)
Oxygen concetration (mgl^{-1})	5.87	(6.7-8.1)	2.87	(7.2-7.7)
Turbidity (NTU - Nephelometric Turbidity Unit)	7.46	(2.02-10.78)	8.15	(8.15-8.51)
pH	7.7	(6.7-8.1)	7.5	(7.2-7.7)
Conductivity (μScm^{-1})	822	(604-1048)	1176	(1310-1083)
NH_4 (mgl^{-1})	0	(0.0-0.0)	0	(0.0-0.0)
Cl (mgl^{-1})	44.38	(39.62-47.92)	55.5	(53.4-58.5)
NO_3 (mgl^{-1})	0.83	(0.02-3.18)	0.14	(0.10-0.17)
PO_4 (mgl^{-1})	0.01	(0.0-0.05)	0	(0.0-0.0)
SO_4 (mgl^{-1})	92.53	(44.16-143.82)	159.9	(0.10-0.17)
Water hardness ($mmoll^{-1}$)	3.15	(3.06-3.78)	2.78	(2.70-2.88)
Carbonate hardness ($mmoll^{-1}$)	2.86	(2.52-3.06)	2.52	(2.52-2.54)

2.2.4 Tree Holes (PT1-PT6)

As phytotelmata (Figure 6) provide important habitats for *Culicidae*, 6 water-filled tree holes were investigated. These breeding sites undergo strong temperature fluctuation due to the small size of the water body (Table 4). As water persistence depends on the amount of precipitation, phytotelmata regularly dry out during periods of high evaporation, as it is common in summer. The water surface is usually shaded throughout the entire day. The bottom of the sampling sites PT1, PT4 and PT5 was covered by leaves and particular organic matter (POM). A high nutrient concentration due high evaporation was measured. Mean turbidity ranged from 12.93 to 499.24 NTU (Table 5) and pH-values were in the 6.7 to 7.4 range. In addition, these sampling sites were characterized by variable oxygen concentrations averaging 3.5 – 12.6 mgL^{-1} (Table 5). From April to September *Prionocyphon serricornis* (P. W. J. Muller 1821) were detected in high abundances at PT3, PT4 and PT5.

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Table 4: Estimated maximum water volume (ml), mean depth (cm) and water persistence (days; entire sampling period encompassed 245 days) of the investigated phytotelmata (PT1 – PT6).

Sampling Site (Species)	Depth (cm)	Water volume (ml)	Water persistence (days)
PT1 (*Fraxinus excelsior* L.1753)	10	32	156
PT2 (*Carpinus betulus* L. 1753)	18	250	180
PT3 (*Carpinus betulus* L. 1753)	7	852	172
PT4 (*Tilia cordata* .Mill. 1768)	12.5	1358	181
PT5 (*Carpinus betulus* L. 1753)	5.3	40	111
PT6 (*Carpinus betulus* L. 1753)	3.9	55	142

Figure 6: Phytotelmata P1 (A, picture taken on August 18, 2011), PT2 (B, picture taken on May 22, 2011), PT3 (C, picture taken on March 26, 2011), PT4 (D, picture taken on August 18, 2011), PT5 (E, picture taken on August 18, 2011) and PT6 (F, picture taken on August 18, 2011).

Table 5: Limnochemical parameters (arithmetic means with range) measured at the investigated phytotelmata (PT1-PT6).

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Parameter	PT1		PT2		PT3	
Water level (cm)	2.3	(0-9.1)	5	(0-15)	5	(0-12.5)
Temperature (°C)	17.8	(12.1-32.2)	15.3	(12.2-20.5)	15.8	(12.3-21.4)
Oxygen saturation (%)	73.3	(69.5-99)	64	(6.2-106.8)	41.6	(4.2-105.6)
Oxygen concetration (mgL^{-1})	12.86	(5.7-22.8)	6.09	(3.4-8.67)	3.69	(0.27-8.8)
Turbidity (**N**ephelometric ***T****urbidity* **U**nit)	454.5	(195.3-500)	134.5	(38.5-365.3)	164.1	(11-274.9)
pH	7.3	(7.1-7.5)	6.7	(5.1-7.9)	7.1	(6.8-7.4)
Conductivity (μScm^{-1})	287	(259-300)	217	(196-228)	429	(224-681)
NH_4 (mgl^{-1})	0.73	(0.0-4.97)	6.1	(1.49-27.75)	9.3	(1.55-21.73)
Cl (mgl^{-1})	20.61	(2.61-54.4)	7.75	(2.2-15.23)	12.29	(7.09-24.14)
NO_3 (mgl^{-1})	5.03	(0.0.-9.69)	10.98	(0.01-32.31)	2.02	(0.01-7.93)
PO_4 (mgl^{-1})	4.73	(0.0-13.44)	2.41	(0.13-6.14)	3.9	(0.31-8.58)
SO_4 (mgl^{-1})	10.19	(2.3-18.6)	10.76	(4.2-19.3)	21.8	(3.93-63.90)
Water hardness ($mmoll^{-1}$)	1.71	(1.26-1.8)	1.65	(1.08-1.62)	3.97	(1.26-4.14)
Carbonate hardness ($mmoll^{-1}$)	1.53	(1.08-1.62)	1.27	(0.9-2.52)	2.38	(1.08-2.52)

Parameter	PT4		PT5		PT6	
Water level (cm)	4.3	(0-12.2)	0.6	(0-6)	1	(0-8)
Temperature (°C)	16	(12.1-27)	12.2	(12.2-12.8)	18.9	(16.1-21.1)
Oxygen saturation (%)	54.6	(14-103)	67.5	(66.5-70.1)	43	(42.1-43.8)
Oxygen concetration (mgL^{-1})	4.57	(1.20-8.30)	5.62	(4-9-5.68)	3.5	(2.4-3.8)
Turbidity (**N**ephelometric ***T****urbidity* **U**nit)	89.05	(20.1-377.8)	12.93	(12.81-14.05)	499.2	(486-509.2)
pH	6.9	(4.5-7.5)	7.2	(6.9-7.3)	7.4	(7.4-7.5)
Conductivity (μScm^{-1})	551	(247-754)	247	(245-247)	536	(491-593)
NH_4 (mgl^{-1})	5.05	(0.0-10.53)	2.93	(0.38-9.03)	3.83	(2.87-5.53)
Cl (mgl^{-1})	14.6	(7.15-54.4)	11.91	(7.45-12.4)	13.56	(10.5-18.75)
NO_3 (mgl^{-1})	9.16	(0-80.14)	6.08	(0.0-22.74)	1.29	(0.0-2.65)
PO_4 (mgl^{-1})	7.88	(0-29.26)	6.87	(0.03-25.17)	13.73	(5.93-16.51)
SO_4 (mgl^{-1})	14.01	(3.94-49.33)	32.45	(14.9-41)	13.71	(7.65-19.4)
Water hardness ($mmoll^{-1}$)	3.56	(2.16-3.96)	1.48	(1.26-1.62)	2.07	(1.98-2.16)
Carbonate hardness ($mmoll^{-1}$)	2.23	(1.80-2.34)	1.08	(1.08-1.09)	1.89	(1.8-1.98)

2.2.5 Temporary stagnant water bodies (T1, T2, T3, T4 and T5)

In temporary water body T1 (Figure 7A) water persisted from March to June; its surface area was 1.57 x 0.79 m in March. The bottom of this water body was mainly covered by leaves and branches. *Impatiens parviflora* DC. (1824), an East Asian neophyte, dominated the shore vegetation. The mean water level was 11 cm, mean conductivity 797 μScm^{-1} and mean

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oxygen concentration 5.59 mgL^{-1}. Turbidity was relatively high, averaging 166 NTU; on the other hand, nutrient concentration was rather low (Table 6).

Sampling site T2 (Figure 7B) was a stagnant water body created by a windthrow and had a maximum surface area of 0.56 x 1.35 m at the beginning of the sampling period. Riparian vegetation consisted mainly by *Impatiens parviflora* DC. (1824).The bottom of the water body was covered with branches. It had a high conductivity (mean = 969 μScm^{-1}) and a mean oxygen concetration of 5.27 mgl^{-1}; in addition, relatively high nutrient levels were measured (Table 6).

Figure 7: Temporary water bodies T1 (A, southern view; picture taken on 22 May, 2011) and T2 (B, western view; picture taken on 26 March, 2011).

From early March to May sampling site T3 (Figure 8A) was filled with water (= 68 days), resulting in a mean water level of 11 cm (Table 6). Its maximum size was 1.52 x 3.15 m in March. *Typha angustifolia* (L. 1753) was highly abundant; *Populus nigra* (L. 1753), *Populus alba* (L. 1735), *Salix alba* (L. 1753), *Alisma lanceolatum* With. (1796) and *Sparganium erectum* L.s.l. (1753) were recorded in lower abundances. Mean conductivity was 779 μScm^{-1} and mean oxygen concentration was relatively high (9.68 mgL^{-1}) (Table 6).

Sampling site T4 (Figure 8B) was located in the alluvial forest and had a mean water depth of 17 cm (Table 6) and a maximum surface area of 3 x 2.5 m. It was deeply shaded by a dense canopy of *Quercus petraea* Liebl. (1784), *Tilia cordata* (Mill. 1768), *Carpinus betulus* (L. 1753) and *Acer campestre* (L. 1753). Aquatic vegetation was lacking. Mean conductivity was relatively low (475 μScm^{-1}), and mean oxygen concentration was relatively high (7.66 mgL^{-1}). Nutrient values were high (Table 6) due to high evaporation rates. This sampling site fell dry for a short period in July and September.

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Figure 8: Temporary water bodies T3 (A, south-western view; picture dated 26 March, 2011), T4 (B, north-eastern view; picture dated 04 May, 2011) and T5 (C, north-eastern view; picture dated 01 March, 2011).

The water level at site T5 (Figure 8C) fluctuated between 8 and 9 cm and its surface area between 2 x 1.5 m in early March to 0.30 – 0.15 m at the end of April. This temporary water body received its water exclusively from precipitation. Site T5 was located in the alluvial forest, shaded by a dense canopy of *Quercus petraea* Liebl. (1784), *Tilia cordata* (Miller 1768) and *Carpinus betulus* (Linnaeus 1753). The bottom of the temporary pond was covered with leaves and branches. Mean conductivity was 866 μScm^{-1} and mean oxygen concentration was 9.74 mgL^{-1} (Table 6). The high concentration of nutrients at the end of April was due to high evaporation rates in spring (Table 6).

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Table 6: Limnochemical parameters (arithmetic means with range) of investigated temporary water bodies (T1-T5)

Parameter	T1		T2		T3	
Water level (cm)	10.5	(0-41.5)	8	(0-29.8)	10.6	(0-16.2)
Water persistence (d)	188		172		68	
Temperature (°C)	13.4	(11.9-19.2)	13.4	(9.5-19.7)	12.1	(11.8-12.9)
Oxygen saturation (%)	58.98	(16.3-99.9)	54.6	(16.3-105.1)	98.89	(96.4-105.1)
Oxygen concetration (mgl^{-1})	5.59	(1.63-10.1)	5.27	(1.63-9.04)	9.68	(9.04-9.94)
Turbidity (NTU)	166.5	(32.38-748.9)	94.3	(32.38-267.9)	14.01	(14.01-15.3)
pH	7.8	(7.6-7.9)	7.8	(7.5-8)	8.1	(7.99-8.2)
Conductivity (μScm^{-1})	796	(357-959)	968	(788-1158)	978	(846-1032)
NH_4 (mgl^{-1})	0.38	(0.0-0.62)	1.77	(0.0-10.86)	0.15	(0.0-0.21)
Cl (mgl^{-1})	19.11	(2.71-22.94)	32.6	(5.6-47.9)	29.25	(19.3-33.24)
NO_3 (mgl^{-1})	0.84	(0.03-5.78)	0.59	(0.01-3.67)	0.08	(0.01-0.11)
PO_4 (mgl^{-1})	0.12	(0.01-0.25)	0.65	(0.0-3.98)	0.06	(0.02-0.08)
SO_4 (mgl^{-1})	38.63	(9.4-58.9)	84.2	(9.2-122.9)	54.44	(9.2-72.54)
Water hardness ($mmoll^{-1}$)	2.09	(1.8-2.7)	2.42	(1.98-2.88)	1.62	(1.62-1.65)
Carbonate hardness ($mmoll^{-1}$)	1.08	(1.08-1.08)	1.05	(0.9-1.26)	1.44	(1.44-1.5)

Parameter	T4		T5	
Water level (cm)	5.5	(0-13)	6.9	(0-9.2)
Water persistence (d)	170		58	
Temperature (°C)	16.8	(12.5-22.2)	13.8	(12.1-15.2)
Oxygen saturation (%)	70.51	(27-106)	92.73	(79.9-97)
Oxygen concetration (mgl^{-1})	7.66	(3.6-10.1)	9.74	(9.62-10.3)
Turbidity (NTU)	37.32	(4.5-135.9)	32.6	(10.2-40.1)
pH	7.7	(7.5-8.4)	8	(7.4-8.1)
Conductivity (μScm^{-1})	475	(355-675)	886	(547-886)
NH_4 (mgl^{-1})	1.34	(0.0-5.24)	0	(0.0-0.0)
Cl (mgl^{-1})	25.27	(3.27-78.6)	45.36	(34.28-78.6)
NO_3 (mgl^{-1})	5.11	(0.14-14.41)	3.64	(0.05-14.5)
PO_4 (mgl^{-1})	0.04	(0.0-0.14)	0.04	(0.0-0.05)
SO_4 (mgl^{-1})	112.6	(15.39-210.6)	82.77	(46.03-193.0)
Water hardness ($mmoll^{-1}$)	3.07	(1.8-4.86)	2.94	(2.88-3.06)
Carbonate hardness ($mmoll^{-1}$)	2.15	(1.62-2.7)	2.7	(2.65-2.76)

2.3.2 Artificial Pond

A water-filled plastic container (80x35 cm, mean water depth = 20 cm) was used as an artificial pond (Figure 9). It was situated at the sampling site F1 and was investigated during the entire study period in order to get additional material of mosquito species breeding

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close to human settlements. Species detected in this artificial breeding habitat were used for life cycle investigations only.

Figure 9: This plastic tray (artificial Pond, AP1), served as an additional breeding habitat (picture taken on 24 May, 2011).

2.3 Methods and sampling design

2.3.1 Sampling design and preservation of *Culicidae*

From 1 March to 31 October, 2011 (245 days), 20 study sites, situated at Orth an der Donau, Lower Austria, were sampled on a regular basis. Culicidae and their potential predators were collected every third day using a catch per unit effort (CPUE) method: an area of 1 m^{-2} was sampled for 1 minute with a handnet (20 cm in diameter, mesh size: 200 µm) at every study site in order to collect all developmental stages of aquatic mosquitoes (eggrafts, larvae and pupae). Adults were caught strictly at the breeding habitats with a handnet (20 cm in diameter, 200 µm) by moving the net back and forwards for 10 times. The phytotelmata were sampled at the same intervals as described above using a syringe (20 ml).

Potential predators were sampled following the same CPUE method used for aquatic stages of mosquitoes. Larvae and pupae were preserved in vials containing ethanol (75%) for further investigations. Eggrafts were kept in a plastic container until hatching in order to get third and fourth larval instars for reliable identifications.

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2.3.2 Sampling design of habitat parameters

Abiotic parameters were measured every second week and additionally after flood and heavy rainfall at each sampling site. Dissolved oxygen, pH- value, electrical conductivity, water temperature and turbidity were measured in the field using a regularly calibrated Oxi 330 (WTW), pH 330i (WTW), LF 330 (WTW) and a Turbiquant 1000IR (MERCK). Water samples of 100 ml were taken at each sampling site for titrimetric determination of total and carbonate hardness in the laboratory. Nutrients (sulphate, chloride, nitrate and phosphate) were photometrically analysed using a 761 CompactIC (Metrohm). In addition, connectivity, water level and persistence were checked.

2.3.3 Laboratory rearings

A water-filled container (80 x 35 cm, water depth = approx. 20 cm) was used as an additional breeding site to get a higher number of *Culex pipiens* individuals and other mosquito species breeding close to human settlements. Individuals from this additional pond were used for life cycle investigations only.

2.3.4. Measurement of head capsule width and body length

Head capsule widths and body lengths were measured to identify the four larval instars and to provide basic data for lifecycle reconstruction as well as for estimating the number of generations per year. The head capsule width was measured at the widest section of the head; body length was measured dorsally, from the head capsule to the eight abdominal segment, using a binocular microscope (Stereo Lumar.V12, Zeiss) This binocular was also used for photographic documentation of common standard characteristics from the egg to the adult (Camera: AxioCamErc5s, Zeiss in combination with image stacking software Combine ZM, Hadley (2008)).

2.3.4. Statistical Analysis

Biometrical data (head capsule widths and body lengths) were used to reconstruct life cycles of the detected species. Dyar's rule (1890) was used to differentiate the 4 larval instars accurately, whereas the growth ratio was determined by dividing the mean head capsule widths of adjacent instars (McDonald et al. 1977).

Environmental data (e.g. electrical conductivity, nutrients, water persistence) were analysed in order to characterize the breeding habitats using a cluster analysis [hierarchical classification, Wards method (SPSS for Windows)] based on euclidean distance. The gradient

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lengths of species data were estimated using a Detrended Correspondence Analysis (DCA); in addition, a Canonical Correspondence Analysis (CCA, Software CANOCO for Windows 4.5) was employed as an unimodal method to explain species data by environmental data. Significant variables ($p<0.05$) were included into the CCA procedure. The quality of the unimodal model was checked by a Monte Carlo Permutation test.

2.4 Identification of Culicidae

The specimens sampled (pre-imaginal stages and imagines) were examined using a binocular microscope (Stereo Lumar.V12, Zeiss) and were identified using the key of Becker et al. (2010) and Zittra (2012). Genera and subgenera abbreviations follow Reinert (2001).

2.4.1. Standard identification characteristics of mosquito larvae

Larvae of Culicidae (Figure 10) are divided into three parts, a fully sclerotized head capsule, a thorax of three fused segments, which are wider than the head capsule, and the abdomen, which consists of ten segments (Cranston et al 1987, Becker et al 2010). Culicid larvae morphologically differ from other dipteran larvae due to their distinct labral brushes (exceptions are found in carnivorous larvae), the expanded thorax and the presence of a respiratory siphon (Becker et al. 2010), which is located at the ninth abdominal segment in all genera, but differs between *Culicinae* and *Anophelinae* (Cranston et al 1987, Becker et al. 2010). In culicine species (Figure 11) the spiracular valves, an external opening of the metapneustic respiratory system, are located on the top of the long, tubular (e.g. genera *Aedes*, *Culex*, *Culiseta*) or cylindrical (e.g. genus *Coquillettidia*) siphon (Becker et al. 2010). In anopheline mosquito species the siphon is almost reduced to a so called spiracular plate (Becker et al. 2010) (Figure 12).

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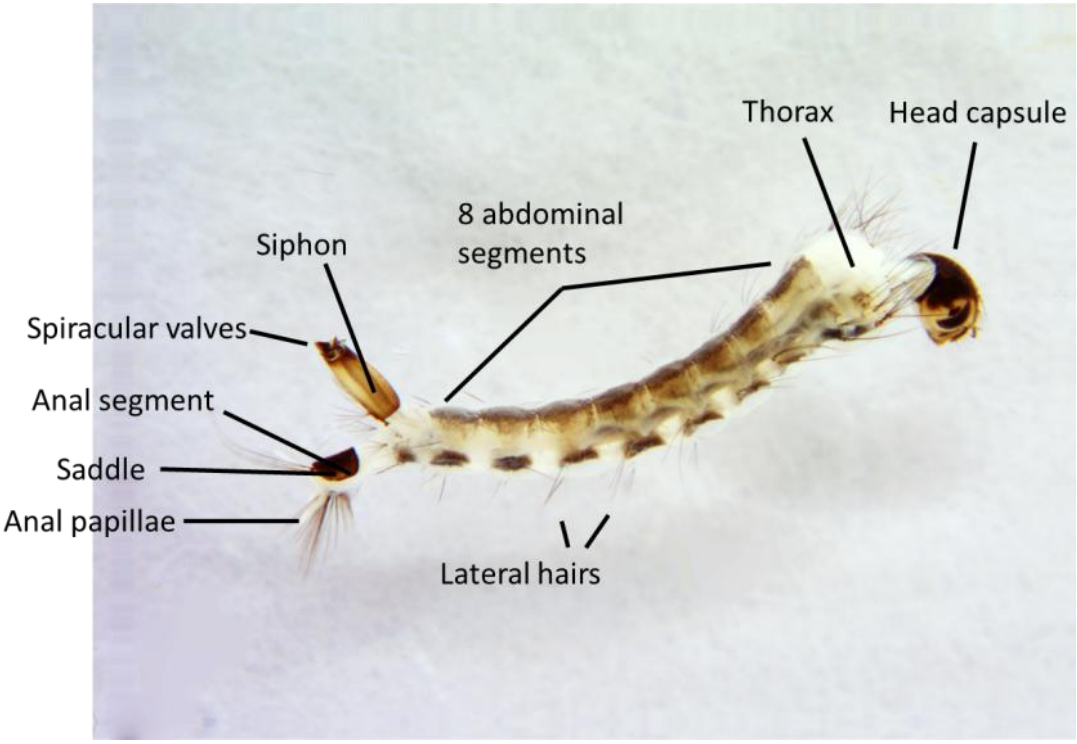

Figure 10: Standard characteristics of mosquito larvae.

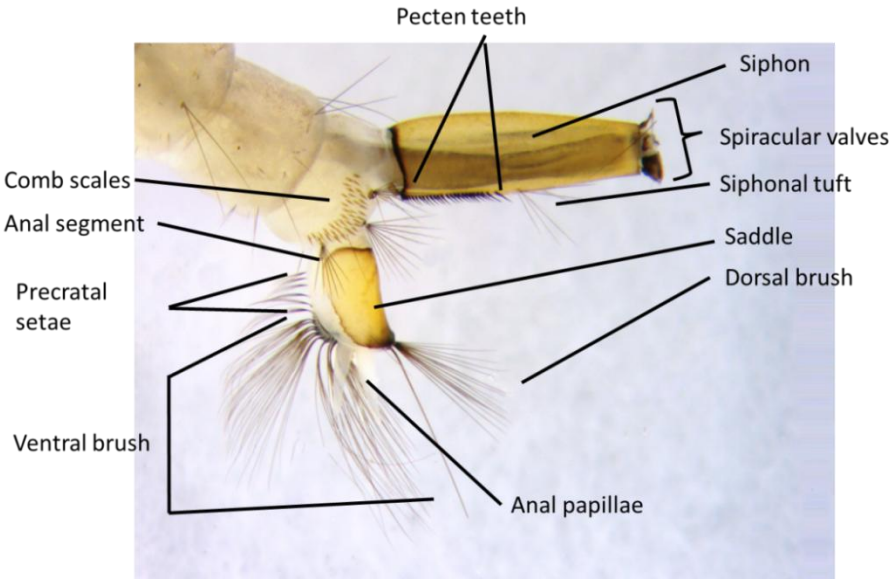

Figure 11: Standard identification characteristics situated at the larval abdomen – (*Culicinae*).

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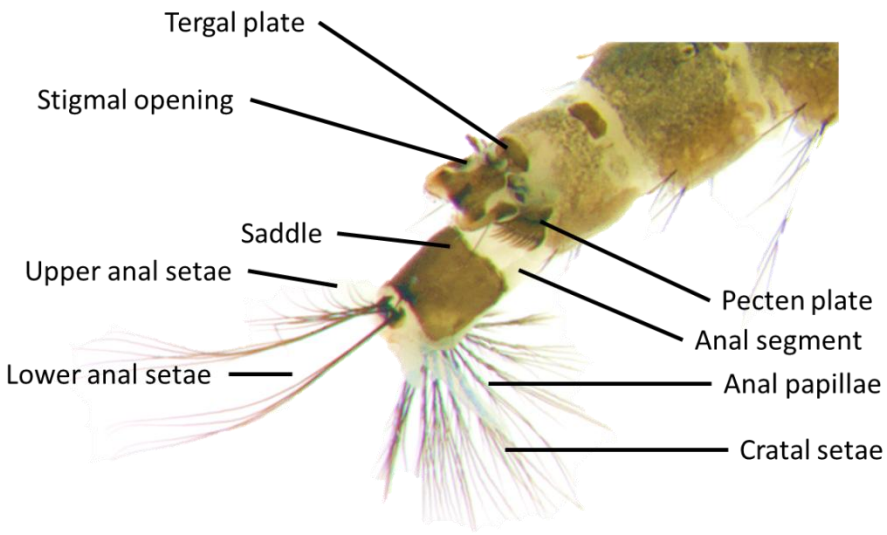

Figure 12: Standard identification characteristics situated at the larval abdomen - (*Anophelinae*).

3.4.2. Standard characteristics of pupal mosquitoes

The mosquito pupa (Figure 13) is comma-shaped and consists of two parts, the cephalothorax and the abdomen, which is further subdivided into 9 segments (Cranston et al. 1987). The last abdominal segment carries the terminal paddles, which are also used for identification (Cranston et al. 1987, Becker et al. 2010). Two respiratory trumpets, located at the cephalothorax, are usually long and cylindrical in culicine mosquito species, but broader and shorter in anophelines (Becker et al. 2010).

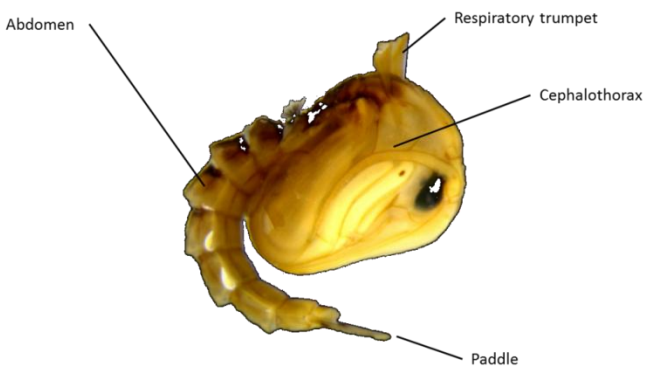

Figure 13: Lateral view of an *Aedes/Ochlerotatus* mosquito pupa.

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3.4.3. Standard characteristics of immature males and females

The proboscis, which is always longer than the thorax, separates adult mosquitoes from other Nematocera (Cranston et al. 1987, Becker et al 2010). The females (Figure 14) have a pilose antenna, whereas a plumose antennae and long hairy maxillary palps are present in males (Cranston et al. 1987). Significant genus and species-specific characteristics are provided by the scales and setae of different shape, length and color at the head, thorax (Figure 15) and abdomen. Furthermore, legs, wings, veins and wing margins are typically covered with scales (Cranston et al. 1987; Becker et al. 2010). Males of mosquito species were further identified using the external genitalia, the hypopygium (Figure 16). It consists of a phallosome surrounded by the aedeagus. The pair of basal gonocoxites, each consisting of a basal lobus with a gonostylus and a gonostylar with a claw on its apex, was used as a further identification standard characteristic (Cranston et al. 1978; Becker et al 2010).

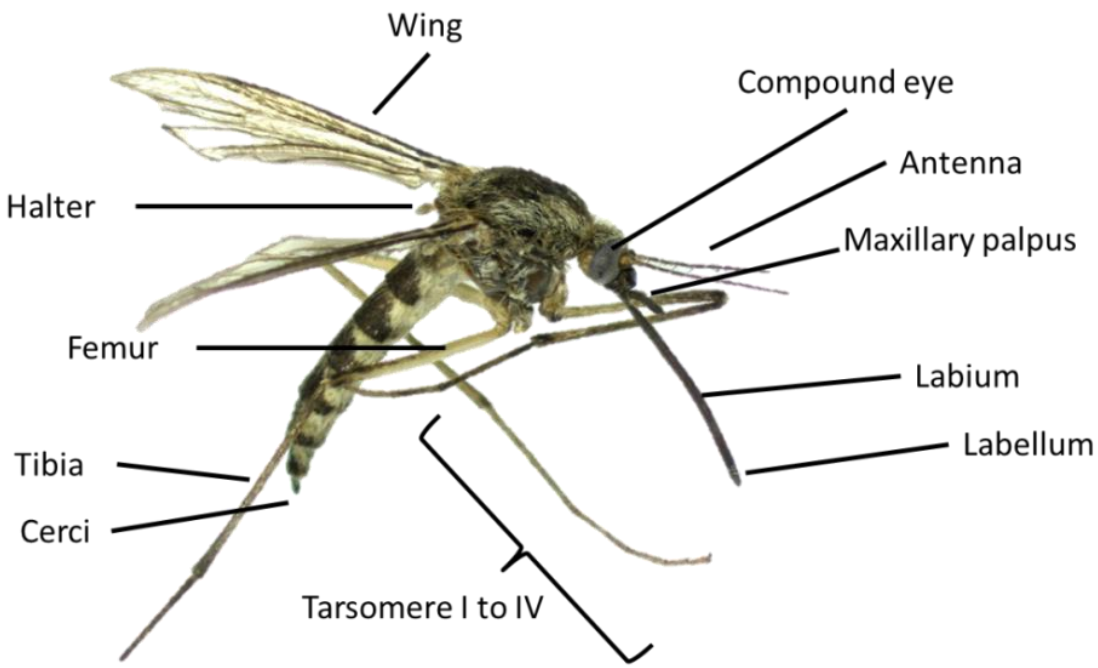

Figure 14: Lateral view of a female mosquito.

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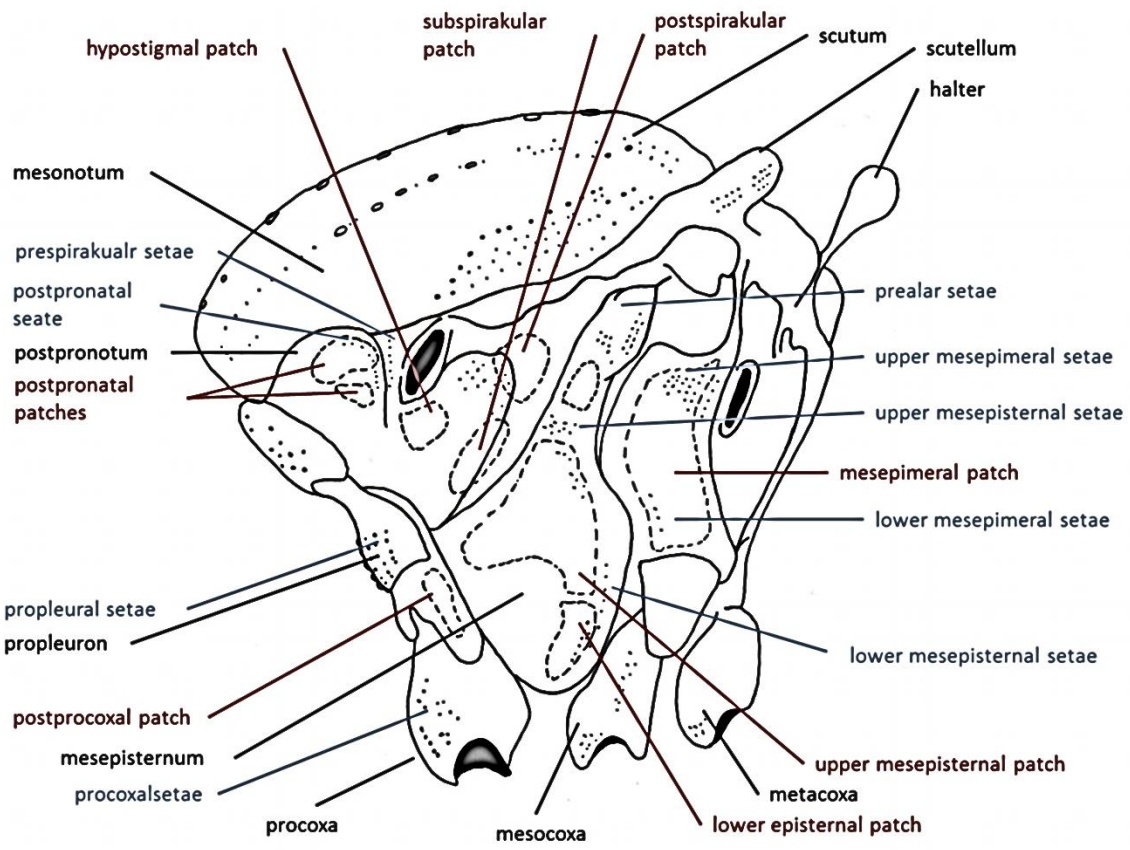

Figure 15: Lateral view of mosquito thorax (pleurites, setation and scale patches).

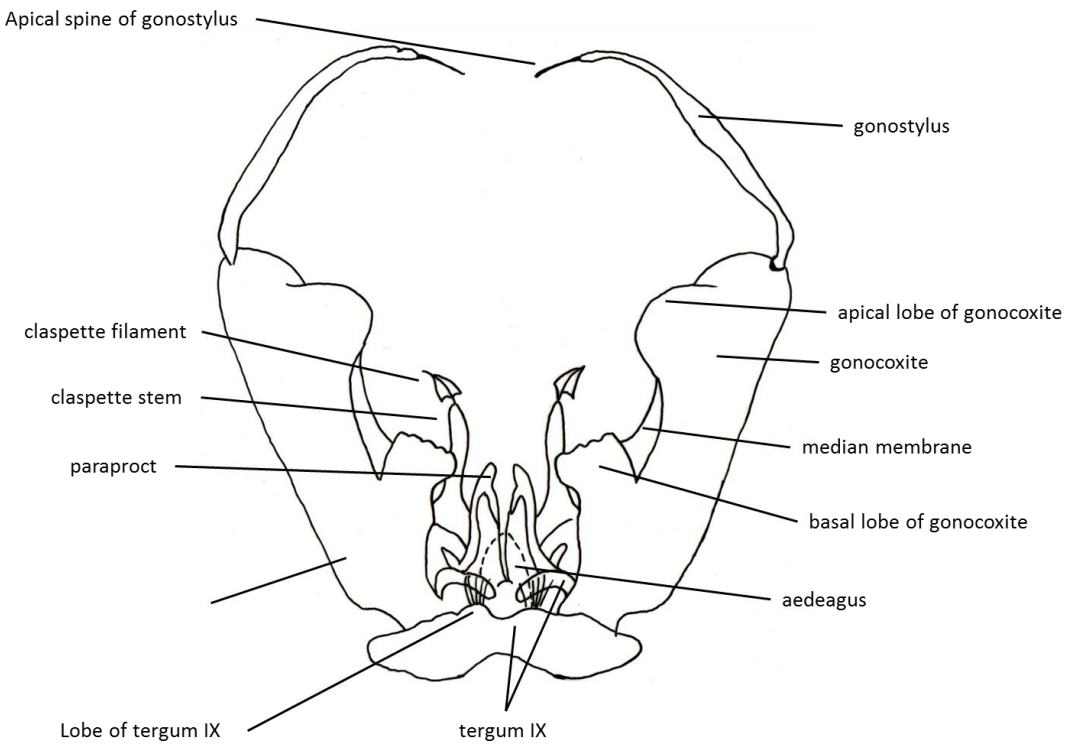

Figure 16: Standard identification characteristics of the male hypopygium of *Aedes/Ochlerotatus.*

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3. Results

3.1 Species inventory, abundance and phenology

A total of 15 Culicidae species (including two morphologically difficult species pairs) were detected from March to October 2011 at 20 sampling sites at Orth and der Donau (Lower Austria). The species inventory consisted to 6 genera, *Anopheles*, *Aedes*, *Ochlerotatus*, *Culex*, *Culiseta* and *Coquilletidia* (Table 7). A total of 34 eggrafts, 1022 larval, 80 pupal and 221 adult mosquitoes (3 males, 218 females) were collected in the study. Adults of *Oc. geniculatus* (40.3%) were most abundant during the sampling period, followed by *Cx. pipiens* (23.1%), *Cx. richiardii* (19.9%) and *Cx. territans* (10.4%) (Table 9). In the larval stage, *Oc. geniculatus* was most abundant (64.1%), followed by larvae of *Cx. territans* (18.7%), *Cx. pipiens* (6.9%) and *Ae. vexans* (2.8%) (Table 8). The remaining species represented 7.5 % of the total catch. Details on site-specific abundances are given in Appendix 1.

Table 7: Culicidae species inventory collected from March to October 2011 at Orth an der Donau at 20 sampling sites.

Anopheles MEIGEN 1818
- *Anopheles maculipennis* complex
- *Anopheles* (*Anopheles*) *plumbeus* STEPHENS 1828

Aedes MEIGEN 1818
- *Aedes* (*Aedes*) *cinereus* MEIGEN 1818 / *rossicus* DOLBESKIN, GORICKAJA & MITROFANOVA 1930
- *Aedes* (*Aedimorphus*) *vexans* MEIGEN 1830

Ochlerotatus LYNCH ARRIBALZAGA 1891
- *Ochlerotatus* (*Finlaya*) *geniculatus* OLIVIER 1791
- *Ochlerotatus* (*Ochlerotatus*) *cantans* MEIGEN 1818/ *annulipes* MEIGEN 1830
- *Ochlerotatus* (*Ochlerotatus*) *cataphylla* DYAR 1916
- *Ochlerotatus* (*Ochlerotatus*) *excrucians* (WALKER 1856)
- *Ochlerotatus* (*Ochlerotatus*) *flavescens* MÜLLER 1764
- *Ochlerotatus* (*Ochlerotatus*) *sticticus* MEIGEN 1838
- *Ochlerotatus* (*Rusticoidus*) *rusticus* ROSSI 1970

Culex LINNAEUS 1758
- *Culex* (*Culex*) *pipiens* LINNAEUS 1758
- *Culex* (*Neoculex*) *territans* WALKER 1856

Culiseta FELT 1904
- *Culiseta* (*Culiseta*) *annulata* SCHRANK 1776

Coquillettidia DYAR 1905

Coquillettidia (*Coquillettidia*) *richiardii* FICALBI 1889

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Table 8: Larval and pupal culicid species inventory (n and percentage) collected from March to October 2011 at Orth an der Donau at 20 sampling sites.

Species	n (l/p)	%	Phenology
Anopheles maculipennis complex	11/0	1.0	May – August
Anopheles (*Anopheles*) *plumbeus* STEPHENS 1828	12/3	2.3	August
Aedes (*Aedes*) *cinereus* MEIGEN 1818 / *rossicus* DOLBESKIN, GORITZKAJA & MITROFANOVA 1930	3/0	0.3	April
Aedes (*Aedimorphus*) *vexans* MEIGEN 1830	30/0	2.8	June – July
Ochlerotatus (*Finlaya*) *geniculatus* OLIVIER 1791	648/36	64.1	April – September
Ochlerotatus (*Ochlerotatus*) *cantans* MEIGEN 1818/ *annulipes* MEIGEN 1818	27/0	2.5	March – April
Ochlerotatus (*Ochlerotatus*) *cataphylla* DYAR 1916	2/0	0.2	March
Ochlerotatus (*Ochlerotatus*) *flavescens* MÜLLER 1764	3/0	0.3	April
Ochlerotatus (*Rusticoidus*) *rusticus* ROSSI 1970	3/0	0.3	March
Culex (*Culex*) *pipiens* LINNAEUS 1758	65/9	6.9	April – September
Culex (*Neoculex*) *territans* WALKER 1856	167/32	18.7	April – September
Culiseta FELT 1904	6/0	0.6	May, July

Table 9: Adult culicid species inventory (n and percentage) collected from March to October 2011 at Orth an der Donau at 20 sampling sites.

Species	n (m/f)	%	Phenology
Anopheles maculipennis complex	0/1	0.5	May
Aedes (*Aedimorphus*) *vexans* MEIGEN 1830	0/1	0.5	August
Ochlerotatus (*Finlaya*) *geniculatus* OLIVIER 1791	0/89	40.3	March to September
Ochlerotatus (*Ochlerotatus*) *annulipes* MEIGEN 1830	0/3	1.4	July – August
Ochlerotatus (*Ochlerotatus*) *cataphylla* DYAR 1916	0/3	1.4	April
Ochlerotatus (*Ochlerotatus*) *excrucians* WALKER 1856	0/2	0.9	May
Ochlerotatus (*Ochlerotatus*) *sticticus* MEIGEN 1838	0/4	1.8	July
Culex (*Culex*) *pipiens* LINNAEUS 1758	3/48	23.1	April-September
Culex (*Neoculex*) *territans* WALKER 1856	0/23	10.4	April – September
Coquillettidia (Coquillettidia) richiardii FICALBI 1889	0/44	19.9	June – August

3.1.1 *Anopheles maculipennis* complex

The *Anopheles maculipennis* complex consists of approximately 12 species which are reproductively isolated, but morphologically very similar; they can be identified using molecular methods (Becker et al. 2010). Anopheline species are characterized by a reduced siphon and the presence of a pecten plate (Figure 17A, arrow) (Cranston et al. 1987; Becker et al. 2010). Larvae of this species complex are separated from other anopheline mosquitoes by their spiculated antennae and long and pinnate frontal setae (Cranston et al. 1987; Becker et al. 2010). The inner clypeal setae are situated close together with long apical branches (Figure 17B, arrows). The palps of female anopheline species are as long as the

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proboscis and the scutellum is evenly rounded. The scutum is dark brown with a longitudinal stripe and the wings are scaled in a species-specific pattern with dense scaling forming spots at some wing vein areas (Becker et al. 2010).

A female of the *Anopheles maculipennis* complex was found on May 16, 2011 at sampling site KB1; Larvae were detected from May to early July at the same sampling site and at the additional breeding habitat AP1 from May to August.

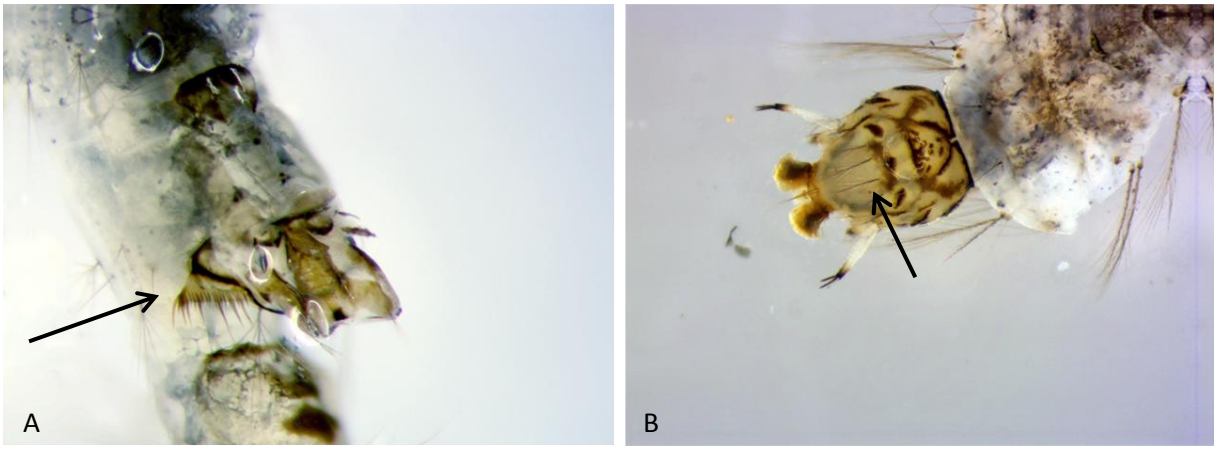

Figure 17: *Anopheles maculipennis* complex; A: reduced siphon with pecten plate (arrow); B: head capsule (arrows: inner clypeal setae).

3.1.2 *Anopheles (Anopheles) plumbeus* (Stephens 1828)

An. plumbeus is separated from other European anopheline mosquito species by the single, tiny frontal setae (Figure 18A, arrow). The antennae are straight and smooth and not covered by spicules when compared with larvae of the *Anopheles maculipennis* complex (Figure 17B, arrows) (Cranston et al. 1987; Becker et al. 2010). Larvae of *An. plumbeus* were abundant at phytotelmata PT2 (6 larvae) and PT4 (6 larvae and 3 pupae) during August 2011, together with larvae of *Cx. pipiens and Oc. geniculatus*.

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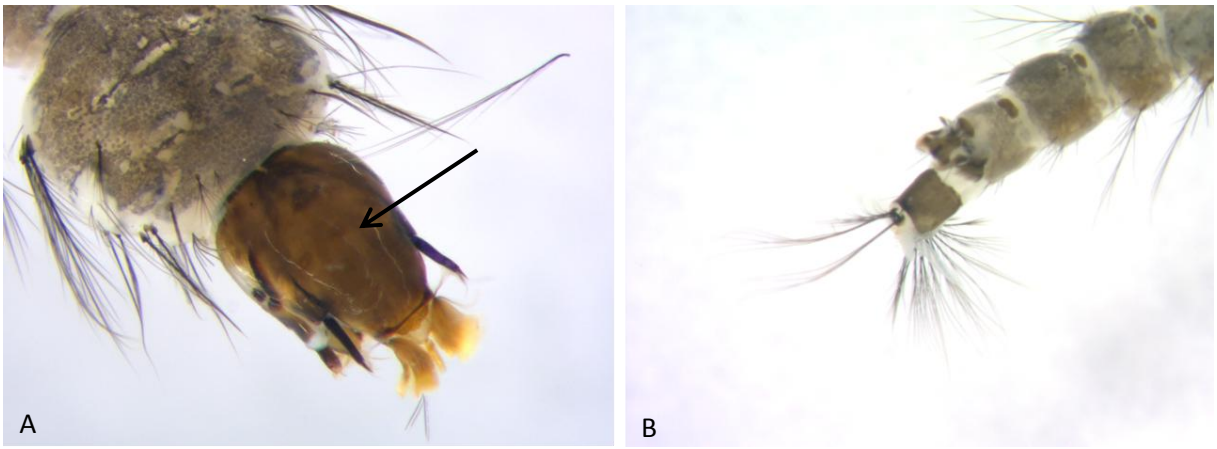

Figure 18: ***Anopheles plumbeus*****; A: head capsule with single, tiny frontal setae (arrow); B: tip of abdomen.**

3.1.3 *Aedes (Aedes) cinereus* (Meigen 1818)/ *rossicus* (Dolbeskin, Goritzkaja & Mitrofanova 1930) (Figure 19)

Larvae of *Ae. cinereus* and *Ae. rossicus* are very similar and differentiation is difficult. The siphonal tuft inserts well beyond the center of the siphon (Mohrig 1969). Larvae of *Ae. cinereus*/ *rossicus* were found in small numbers at sampling sites at temporary water bodies, T1 (1 larva) and T2 (2 larvae).

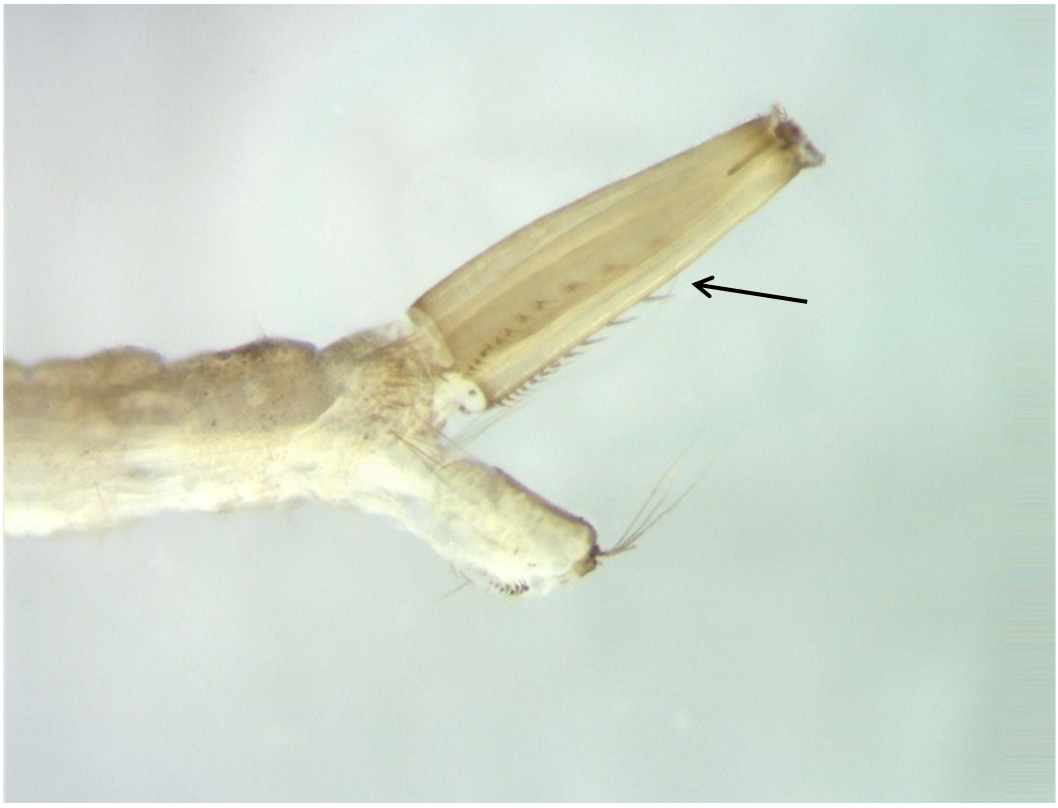

Figure 19: ***Aedes cinereus*****/** ***rossicus*****; tip of abdomen with detached pecten spines (arrow).**

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3.1.4. *Aedes (Aedimorphus) vexans* (Meigen 1830)

Ae. vexans can be distinguished from other mosquito species by a siphonal index of 2.3 to 3.0, with a pecten consisting of 13 to 18 teeth where the apical 2 to 3 teeth are larger than the others and detached (Figure 20A, arrow) (Cranston et al. 1987; Becker et al. 2010). The siphonal tuft is small and situated distally of the siphon center. Comb scales vary from 7 to 13 and are arranged in 1 to 2 irregular rows (Figure 20C, arrow) (Becker et al. 2010). Adults can be seperated from other species by the very narrow pale rings on the tarsi and white transversal basal bands on the terga, constricted in the middle (bilobed pattern) (Becker et al 2010). Larvae of *Ae. vexans* were found at temporary water bodies from June to July; 30 larvae at sampling site T1 and 2 larvae at T2 and T5. One female adult was collected at the Kleine Binn (KB1) in August.

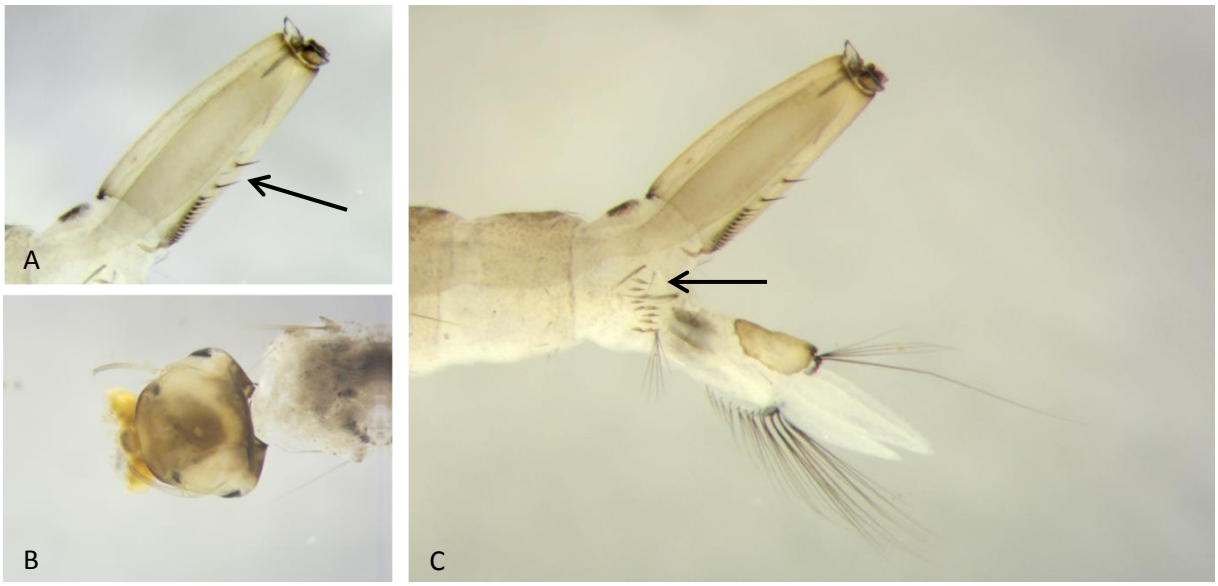

Figure 20: *Aedes vexans*; A: siphon with pecten spines (arrow); B: head capsule with mouthbrush; C: tip of abdomen (arrow: comb scales).

3.1.5 *Ochlerotatus (Finlaya) geniculatus* (Olivier 1791)

Larvae of *Oc. geniculatus* differ from all other indigenous *Aedes*/*Ochlerotatus* species by the presence of numerous stellate seta (Figure 21 C, arrows) spread over the thorax and the abdomen. The antenna is not covered with spicules (Mohrig 1969), and there is only a single antennal seta (Becker et al. 2010). Further standard identification characteristics are the unequally long anal papillae (Figure 21A, arrow) and the single row of large comb scales at the siphon (Figure 21 B, arrow). Adults are identified by white spots of the cerci, white

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triangular lateral patches at the black-scaled terga (Figure 22B, arrow) and a narrow band of white scales around the eyes (Becker et al. 2010). The legs are dark with a white spot at the femora. Adults and aquatic stages of *O. geniculatus* were found exclusively at phytotelmata (PT1 to PT6) during the entire sampling period from March to October 2011. Adult *Oc. geniculatus* were collected in high abundances at PT4 and in low abundances at other phytotelmata. A total of 883 larvae, 36 pupae and 89 female adults were recorded from late March to late September 2011. Larvae and pupae were most abundant at sampling sites PT3 and PT4. In the aquatic stages this species was associated with larvae of *Cx. pipiens* and *An. plumbeus* at PT4.

Figure 21: *Ochlerotatus geniculatus*; A: tip of abdomen (arrow: anal papillae); B: siphon (arrow: comb scales); C: stellate seta (arrows).

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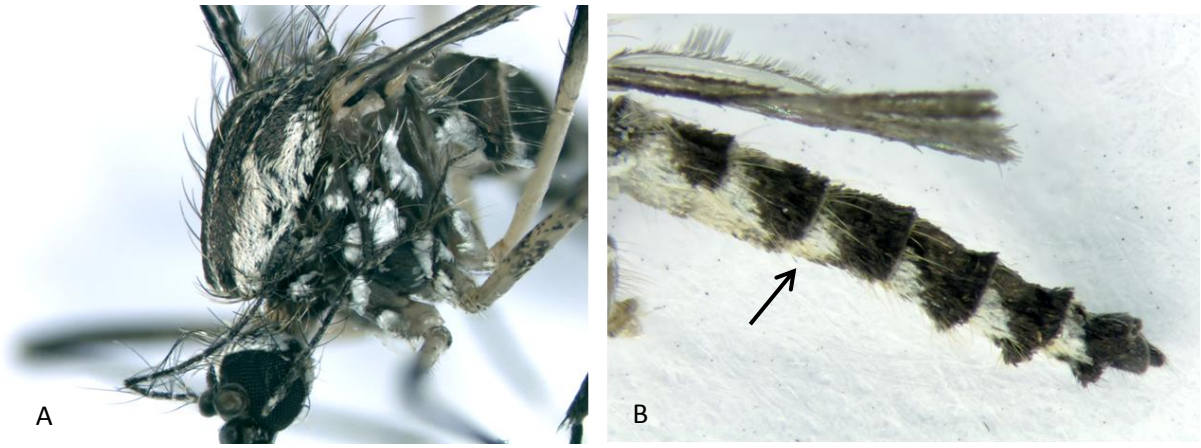

Figure 22: *Oc. geniculatus*; A: head and thorax; B: white patches of the abdominal terga (arrow).

3.1.6 Ochlerotatus (Ochlerotatus) cantans (Meigen 1818) */annulipes* (Meigen 1830)

The close species pairs *Oc. cantans/ Oc. annulipes* has a saddle seta which is as long as the saddle, and the siphonal tuft is approximately as long as the siphon width at the insertion point (Figure 23A, arrow). Anal papillae are as long as or longer than the saddle, and the siphonal index is less than 3.0. The ventral brush of these two species consists of 4 to 6 precratal setae and 10 to 20 cratal setae (Figure 23B, arrow) (Mohrig 1969; Cranston et al. 1987). Larval stages of *Oc. cantans* and *Oc. annulipes* are difficult to separate from each other and were found exclusively at temporary ponds with high abundances at T2 and T5 from early March to April. In addition, 3 female adults originating from Phytotelmata PT1 could be identified as *Oc. annulipes*.

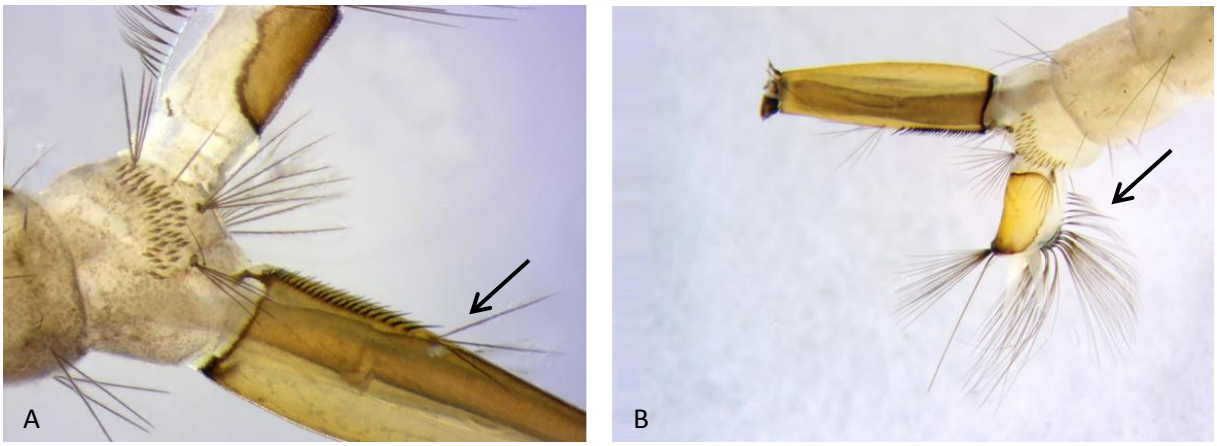

Figure 23: *Ochlerotatus cantans/ annulipes*; A: pecten and siphonal tuft (arrow); B: tip of abdomen (arrow: precratal setae).

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3.1.7 *Ochlerotatus (Ochlerotatus) cataphylla* (Dyar 1916)

Oc. cataphylla has 10 to 30 (usually 25) comb scales arranged in 2 to 3 irregular rows. The siphonal index is around 3. The pecten is made up of 13 to 25 teeth with the last 2 to 4 pecten teeth located beyond the siphonal tuft, which is located approximately at siphon center (Figure 24, arrow) (Becker et al. 2010). Two larvae were found at temporary pond T5 on March 26, two females at T2 on April 19 and 1 female at the Wachtelgraben S1 on April 21, 2011.

Figure 24: *Ochlerotatus cataphylla*; tip of abdomen with siphonal tuft (arrow).

3.1.8 *Ochlerotatus (Ochlerotatus) excrucians* (Walker 1856)

Adults of *Oc. excrucians* have white rings on their palps. White subspiracular and postspiracular scales as well as a postprocoxal patch are present. The fore unguis is bent in a sharp angle (Mohrig 1969). Two females of *Oc. excrucians* were found at sampling site PT1 on May 31, 2011.

3.1.9 *Ochlerotatus (Ochlerotatus) flavescens* (Müller 1764)

Larvae of *Oc. flavescens* have similar identification characteristics than *Oc. cantans,* but the anal papillae are half as long as the saddle and the siphonal index is ≥ 3.0. The saddle seta is nearly as long as the saddle, and the siphonal tuft is as long as the siphon width at the point of insertion (Becker et al. 2010). Larvae of this species were collected at temporary pond T1 (3 individuals) on April 15, 2011.

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3.1.10 *Ochlerotatus (Ochlerotatus) sticticus* (Meigen 1838)

Proboscis and palps of adult Oc. sticticus are dark-scaled. The hypostigmal patch is absent, but subspiracular and postspiracular scale patches are present. Abdominal terga are dark-scaled with pale basal bands (Becker et al 2010). Four adult females were found at sampling site PT1 on July 29, 2011.

3.1.11 *Ochlerotatus (Rusticoidus) rusticus* (Rossi 1790)

The number of comb scales of *Oc. rusticus* varies from 10 to 18 (Figure 25 B, arrow), usually arranged in 2 irregular rows. The siphonal tuft is located approximately at the center of the siphon (Figure 25A, arrow) (Becker et al. 2010). One to three of the pecten spines are detached. Three larvae were collected at temporary pond T5 on March 23, 2011.

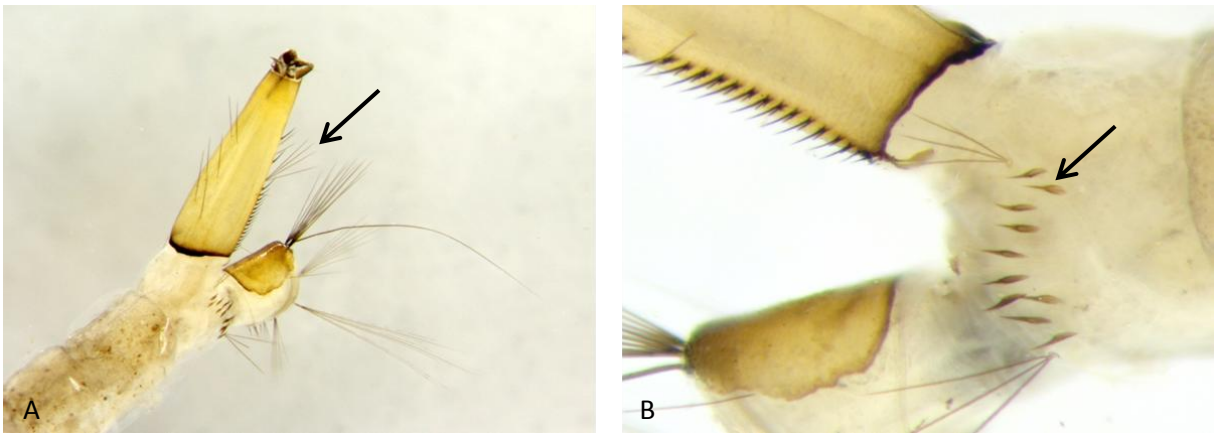

Figure 25: *Ochlerotatus rusticus*; A: siphon with siphonal tuft (arrow); B: pecten teeth (arrow).

3.1.12 *Culex (Culex) pipiens* (Linnaeus 1785)

The larval head of *Cx. pipiens* is wider than long. The siphonal index ranges from 4.8 to 5.0, with the siphon itself being slender (Figure 26B) and tapered toward the apex while the number of pecten teeth is 13 - 17. The species is further characterized by the presence of several paired basal siphonal tufts (Figure 26B, arrows) and the seta at the saddle is usually a single one. The number of the short comb scales at the last abdominal segment (Figure 26C, a) is about 40. The dorsal pair of anal papillae is approximately twice as long as the saddle (Figure 26C, b). The scutellum of females has narrow pale scales and dark setae (Figure 26D, arrow), white scales patches are situated at the mesepisternum, and postspiracular and prespiracular setae are rarely present. The abdomen is dark-scaled with mainly yellowish basal bands (expanding laterally) (Cranston et al. 1987; Becker et al. 2010). A total of 733

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larvae, 9 pupae and 51 adults (48 females and 3 males) were collected during the entire sampling period from April to September at a variety of breeding habitats.

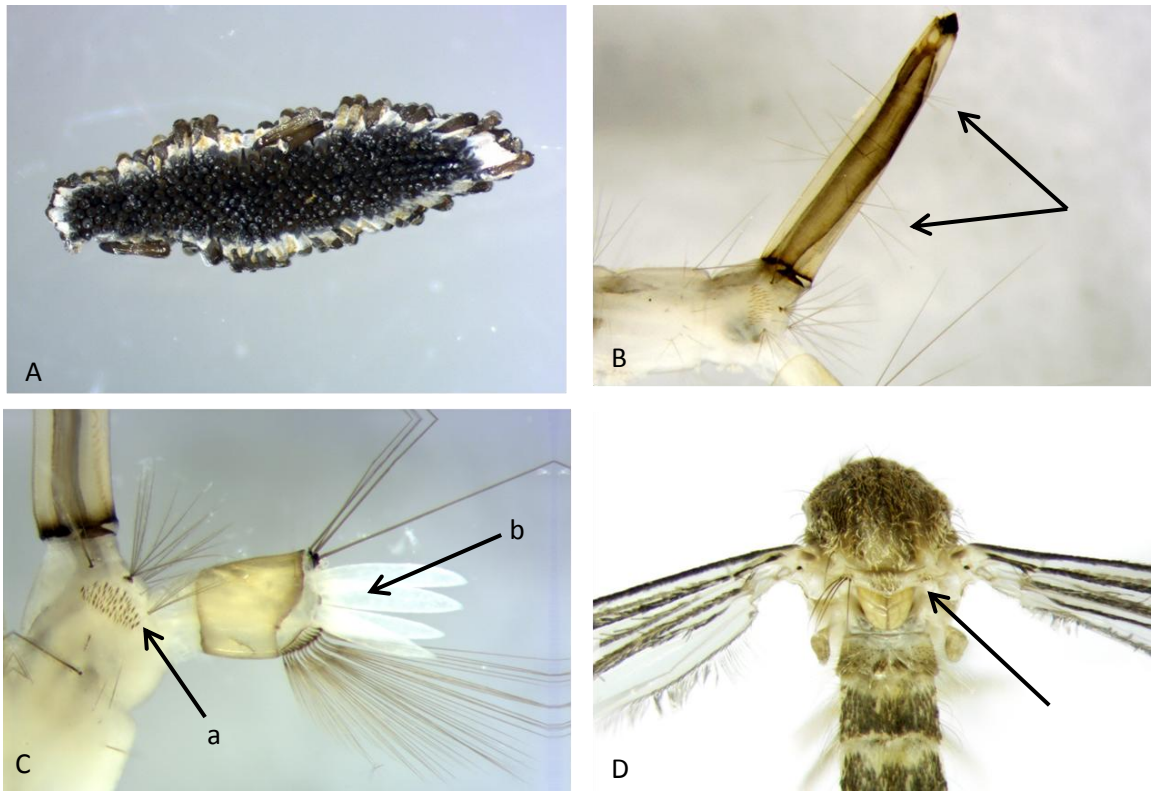

Figure 26: *Culex pipiens;* A: eggraft; B: siphon with several siphonal tufts (arrows); C: comb scales (a) and anal papillae (b); D: scutellum of female (arrow).

3.1.13 Culex (Neoculex) territans (Walker 1856)

Cx. territans has about 50 comb scales (Figure 27A, a) and the siphonal index ranges from 6.0 to 7.0 (Figure 27A). The siphon is long and slender, tapering to the apex (Cranston et al. 1987; Becker et al. 2010), where it distingly expands (Figure 27A, b) in comparison with *Cx. pipiens* (Figure 26B). The larval head is distinctly broader than long and the antennae are as long as the head (Figure 27B, arrow) (Cranston et al. 1987; Becker et al. 2010). The abdomen of female *Cx. territans* is relatively narrow with no widening in the middle (Figure 27C) when compared with females of *Cx. pipiens* (Figure 26D). *Cx. territans* (89 larvae, 32 pupae and 23 female adults) were found from early April to early September at 7 different sampling sites. Aquatic stages and adults were most abundant at the Kleine Binn (KB1 and KB2), mostly associated with species of the *Anopheles maculipennis* complex, and the Fadenbach (F1 and

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F2). Lower numbers were detected at the Wachtelgraben (S1) and at temporary ponds (T2 and T4).

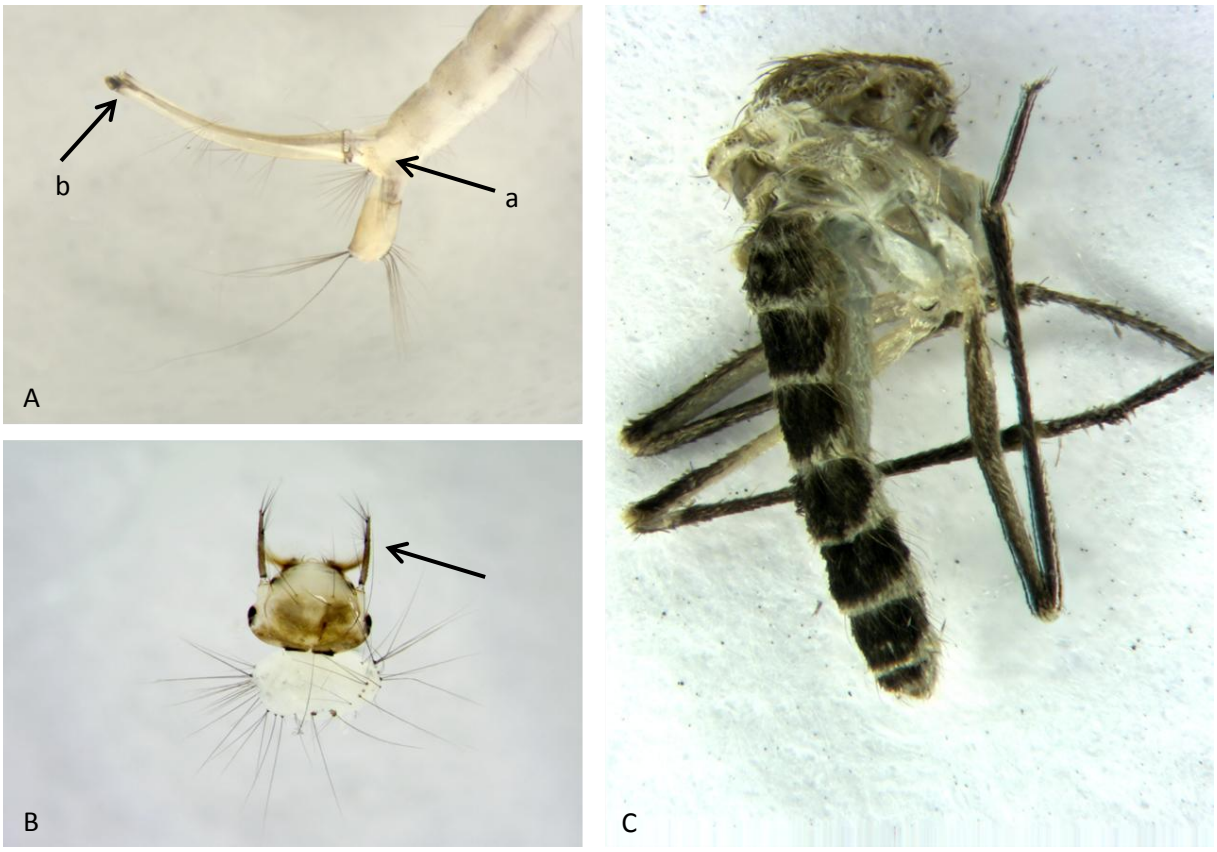

Figure 27: *Culex territans*; A: tip of larval abdomen; B: head capsule with antennae (arrow); C: female adult.

3.1.14 *Culiseta sp.* (Felt 1904)

Larvae of *Culiseta* sp. differ from other mosquito genera by the siphonal tuft (Figure 28, arrow), which is situated at the base of the respiratory siphon (Mohrig 1969). Six larvae of this genus were detected at 4 sampling sites (T1, T2 and KB1) in May and early July, with one larva being identified as *Culiseta annulata* (Schrank 1776).

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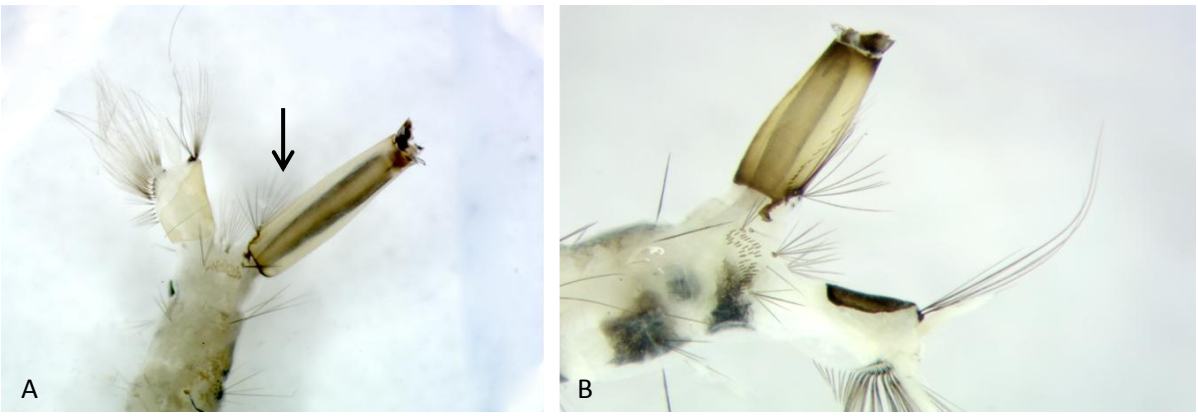

Figure 28: *Culiseta* sp.; A: tip of larval abdomen with siphonal tuft (arrow), B: respiratory siphon detail.

3.1.15 *Coquilletidia (Coquilletidia) richiardii* (Ficalbi 1889)

Adults of *Cq. richiardii* are characterized by a trilobed scutellum, an apically rounded abdomen with short, hardly visible cerci, broad and conspicuous wing scales and the absence of prespiracular and postspiracular setae at the thorax. The claws are simple and pulvilli are absent (Mohrig 1969). Adults of this species were present at temporary pond T4 and at 4 phytotelmata (PT2, PT3, PT4 and PT6) from late June to early August.

3.2 Life cycles

Biometrical data (larval head capsule widths, body lengths) of the most abundant species (*Cx. pipiens*, *Cx. territans* and *Oc. geniculatus*) were measured to define larval instars for life cycle reconstruction. Size-frequency histograms for head capsule widths clearly separated the 4 larval instars of the three mosquito species and are shown in Figure 29.

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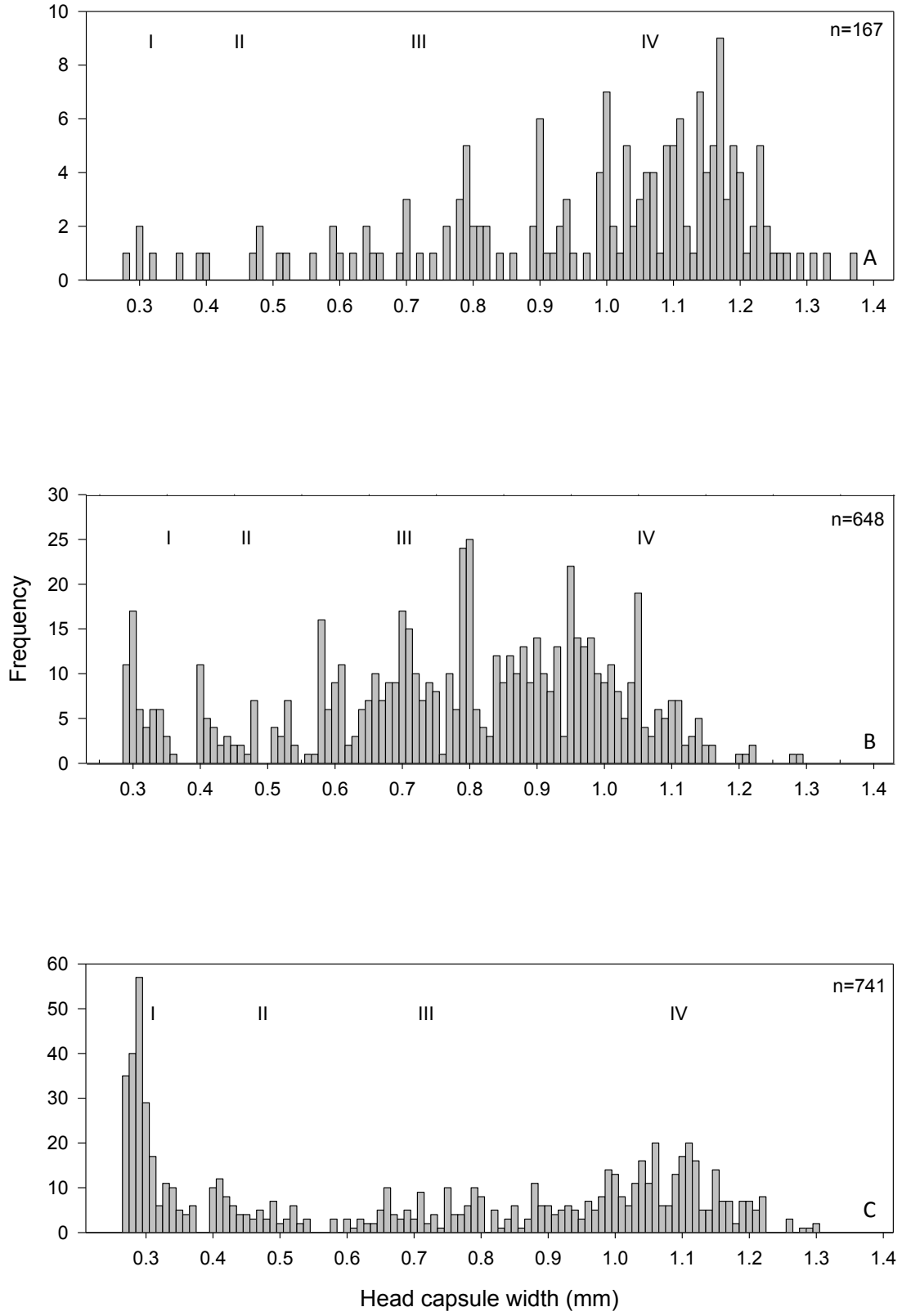

Figure 29: Size-frequency histograms of larval head capsule width measurements of the three most abundant Culicidae species *Cx. territans* (A), *Oc. geniculatus* (B) *and Cx. pipiens* (C) collected at Orth an der Donau. Roman numerals indicate the larval instars.

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Growth patterns of Culicidae reflect the interaction of the size increment per moult and the moulting frequency. In the three most abundant Culicidae species, the average head width increment per moult was proportionately constant at 33 – 56 % (Figure 30, Table 10); therefore Dyar's rule was applicable. Average head capsule width increments, reflected by the growth ratios shown in Table 10, were 43-52 % in *Cx. territans* and 49-60% in *Oc. geniculatus*; lowest increments were observed in *Cx. pipiens* (31-34%).

Table 10: Range of head capsule widths (mm) and growth ratios of the three most abundant mosquito species.

Species	***Cx. territans***		***Cx. pipiens***		***Oc. geniculatus***	
	Head capsule width		**Head capsule width**		**Head capsule width**	
Instar	**range (mm)**	**growth ratio**	**range (mm)**	**growth ratio**	**range (mm)**	**growth ratio**
1	0,26-0,40		0,29-0,36		0,25-0,37	
		1.50		1.33		1.60
2	0,47-0,52		0,40-0,48		0,40-0,54	
		1.43		1.34		1.58
3	0,56-0,86		0,51-0,80		0,58-0,80	
		1.52		1.31		1.49
4	0,89-1,37		0,81-1,37		0,82-1,36	

Head capsule widths were clearly separated (Table 10) and did not overlap within a given species. The ranges of larval head capsule widths of *Cx. territans*, *Cx. pipiens* and *Oc. geniculatus* vary slightly within each instar. The lowest head capsule width observed was 0.25 mm, the highest was 1.37mm (Table 10).

A total of 222 specimens (larvae, pupae and adults) of *Cx. territans* were used for life cycle reconstruction (Figure 31). Larvae were detected from April to June 2011 in high abundance, when compared to September (3 larvae). Larvae pupated in May and July in low abundances; adults were sampled from May to July 2011. The sampling sites dried up in October. This species most probably had 2 generations emerging in May/June and June/July.

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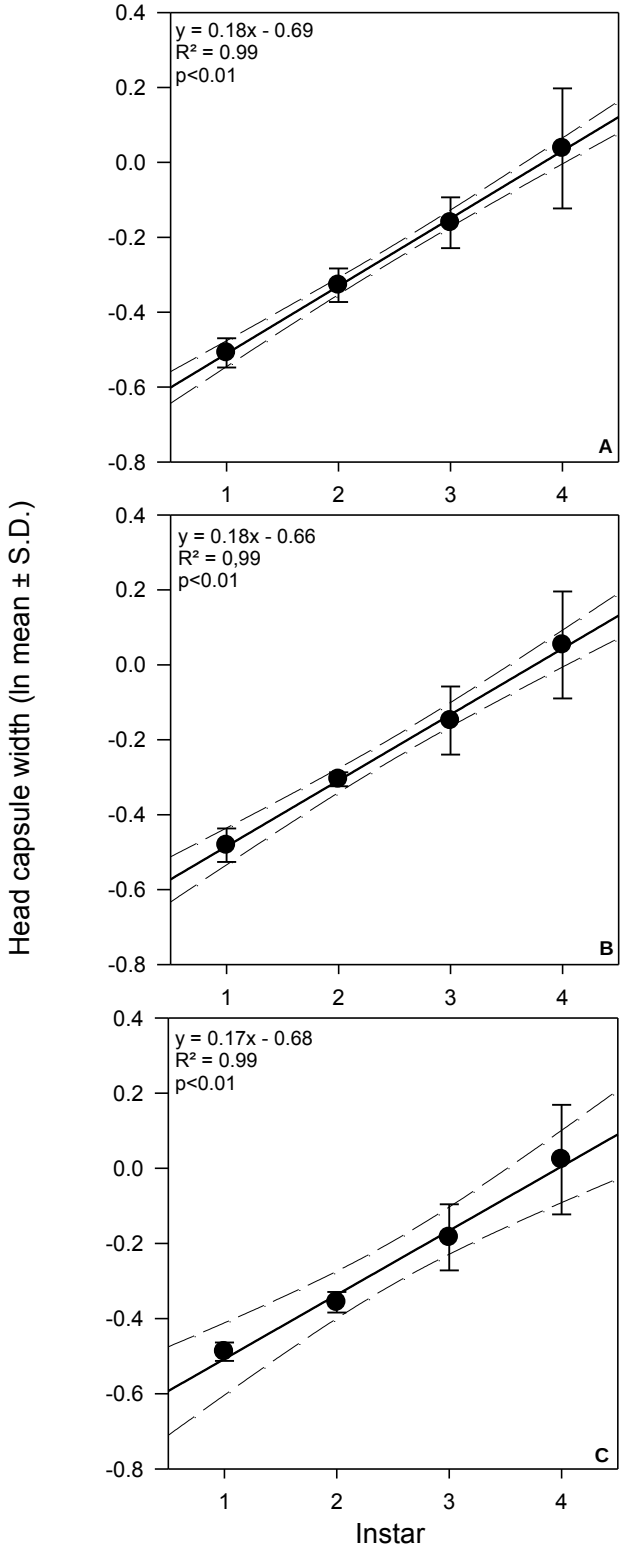

Figure 30: Dyar's rule was applicable for instar versus ln head width regressions in *Ochlerotatus geniculatus* (n=648) (A), C*ulex territans* (n=167) (B) and *Culex pipiens* (n=741)(C).

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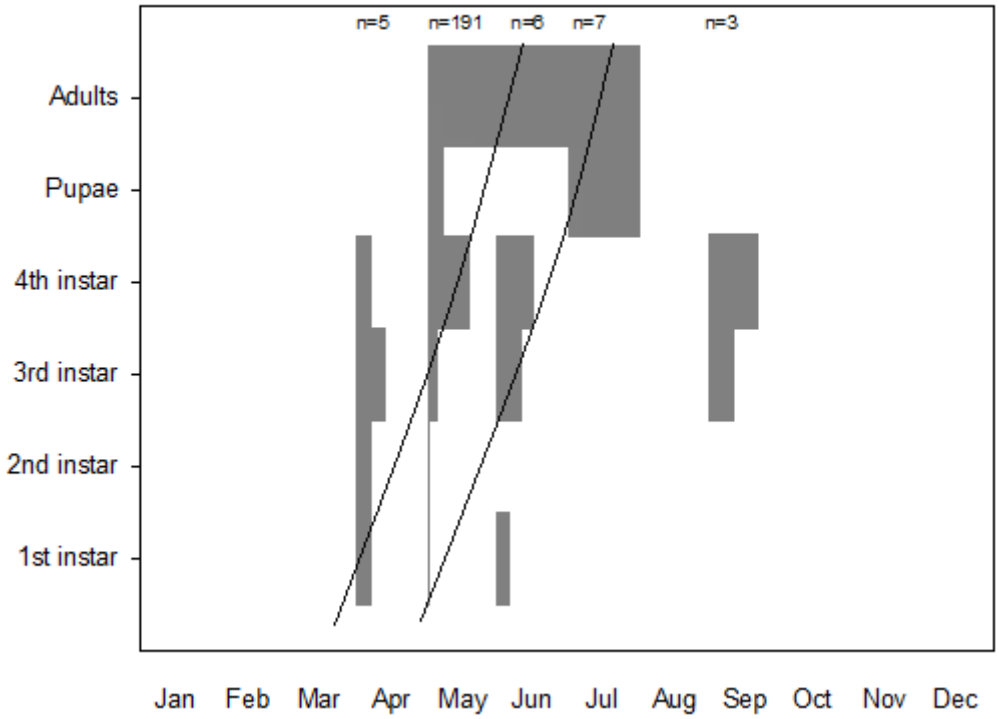

Figure 31: Life cycle diagram of *Cx. territans*, showing the flight period (=Adults) and the percentage of larval instars 1-4 and pupae. The number of individuals sampled at each month is shown at the top of the diagram. Generations are indicated by straight lines.

In *Oc. geniculatus*, a total of 868 individuals (larvae, pupae and adults) sampled from early March to the end of October 2011 were included in life cycle reconstruction. This species had 2 generations emerging in May and July/August. As sampling sites dried up at the end of September, a third generation was unable to emerge. Pupae were detected from April to May and from July to August; larvae were abundant from March to late September (Figure 32). A total of 759 larvae, pupae and adults were collected in *Cx. pipiens* (Figure 33). Larvae were detected from March to September, a small number of pupae in March and from June to August. The flight time ranges from May to the end of July. Sampling sites dried up in September, terminating the development of larvae detected this month. Two generations emerged in May/June and July with the possibility of a spring generation in March/April.

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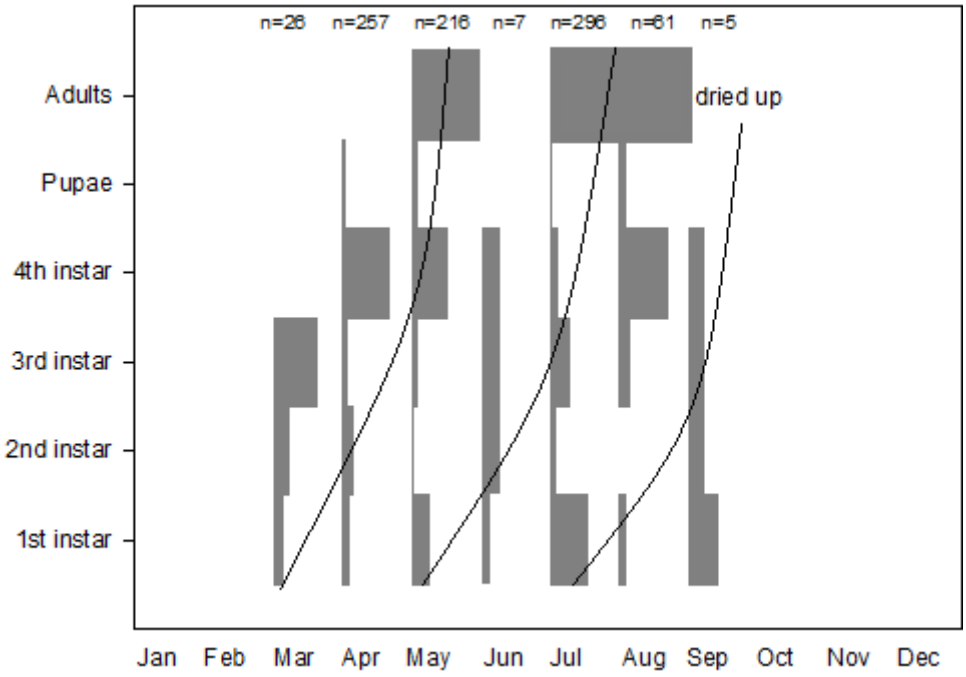

Figure 32: Life cycle diagram of *Oc. geniculatus*, showing the flight period (=Adults) and the percentage of larval instars 1-4 and pupae. The number of individuals sampled at each month is shown at the top of the diagram. Generations are indicated by straight lines.

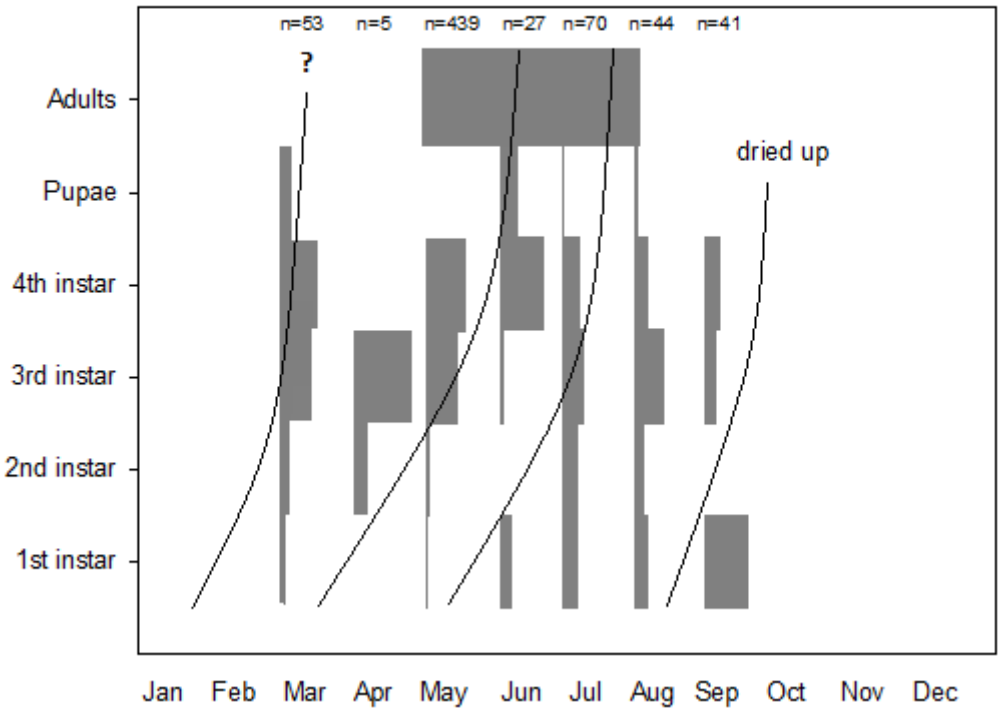

Figure 33: Life cycle diagram of *Cx. pipiens*, showing the flight period (=Adults) and the percentage of larval instars 1-4 and pupae. The number of individuals sampled at each month is shown at the top of the diagram. Generations are indicated by straight lines.

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3.3 Microhabitat characteristics

In order to explore the effects of environmental parameters on spatial distribution of larval Culicidae of the Danube floodplain, a cluster analysis (hierarchical classification, Wards method) based on the euclidean distance was performed using the data listed in chapter 2.2 (Figure 34). Four groups of habitats were extracted (Table 11).

Group 1 (Table 11) consists of sampling sites PT5, S2 and temporary water bodies (T1-T5). Here the highest amount of larval mosquitoes with 1044 individuals out of 8 different species (Figure 35A) was found. The breeding sites are characterized by a low water level (0.50 to 10.53 cm), very low oxygen concentrations (0.80 to 10.10 mgL^{-1}) and the highest loads of chloride (10.99 to 78.60 mgL^{-1}) and sulphate (7.65 to 193.12 mgL^{-1}) of all groups (Table 11).

Phytotelmata (PT1-PT6) with exception of PT5 belong to group 2 (Table 11) which consists of temporary water bodies with a very low water level (only 3 - 9 cm), with the lowest electric conductivity (214 to 574 μScm^{-1}) and the highest amounts of nitrate (1.22 to 32.44 mgL^{-1}) of all groups (Table 11). A total of 871 larvae were detected at these special breeding habitats. Larvae of *Oc. geniculatus* were found in high numbers at these sampling sites during the entire sampling period, and some larvae of *Cx. pipiens* and *An. plumbeus* occurred in autumn (Figure 35B).

Group 3 (Figure 35C) combines sampling sites at the Kleine Binn (KB1 and KB2) and the Große Binn (GB1). The species inventory consists of *Cx. territans* (77 larvae), *An. maculipennis* (12 larvae) and *Cs. annulata* (1 larvae) at KB1 and KB2 and of one larva of *Cx. territans* at GB1 (larvae), but no mosquitoes were detected at GB1. GB1 and both KB1 and KB2 are permanent water bodies with a high water level when compared with groups 1, 2 and 4. In addition, comparatively high oxygen concentrations ranging from 7.16 to 10.17 mgL^{-1} and lower nutrient concentrations than at the breeding sites of group 1 were measured (Table 11).

Group 4 (Table 11) consists of the sampling sites located at the Fadenbach (F1, F2, and F3) and one sampling site at the Wachtelgraben (S1). These sites were characterized by water persistence, a nearly constant water level during the entire sampling period, a high conductivity ranging from 408 to 999 μScm^{-1} and chloride concentrations ranging from 18.86 to 68.44 mgL^{-1}. A high predator density (*Dytiscus marginalis* (Linnaeus 1758), *Chaoborus crystallinus* (De Geer 1776)) was detected in comparison to groups 1 to 2. Twenty-five larval

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mosquitoes (Figure 35D) were detected at these breeding habitats, mostly *Cx. territans* and less *Cx. pipiens*.

To detect habitat parameters associated with the distribution of Culicidae species, a Canonical correspondence analysis was performed, based on larval abundance and five environmental variables. Monte-Carlo permutation tests revealed that conductivity, water level, water persistence, pH and phosphate concentrations contributed significantly ($P<0.05$) to the model (Figure 36). The model explains in total 99.8 % of the variance of the 5 selected variables. The first axis discriminated between species in short-living temporary water bodies and species in stagnant water bodies, with *Culex territans* preferring stagnant water bodies with high water levels. In fact, aquatic stages of *Culex territans* were found in high abundances in stagnant water bodies (KB1, KB2 and F3) where water levels ranged from 46 to 103 cm, and only in very low abundances in temporary water bodies. *Ochlerotatus geniculatus* and A*nopheles plumbeus* were exclusively found at phytotelmata sampling sites where concentrations of PO_4 were high, ranging from 1.10 to 16.18 mgL^{-1}. Larvae of *Ochlerotatus annulipes/cantans* and *Aedes vexans* are constricted to short-living temporary ponds. *Cx. pipiens*, on the other hand, is abundant at a variety of sampling sites, ranging from slowly-flowing running waters to phytotelmata and temporary water bodies. The results of the Canonical Correspondence Analysis of environmental variables versus sampling sites (Figure 36B) underline the results of the Cluster analysis (Figure 35) which split the sampling sites into four groups: phytotelmata, temporary water bodies, Kleine and Große Binn and Fadenbach sampling sites.

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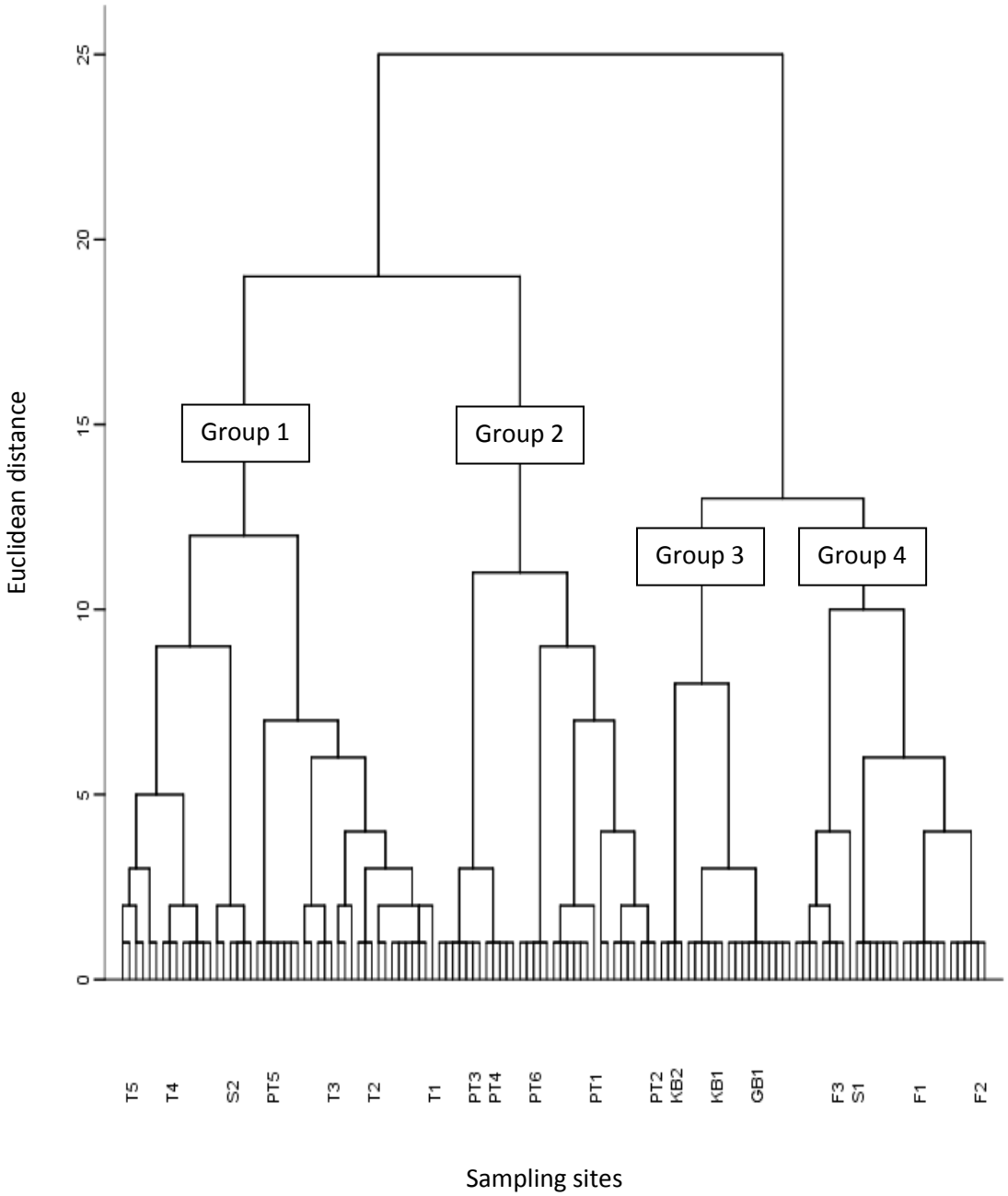

Figure 34: Cluster analysis (hierarchical classification, Wards method), based on abiotic parameters given in chapter 2.2; showing euclidean distances of Culicidae sampling sites in Danubean floodplain water bodies within the Nationalpark Donau-Auen.

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Table 11: Habitat characteristics of the Culicidae species sampled in Danubean floodplain water bodies within the Nationalpark Donau-Auen, based on cluster analysis.

Parameter	Group 1	Group 2	Group 3	Group 4
Water level (cm)	0.50-10.53	3.50-9.16	34.05-74.33	46.10-103.53
Water supply (days)	58-245	114-181	245	245
Conductivity (μScm^{-1})	247-1310	214-574	451-631	408-999
O^2 (mgL-1)	0.80-10.10	1.50-22.80	7.16-10.17	3.44-8.11
O^2 (%)	7.90-105.10	18-87	82.46-106.39	41.15-90.74
pH	7.20-8.20	5.92-7.53	7.57-9.6	7.31-7.89
Temperature (°C)	9.20-21.77	12.87-22.80	8.21-19.39	14.30-21.77
NH4 (mgL^{-1})	0-5.43	0-10.60	0-0.05	0-0.89
Cl (mgL^{-1})	10.99-78.60	2.61-28.51	15.22-18.30	18.86-68.44
NO_3 (mgL^{-1})	0-14.41	1.22-32.44	1.86-5.49	0.59-25.08
PO_4 (mgL^{-1})	0-16.18	1.10-16.18	0-0.04	0.01-4.15
SO_4 (mgL^{-1})	7.65-193.12	2.30-28.56	32.25-45.56	22.93-139.20
Water hardness ($mmolL^{-1}$)	1.26-3.48	1.20-4.14	2.60-3.06	1.73-4.09
Carbonate hardness ($mmolL^{-1}$)	0.95-2.84	1.02-2.52	2.01-2.67	1.51-3.65
Mosquito species inventory	*Cx. pipiens* *Cx. territans* *Ae. vexans* *Oc. cataphylla* *Oc. geniculatus*	*An. plumbeus* *Cx. pipiens* *Oc. geniculatus*	*An. maculipennis* complex *Cx. territans* *Cs. annulata*	*Cx. pipiens* *Cx. territans*
Sampling sites	PT5, S2, T1, T2, T3, T4, T5	PT1, PT2, PT3 PT4, PT6	GB1, KB1, KB2	F1, F2, F3, S1

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Table 12: Arithmetic means of predator density (CPUE; m^{-2} min^{-1}) of four groups of breeding habitats, grouped according to Cluster Analysis (+ = present; - = absent)

Predator	Group1	Group2	Group3	Group4
Invertebrate Predators				
Chaoborus crystallinus	0.25	0	0.06	15.23
Asilius sulcatus	0.09	0	0	0.15
Nepa cinerea	0	0	0.11	0.23
Ranatra linearis	0	0	0	0.23
Notonecta glauca	0.05	0	0.09	0.05
Gerris sp.	0.78	0	0.39	0
Hydrometra stagnorum	0.44	0	1.27	0
Zygoptera	0.09	0	0.36	0
Anisoptera	0.35	0	0	0
Hyphydrus ovatus	0.04	0	0	0.15
Ilyocoris cimicoides	0	0	0	0.09
Dytiscus marginalis	0.3	0	0	0.12
Newts	+	-	-	+
Fish	-	-	+	+
Range	0-0.78	0	0-1.27	0-15.23

Figure 36 B highlights the high conductivity of the temporary waters due to high evaporation rates. Phytotelmata sampling sites, where *Anopheles plumbeus* and *Ochlerotatus geniculatus* were found, are characterised by high phosphate concentrations. Große and Kleine Binn as well as the Fadenbach sampling sites were mainly split according to water persistence, water level and pH.

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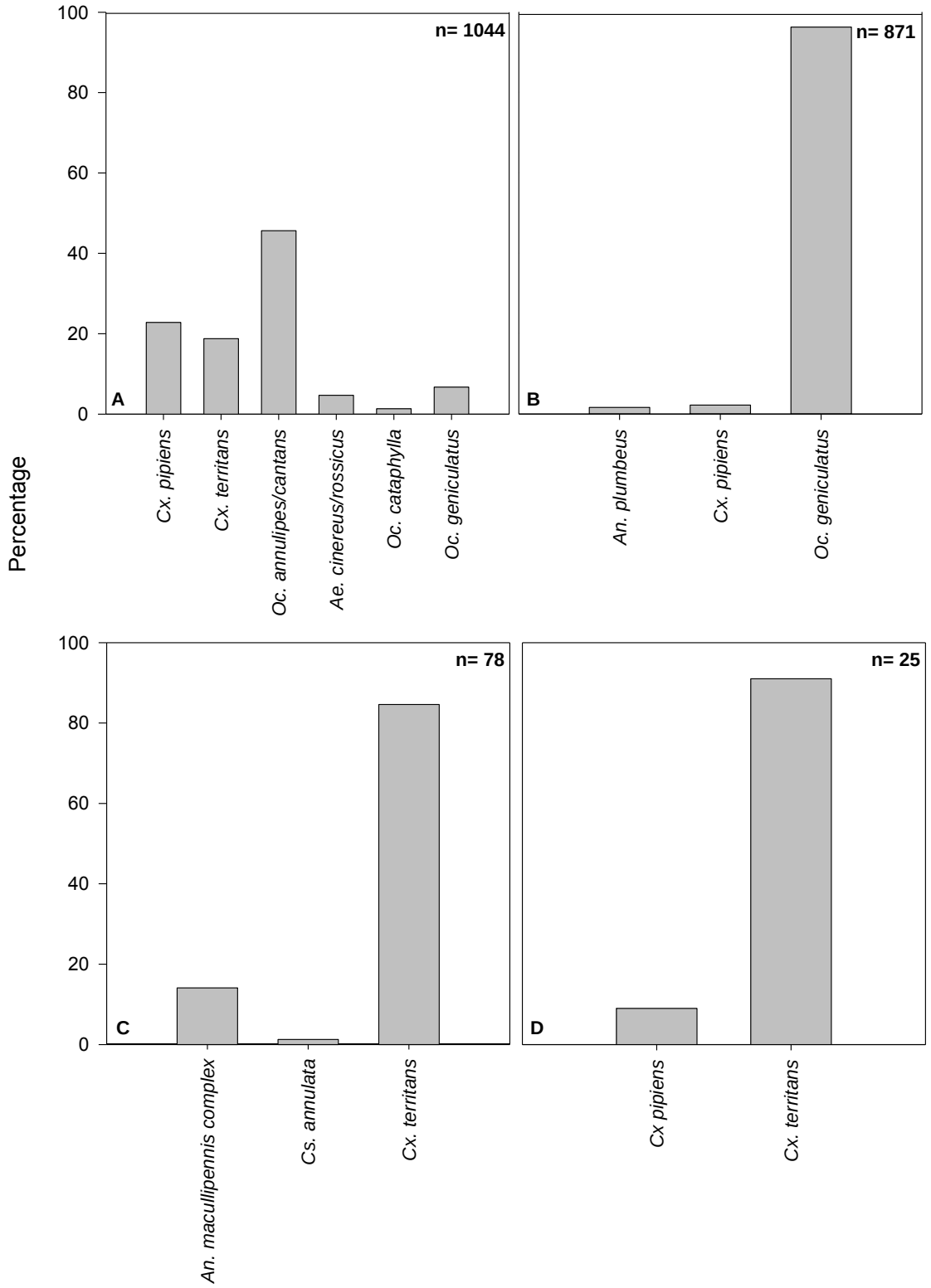

Figure 35: Percentage of species of Group 1 (A), Group 2 (B), Group 3 (C) and Group 4 (D).

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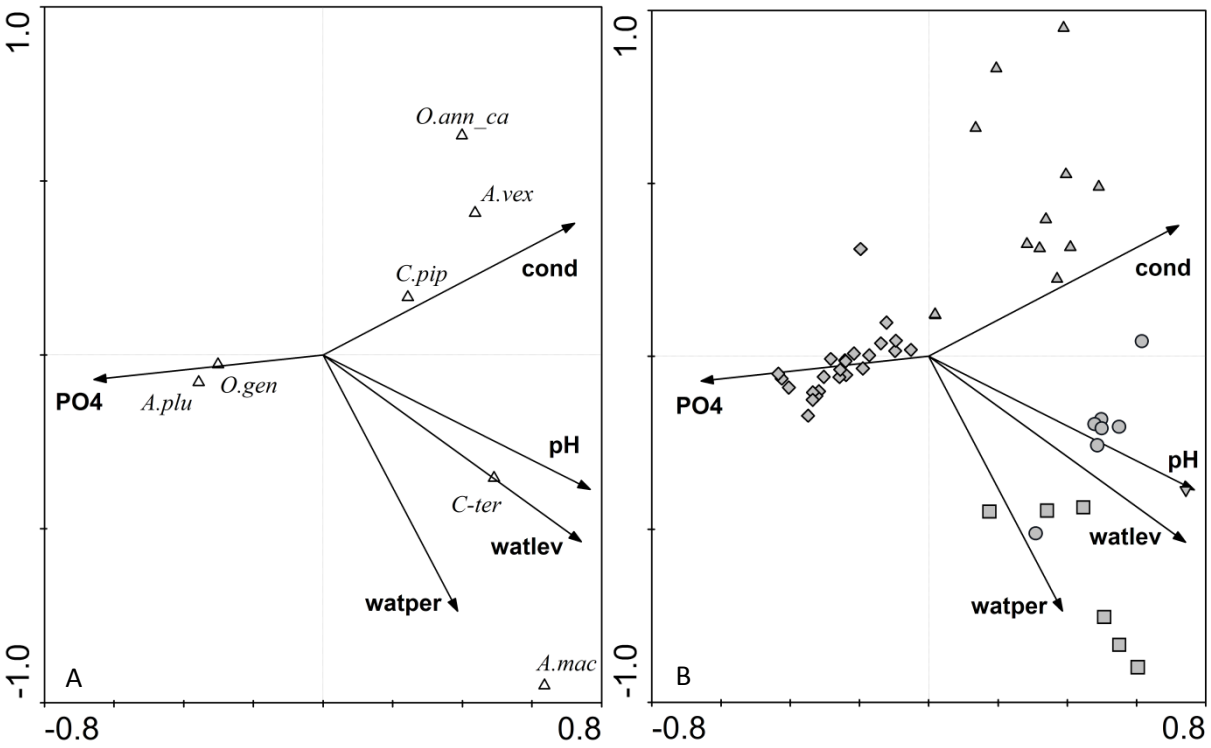

Figure 36: A; Canonical correspondence analysis biplot of five significant (P<0.05) environmental variables versus Culicidae species abundance. Arrows indicate environmental variables; Cond = Conductivity, watper = water persistence, watlev = water level, PO_4 = phosphate; A.plum = *Anopheles plumbeus*, A.mac = *Anopheles* m*aculipennis* complex, C.ter = *Culex territans*, C.pip = *Culex pipiens*, A.vex= *Aedes vexans*, O.gen = *Ochlerotatus geniculatus*, O.ann_can = *Ochlerotatus annulipes/ cantans*. B; Canonical correspondence analysis biplot of five significant (P<0.05) environmental variables versus sampling sites. Down triangles = Wachtelgraben sampling sites (S1, S8); up triangles = temporary water bodies (T1-T5); diamonds = phytotelmata; circles = Kleine Binn and Große Binn sampling sites; squares = Fadenbach sampling sites (F1-F3).

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4. Discussion

4.1 Sampling methods

A wide range of apparatus and techniques have been developed in the last years to collect mosquitoes. Different methods were used depending on whether the interest focuses on the ecology and population dynamics of mosquitoes or on pest controls. Most commonly used for collecting larval and pupal mosquitoes in a variety of habitats is the dipper (Silver 2008). The often-used standard pint dipper consists of a white plastic container (11cm in diameter; capacity: 350 ml) with an attached handle (Dixon and Brust 1972; Lemenager et al. 1986; Amerasinghe & Ariyasena 1990). In the case of our study, the usage of a CPUE method, performed with a handnet (chapter 2.3.1) was a better choice due to the low density of larval and pupal Culicidae. Both sampling methods, however, fail to sample aquatic stages of *Coquillettidia richiardii*. Species of this genus use their highly modified siphon to pierce the roots of a variety of macrophytes (Mohrig 1969, Cranston et al 1987, Becker et al. 2010) and are likely to be found at breeding sites with high abundances of *Typha* sp., *Phragmites* sp. and *Juncus* sp. (Sérandour 2010). Larvae and pupae of *Coquillettidia* are not detectable from the water surface (Brothers 2005, LaPointe 2007). The Morozow method, where larvae and pupae are collected manually from removed host plants is known to be effective in detecting aquatic stages of Coquillettidia, but this technique is not usable in a nature reserve such as the Danubean floodplain National Park. The newest method to monitor mosquito populations (e.g. for pest control) is the usage of carbon dioxide traps (e.g. BG-Sentinel, Biogents) which leads to higher capture rates of Culicidae than traditional trapping techniques (e.g. Light traps). The field efficiency of the BG-Sentinel Trap (BGS) used with carbon dioxide in combination with a lure is higher when compared with industry-standard traps (CDC light traps and gravid traps) for the invasive mosquito species *Aedes albopictus* (Farajollahi et al. 2009). Meanwhile, the BGS Trap is used in many mosquito surveillance programs and the high capture rates of this trapping technique are well documented (Azil et al. 2010; Krueger & Hagen 2007; Werner et al. 2012). The BGS Trap in combination with the BG-Lure (Biogents) as an additional attractant to carbon dioxide increases the detection of invasive mosquito species like *Aedes aegypti* and *Aedes albopictus* (Irish et al. Nguyen et al. 2010; Whelan et al. 2011), and the usage of this trap is highly recommended for surveillance programs from institutions like the Armed Forces Pest Management Board (Dengue and Chikungunya Vector Control Pocket Guide 2012). Aside from high acquisition and running

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costs of a carbon dioxide sampling trap, our sampling design (chapter 2.3.1) favoured the usage of a handnet to collect female and male mosquitoes during the sampling period. The traditional hand-netting method was easier to handle at 20 sampling sides which had to be investigated every third day. Moreover, the aim of the adult mosquito sampling was the detection of females using the sampling sites as breeding grounds. The handling of additional lures would have attracted females over long distances and, therefore, increased the possibility of flawing results.

4.2 Species inventory, abundance and phenology

In Austria 39 mosquito species belonging to 7 genera (*Aedes*, *Anopheles*, *Culex*, *Coquillettidia*, *Culiseta, Ochlerotatus* and *Uranotaenia*) are reported (Mohrig & Car 2002); three further species (*Ochlerotatus nigrinus, Aedes albopictus* and *Anopheles hyrcanus*) were detected by Seidel 2011 and Walter 2012. In our study, 15 species including two close species pairs (*Aedes cinereus*/*rossicus* and *Ochlerotatus cantans*/ *annulipes*) (Table 7) belonging to 6 genera were detected. Genus *Uranotaenia* and invasive mosquito species such as *Aedes albopictus, Aedes aegypti and Ochlerotatus japonicus* were not present in the study area. The recorded mosquito inventory basically is in line with previous studies. Rötzer (1995) detected 12 species out of 5 genera in the Danube Floodplain at Stockerau (Lower Austria) (Table 13). In this case floodplain species were predominant, with *Aedes vexans* (49.9 %) and *Ochlerotatus flavescens* (28.8 %) being abundant, followed by the phytotelmata-breeding species *Ochlerotatus geniculatus* (14.6 %). The most abundant species in the Danube Floodplain at Orth an der Donau were *Ochlerotatus geniculatus* (64.1 %), *Culex territans* (18.7 %) and *Culex pipiens* (6.9%) (Table 8).

Floodplain dynamics influence the mosquito species composition (Rettich et al. 2007; Becker et al. 2010; Strelková et al. 2012). This is confirmed by the results of the canonical correspondence analysis (Figure 36) where water level as well as water persistence were key factors for larval development in 2011 and species distribution. Therefore the low flooding frequency in 2011 consisting of one spring flood is considered to be responsible for the lack of typical flood-plain mosquitoes in 2011. Larvae of mosquito species predominant in frequently inundated areas, floodplains and temporary water bodies like *Ae. vexans*, *Ae. sticticus*, *Ae. cinereus*, *Ae. rossicus*, *Oc. rusticus*, *Oc. annulipes* and *Oc. cantans* (Becker 2003, Medlock 2006, Strelková 2012,) were detected in unexpected low numbers (5.9 % of total abundance). The most abundant species in the larval stage were phytotelmata species

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(*Ochlerotatus geniculatus*, *Anopheles plumbeus*) (66.4 % of the total), followed by mosquito species associated with urban areas (*Culex pipiens* and *Culex territans*) (26.6 % of total larval abundance).

Culicidae species richness and abundance of the Danubean floodplain in the area of Orth an der Donau are relatively low when compared with floodplain regions of other countries (Table 13). As in the National Park Donau-Auen, the South-Moravian region is mainly characterized by floodplain forests and meadows, influenced by annual floods of the rivers Morava and Dyje, thereby providing a variety of breeding habitats for mosquito development (Rettich et al. 2007, Sebesta et al. 2012a). Mosquito sampling of the flood plain regions of Bohemia and Moravia (Czech Republic) in 2005 and 2006 recorded 11 307 adults and 38 869 larvae belonging to 22 species and 6 different genera (*Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Coquillettidia* and *Ochlerotatus*) (Rettich et al 2007). In the region of the Lower Dyje River Basin (Podyji) at the Czech-Austrian border, 30 mosquito species belonging to 7 genera (*Anopheles*, *Aedes, Ochlerotatus*, *Coquillettidia*, *Culex*, *Culiseta* and *Uranotaenia*) were detected in a 3 year study from 2009 to 2011. Two floodplain species, *Aedes vexans* (56.5 %) and *Aedes sticticus* (16.4 %) were most abundant, followed by *Culex (Barraudius) modestus* (Ficalbi 1890) (8.9 %), *Culex pipiens* (7 %) and *Aedes rossicus* (5.2%). *Ochlerotatus geniculatus*, the most prevalent mosquito species (44% of the total catch) in 2011 at Orth an der Donau was represented by only 0.19 % of the total catch in the Lower Dyje River Basin (Sebesta et al. 2012a). The high occurrence of flood-plain species the South-Moravian region might be explained by the higher frequency of floods between 2009 and 2011 (Sebesta et al. 2012a), which are necessary for the development of *Aedes vexans* and further floodplain species (Kenyeres et al. 2011; Becker et al. 2010; Mohrig 1969). The mosquito species inventory and distribution of urban, wetland and forest habitats in Osijek (north-eastern Croatia) were investigated in a ten-year study from 1995-2004. This wetland region is located near the Nature Park Kopački rit, which is influenced by the floods of the Danube and the Drava River (Merdic 1993). In this study 207136 adult mosquitoes belonging to 20 species and 7 genera were sampled: *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Coquillettidia*, *Ochlerotatus* and *Uranotaenia.* Most common species were *Aedes vexans* (75.6%) and *Ochlerotatus sticticus* (13.3 %), followed by species of the *Culex pipiens* complex (5.9 %) and the *Anopheles maculipennis* complex (1.9 %). This long-term study confirmed that seasonal

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population dynamics were influenced by the water level of the Drava and Danube River (Sudarić Bogojević et al. 2009).

The knowledge that 40 species have been imported to Europe in the last 40 years mainly via international trade with used tires and bamboo (Medlock et al. 2012), among them *Aedes albopictus* and *Aedes japonicus* (Seidel 2011; Werner 2012; Šebesta 2012) as well as *Anopheles hyrcanus* (Šebesta 2009; Walter 2012) in Austria and bordering countries, highlight the need to verify and revise the mosquito fauna inventory of Austria.

Table 13: Synopsis of faunistic mosquito studies in Austria and other floodplain regions. A = Adult numbers; L = Larval numbers. [1] Rötzer (1995); [2] Sudarić Bogojević et al. (2009); [3] Rettich et al. (2007); [4] Strelková et al. (2012); [5] Sebesta et al. (2012).

Genera	Species	Individuals	Study area
5	12	296 (A)/ 3272(L)	Danube Floodplain 1994-1995 [1]
7	22	207 136 (A)	Osijek (Croatia) 1995-2004 [2]
6	22	38 869 (L)/ 11 307 (A)	Bohemia & Morava (Czech Rep.) 2005-2006 [3]
6	28	5864 (A)	Morava River Basin (Slovakia) 2009-2010 [4]
7	30	415 218 (A)	South Moravia (Czech Rep.) 2009-2011 [5]
6	15	1022 (L)/221 (A)	Danube Floodplain 2011

4.4 Life cycles

Generally, life cycles of Culicidae are well studied. In the National Park Donau-Auen only three species were detected throughout the sampling period. Larvae of *Ochlerotatus geniculatus* were found from March to September, *Culex pipiens* and *Culex territans* from April to September.

Ochlerotatus geniculatus is considered to be polycyclic (Mohrig 1969). A synchronized hatch was investigated in the Danube floodplain in the area of Stockerau, Lower Austria, whereby the simultaneous occurrence of different larval instars was explained by the increased food competition in the small breeding habitats (Rötzer 2005). Prolonged and asynchronous emergence patterns were explained as an adaption to dry periods (Mohrig 1969), whereas Rötzer (1995) detected that larvae and pupae of *Ochlerotatus geniculatus* were able to survive dry periods in the moist substrate. During the sampling period from March to October 2011 synchronous as well as asynchronous emergence were observed at the phytotelmata in the National Park Donau-Auen, depending on the sampling site. At two phytotelmata sampling sites (PT1 and PT4) only synchronous emergence was observed,

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whereas more than one emergence peak was noticed at the remaining phytotelmata (PT2, PT3, PT5 and PT6). It was not possible to detect any larvae or pupae surviving dry periods in the substrate; this might be explained by extensive droughts which eliminated any moisture from the substrate. The second phytotelmata-inhabiting mosquito species, *Anopheles plumbeus,* is known to breed several generations per year (Kenyeres et al. 2012), but larvae were only found in August 2011. The high abundance of *Ochlerotatus geniculatus* with two generations in 2011 possibly influenced *Anopheles plumbeus* population size. Hard et al. (1989) showed that tree-hole species have different habitat requirements and responses to food quality and density. Given their different feeding strategies, with *Oc. geniculatus* being filter-feeders and *An*. *plumbeus* beeing browsers who feed on the microbial biofilm of the air-water interface (Becker et al. 2010), there is a significant effect on species density in the low water volume of these habitats ranging from 32 to 1358 ml (Table 4). Larvae of *An. plumbeus* were only present when the abundance of *Oc*. *geniculatus* decreased.

Culex pipiens and *Culex territans* have the same life-cycle strategy (Kenyeres et al. 2012; Becker et al. 2010; Mohrig 1969). Both species hibernate exclusively as females and start to select their oviposition sites in early spring (Becker et al. 2010; Mohrig 1969), resulting in the first larvae being present as early as April 2011. Larvae of *Culex pipiens* were found in spring near their hibernation sites in houses and stables in Orth an der Donau: at the Fadenbach sampling sites, in the artificial pond and additionally in rain barrels. In late summer and autumn their breeding sites shifted to more remote areas in phytotelmata as well as temporary water bodies. Metamorphosis could not be observed during the investigation period. In contrast, *Culex territans* which is not bound to urban areas was mainly collected at the Große and the Kleine Binn, but also at temporary water bodies and the Fadenbach. The development of both species is not strongly correlated with floodplain dynamics, and suitable breeding sites were present from early spring to September. Therefore, two generations of *Culex territans* and *Culex pipiens* were observed in 2011. Rötzer (1995) reported only a low number of *Culex territans* in the Danubean floodplain in Stockerau (Lower Austria). When compared with our investigations in 2011, where *Culex territans* was one of the most abundant species. *Culex territans* is known to be polycyclic and the larval development usually starts in May or June (Mohrig 1969). *Culex territans* is likely to be found in shaded waterbodies (Mohrig 1969), often covered with *Lemna minor* L. (Becker et al. 2010; Mohrig 1969), which is in line with our findings in the Danubean Floodplain. Rötzer

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(1995) did not collect any *Culex pipiens* in the Danubean floodplain, but in artificial containers bordering the floodplain. *Ochlerotatus cantans*, *Ochlerotatus cataphylla* and *Ochlerotatus rusticus* were detected in temporary ponds in March. These three species had only one generation per year and were exclusively found in temporary water bodies; they share the same lifecycle strategy, hibernating as eggs and hatching after their breeding habitats get filled with water from snowmelt or rainfall. Data of Kenyeres et al. (2012) confirm these findings. Floodplain species like *Aedes vexans* and *Aedes sticticus* were found from June to July in low abundances in temporary water bodies, yielding to one generation in 2011 for both species; however, they are known to be bi- or multivoltine elsewhere (Kenyeres et al. 2012; Becker et al. 2010; Mohrig 1969).

4.5 Microhabitat characteristics

Ecological data of mosquito breeding habitats were mainly compiled in order to improve vector control measurements of the larval population (Brothers 2005; LaPointe 2007; Azari Hamidian 2011). Research focused, therefore, on species which are known to be a potential health risk such as species belonging to genera *Anopheles*, *Aedes* and *Toxorhynchites* (Sérandour 2010; Rey 2006; Clements 1999), vectors of dirofilariasis for (Azari-Hamidian 2009) and invasive species like *Ochlerotatus japonicus*, *Aedes albopictus* and *Aedes aegypti* (Bartlet-Healy et al. 2012; Juliano and Lounibos 2005; Braks et al. 2003). Laboratory studies highlighted that breeding habitat selection is species-dependent and that different mosquito development strategies favour different microhabitats (Becker et al. 2003, Mohrig 1969). However, the study of natural habitat requirements were poorly neglected in Austria and the neighbouring countries in the last years. Regions where floods were followed by mosquito mass productions were observed in order to detect breeding habitats of species declared as a potential health risk (Clements 1999). Subsequently, such species were reduced using chemical and biological methods (Chandra et al. 2008). Besides water level fluctuations (Sudarić Bogojević et al. 2009), water surface area, presence of pondweed vegetation, water depth, water transparency and temperature of the breeding habitats were identified as basic indicators of larval mosquito distribution (Kenyeres 2011). Furthermore, floodplain species like *Aedes vexans* and *Ochlerotatus sticticus* as well as snow-melt species like *Ochlerotatus annulipes*, *Ochlerotatus cantans* and *Aedes rossicus* were associated with shallow, astatic, clear waterbodies (Kenyeres et al. 2011). These investigations confirm our results in the Nationalpark Donau-Auen, where these species were only collected in

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temporary water bodies with water levels ranging from 0.50 to 10.53 cm. *Aedes flavescens* has been reported to avoid forests as breeding habitats (Mohrig 1969); nevertheless, Rötzer (1995) found larvae of *Ae*. *flavescens* in high abundances in the Danube floodplain due to a lack of adequate breeding habitats outside the alluvial forests. In contrast to Rötzer (1995) we collected only three larvae at temporary water body T1 which confirms the findings of Mohrig (1969). *Ochlerotatus geniculatus* was found in all phytotelmata sampled, a pattern also confirmed by Kapeszky (1938). In contrast, *Anopheles plumbeus* is known to breed primarily in tree holes of *Quercus* sp. (L. 1753) and *Fagus sylvatica* (L.)(Mohrig 1969); in the present study this species was also recorded in a water-filled tree hole of *Carpinus betulus* (L.) (PT2) and *Tilia cordata* (Mill. 1768) (PT4). The tolerance of both species to acidic pH values and high NH_4 concentrations can be confirmed. Both species, *An*. *plumbeus* and *Oc*. *geniculatus,* were found in habitats with pH-values ranging from 4.5 to 7.9 and NH_4 concentration up to 27.75 mgl^{-1}. These findings were also observed by Kapeszky (1938) who detected both species in water-filled tree holes with NH_4 concentrations up to 30mgl^{-1}. Although *Oc. geniculatus* was the most abundant species collected in our study, this species is not prone for mass production, because of their strictly limited breeding habitats (Mohrig 1969).

In the case of the relatively immobile aquatic stages of *Coquillettidia richiardii* (Laurence 1960), vegetation cover is an important biotic habitat requirement in addition to abiotic parameters such as oxygen concentration and turbulence (Sérandour et al. 2010). These findings could not be confirmed in our study due to a lack of larvae and pupae of this highly-adapted mosquito species. *Coquillettidia richiardii* was also lacking in the Wachtelgraben (S1), a permanent stagnant water body with dense stands of *Typha latifolia (*L. 1753) and *Typha angustifolia* which are reported to be favoured by this genus (Sérandour 2010). Nevertheless, 44 females of *Cq. richiardii* were detected from June to August 2011, belonging to the three most abundant species collected in the adult stage (19.9% of the total catch; Table 9) after *Ochlerotatus geniculatus* (40.3%) and *Culex pipiens* (23.1%).

Intraspecific competition plays an important role in container-breeding mosquito species and is well-studied in invasive and indigenous Culicidae (Barbosa et al. 1972; Carpenter 1983). The ability to survive at times of low food availability as a kind of larval competition was tested in three container species (*Aedes aegypti* (L.), *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say)) under laboratory conditions using decomposing leaf litter as a natural food

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(Barrera 1996). In addition competition was tested in the invasive mosquito species *Aedes albopictus* and the indigenous container species *Culex pipiens* in Italy, showing that *Ae. albopictus* performed better in exploiting food resources than *Cx. pipiens* (Carrieri et al. 2003). The phytotelmata sampling sites investigated in 2011 were not strongly variable in abiotic parameters; this is reflected by the results of the canonical correspondence analysis (Figure 36A). Furthermore, the lack of predators in water filled-containers and tree holes (chapter 3.3) as well as the high adaption of mosquito species inhabiting these waterbodies suggests that intraspecific competition might be a key factor influencing mosquito populations of such habitats.

In the National Park Donau Auen *Ochlerotatus geniculatus* was found in high abundances in all phytotelmata sampling sites (PT1-PT6), together with low numbers of *Anopheles plumbeus* and *Culex pipiens* at two phytotelmata only (PT2 and PT4). Invasive species like *Aedes albopictus,* reported for the first time in the Czech Republic in Mikulov near of the Austrian border (Sebestá et al. 2012b) and meanwhile widely established in Italy as well as parts of France and Spain (Medlock et al. 2012), invades both water-filled containers as well as phytotelmata (Barrera 1996; Livdahl and Willey 1991). The latter species, breeding mainly in rural areas (Rey 2006), has the potential to replace our indigenous species.

Aquatic stages of floodplain species (e.g. *Aedes vexans*) were mainly detected at temporary water bodies; this is in accordance with their lifecycle strategy where females select oviposition sites during dry seasons and larvae hatch after flooding. The small number of floodplain species can be explained by the unavailability of adequate breeding habitats in 2011. In contrast, Culicidae species where hibernation takes place in the adult stage and which are strongly bound to human settlements (Becker et al. 2010) occurred in unexpected high abundances. The females select their oviposition sites like rain barrels and flower vases near the hibernation place in early spring. Larvae of *Culex pipiens* were found in early spring at sampling sites situated near human settlements shortly after snowmelt with adults being were detected in temporary water bodies (T1 and T2) at the end of March. *Aedes vexans* and *Ochlerotatus sticticus* were expected to be typical outbreak species, observed in high abundances (Aspöck 1969; Strelková et al. 2012; Vujić et al. 2010; Sudarić Bogojević et al. 2009) in floodplains. One of the main reasons for the low numbers sampled were the low flood frequencies which significantly shortened water persistence of the breeding habitats. Previous studies also confirm these findings (Schäfer et al. 2004; Service 1977, Pritchard &

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Scholefield 1983). In addition, the decreasing water volume and the increasing concentration of dissolved organic matter of temporary water bodies can result in a smaller size of emerging mosquitoes due to premature metamorphosis (Schäfer 2004; Juliano & Stoffregen 1994). Subsequent studies revealed that decreasing larval density is the key factor for an earlier metamorphosis in drying habitats (Chodorowski 1969). Investigations of Schäfer and Lundström (2006) showed that floodplain species reacted differently when habitats dried up: in *Ae. vexans* the duration until metamorphosis increased whereas it decreased in *Oc. sticticus.*

In the *Anopheles maculipennis* complex, collected in the Kleine Binn and in the artificial pond, the canonical correspondence analysis (Figure 36A) revealed that pH, water persistence and water level were key factors influencing habitat choice. In contrast, Sattler et al. (2005) did not identify any habitat requirements which influence the spatial distribution of *Anopheles* sp., since every stagnant open water body had been colonized by larvae. In our study, the Kleine Binn was populated by anopheline larvae when the mostly low-flowing water was cut off from the Danube and subsequently transformed into a short-term stagnant water body.

Predation pressure on mosquitoes is hardly detectable in field investigations. The results of our study show that the habitat groups were inhabited by different sets of aquatic predator species. In our CPUE sampling we recorded only aquatic predators (chapter 2.3.1) which are known to feed on mosquitoes and are used as biocontrol agents (Chandra et al. 2008). Numbers of predator were negatively correlated with mosquito abundance. Fadenbach and Wachtelgraben sampling sites (Group 4) were characterized by a high density of *Chaoborus crystallinus* (DeGeer 1776) *and Dytiscus marginalis* (Linnaeus 1758) resulting in only 25 mosquito larvae sampled (Table 1). In habitat group 3 (Große and Kleine Binn sampling sites) also high density of *Gerris* sp. (Fabricius 1794) and *Hydrometra stagnorum* (Linnaeus 1758), but only 78 larval mosquitoes were detected (Figure 35). On the other hand, the highest larval mosquito density (n=1044; Figure 35) was recorded at temporary water bodies despite the presence of invertebrate predators. These findings suggest that also newts and fish might play an important role, since fish generally lack in temporary waters. In completely predatorless phytotelmata up to 871 Larvae were recorded; here the larval density is regulated by habitat size, water persistence and intraspecific competition, not by predator pressure. Laboratory studies of DuRant and Hopkins (2008) have shown that amphibians are

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able to consume a large amount of mosquito larvae per day, indicating the important role of mosquitoes in the food chain as well as the role of amphibians in mosquito predation control (Brodman et al. 2003). Other laboratory studies stress the importance of many other taxa like Odonata (Kögel 1984), Corixidae (Kögel 1984), *Hydrometra stagnorum* (Purthi 1928), Chaoboridae (Chodourowski 1968, Skierska 1969) or *Dytiscidae* (Nelson 1977, Chandra et al. 2008) as predators with the capacity of effectively reducing mosquitoes in large numbers. The influence of predators feeding on adult mosquitoes like dragonflies (Becker et al. 2010; Lamborn 1980), birds (Blotzheim 1985) or bats (Arnold et al. 1998) are hardly to examine in field studies, because flight-hunting predators have a wide activity range where the activity patterns of prey and predator overlap (Becker et al. 2010). Culicidae react by a number of avoidance strategies. Ovipositioning Culicidae, e.g. *Culiseta (Allotheobaldia) longiareolata* (Macquart 1838), *Culex (Culex) laticinctus* (Edwards 1913) and *Anopheles gambiae* (Giles 1902) avoid predator-rich water bodies (Spencer et al. 2002; Kiflawi et al. 2003; Kershenbaum et al. 2011) because they are able to detect predator-released kairomones (Warburg et al. 2011). In fact, the active avoidance of water bodies with a high abundance of predators such as *Notonecta maculata* (Fabricius 1794) can strongly support a mosquito population (Warburg et al. 2011).

Floodplain areas are dynamic systems with distinct mosquito species pools, but the yearly composition of the mosquito fauna varies, depending on hydrological conditions, weather (especially the amount of precipitation and possible dry seasons), water level fluctuations and flood frequencies (Schäfer et al. 2008, Sebesta et al. 2012; Sudarić Bogojević et al. 2009). Species abundance is further influenced by intraspecific competition and predator pressure (Sunahara et al. 2002). In order to fully elucidate these complex interactions, long-term studies in floodplain areas such as the National Park Donau-Auen are strictly necessary.

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Abstract

Mosquitoes are known as hosts for a variety of parasites and pathogens and are therefore considered as nuisance and as vectors of human diseases. Until recently not much attention had been paid to their ecology although they play an important but poorly understood role in food chains (Poulin 2010). In order to understand the ecological function of Culicidae in an ecosystem it is imperative to update information on culicid species distribution and to investigate the factors controlling it. We monitored abiotic parameters such as water level, nutrients, oxygen concentration and conductivity as well as biotic parameters (Culicidae and potential predators) from March to October 2011 at 20 sampling sites in the National Park Donau-Auen. A total of 34 eggrafts, 1927 larval, 80 pupal and 200 adult Culicidae were collected. We detected 15 mosquito species belonging to 6 genera (*Anopheles, Culex*, *Culiseta*, *Coquillettidia*, *Aedes* and *Ochlerotatus*), whereas *Ochlerotatus geniculatus* (68 %) and *Culex territans* (13 %) were most abundant, followed by *Culex pipiens* and *Aedes vexans* with approximately 5% and 4 % of total abundance. Biometrical data were used to reconstruct life cycles; at the study area *Cx. pipiens* and *Cx. territans* were bivoltine and *Oc. geniculatus* multivoltine. Based on abiotic and biotic parameters, sampling sites were grouped into 4 clusters. The results show that water level and persistence, pH, electric conductivity and phosphate concentrations had a significant influence on species distribution and that flood plain dynamics are a key factor for the seasonal and spatial distribution of mosquito larvae in the National Park Donau-Auen.

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Zusammenfassung

Stechmücken haben aufgrund ihrer human- und veterinärmedizinischen Bedeutung als Krankheitserreger sowie als Vektor einer Vielzahl von Parasiten und Pathogenen eine große Bedeutung und werden daher hauptsächlich als Gesundheitsrisiko und Plage wahrgenommen. Die Ökologie der Stechmücken und in Folge auch ihre wichtige und bisher spärlich untersuchte Rolle in aquatischen und terrestrischen Nahrungsketten wurde bisher stark vernachlässigt. Um die ökologische Funktion der Stechmücken innerhalb eines Ökosystems nachvollziehen zu können, ist es unerlässlich, den derzeitigen Wissensstand über die Artverteilung und die jeweiligen Einflussfaktoren zu erheben und zu aktualisieren. Abiotische Parameter wie Wasserstand, Wasserführung, Sauerstoffkonzentration und Leitfähigkeit sowie biotische Parameter (Stechmücken und deren Predatoren) wurden an 20 Untersuchungsstellen im National Park Donau-Auen regelmäßig erhoben. Insgesamt wurden 34 Eischiffe, 1927 Larven, 80 Puppen und 200 adulte Culicidae während der Untersuchung gesammelt. Es wurden 15 Stechmückenarten aus 6 Gattungen (*Anopheles*, *Culex*, *Culiseta*, *Coquillettidia*, *Aedes* und *Ochlerotatus*) bestimmt, wobei die häufigsten Arten *Ochlerotatus geniculatus* (68%) und *Culex territans* (13%), gefolgt von *Culex pipiens* und *Aedes vexans* mit 5% und 4% der Gesamtabundanz darstellten. Biometrische Daten wurden verwendet, um die Lebenszyklen der abundantesten Arten zu rekonstruieren. *Culex pipiens* und *Culex territans* zeigten mit 2 Generationen einen bivoltinen und *Ochlerotatus geniculatus* mit 3 Generationen einen multivoltinen Entwicklungszyklus. Basierend auf den erhobenen abiotischen und biotischen Parametern wurden die Untersuchungsgebiete in vier Habitatgruppen zusammengefasst. Die Resultate ergaben, dass Wasserstand, Wasserführung, pH, elektrische Leitfähigkeit und Phosphatkonzentration einen signifikanten Einfluss auf die Artverteilung ausüben und somit die Auendynamik einen Schlüsselfaktor für die saisonale und räumliche Verteilung von Stechmückenpopulationen im National Park Donau-Auen darstellt.

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References

ASPÖCK H: (1969): Die Stechmücken Ostösterreichs und ihre Bedeutung als Überträger von Arboviren. Wien. Klein. Wschr. 31:107-108.

AMERASINGHE F.P., ARIYASENA T.G. (1990) Larval survey of surface water breeding mosquitoes during irrigation development in the Mahaweli project, Sri Lanka. J. Med. Entomol. 27: 789-802.

AZARI-HAMIDIAN S. (2011) Laval Habitat Characteristics of the Genus Anopheles (Diptera: Culicidae) and a Checklist of Mosquitoes in Guilan Province, Northern Iran. Iran J. Arthropod-Borne Dis. 5(1): 37-53.

AZARI-HAMIDIAN S. (2009) Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. Medical and Veterinary Entomology. 23: 111-121.

AZIL A.H., LONG S.A., RITCHIE S.A., WILLIAMS C.R. (2010) The development of predictive tools for pre-emptive dengue vector control: a study of *Aedes aegypti* abundance and meteorological variables in North Queensland, Australia. Tropical Medicine and International Health. 15(10): 1190-1197.

BACK C., BOISVERT J., LACOURSIÉRE J.O., CHARPENTIER G. (1985) High-dosage treatment of a Quebec stream with Bacillus thuringiensis serovar. Israelensis: Efficiancy against blackfly larvae (Diptera: Simuliidae) and impact on non-target insects. Canadian Entomologist 117: 1523-1534.

BARRERA R. (1996) Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. Ecological Entomology. 21: 117-127.

BARTLET-HEALY K., UNLU I., OBENAUER P., HUGHES T., HEALY S., CREPEAU T., FARAJOLLAHI A., KESAVARAJU B., FONESCA D., SCHOELER G., GAUGLER R., STRICKMAN D. (2012) Larval

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

Habitat Utilization and Community Dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). J.Med.Entomol. 49(4): 813-824.

BAUER N., KENYERES Z., TÓTH S., SÁRINGER-KENYERES T. (2011) Connections between the habitat pattern and the pattern of the mosquito larval assemblages. Biologica. 66(5): 877-885.

BECKER N. (1998) The use of *Bacillus thuringiensis* subsp. *israelensis (BTI)* against mosquitoes, with special emphasis on the ecological impact. Israel Journal of Entomology. 32: 63-69.

BECKER N., MARGALIT J. (1993) Use of *Bacillus thuringiensis israelensis* against mosquitoes and blackflies, in *Bacillus thuringiensis*, an environmental Biopesticide: Theory and Practice (Entwistle PF, Cory JS, Bailey MJ & Higgs S, Eds) John Wiley, Chichester UK pp. 147-170.

BECKER, N., PETRIC D., ZGOMBA M., BOASE C., MADON M., DAHL, C., KAISER A. (2010) Mosquitoes and their control. 2nd ed. Heidelberg: Springer.

BENEDICT M.Q., LEVINE R.S., HAWLEY W.A., LOUNIBOS L.P. (2007) Spread of the Tiger: Global Risk of Invasion by the Mosquito *Aedes albopictus*. Vector Borne Zoonotic dis. 7(1): 76-85.

BLOTZHEIM U.N.G. (1985) Handbuch der Vögel Mitteleuropas. Aula Verlag, Wiesbaden 14 Vols.

BOISVERT M., BOISVERT J. (2000) Effects of *Bacillus thuringiensis* var. *israelensis* on Target and Nontarget Organisms: A Review of Laboratory and Field Experiments. Biocontrol Science and Technology. 10(5): 517-561.

BRAKS M.A.H., HONORIO N.A., LOURENCO-DE-OLIVEIRA R., JULIANO S.A., LOUNIBOS L.P. (2003) Convergent Habitat Segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Southeastern Brazil and Florida. J. Med. Entomol. 40(6):785-794.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

BRODMAN R., OGGER J., KOLACZKY M., PULVER R., LONG A.J., BOGARD T. (2003) Mosquito control by pond-breeding salamander larvae. Herpetol. Rev. 34: 116-119.

BROTHERS, D. R. (2005) New distributional records for *Coquillettidia perturbans* (Walker) (Diptera, Culicidae) in Idaho. Journal of Vector Ecology. 30(1): 163–164.

CAPELLI G., DRAGO A., MARTINI S., MONTARSI F., SOPPELSA M., DELAI N., RAVAGNAN S., MAZZON L., SCHAFFNER F., MATHIS A., DI LUCA M., ROMI R., RUSSO F. (2011) First report in Italy of the exotic mosquito species *Aedes (Finlaya) koreicus*, a potential vector of arboviruses and filariae. Parasites and Vectors. 4: 188.

CARPENTER S.R. (1983) Ressource Limitation of Larval Treehole Mosquitoes Subsisting on Beech detritus. Ecology. 64(2): 219-223.

CARRIERI M., BACCHI M., BELLINI R., MAINI S. (2003) On the Competition Between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. Environmental ecology. 32(2): 1313-1321.

CHANDRA G., MANDAL S.K., GHOSH A.K., DAS D., BANERJEE S.S. (2008) Biocontrol of larval mosquitoes by *Acilius sulcatus*. (Coleoptera: Dytiscidae).

CHODOROWSKI A. (1969) The desiccation of ephemeral pools and the rate of development of *Aedes communis* larvae. Pol. Arch. Hydrobiol. 16: 79-91.

CLEMENTS, A.N. (1999) The Biology of Mosquitoes: Vol. 2: Sensory Reception and Behaviour. Chapman & Hall, London, United Kingdom. pp. 756.

CLEMENTS, A.N. (2012) The Biology of Mosquitoes: Vol. 3: Transmission of viruses and interactions with bacteria. Chapman & Hall, London, United Kingdom. pp. 571.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

CRANSTON P.S., RAMSDALE C.D., SNOW K.R., WHITE G.B. (1987) Adults, larvae and pupae of British mosquitoes (Culicidae): A key. Scientific Publications of the Freshwater Biological Association 48: 1-152.

DIXON R.O., BRUST R.A. (1972) Mosquitoes of Manitoba. III. Ecology of larvae in the Winnipeg area. Can Entomol. 104: 961-968.

DURANT A.E., HOPKINS W.A. (2008) Amphibian predation on larval mosquitoes. Can. J. Zool. 86: 1159-1164.

DYAR H.G. (1890) The number of moults of lepidopterous larvae. Psyche. 5: 420-422.

EDMAN, J.D., 2000. Arthropod Transmission of Vertebrate Parasites. In: Eldridge, B.F., Edman, J.D. (Eds.), Medical Entomology. Kluwer Academic Publishers, Dordrecht/ Boston/ London, pp. 51–163.

European Centre for Disease Prevention and Control. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC; 2012. http://ecdc.europa.eu/en/healthtopics/vector-borne_diseases/public_health_measures/Pages/mosquito-guidelines.aspx
Zugriff: 23.09.2012, 20:35

FARAJOLLAHI A., KESAVARAJU B., PRICE D.C., WILLIAMS G.M., HEALY S.P., GAUGLER R., NELDER M.P. (2009) Field Efficacy of BG-Sentinel and Industry-Stan dard Traps for Aedes albopictus (Diptera: Culicidae) and West Nile Virus Surveillance. J. Med. Entomol. 46(4): 919-925.

Fauna Europaea (2011) Fauna Europaea version 2.4. Web Service available online at http://www.faunaeur.org.

FINK M., MOOG O. & WIMMER R. (2000) Fließgewässer – Naturräume Österreichs. UBA Monographien 128, Wien, 110 pp.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

GARCIA R., DESROCHERS B., TOZER W. (1980) Studies on the toxicity of Bacillus thuringiensis var. israelensis against organisms found in association with mosquito larvae. Proceeding & Papers of the California Mosquito and Vector Control Association. 48: 33-36.

HADLEY, A. 2008. CombineZM. An image stacking software program available at http://www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm.

HARD J.J., BRADSHAW W.E., MALARKEY D.J. (1989) Resource- and density dependent development in tree-hole mosquitoes. OIKOS. 54: 137-144.

IRISH S.R., CHANDRE F, N`GUESSAN R. (2008) Comparison of octenol- and BG Lure©-baited Biogents sentinel traps and an encephalitis virus surveillance trap in Portland, OR. Journal of the American Mosquito Control Association. 24(3): 393-397.

IRWIN P., ARCARI C., HAUSBECK J., PASKEWITZ S. (2008) Urban Wet Environment as Mosquito Habiat in the Upper Midwest EcoHealth. 5: 49-57.

JULIANO S.A., STOFFREGEN T.L. (1994) Effects of habitat drying on size at and time to metamorphosis in the tree hole mosquito Aedes triseriatus. Oecologia. 97(3): 369-376.

JULIANO S.A., LOUNIBOS L.P. (2005) Ecology of invasive mosquitoes: effects on resident species and human health. Ecol. Lett. 8(5): 558-574.

KAPESZKY E. (1938) Die Culicidenfauna der engeren Umgebung Wiens und ihre Abhängigkeit von der physikalisch-chemischen Beschaffenheit des Mediums. Diplomarbeit/Dissertation Universität Wien pp. 56.

KENYERES Z., BAUER N., TÓTH S, SÁRINGER-KENYERES T. (2011) Habitat requirements of mosquito larvae. Rom.J.Biol.-Zool. 56: 147-162.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

KENYERES Z., TÓTH S., BAUER N., SÁRINGER-KENYERES T. (2012) Life-strategy based structural features of the larval mosquito metacommunities in Hungary. Ekológia (Bratislava). 31(2): 210-230.

KERSHENBAUM A., SPENCER M., BLAUSTEIN L., COHEN J.E. (2011) Modelling evolutionarily stable strategies in oviposition site selection, with varying risks of predation and intraspecific competition. Evol. Ecol. doi:10.1007/s10682-011-9548-9).

KIFLAWI M., BLAUSTEIN L., MANGEL M. (2003) Oviposition habitat selection by the mosquito Culiseta longiareolata in response to risk of predation and conspecific larval density. Ecological Entomology. 28: 168-173.

KÖGEL F. (1984) Die Predatoren der Stechmückenlarven im Ökosystem der Rheinauen. Ph.D. thesis, University of Heidelberg pp. 347.

KONDO S., FUJIWARA M., OHBA M., ISHI M. (1995) Comparative activities of the four Bacillus thuringiensis serovars against a chironomid midge *Paratanytarsus grimmii* (Diptera: Chironomidae). Microbiological Research. 150: 425-428.

KRUEGER A., HAGEN R.M. (2007) First record of Aedes albopictus in Gabon, Central Africa. Tropical Medicine and International Health. 12(9): 1105-1107.

LAMBORN R.H. (1980) Dragon flies vs. mosquitoes. Can the mosquito pest be mitigated? Studies in the life history of irritating insects, their natural enemies, and artificial checks by working entomologists. D Appleton Co, New York, pp22.

LAPOINTE, D. A. (2007) Current and potential impacts of mosquitoes and the pathogens they vector in the Pacific Region. Proceedings of the Hawaiian Entomological Society. 39: 75–81.

LAURENCE, B. R., 1960. The biology of two species of Mosquito, Mansonia Africana(Theobald), and Mansonia uniformis (Theobald) belonging to the subgenus Mansoniodes (Diptera, Culicidae). Bulletin of Entomological Research. 51: 491–517.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

LAZOWSKI, W. (1997). Auen in Österreich. Monographien Band 81. Wien, Umweltbundesamt: 251.

LEBL K., NISCHLER E.M., WALTER M., BRUGGER K., RUBEL F. (2013) First record of the disease vector *Anopheles hyrcanus* in Austria. Journal of the American Mosquito Control Association, in print.

LEMENAGER D.C., BAUER S.D., KAUFFMANN E.E. (1986) Abundance and distribution of immature Culex tarsalis and Anopheles freeborni in rice fields of the Sulter-Yuba MAD. 1. Initial sampling to detect major mosquito producing rice fields, augmented by adult light trapping. Proc. Calif. Mosq. Vect. Control Assoc. 53:101-104.

LIVDAHL T.P., WILLEY M.S. Prospects for an Invasion: Competition Between *Aedes albopictus* and Native *Aedes triseriatus*. Science. 253: 189-191.

MEDLOCK J.M., SNOW K.R., LEACH S. (2006) Possible ecology and epidemiology of medically important mosquito-borne arboviruses in Great Britain. Epidemiol. Infect. 135(3): 446-482.

MEDLOCK J.M., HANSFORD K.M., SCHAFFNER F., VERSTEIRT V., HENDRICKX G., Zeller H, VAN BORTEL W. (2012) A Review of the Invasive Mosquitoes in Europe: Ecology, Public Health Risks, and Control Options. Vector Borne and Zoonotic diseases. 12(6): 435-447.

MERDIĆ E., JELIČIĆ Z., KRCMAR S., HACKENBERGER-KUTOZOVIC B., TURIĆ N., SUDARIĆ BOGOJEVIĆ M., ZAHIROVIĆ Z. (2010) Efficacy of mosquito attractants in various habitats of a flood. Biologica. 65(3): 545-551.

MERDIĆ E. (1993) Mosquitoes (Diptera: Culicidae) of Special Zoological reserve Kopacki rit (NE Croatia). Nat. Croat. 2: 47-54

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

MINAKAWA N., MUTERO C.M., GITHURE J.I., BEIER J.C., Yan G. (1999) Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. Am. J. Trop. Med. Hyg. 4(4):1010–1016.

MIURA T., TAKAHASI R.M., MULLIGAN F.S. (1980) Effects of the mosquito larvicide Bacillus thuringiensis serotype H-14 on selected aquatic organisms. Mosquito News. 40(4) : 619-622.

MOHRIG W., CAR M. (2002) Diptera: Culicidae.- Teil III, 9pp., in Moog, O. (Ed) (2002): Fauna Aquatica Austriaca, Lieferung 2002.- Wasserwirtschaftkataster, Bundesministerium für Land und Forstwirtschaft, Umwelt- und Wasserwirtschaft. Wien.

MOHRIG W., 1969. Die Culiciden Deutschlands. Untersuchungen zur Taxonomie, Biologie und Ökologie der einheimischen Stechmücken, Parasitologische Schriftenreihe 18. Gustav Fischer Verlag, Jena, Germany, 260 pp.

MOOG O., SCHMIDT-KLOIBER A., OFENBÖCK T., GERRITSEN J. (2001) Aquatische Ökoregionen und Fließgewässer – Bioregionen Österreichs – eine Gliederung nach geoökologischen Milieufaktoren und Makrozoobenthos-Zönosen, BMLFUW, Wien. pp. 106

MOOG O., NESEMANN H., OFENBÖCK, T. (2001) Österreichs Anteil an den österreichischen Ökoregionen gemäß EU-Wasserrahmenrichtlinie- eine deduktive Analyse landschaftsprägender Milieufaktoren. Österr. Wasser-und Abfallwirtschaft Wien 52(7/8): 204-209.

NAEEM-ULLAH U., AKRAM W., SUHAIL A., RANA S.A. (2010) Grouping of different mosquito species on the bases of larval habitats. Pak.J.Agri.Sci. 47(2): 124-131.

NGUYEN H., WHELAN P., FINLAY-DONEY M., SOONG S.Y. (2010) Interceptions of Aedes aegypti and Aedes albopictus in the port of Darwin, NT, Australia, 25 January and 5 February 2010. The Northern Territory Disease Control Bulletin. 17(1): 29-35.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

NIEMI G.J., HERSHEY A.E., SHANNON L., HANOWSKI J.M., LIMA A., AXLER R.P., REGAL R.R. (1999) Ecological effects of mosquito control on zooplankton, insects, and birds. Environ. Toxicol. Chem. 18(3): 549-559.

PAUPY C., DELATTE H., BAGNY L., CORBEL V., FONTENILLE D. (2009) Aedes albopictus, an arbovirus vector: from the darkness to the light. Microbes and Infections. 11: 1177-1185.

POULIN B., LEFEBVRE G., Paz L (2010) Red flag for green spray: adverse trophic effects of Bti on breeding birds. J. Appl. Ecol. 47: 884-889.

PRITCHARD G., SCHOLEFIELD P.J. (1983) Survival of *Aedes* larvae in constant area ponds in Southern Alberta (Diptera: Culicidae). Can. Entomol. 115: 183-188.

REINERT J.F. (2001) Revist list of abbreviations for genera and subgenera of Culicidae (Diptera) and the notes on generic and subgeneric changes. J. am. Mosq. Control. Assoc. 17: 51-55.

RETTICH F., IMRICHOVA K., SEBESTA O. (2007) Seasonal comparisons of the mosquito fauna of the flood plains of Bohemia and Moravia, Czech Republic. EMB. 23: 10-16.

REY J.R., NISHIMURA N., WAGNER B., BRAKS M.A.H., O'CONNEL S.M., LOUNIBOS L.P. (2006) Habitat Segregation of Mosquito Arbovirus Vectors in South Florida. J. Med. Entomol. 43(6): 1134-1141.

RÖTZER K. (1995) Ökologische Untersuchungen an Culiciden (Insecta:Diptera) in den Donauauen bei Stockerau (NÖ). Diplomarbeit Universität Wien. pp. 120

SATTLER M.A., MTASIWA D., KIAMA M., PREMJI Z., TANNER M., KILLEEN G.F., LENGELER C. (2005) Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. Malaria Journal. 4:4. doi:10.1186/1475-2875-4-4

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SCHÄFER M. (2004) Mosquitoes as a part of Wetland Biodiversity. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. 1042. pp. 63. Uppsala. ISBN 91-554-6094-1.

SCHÄFER M.L., LUNDSTRÖM J.O., PETERSSON E. (2008) Comparison of mosquito (Diptera: Culicidae) populations by wetland type and year in the lower River Dalälven region, Central Sweden. Journal of Vector Biology. 33(1): 150-157.

SCHÄFER M.L., LUNDSTRÖM J.O. (2006) Different responses of two floodwater mosquito species, Aedes vexans and Ochlerotatus sticticus (Diptera: Culicidae), to larval habitat drying. Journal of Vector Ecology. 31 (1): 123-128.

SCHAFFNER F., CHOUIN S., GUILLOTEAU J. (2003) First record of *Ochlerotatus* (*Finlaya*) *japonicus japonicus* (Theobald, 1901) in metropolitan France. Journal of the American Mosquito Control Association. 19(1): 1-5.

SCHAFFNER F., KAUFMANN C., HEGGLIN D., MATHIS A. (2009) The invasive mosquito Aedes japonicus in Central Europe. Med Vet Entomol. 23: 448-51.

SCHIEMER F., BAUMGARTNER C., TOCKNER K. (1999) Restoration of Floodplain Rivers: The Danube Restoration Project. Regul. Rivers: Res. Mgmt. 15: 231-244.

SCHOLTE E.J., SCHAFFNER F. (2007) Waiting for the tiger: establishment and spread of the Aedes albopictus mosquito in Europe. In Takken W, Knols BGJ, editors. Emerging pests and vectorborne diseases in Europe. Wageningen: Wageningen Academic Publishers. pp. 241-260.

SCHOLTE E., DEN HARTOG W., DIK M., SCHOELITSZ B., BROOKS M., SCHAFFNER F., FOUSSADIER R., BRAKS M., BEEUWKES J. (2010) Introduction and Control of three invasive mosquito species in the Netherlands, July-October 2010. Euro. Surveill. 15:11.

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ŠEBESTA, O., Rettich F., MINÁŘ J., HALOUZKA J., HUBÁLEK Z., JUŘICOVA Z., RUDOLF I., ŠIKUTOVÁ S. GEBLIĆ I., REITER P. (2009) Presence of the mosquito Anopheles hyrcanus in South Moravia, Czech Republic. Medical and Veterinary Entomology 23: 284-286.

ŠEBESTA, O., GELBIČ, I., & MINÁŘ, J. (2012a): Mosquitoes (Diptera: Culicidae) of the Lower Dyje River Basin (Podyji) at the Czech-Austrian border. Cent. Eur. J. Biol. 7: 288-298.

ŠEBESTA O., RUDOLF I., BETÁŠOVÁ L., PEŠKO J., HUBÁLEK Z. (2012b) An invasive mosquito species *Aedes albopictus* found in the Czech Republic. Euro Surveill. 17(43)
Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20301

SEIDEL B., DUH D., NOWOTNY, N., ALLERBERGER F. (2012) Erstnachweis der Stechmücken *Aedes* (*Ochlerotatus*) *japonicus japonicus* (Theobald, 1901) in Österreich und Slowenien in 2011 und für *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1895) in Österreich 2012 (Diptera: Culicidae) Entomologische Zeitschrift, 122, 223-226.

SÉRANDOUR J., WILLISON J., THUILLER W., RAVANEL P., LEMPÉRIÈRE G., RAVETON M. (2010) Environmental drivers for Coquillettidia mosquito habitat selection: a method to highlight key field factors. Hydrobiologica. 652(1): 377-388

SERVICE M. W. (1977) Ecological and biological studies on *Aedes cantans* (Meig.) (Diptera: Culicidae) in southern England. Journal of Applied Ecology 14:159–196.

SILVER J.B. (2008) Mosquito Ecology. Field sampling methods. Third Edition. Springer New York. pp. 1498.

SKIERSKA B. (1969) Larvae of Chaoborinae occurring in small reservoirs. I. Some observations of larvae of *Chaoborus crystallinus* (DeGeer 1776) and on the possibility of their predacity in relation to larvae of biting mosquitoes. Bull.Inst.Mar.Med.Gdansk. 20: 101-108.

SPENCER M., BLAUSTEIN L., COHEN J.E. (2002) Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. Ecology. 83(3): 669-679.

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SUDARIĆ BOGOJEVIĆ M., MERDIĆ E., TURIĆ N., JELČIĆ Z., ZAHIROVIĆ Z., VRUĆINA I., MERDIĆ S. (2009) Seasonal dynamics of mosquitoes (Diptera: Culicidae) in Osijek (Croatia) for the period 1995-2004. Biologica. 64(4): 760-767.

STRELKOVÁ L., HALGOŠ J. (2012) Mosquitoes (Diptera, Culicidae) of the Morava River floodplain, Slovakia. Cent. Eur. J. Biol. 7(5): 917-926.

SUNAHARA T., ISHIZAKA K., MOGI M. (2002) Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. Journal of Vector Ecology. 27(1): 8-20.

TOCKNER, K. SCHIEMER, F, WARD J.V. (1998) Conservation by restoration: the Management concept for a river–floodplain system on the Danube River in Austria. *Aquat*. *Conser.* 6(8): 71–86.

VUCIĆ A.; STEFANOVIĆ A., DRAGIČEVIĆ I, MATIJEVIĆ T., PEJČIĆ L., KNEŽEVIĆ M, KRAŠIĆ D., VESELIĆ S. (2010) Species composition and seasonal dynamics of mosquitoes (Diptera: Culicidae) in flooded areas of Vojvodina, Serbia. Arch. Biol. Belgrade. 62(4): 1191-1206.

WALKER E.D., KAUFMANN M.G., AYRES M.P., RIEDEL M.H., MERRITT R.W. (1997) Effects of variation on quality of leaf detritus in growth of the eastern tree-hole mosquito, Aedes triseriatus (Diptera: Culicidae). Can. J. Zool. 75: 706-718.

WALTER M. (2012) Stechmücken der Familie Culicidae als vektoren für zoonotische Infektionskrankheiten im urbanen Raum Wien. Bachelor thesis, University of Veterinary Medicine, Vienna pp. 56.

WARBURG A., FAIMAN R., SHTERN A., SILBERBUSH A., MARKMAN S., COHEN J.E., BLAUSTEIN L. (2011) Oviposition habitat selection by *Anopheles gambiae* in response to chemical cues by *Notonecta maculata*. Journal of Vector Ecology. 32(2): 421-425.

©Nationalpark Donau-Auen GmbH, download www.biologiezentrum.at

WERNER D., KRONEFELD M., SCHAFFNER F. & KAMPEN H. 2012. Two invasive mosquito species, *Aedes albopictus* and *Aedes japonicus*, trapped in south-west Germany, July to August 2011. Euro Surveill. 17(4).
Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20067

WERNSDORFER W.H. (2002) Malaria in Mitteleuropa. Denisia 6: 184: 201-212.

WHELAN P.I., NGUYEN H., FINLEY-DONEY M. (2011) Another exoctic mosquito interception at Frances Bay port facility, Darwin, January 2011. The Northern Territory Disease Control Bulletin. 18(2): 24-26.

ZELL R., KRUMBHOLZ A., WUTZLER P. (2008) Impact of global warming on viral diseases: what is the evidence? Current Opinion in Biotechnology. 19: 652-660.

ZITTRA, C. (2012) Bestimmungsschlüssel der Culiciden (Insecta: Diptera) im Nationalpark Donau-Auen. Wissenschaftliche Reihe Nationalpark Donau-Auen, Heft 27.

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Appendix

Tables

Table 14: Number of mosquitoes sampled at the National Park Donau-Auen from March to October 2011.. E= Eggraft; L=Larvae; P=Pupae; A=Adults.

Mosquito species	Sampling site	E	L	P	A
Anopheles (Anopheles) maculipennis s.l. MEIGEN 1818	AP1	0	36	0	0
	F3	0	11	0	0
Anopheles (Anopheles) plumbeus STEPHENS 1828	PT2	0	6	0	0
	PT4	0	6	3	0
Aedes (*Aedes*) *cinereus* MEIGEN 1818 / *esoensis* DOLBESKIN, GORITZKAJA & MITROFONA 1930	T1	0	1	0	0
	T2	0	2	0	0
Aedes (*Aedimorphus*) *vexans* MEIGEN 1830	T1	0	30	0	0
	T2	0	2	0	0
	T5	0	2	0	0
	KB1	0	0	0	1
Ochlerotatus (Finlaya) geniculatus OLIVIER 1791	PT1	0	77	3	6
	PT2	0	101	4	0
	PT3	0	238	8	4
	PT4	0	396	21	77
	PT5	0	11	0	0
	PT6	0	60	0	2
Ochlerotatus (Ochlerotatus) cantans MEIGEN 1818 /*annulipes* MEIGEN 1830	T1	0	9	0	0
	T2	0	23	0	0

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	T3	0	1	0	0
	T4	0	2	0	0
	T5	0	29	0	0
	PT1	0	0	0	3
Ochlerotatus (*Ochlerotatus*) *cataphylla* DYAR 1916	T2	0	0	0	2
	T5	0	2	0	0
	S1	0	0	0	1
Ochlerotatus (*Ochlerotatus*) *excrucians* WALKER 1856	PT1	0	0	0	2
Ochlerotatus (*Ochlerotatus*) *flavescens* MÜLLER 1764	T1	0	3	0	0
Ochlerotatus (*Ochlerotatus*) *sticticus* MEIGEN 1838	PT1	0	0	0	4
Ochlerotatus (*Rusticoidus*) *rusticus* ROSSI 1790	T1	0	3	0	0
Culex (*Culex*) *pipiens* LINNAEUS 1758	T1	0	31	1	6
	T2	0	3	2	0
	T4	10	1	0	12
	F1	0	36	0	7
	F2	0	0	0	4
	AP1	23	668	3	18
	PT1	0	4	0	0
	PT4	0	15	1	0
	T1	0	23	2	4
Culex (*Neoculex*) *territans* WALKER 1856	T2	0	4	1	1
	T4	0	1	0	0
	F1	0	10	0	4
	F2	0	7	0	2
	KB1	0	7	3	3
	KB2	1	58	28	13

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	S1	0	2	0	0
Culiseta (*Culiseta*) *annulata* SCHRANK 1776	T1	0	2	0	0
	T2	0	3	0	0
	KB2	0	1	0	0
	PT2	0	0	0	2
Coquillettidia (*Coquilletidia*) *richiardii* FICALBI 1889	T1	0	0	0	1
	T2	0	0	0	2
	T5	0	0	0	2
	F1	0	0	0	1
	PT1	0	0	0	12
	PT2	0	0	0	2
	PT3	0	0	0	8
	PT4	0	0	0	8
	PT6	0	0	0	3
Total:		34	1927	80	217

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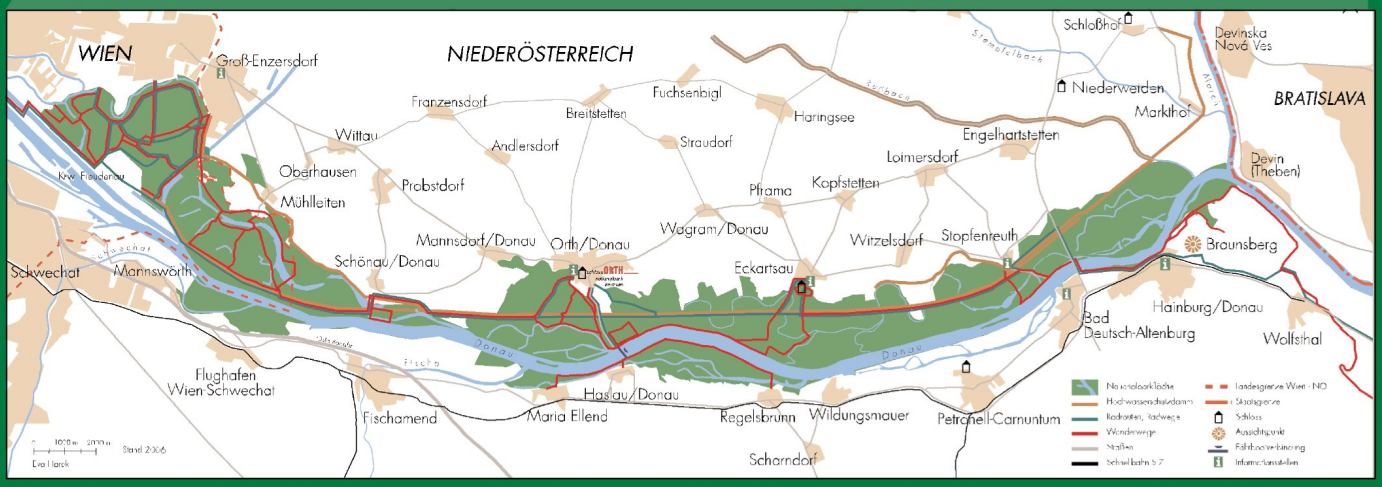

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