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Short communication

Investigation of mosquito larvae (Diptera, Culicidae) in the coastal area of Dalmatia, Croatia

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Abstract

Background and Purpose: *The coastal region of Croatia was an autochthonous area of malaria until the first half of the 20th century. An investigation of mosquitoes in Dalmatia was carried out in order to obtain a better insight into the composition and distribution of mosquito species. Dalmatia is a very specific region with a high diversity of breeding sites.*

Material and Methods: *Mosquitoes were collected in the larval stage with a larva net (20 cm in diameter), small glasses and an aspirator. The sampling took place from April to November during the period from 2001 to 2004 at 41 localities from Maslenica bridge to Prevlaka.*

Results and Conclusions: *The investigation of mosquito fauna showed the presence of 25 species in natural and artificial breeding habitats. According to this investigation the most abundant mosquito species in Dalmatia were: Cx. pipiens complex (55,58 %), Cs. longiareolata (37,91 %) and Cs. annulata (33,34 %). The species whose presence in different types of breeding habitats varied between 10 % and 30 % were: Ae. vexans, An. claviger, An. maculipennis kompleks, An. plumbeus, Cx. hortensis, Oc. caspius and Or. pulcripalpis. The remaining 15 species were rare for the region of Dalmatia.*

INTRODUCTION

Dalmatia is a part of the coastal area of Croatia that consists of an elongated marine belt 400 km long and 70 km wide. The larger northwest part inland is bordered by the mountain chains of Velebit, Dinara and Kamešnica, whereas towards the southeast the natural boundary is less rugged and is situated in the near hinterland of the coastal belt. The region of Dalmatia covers an area of 11,960 km^2. It also includes the islands, which were not part of this investigation.

The prevailing karst substraum reveals an abundant and complex network of underground water circulation and comparatively scanty surface waters. Among the longer watercourses in the area (to be singled out) are the Cetina, Krka, Zrmanja and Neretva rivers. There are several lakes in Dalmatia: Baćinska jezera (a group of 5 interconnected lakes), Modro and Crveno jezero, Prokljansko jezero and finally Vransko jezero, the largest in Croatia *(1)*.

The average annual rainfall in Dalmatia is about 870 to 980 mm, with most rainfall occurring during the winter period. Since the region has adequate rainfall the listed potential breeding sites can be active during the whole year.

Despite the Dalmatian biodiversity potential, there have only been a few entomological investigations. Most papers date back to the early 20th century and the only written records deal with the species of genus Anopheles. After the eradication of malaria interest in mosquitoes generally decreased in this region.

Exceptions are the investigations of Adamović and Paulus *(2, 3)* from this area, although they only deal with the Anopheles species.

More recent investigations in Dalmatia began in the mid-nineties of the past century *(4, 5)* and the beginning of the 21st century *(1, 6–10),* not dealing with the potential role of the vector of a particular species.

Despite malaria eradication in the 1960s (the last case of malaria was recorded in Croatia in 1958), mosquitoes still represent a serious threat to humans and animals in rural as well as in urban areas. The major concern is the possibility of the re-activation of a vector born disease in areas suitable for the development of arthropod vectors *(11–13)* as well as the entrance of *mosquitoes of medical concern in new areas due to modern means of transportation (14, 15)*. This proved to be vindicated when it came to the species *Ae. albopictus*, representing a potential danger for the appearance and spread of diseases not specific for the area.

MATERIAL AND METHODS

In the four-year period (2001–2004) 1065 samples were analyzed, collected from 41 localities in Dalmatia.

Mosquito larvae were collected with a larva net (25 cm in diameter), small glasses and an aspirator according to the size of breeding sites. A part of the sampled larvae were stored in 50 % alcohol.

The preparation was conducted using several alcohol solutions (50 %, 70 %, 96 %), immersed in xilol and fixed in Canada balsam.

A total of 10,090 mosquito larvae were counted.

Determination of the species was carried out by the key presented in Gutsevich *et. al*, 1974 *(16)*. The collection is stored at the Department of Epidemiology and Health Ecology, the Naval Medicine Institute, Croatian Navy, Split.

In ecological investigations the calculation method of the abundance of specific mosquito species has to take at least two elements into account. These are the number of individuals of a specific species as well as the number of specific types of breeding habitats wherein they have been proportioned 1:1. A more real estimation of the mosquito abundance expressed in percentages has been obtained in this way.

RESULTS

The investigation of the Dalmatian mosquito fauna represents 25 species which is shown in Table 1.

TABLE 1

List of mosquitoes sampled in Dalmatia.

Species	The presence of the mosquito species in different habitats of the study area (%)
Aedes cinereus Meigen, 1818.	4,95
Aedes vexans Meigen, 1830.	17,45
Anopheles (Anopheles) algeriensis Theobald, 1903.	2,42
Anopheles (Anopheles) claviger Meigen, 1804.	17,98
Anopheles (Anopheles) maculipennis kompleks Meigen, 1818.	14,15
Anopheles (Anopheles) plumbeus Stephanus, 1828.	12,60
Coquillettidia (Mansonia) richiardii Ficalbi, 1889.	0,95
Culex (Neoculex) hortensis Ficalbi, 1889.	17,34
Culex (Culex) pipiens kompleks Linnaeus, 1758.	55,58
Culex (Neoculex) territans Walker,1856.	9,11
Culiseta (Culiseta) alascaensis Ludlow, 1906.	1,26
Culiseta (Culiseta) annulata Schrank, 1776.	33,34
Culiseta (Allotheobaldia) longiareolata Macquart, 1838.	37,91
Culiseta (Culicella) morsitans Theobald, 1901.	1,95
Ochlerotatus (*Ochlerotatus*) *annulipes* Meigen, 1830.	2,54
Ochlerotatus (Ochlerotatus) cantans Meigen, 1818.	7,20
Ochlerotatus (Ochlerotatus) caspius Pallas, 1771.	22,40
Ochlerotatus (Ochlerotatus)) detritus Haliday,1833.	3,15
Ochlerotatus (Finlaya) geniculatus Olivier, 1791.	6,45
Ochlerotatus (Ochlerotatus) leucomelas Meigen, 1804	2,88
Ochlerotatus (Ochlerotatus) mariae Sergent Sergent, 1903	1,10
Ochlerotatus (Ochlerotatus) pulchritarsis Rondani, 1872.	9,91
Ochlerotatus (Ochlerotatus) rusticus Rossi, 1790	1,41
Ochlerotatus (Ochlerotatus) sticticus Meigen, 1838	9,32
Orthopodomya pulcripalpis Rondani, 1872.	15,67

The research and sampling were conducted in the coastal area of Dalmatia. Figure 1. shows the 41 localities where the mosquitoes were sampled in larval stage. As the investigated area abounds in different habitats that are favorable for the development of mosquitoes, we managed to establish a great diversity of species.

This research covered 16 types of mosquito breeding habitats (natural and artificial). Natural breeding habitats were: areas of the lakes, flooded areas, ditches, swamps, banks of rivers, tree-holes, channels, waterholes, and marshes prevailing in rural areas. Artificial breeding habitats were: stone-holes, barrels, tires, abandoned vessels, cisterns, wells, water storage sites, fountains prevailing in suburban and urban areas.

The species with the greatest distribution across Dalmatia was *Cx. pipiens* complex. It was found that *Cx. pipiens* complex used all available breeding habitats (except tree-hole habitats) and in many cases coexisted with other mosquito species. *Cx. pipiens* complex did not have a specific habitat preference and could breed in almost all of the aquatic habitats. The mosquitoes of this complex produce up to 10 generations a year and spend the winter as fertilized females *(17, 18, 19)*.

The next most abundant mosquitoes across Dalmatia were *Cs. longiareolata* and *Cs. annulata*. The presence of *Cs. longiareolata* in breeding habitats of the investigated area was 37.91 %, while the presence of *Cs. annulata* in breeding habitats was 33.34%.

Like the *Cx. pipiens* complex, species *Cs. longiareolata* and *Cs. annulata* were also not specific in their choice of breeding habitats (natural and artificial) for larval growth occurring in the investigated area. In fact, neither of the species was fastidious regarding types of breeding habitats. Obviously, in view of their specific adaptation mechanism these species are capable of thriving in many types of breeding habitats, which makes them one of the most widespread species across Dalmatia.

The species whose presence in different types of breeding habitats varied between 10 % and 30 % are: *Ae. vexans* (17,45 %), *An. claviger* (17,98 %), *An. maculipennis* complex (14,15%), *An. plumbeus* (12,60 %), *Cx. hortensis* (17,34 %), *Oc. caspius* (22,40 %) and *Or. pulcripalpis* (15,67 %).

Species *Ae. vexans* and *Oc. caspius* prefer exclusively natural habitats, such as the banks of rivers, flooded areas, swamps etc. The presence of both species varied from 17,45% (*Ae. vexans*) to 22,40% (*Oc. caspius*). Due to the fact that *Oc. caspius* is tolerant to certain salt concentrations, we also found this species in lagoons.

The species of *An. maculipennis* complex were found to prefer certain salt concentrations in breeding habitats of the investigated area. Since sibling species of *An. maculipennis* complex cannot be distinguished at the larval stage in this study they were presented as *An. maculipennis* complex.

The presence of the remaining four species (*Cx. hortensis*, *An. claviger*, *An. plumbeus*, *Or. pulcripalpis*) varied

Figure 1. *Location of the study area (Dalmatia, Croatia), blue dots represent 41 sampling locations.*

from 12,60 % (*An. plumbeus*) to 17,98 % (*An. claviger*). Although all the mentioned species used some natural types of habitats, species *An. claviger*, *Cx. hortensis* and *An. plumbeus* preferred artificial types of habitats, so we found them mostly in urban and suburban areas. An exception to this was *Or. pulcripalpis*, due to its capability of larval growth in specific types of natural breeding habitats such as tree-holes. This species was found mostly in rural zones of the investigated area.

The remaining 15 determined species in Dalmatia were very fastidious regarding the breeding habitats used for larval growth. Certainly, among other reasons, this exclusiveness is very important as to why these species are rare for the region of Dalmatia.

DISCUSSION

The fauna research of mosquitoes in Dalmatia showed the presence of 25 mosquito species which comprises 50 % of the Croatian mosquito fauna *(1, 20–22)*.

Due to their specific adaptation mechanisms, mosquitoes are capable of thriving in various environments such as permanent or temporary bodies of water as well as in large or small accumulations of water. This makes mosquitoes one of the most successful insect groups.

The investigation showed a greater abundance of sinantropic species such as species of *Cx. pipiens* complex. This complex has several species, subspecies, forms, races, physiological varieties of biotypes depending on different authors *(23, 24)*. As they do not differ morphologi-

cally it was supposed that, according to the breeding habitats, captured individuals belonged to the subspecies *Cx. pipiens pipiens* and *Cx. pipiens* biotype *molestus*. They were found in small breeding sites in urban and suburban zones usually preferred by *Cx. pipiens molestus* as well as in natural breeding habitats away from urban zones, usually preferred by *Cx. pipiens pipiens*.

The species *Cs. longiareolata* and *Cs. annulata* also belong to more abundant and more widespread species in the region of Dalmatia. Zoogeographically, *Cs. longiareolata* is a typically Mediterranean species, *Cs. annulata* Paleartic, while *Cx. pipiens* complex is a cosmopolitan species.

Cs. longiareolata and *Cs. annulata* prefer artificial habitats for their larval growth. In fact, offspring survival and growth depend strongly on the quality of the habitat in which they are oviposited. Thus, when potential habitats vary in their suitability for larvae, females are expected to choose habitats that maximize their fitness *(25),* which is proved in our investigation. Generally speaking, in mosquitoes such oviposition habitat, selection has been demonstrated in response to physical and chemical suitability for larval development *(26),* habitat size and resource availability *(27, 28),* the presence and density of specific competitors *(29)* and presence of predators *(30)*.

On the other hand, density dependence on fitness components such as survival and growth *(31–33)* implied that the more females chose the same subset of available habitats the sooner the preferred habitats will decline in numbers. Nevertheless, *Cs. longiareolata* and *Cs. annulata* females oviposited preferentially in predator-free habitats such as artificial habitats which prevailed in the investigated area.

Other species whose presence varied between 10%–30% (*Ae. vexans*, *An. claviger*, *An. maculipennis* complex, *An. plumbeus*, *Cx. hortensis*, *Oc. caspius*, *Or. pulcripalpis*) also preferred mainly artificial habitats. The exceptions were *Or. pulcripalpis*, *An. maculipennis* complex, *Ae. vexans* and *Oc. caspius*.

The development of *Or. pulcripalpis* larvae is linked to the water collected in tree-holes, which are specific breeding sites for the mosquitoes of this species. Mosquitoes, whose usual breeding sites are tree holes, were *Oc. pulchritarsis*, *Oc. geniculatus* and *An. Plumbeus,* but they were less abundant.

Species belonging to *An. maculipennis* complex caused many problems in the past as malaria vectors in Dalmatia. Today, we know 12 *(34)* morphologically identical species that belong to the *An. maculipennis* complex, six of which have been registered in the Croatian fauna: *An. atroparvus*, *An. labranchiae*, *An. messeae*, *An. maculipennis*, *An. sacharovi* and *An. subalpinus*. *An. maculipennis* complex can breed among emergent vegetation at the edges of impoundments (?) (ponds) and ditches where larvae are protected from currents *(8, 9.)*. We found them in temporary rain pools which are widely scattered in the environment and impossible to remove. However, such breeding sites may be very seasonal *(1, 8, 9)*. The majority of *An. maculipennis* complex were found in the estuary of the Neretva river, and at the edges of lakes (Baćina, Vrana). Since the Neretva estuary has some salt concentration, we (presumed to have found) (expected to find) *An. labranchiae* and *An. maculipennis*, the species that tolerate certain salt concentrations. In urban areas anopheline usually breed in artificial water containers such as barrels, rain pools, metal drums, but in these sites they were less abundant *(1, 9)*.

The species *Oc. Caspius,* whose presence in the habitats of the researched area was 22,40 %, preferred exclusively natural habitats, the majority with certain salt concentrations such as the estuary of the Neretva river and the flooded area near the city of Trogir (Pantan).

The remaining 15 species as previously mentioned are rare species for the region of Dalmatia. The reason for their poor distribution lies in fastidiousness regarding breeding habitats and competition with other species for these habitats *(1)*.

The presence of one of the most important mosquito-disease vectors (*Ae. albopictus*) in Croatia *(21)* and specifically in Dalmatia, and the favorable geo-climate characteristics of the Croatian coastal area qualify Dalmatia as a risky area, not only regarding present species such as *Ae. albopictus*, but also because of the possibility of reactivation of vector born disease, which is the case in some countries *(35)*.

Because of all the facts previously mentioned and some (certain) other biological and ecological characteristics that make *Ae. albopictus* a very aggressive species with strong biological potential that can displace *Cx. pipiens* complex (the most abundant and widespread complex in Dalmatia) from its habitats, it is strongly recommended that Dalmatia should be constantly monitored in the future.

To sum up the results of all the research (investigations) in the past 7 years, the Dalmatian fauna is found to have 27 species.

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