Conference Volume	5th Symposium for Research in Protected Areas 10 to 12 June 2013, Mittersill	pages 837 - 842

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

Carina Zittra[1] & Johann Waringer[2]

[1]Nationalpark Donau-Auen GmbH., Orth an der Donau, Austria
[2]Department of Limnology, University of Vienna, Austria

Abstract

Culicidae are known as hosts for a variety of pathogens and are therefore considered as nuisance and as vectors of human diseases, such as malaria, dengue and yellow fever. Until recently mosquito ecology had been neglected although they play an important but poorly understood role in food chains. In order to understand the ecological function of Culicidae it is imperative to investigate species distribution patterns and the factors controlling it. Abiotic parameters such as water level, nutrients, oxygen concentration and conductivity as well as biotic parameters (Culicidae and potential predators) were monitored 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 221 adult Culicidae were collected. We detected 15 Culicidae species belonging to 6 genera (*Anopheles, Culex*, *Culiseta*, *Coquillettidia*, *Aedes* and *Ochlerotatus*), with*Oc. geniculatus* (68 %) and *Cx. territans* (13%) being most frequent, followed by *Cx. pipiens* and *Ae. vexans* with approximately 5% and 4% of total abundance. Biometrical data were used to reconstruct life cycles; *Cx. pipiens* and *Cx. territans* were bivoltine and *Oc. geniculatus* multivoltine in the study area. Based on abiotic and biotic parameters, sampling sites were grouped into 4 separated clusters. Water level and persistence, pH, electric conductivity and phosphate concentrations significantly influenced species distribution patterns and revealed that flood plain dynamics are a key factor for the seasonal and spatial distribution of mosquito larvae in the National Park Donau-Auen.

Keywords

Culicidae, species inventory, phenology, ecology, Nationalpark Donau-Auen

Introduction

Worldwide, more than 3,500 mosquito species have been recorded and more than 40 species are known to be endemic in central Europe (BECKER et al. 2010). The species inventory of Austria consists of 43 published Culicidae species belonging to 7 genera (*Aedes*, *OchlerotatusAnopheles*, *Culex*, *Coquillettidia*, *Culiseta* and *Uranotaenia*) (MOHRIG & CAR 2002, SEIDEL et al. 2012, LEBL et al. 2013). Culicidae are mainly known as vectors for medically important pathogens and parasites and as transmitters of diseases.The ecology of Culicidae has been neglected with exception of some investigations on population and community ecology of invasive Culicidae species like *Ae. albopictus*, which is considered as a major threat to public health (MEDLOCK et al. 2006). Studies on mosquito life-strategies and distribution patterns gain importance again since global change supports the arrival of invasive, non-indigenous Culicidae species and new emerging vector-borne diseases in Europe. A main topic of mosquito research was pest control; i.e. reduction of culicid populations by using methods like *Bacillus thuringiensis* ssp. *israelensis* (*Bti*) to protect people from mosquito-borne diseases, whereas 200 tonnes of *Bti* are used annually for mosquito control worldwide (BECKER 1998). Since 1990 the effects of *Bti* on target (mosquitoes and black fly larvae)and non-target organisms were investigated,the results showed that nematoceran species, such as Ceratopogonidae and Chironomidaeare susceptible to *Bti* (GARCIA et al. 1980, BOISVERT & BOISVERT 2000). Meanwhile it is known that the usage of *Bti* has negative effects on breeding success of birds. POULIN et al. 2010 showed that the clutch size and fledging survival was lower at the Bti treated siteswhen compared to control sites in southern France. In contrast,no negative trophic effects could be attributed to the change in the insect community by a 3 year study in wetlands of central Minnesota (NIEMI et al. 1999). These studies underline the necessity for long-term investigations to fully predict the consequences of mosquito control on floodplain communities. Despite increasing interest in mosquitoes there is a lack of information on species inventoriesand distribution patterns in Austria and the bordering countries (European Centre for Disease Prevention and Control 2012).The National Park Donau-Auen, one of the last remaining large wetlands in Central Europe was chosen for this study, because it provides many different habitats for the larval development of mosquitoes. The goal of this study was to update the information on species inventory, ecology and spatial distribution patterns of mosquitoes in the area of the National Park Donau-Auen, which serves as a basis for further investigations in this region.

Methods

From 1 March to 31 October, 2011, the 20 study sites (phytotelmata, one artificial pond, temporary and stagnant water bodies) at Orth an der Donau were sampled at regular intervals (Fig.1). Culicidae (eggrafts, larvae and pupae) and their potential aquatic predators were sampled every third day using a catch per unit effort (CPUE) method (KREBS 1989). Adults were caught strictly above the water surface of their breeding habitats with a handnet (20 cm in diameter,200 µm). Phytotelmata were sampled at the same intervals using a syringe (20 ml). The catch was preserved in vials containing ethanol (75%). Pre-imaginal stages and adults were identified using the key of BECKER et al. (2010). Head widths and body lengths were measured to identify the four larval instars and to provide basic data for life cycle reconstruction. Dyar's rule was used to ensure instar definition (MCDONALD et al. 1977). Abiotic parameters (dissolved oxygen, pH, conductivity, water temperature, nutrients, total and carbonate hardness) were measured every second week and additionally after flood and heavy rainfall at each sampling site. Hydrological connectivity, water level and persistence were noted additionally. These were used to characterize the breeding habitats employing cluster analysis based on euclidean distances. A Canonical Correspondence Analysis (CCA) was employed as an unimodal method to explain species data by environmental data.

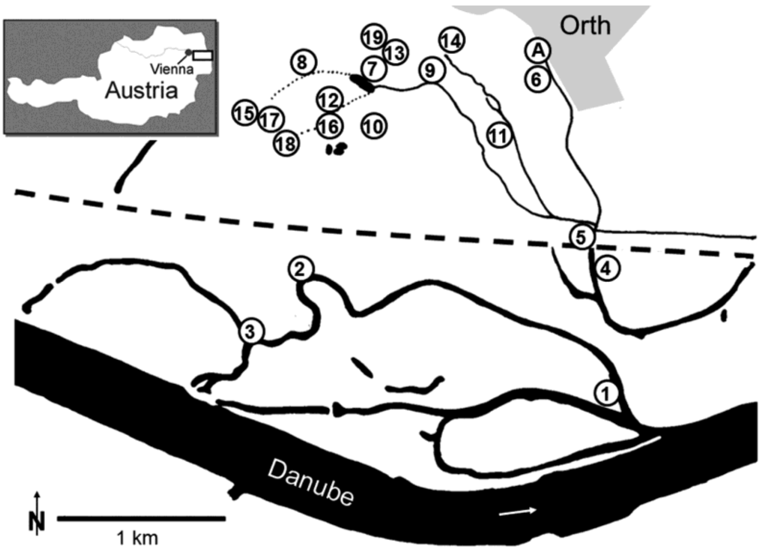

Figure 1: Map of the study area in the National Park Donau-Auen at the northern bank of the Danube near the city of Orth, showing the sampling sites covering a wide range of water body types (large permanent waters: sites1 – 3 (Große Binn, kleine Binn); intermediate permanent waters: sites 4 – 6 (Fadenbach); small permanent (7) and temporary (8) waters: sites 7 – 8 (Wachtelgraben); small temporary waters: sites 9 -13; phytotelmata: sites 14 – 19; plastic container: site A). Dotted line: dam for flood control. The upper left insert indicates the location of the National Park Donau-Auen within the borders of Austria.

Results

A total of 34 eggrafts, 1927 larval, 80 pupal and 221 adult mosquitoes (3 males, 218 females) were collected, belonging to 15 Culicidae species (including two morphologically difficult species pairs). The species inventory consisted of 6 genera: *Aedes, Anopheles*, *Coquillettidia*, *Culex*, *Culiseta* and*Ochlerotatus*.*Oc. geniculatus* was most abundant (64.1%) in the larval stage, followed by larvae of *Cx. territans* (18.7%), *Cx. pipiens* (6.9%) and *Ae. vexans* (2.8%) (Tab.1). The remaining species represented 7.5% of the total catch. Adults of*Oc. geniculatus* (40.3%) were most abundant, followed by *Cx. pipiens* (23.1%), *Cq. richiardii*(19.9%) and *Cx. territans* (10.4%) (Tab.2). A total of 679 larvae, pupae and adults of *Cx. pipiens* were collected with the majority of the aquatic stages originating from the artificial pond (Figure 1, A).Two generations emerged in May/June and July with a possible spring generation in March/April. *Cx. territans* had two generations emerging in May/June and June/July 2011; a possible third generation was not completed as sampling sites dried up in October.*Oc. geniculatus* had two generations emerging in May and July/August. As sampling sites dried up at the end of September, a third generation was unable to emerge. A Cluster analysis was performed in order to explore the effect of environmental parameters on Culicidae species distribution.Four groups of habitats were extracted. Group 1 consisted of temporary water bodies (sites 8-13) and one phytotelma (site 18) with the highest amount of larval Culicidae (1044 individuals out of 8 species). These breeding sites were characterized by a low water level (0.5 to 10.5 cm), very lowoxygen 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. Group 2 combined phytotelmata (sites 14-19 except site 18). These temporary water bodies had a very low water level (only 3.0 – 9.0 cm), the lowest conductivity (214 to 574 μScm^{-1}) and the highest amounts of nitrate (1.22 to 32.44 mgl^{-1}) ofall groups. A total of 871 larvae of two tree-hole species, *Oc. geniculatus* and *An. plumbeus*,were detected. Group 3 consisted ofsampling sites of two permanent, large water bodies, the Kleine Binn (sites 2 and 3) and the Große Binn (site 3), characterized by comparatively high oxygen concentrations ranging from 7.16 to 10.17 mgl^{-1} and lower nutrient concentrations. The species inventory consisted of *Cx. territans*, *An. maculipennis* and *Cs. annulata*. Group 4 consisted of the sampling sites located along the Fadenbach (sites 4-6) and one sampling site at the Wachtelgraben (site 7), characterized by water persistence, a nearly constant water level during the entire sampling period, high conductivity ranging from 408 to 999 μScm^{-1} and chloride concentrations ranging from 18.86 to 68.44 mgl^{-1}.The high predator density at these breeding habitats resulted in only 25 larval Culicidae. To detect habitat parameters associated with the distribution of Culicidae species, a Canonical Correspondence Analysis was performed. Habitat parameters likeconductivity, pH, phosphate concentrations, water level and persistence significantly (CCA, $p<0.05$) influenced larval distribution of Culicidae species (Fig. 2). In total, the model explained 99.8 % of the variance of the five selected variables. In general the results of the Canonical Correspondence Analysis of environmental variables (Fig. 2b) support the results of the Cluster analysis.The first axis discriminated between species in short-living temporary water bodies and species in stagnant water bodieswith high water levels (Figure 2a). In fact, aquatic stages of *Cx. territans* were found in high abundances in stagnant water bodies (sites 1, 3, 5) where water levels ranged from 46 to 103 cm, and only in very low abundances in temporary water bodies. *Oc. geniculatus* and *An. plumbeus* were found in phytotelmata (14-19) where concentrations of PO_4 were high, ranging from 1.10 to 16.18 mgl^{-1}. Larvae of *Oc. annulipes/cantans* and *Ae.vexans*are constricted to short-living temporary ponds. *Cx.pipiens*, on the other hand, was abundant at a variety of sampling sites, ranging from slowly-flowing running waters to phytotelmata and temporary water bodies.

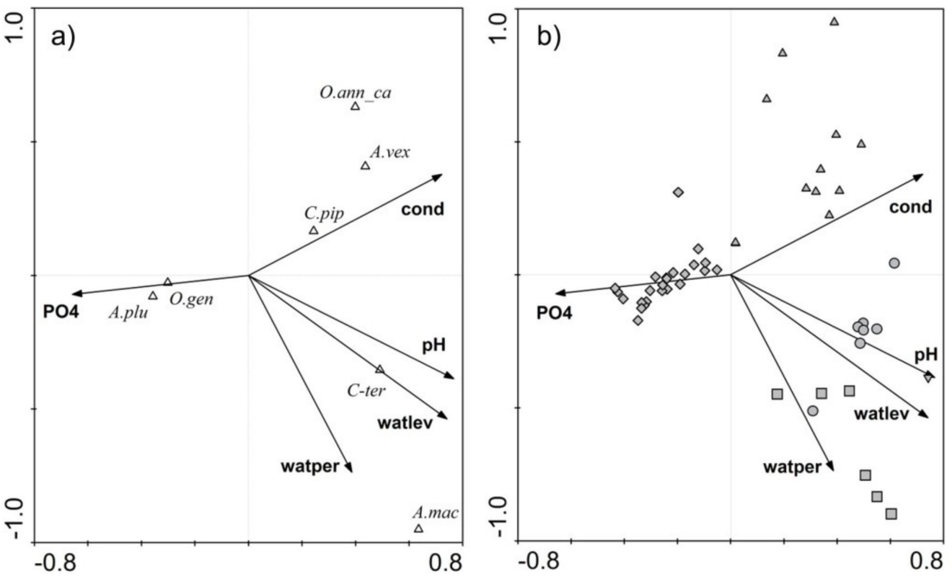

Figure 2: a) Canonical Correspondence Analysis biplot of five significant (P<0.05) environmental variables versus Culicidae species abundance. Arrows indicate environmental variables; cond = conductivity, watper = water persistence, watlev = water level, PO4 = phosphate concentration; A.plu = *Anopheles plumbeus*, A.mac = *Anopheles maculipennis* complex, C.ter = *Culex territans*, C.pip = *Culex pipiens*, A.vex= *Aedes vexans*, O.gen = *Ochlerotatus geniculatus*, O.ann_ca = *Ochlerotatus annulipes* / *cantans*. b) Canonical Correspondence Analysis biplot of monthly means of five significant (P<0.05) environmental variables versus sampling sites. Down triangles = Wachtelgraben sampling sites (7 - 8); up triangles = temporary water bodies (9 - 13); diamonds = phytotelmata (sites 14 - 19); circles = Kleine Binn and Große Binn sampling sites (1 - 3); squares = Fadenbach sampling sites (4 - 6).

Discussion

In our study at the National Park Donau-Auen the detected Culicidae species inventory is equivalent to 36% of the Austrian species inventory (MOHRIG & CAR 2002; SEIDEL et al. 2012; LEBL et al. 2013). Species like *Ae.vexans*, *Ae. sticticus*, *Ae. cinereus*, *Ae. rossicus*, *Oc. rusticus*, *Oc. annulipes* and *Oc. cantans*, which are typical for frequently inundated areas were detected in unexpected low numbers (5.9 % of total abundance). Larvae of tree-hole species (*Oc. geniculatus*, *An. plumbeus*) (66.4 % of the total) were most abundant in our study, followed by species which are associated with urban areas such as *Cx. pipiens* and *Cx. territans* (26.6 % of total larval abundance). In contrast, RÖTZER (1995) detected 12 species belonging to 5 genera in the Danube Floodplain at Stockerau (Lower Austria) with floodplain species being predominant. The low flood frequency in 2011 with only one spring flood is presumably the reason why we lacked many floodplain species, since floodplain dynamics are known to be a key factor for Culicidae species composition(ŠEBESTA et al. 2012; SUDARIĆ BOGOJEVIĆ et al. 2009). The most abundant species in the larval stage were tree-hole species (*Oc. geniculatus and An. plumbeus*),because suitable breeding habitats were present throughout the sampling period. Species like *Cx. pipiens* and *Cx. territans* which are often associated with urban areas were present from early spring to September. Therefore, two generations of these specieswere observed in 2011 with two further generations terminated by desiccation of the breeding habitats. In contrast, RÖTZER (1995) reported only a low amount of *Cx. territans* in the Danubean floodplain in Stockerau; *Cx. pipiens* was completely lacking in this study. *Oc. cantans*, *Oc.cataphylla* and *Oc. rusticus* were detected in temporary ponds in March and had only one generation in 2011. The results of the Canonical Correspondence Analysis revealed that conductivity, water level, water persistence, pH and phosphate concentrations contributed significantly ($p < 0.05$) to species distribution. Aquatic stages of floodplain species 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. Concurrently, Culicidae species that hibernate in the adult stage and that are strongly bound to human settlements (BECKER et al. 2010) occurred in high abundances. Aquatic predators which are also used as biocontrol agents (CHANDRA et al. 2008) were sampled in our CPUE sampling method.Sampling sites at the Wachtelgraben and the Fadenbach (Group 4) were characterized by a high predator densityresulting in only 25 mosquito larvae sampled. In habitat group 3 (Grosse and Kleine Binn sampling sites) high predator densities, but only 78 larval mosquitoes were detected. However, highest larval mosquito densities of 1044 specimens were recorded at temporary water bodies although invertebrate predators were present. Newts and fish might play an important role, since fish generally lack in temporary waters. A high number of Culicidae (871 larvae) were recorded in predatorless phytotelmata sampling sites; here the larval density was regulated by habitat size and water persistence, not by predator pressure.

Table 1. Culicidae species inventory (larvae and pupae; n and percentage) collected from March to October 2011 at 19 sampling sites within the National Park Donauauen.

Species	Larvae / pupae	%	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, Gorickaja & 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, 1830)	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, 1790)	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 (*Culiseta*) *annulata* (Schrank, 1776)	6 / 0	0.6	May, July
Total	**977 / 80**	**100.0**	

Table 2: Culicidae species inventory (adults; n and percentage) collected from March to October 2011 at 19 sampling sites within the National Park Donauauen.

Species	males / females	%	Phenology
Anopheles maculipennis complex	0 / 1	0.4	May
Aedes (*Aedimorphus*) *vexans* (Meigen, 1830)	0 / 1	0.4	August
Ochlerotatus (*Finlaya*) *geniculatus* (Olivier, 1791)	0 / 89	40.3	March – 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
Total	**3 / 218**	**100.0**	

Conclusion

Floodplain areas are dynamic systems with distinct Culicidae species pools. However, the yearly composition of the culicid speciesinventory varies, depending on hydrological conditions, weather (especially the amount of precipitation and possible dry seasons), water level fluctuations and flood frequencies (ŠEBESTA 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.

References

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

BECKER, N., PETRIC, D., ZGOMBA, M., BOASE, C., MADON, M., DAHL, C., KAISER, A. 2010. Mosquitoes and their control (2nd ed.). Heidelberg: Springer 578 pp.

BOISVERT, M. & J. BOISVERT 2000. Effects of *Bacillus thuringiensis var. israelensis* on target and non target organisms: a review of Laboratory and field experiments, Biocontrol Science and Technology, 10:517-561.

CHANDRA, G., MANDAL, S.K., GHOSH, A.K., DAS, D., BANNERJEE, S.S. 2008. Biocontrol of larval mosquitoes by *Acilius sulcatus* (Coleoptera: Dytiscidae), BMC Infectious Diseases, 8: 138.

European Centre for Disease Prevention and Control. 2012. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC, http://ecdc.europa.eu/en/healthtopics/vector-borne_diseases/public_health_measures /Pages/mosquito-guidelines.aspx accessed 23 September 2012.

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.

KENYERES, Z., TOTH, S., BAUER, N. & SARINGER-KENYERES, T. 2012. Life-strategy basedstructural features of the larval mosquito metacommunities in Hungary, Ekológia,31:210-230.

KREBS, C.J. 1989. Ecological Methodology, Harper Collins Publishers, 654pp.

LEBL, K., NISCHLE,R 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, 29(10):59-60.

MCDONALD, G., SMITH, I.R., SHELDEN, G.P. 1977. Identification of instars of *Culex annulirostris* Skuse (Diptera: Culicidae), Journal of the Australian Entomological Society, 16: 359-360.

MEDLOCK, J.M., SNOW, K.R. & LEACH, S. 2006. Possible ecology and epidemiology ofmedically important mosquito-borne arboviruses in Great Britain, Epidemiology and Infection, 135:446-482.

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

MOHRIG, W. & M. CAR 2002. Diptera: Culicidae in Fauna Aquatica Austriaca, Teil III, Lieferung 2002, ed O. Moog, Wien: Wasserwirtschaftskataster, Bundesministerium für Land und Forstwirtschaft, Umwelt- und Wasserwirtschaft, 1-9.

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.

POULIN, B., LEFEBVRE, G., PAZ, L. 2010. Red flag for green spray: adverse trophic effectsof Bti on breeding birds, Journal of Applied Ecology,47:884-889.

RETTICH, F., IMRICHOVA, K., ŠEBESTA, O. 2007. Seasonal comparisons of the mosquitofauna of the flood plains of Bohemia and Moravia, Czech Republic, European Mosquito Bulletin, 23:10-16.

RÖTZER, K. 1995. Ökologische Untersuchungen an Culiciden (Insecta: Diptera) in den Donauauen bei Stockerau (NÖ), Diplomathesis, University of Vienna 120 pp.

ŠEBESTA, O., GELBIC, I., MINAR, J. 2012. Mosquitoes (Diptera: Culicidae) of the Lower Dyje River Basin (Podyji) at the Czech-Austrian border,Central European Journal of Biology,7:288-298.

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 Osterreich 2012 (Diptera: Culicidae), Entomologische Zeitschrift, 122:223-226.

STRELKOVA, L. & J. HALGOS 2012. Mosquitoes (Diptera, Culicidae) of the Morava River floodplain, Slovakia, Central European Journal of Biology, 7:917-926.

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, 64:760-767.

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, 27:8-20.

Contact

Carina Zittra
c.zittra@donauauen.at

Nationalpark Donau-Auen GmbH.
Schloss Orth
2304 Orth an der Donau
Austria