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DYNAMICS OF MOSQUITO (DIPTERA, CULICIDAE) SPECIES RICHNESS IN SAMPLES OF DRY-ICE BAITED CDC TRAPS IN THE URBAN AREA OF OSIJEK, CROATIA

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In this paper the results of a ten year investigation of mosquitoes using dry ice baited CDC traps in the city of Osijek, Croatia are presented. In the first year we recorded 16 species. During these ten years the total of 20 species were determined. The single method, dry ice baited CDC traps, makes it possible to determine almost the entire fauna in a given area. The estimated fauna is statistically calculated. Five non-parametric estimators were chosen for evaluation: one-factorial Jackknife (Jackknife-1); two-factorial Jackknife (Jackknife-2); one-factorial Chao (Chao-1); two-factorial Chao (Chao-2) and Bootstrap. The estimated fauna contains between 22 and 29 species. The method used turned out to be very good when used over a long period of time, because new species were registered even in years without many mosquitoes.

Key words: mosquitoes, dry ice baited CDC traps, species richness, non-parametric estimators, Croatia

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U ovom radu govori se desetgodišnjim rezultatima istraživanja komaraca, koji su hvatani metodom CDC klopke uz suhi led kao atraktant u Osijeku. Prve istraživačke godine zabilježeno je 16 vrsta. Tijekom deset istraživačkih godina broj poznatih vrsta popeo se na 20. Predpostavka ovog rada je da se korištenjem samo jedne metode uzorkovanja može se doći do skoro kompletne faune nekog područja. Očekivana fauna izračunata je statistički. U tu svrhu korišteno je pet neparametar-

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skih estimatora: jedanfaktorijali Jackknife (Jackknife-1); dvofaktorijalni Jackknife (Jackknife-2); jedanfaktorijali Chao (Chao-1); dvofaktorijalni Chao (Chao-2) i Bootstrap. Ovim statističkim metodama je određeno da se broj vrsta komaraca u Osijeku kreće od 22 do 29 vrsta. Korištena metoda se pokazuje kao dobra ako se koristi kroz duže vremensko razdoblje, a naročita joj je vrijednost u tome što su se nove vrste evidentirale u godinama kada i nije bilo puno komaraca. Do otkrivanja kompletne faune ovom metodom, potrebno je uzorkovati još neko vrijeme.

Ključne riječi: komarci, CDC klopka uz suhi led kao atraktant, bogatstvo vrsta, neparametarski estimatori, Hrvatska

INTRODUCTION

The efficiency of various mosquito traps has been investigated by many researchers. New Jersey Light Traps (NJLT), gravid Reiter traps, bed net traps, traps baited with synthetic oviposition pheromone (MBOREA *et al.*, 2000) and more recently the propane-powered mosquito trap (KLINE, 2002), have demonstrated different results, with dry ice-baited CDC traps providing the best results (REISEN *et al.*, 2000, REISEN *et al.*, 1999). Dry ice, octenol and light are among the most frequently used attractants (BURKETT *et al.*, 2001, RITCHIE & KLINE 1995), of which dry ice proved to be the best for host-seeking females. The CDC trap has the best results when a combination of dry ice plus octenol is offered as attractant (BECKER *et al.*, 1995, WHITT *et al.*, 2001).

Because of their versatility, battery-operated CDC traps can be used in a variety of situations (in inhabited or uninhabited areas, marshland, forests, and even at different elevations in forests, (LUNDSTROM *et al.*, 1996), making it possible to collect data on ornithophilic species. Much research has been carried out on the emission of CO_2 which actually imitates the presence of a host. In the past few years many research papers on the standardization of CDC traps in Europe under the auspices of EMCA have been published (BELLINI *et al.*, 2003).

It is evident that none of the methods used singly can provide absolute results, therefore, in order to collect as many species of mosquitoes as possible from an area in the shortest possible time, a combination of trapping methods is recommended. In this paper we wish to demonstrate the ability of dry ice-baited CDC traps to provide data on most of the mosquito fauna, even if the method is more time-consuming. Statistical estimators are used to support this thesis. The only way to estimate species richness from the field investigation results is the use of various species richness estimators. The better to describe species richness five common nonparametric estimators were used.

MATERIAL AND METHODS

The investigation was carried out in the broader area of the city of Osijek, (northeastern Croatia, UTM CR25 and CR26), during a period of 10 years. This study discusses the data obtained from 1995 to 2004. Battery-operated, dry ice-baited CDC (Center for Disease Control) traps were set in 9 locations (Donji grad N 45 33 41,2 E

18 43 37,4; Jug II N 45 33 7,2 E 18 42 34,3; Sjenjak N 45 33 7,2 E 18 42 34,3; Iktus N 45 33 44,7 E 18 41 46,7; Županijska N 45 33 20 E 18 40 49,8; Strma N 45 33 57,9 E 18 40 7,2; Retfala S N 45 34 2,5 E 18 39 20,3; Retfala J N 45 33 25,4 E 18 39 13,3 and Josipovac N 45 34 23 E 18 36 16,9) and operated twice a month from May to September each year. The traps were put in yards of houses 1 m from ground and active for over 24 hours (± 2h), with a 9 kg dry ice cube. A total of 207,136 mosquitoes belonging to 7 genera and 20 species were collected in 900 samplings during this period.

All statistical calculations were performed using the statistical package »vegan« under the R software environment and Estimates.

RESULTS AND DISCUSSION

In the investigated area we collected a total of 20 species in 7 genera. The species *Aedes vexans*, *Culex pipiens* c., *Anopheles maculipennis* c., *Ochlerotatus cantans*, *Ochlero-*

Tab. 1. Mosquito species recorded in Osijek (1995–2004)

Nr.	Species/Year	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
1	*Aedes vexans*	+	+	+	+	+	+	+	+	+	+
2	*Anopheles maculipennis* c.	+	+	+	+	+	+	+	+	+	+
3	*Coquillettidia richiardii*	+	+	+	+	+	+	+	+	+	+
4	*Culex modestus*	+	+	+	+	+	+	+	+	+	+
5	*Culex pipiens* c.	+	+	+	+	+	+	+	+	+	+
6	*Culiseta annulata*	+	+	+	+	+	+	+	+	+	+
7	*Ochlerotatus cantans*	+	+	+	+	+	+	+	+	+	+
9	*Ochlerotatus excrucians*	+	+	+	+	+	+	+	+	+	+
8	*Ochlerotatus sticticus*	+	+	+	+	+	+	+	+		+
10	*Ochlerotatus caspius*	+	+		+	+	+	+	+	+	+
11	*Aedes cinereus*	+	+	+		+	+		+	+	
14	*Anopheles hyrcanus*	+	+			+	+	+	+	+	
12	*Aedes rossicus*	+	+			+	+	+	+		
13	*Ochlerotatus rusticus*		+	+	+	+	+		+		
15	*Anopheles claviger*	+	+		+	+	+				
17	*Ochlerotatus cataphylla*	+									
19	*Uranotenia unguiculata*	+									
16	*Culex territans*				+						
18	*Ochlerotatus leucomelas*							+			
20	*Anopheles plumbeus*									+	
	No of species per year	16	15	11	13	15	15	13	14	12	10

tatus excrucians, *Coquillettidia richiardii*, *Culex modestus* and *Culiseta annulata* were found every year, and can therefore be referred to as »frequent species«. *Ochlerotatus sticticus* and *Ochlerotatus caspius* were recorded in nine seasons; *Aedes cinereus* and *Anopheles hyracanus* were recorded in seven seasons; *Aedes rossicus* and *Ochlerotatus rusticus* in six, and *Anopheles claviger* in five. *Ochlerotatus cataphylla*, *Uranotenia unguiculata*, *Culex territans*, *Ochlerotatus leucomelas*, and *Anopheles plumbeus* (»rare species«) occurred in one season only.

The abundance analysis demonstrated that the dominant species breeding in the flooded area were *Aedes vexans* and *Ochlerotatus sticticus*. These two species made up 88.91% of the mosquito fauna in the investigated area, as they did in previous research (MERDIĆ & LOVAKOVIĆ, 2001).

The number of species found differs from year to year, varying from 10 to 16 species (Tab. 1). In 1996, 1999, and 2000, we had a qualitatively identical composition of mosquito fauna, with 15 species. The largest number of species, 16, was recorded in 1995, the first year of investigation.

The number of species trapped using this method increased to 17 the following year (1996, *Ochlerotatus rusticus*). Two years later, another species (1998, *Culex territans*) was found, and three years later, yet another (2001, *Ochlerotatus leucomelas*), and finally, two years later one more rare species was found (2003, *Anopheles plumbeus*). The probability of recording the entire mosquito fauna was calculated based on the dependence of each particular year and the probability of recording most of the mosquito fauna in the years of investigation.

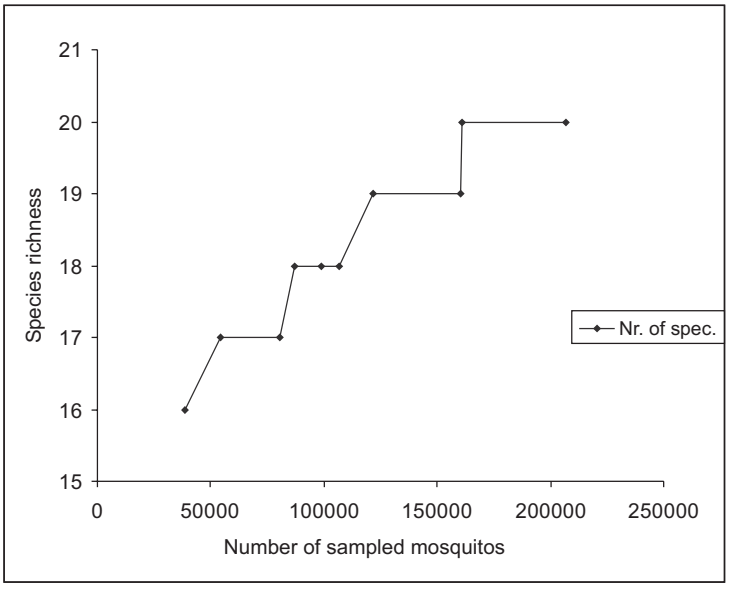

Fig. 1. Increase of found species according to number of specimens

The increase in the number of rare species is directly connected with the number of sampled specimens (Fig. 1). A new species appears at regular intervals (every two years). The value of the method used is additionally proved by the fact that a new species appeared even in the dry season.

The estimation of total species richness in a biological community using a limited number of survey units has drawn a great deal of attention (BUNGE & FITZPATRICK, 1993; COLWELL & CODDINGTON, 1994; WILLIAMS *et al.*, 2002; CAO *et al.*, 2004). Recent evaluations have suggested that non-parametric estimators perform better than methods based on either species accumulation curves or species-abundance distributions (PALMER, 1990; BALTANAS, 1992; COLWELL & CODDINGTON, 1994; WALTHER & MORAND, 1998; KREBS, 1988; WALTHER & MARTIN, 2001, CAO *et al.*, 2004) For some insect species Chao-2 was the most accurate and precise estimator, the Bootstrap estimator performed only slightly better than the number of observed species. Jacknife-1 and 2 performed intermediately. For such populations only Chao2 performed more precisely than the observed number of species. Since, however, for mosquito populations it is still unknown which estimator is best to describe species richness, for this paper five estimators have been used.

Nonparametric estimators are sampling theoretical extrapolation methods that only require the number of samples in which each species is found rather than any parametric information about their abundance. In this paper five non-parametric estimators that are often recommended in the literature were chosen for our evaluation: one-factorial Jackknife (Jackknife-1); two-factorial Jackknife (Jackknife-2);

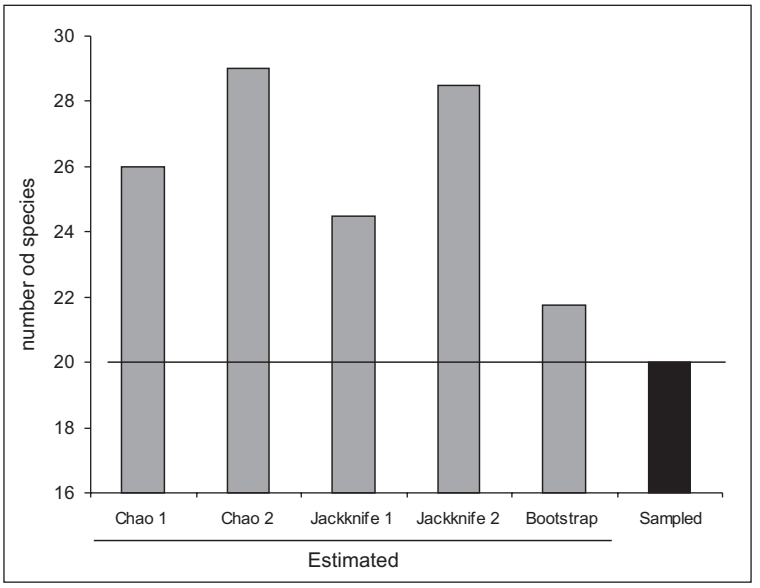

Fig. 2. Number of predicted species using different estimators

Tab. 2. Theoretical increase of species using different estimators

Sampling year	Chao 1 (mean)	Chao 2 (mean)	Jackknife 1 (mean)	Jackknife 2 (mean)	Bootstrap (mean)	Real number
1996	16	16	17	17	16	17
1997	17	19	19	19	18	17
1998	18	20	19	20	18	18
1999	19	21	20	21	19	18
2000	21	23	21	23	19	18
2001	22	24	22	24	20	19
2002	23	25	23	26	20	19
2003	24	27	23	27	21	20
2004	26	29	25	29	22	20

one-factorial Chao (Chao-1); two-factorial Chao (Chao-2) and Bootstrap. The results of these analysis show that the estimated fauna is between 22 (Bootstrap) and 29 (Chao-2 and Jackknife-2) (Fig. 2, Tab. 2). A good basis for this hypothesis is the data that in the neighbouring countries Hungary (MIHALY, 1963) and Serbia, in Vojvodina, 42 and 35 species, respectively (PETRIĆ, 1989) were found.

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