CULICIDAE AND WATER MITES : PARASITISM UNDER MEDITERRANEAN CLIMATIC CONDITIONS

B. MATHIEU [1], L. BERTRAND [2], V. PEYRUSSE [3],
F. SCHAFFNER [1], M. BERTRAND [3]

(Accepted November 2006)

WATER MITES, MOSQUITOES, PARASITE, *CULEX PIPIENS, ARRENURUS,* DISPERSION. *OCHLEROTATUS CASPIUS, THYAS,* HOST-PARASITE INTERACTIONS.

SUMMARY: Under Mediterranean climatic condition and by a large survey of parasitism on adult Culicidae, two models of host and parasite relationships are compared. *Culex pipiens* (wintering female) and *Arrenurus* sp. are characteristic of permanent inland water and *Ochlerotatus caspius* (wintering eggs) and *Thyas barbigera*, from salt or brackish water and temporary ponds. The seasonnal variation of load (nb mites/indiv.) can be explained by the shorter development of the host, and by traits of life in more or less unpredictible environment. Parasitism cannot control the pullulating mosquitoes except during some weeks, at the end the most favourable periods for culicids.

The hematophagous Culicidae *Culex pipiens* Linnaeus, 1758 and *Ochlerotatus caspius* (Pallas, 1771) are commonly found hosting larval water mites. During a large survey of adult mosquitoes carried out in Southern France and Corsica (spring to autumn) since 2000, seven species were found infested by terrestrial Parasitengona (notably *Anopheles* spp. and *Aedes albopictus*), and by water mite larvae: *Culex pipiens* and *C. modestus* by two arrenurids (*Arrenurus* spp.) and *Ochlerotatus caspius* and *detritus* by *Thyas barbigera*.

It is obvious that heavy load of parasites is damaging, reduces host's fitness, and increases mortality (LANCIANI 1979): the parasite is suspected to be involved in the natural control of culicids (all the more because the adults prey on first stages of culicid larvae in experimental condition: SMITH, 1983). In field condition, infestation (% individuals infested in a population) estimates the "intensity" of the host-parasite interactions (ROLFF *et al*. 2001). It depends first on the relative number of the both populations, and consequently on the parasite's chance to find the most favourable host at the right time. If population fluctuates all along the period of host's activity, highest load (number of parasites/individual) reveals the key-periods (when largest parasitism on the target is observed).

The model [Water mite-Diptera] is characterized notably by differences in host and parasite generation time (delay between consecutive cohorts): short development of the host, (the aquatic time life is less than one week to 20 days, depends on temperature), longer for water mites (water mites are aquatic and free more than 50% of the time life). Culicid species are distributed in different habitats (permanent pond or temporary ponds), differ by behaviour (laying eggs at water surface = *Culex,* or on wet mud = *Ochlerotatus caspius*). The host dynamic and parasitism pressure

1. EID Méditerranée, 165 Av. Paul Rimbaud, 34184 Montpellier Cedex 4, France.
2. Domaine du Rayol 83820 Le Rayol Canadel, France
3. UMR5175, Departement Ecologie des Arthropodes des Agro-écosystèmes méditerranéens, Université Montpellier III, route de Mende, F-34199 Montpellier Cedex 5, France

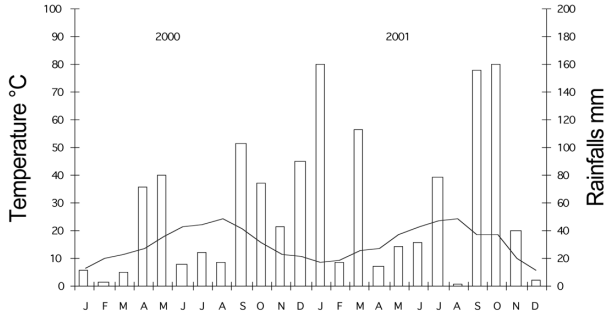


Fig. 1. Climatic conditions: (Mauguio) Mean temperatures (°C) and rainfalls (mm) 2000-2001 (Meteo France

can be estimated by surveys of the emerging hosts (Rolff 2000), and by the infestation rate (Smith & McIver 1984). With a good knowledge of host biology and ecology, is it possible to estimate if damaging interactions are significant? And is it possible to point out the differences between host parasite communities in stable (permanent water piece = *C. pipiens* dominant) or in fluctuating or unpredictable environment (*O. caspius* = temporary ponds in salt marshes, filled by rainfalls).

Material and Methods

Two campaigns of survey of anthropophilous mosquitoes were carried out from 2000 in Southern France by the "*Entente Interdépartementale pour la Démoustication*" (EID: public agency in charge of the mosquito control) in Camargue, the delta region of the Rhône River (140,000 ha of wetlands, marshes pastures and salt flats (43°30'N, 4°30'E) before the

Mosquitoes		Number per sampling period	
		2000	2001
Genera	species	18170	20360
Culex	*pipiens**	5862	8071
	*modestus**	1679	178
Ochlerotatus	*caspius**	9000	11618
Aedes	*detritus*	+	0
Anopheles	*vexans*	+	0
	maculipennis	+	+
	hyrcanus	+	0
Coquillettidia	*richardii*	+	0
Total with parasites (%)		91 (0.5%)	69 (0.3%)

(* found with parasites).

Table 1: Captures during the sampling period.

Hosts	nb. Culicidae		Mean infestation rate			Water Mite
	2000	2001	2000	2001	Mean	
C. pipiens	5862	8071	0.9	0.74	0.81	*Arrenurus*
C. modestus	1679	178	0.3	0.56	0.32	
O. caspius	9000	11618	0.4	0.06	0.21	*Thyas*

Table 2: Mean infestation rate during the sampling campaigns..

French episode of West Nile arbovirus (from June to October 2000) and during the following years (April to October). Only two years will be considered here : 2000 &2001.

Natural history of the [mite–mosquito] system: 1 — The mites: larvae of two genera (*Arrenurus* and *Thyas*) are common parasites in southern France. a) *Thyas*: the larvae are known to climb or to jump on culicid females when they are close to the water surface (notably during egg laying) (Mullen 1974; 1975a; 1975b). Larvae are often collected on *Aedes* sensu lato. b) *Arrenurus* genus is parasite on various insects, and some species are found on *Culex* spp.. 2 — the hosts: a) *C. pipiens* : larvae are found in permanent pools where the non desiccation resistant eggs are laid on water with high organic content, wintering females, b) *O. caspius*: larval habitat is temporary ponds, eggs hatch after rainfall from wintering and desiccation resistant eggs, female deposits eggs in mud in ground depressions; the development time depends on the temperature (four-six days in summer), the resistant stage being the wintering and the non re-hydrated eggs all along the dry season. (http://www.mosquitocatalog.org/pdfs/0934806.PDF). 3 — Seasonal abundance: a) *Culex:* adults are active as soon as temperature are hot enough, with overlapping cohorts if none limiting factor ; b) *O. caspius*: variation in adult population is characterized by peaks (rhythm of cohorts given by successive rainfalls filling or not larval biotopes). Under Mediterranean climatic conditions, peaks of active adults (i.e. captured by traps) correspond to the successive cohorts, non overlapping cohorts because of the delays between successive rainfalls from spring to october under Mediterranean conditions (because of the "continuous" or "interrupted" functioning of larval biotopes).

Trapping mosquitoes and collection of the mites: Traps were attractive CO_2 traps functioning one

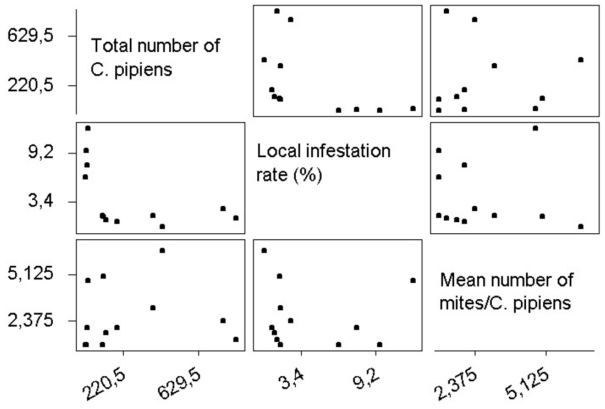


FIG. 2 : 2001, Two weeks variation of Frequency Indexes (see text) of trapped *Culex* spp., local infestation rate and mean number of larval mite per *Culex*. Cross diagrams.

night, collecting females. Mosquitoes were trapped monthly with 3 traps per site placed in triangle and collecting a population from a marsh (2000); or weekly (2001) with a single trap per site with a total of eleven sites trapped. The capture of mosquitoes was based mostly on a testing program on traps efficiency (2000) and on the routine survey of culicid populations potentially involved in the transmission of the West Nile Arbovirus (2001). Insects were kept frozen, then identified by EID (L.B. & B.M.) and counted. Mosquitoes were stored in ethanol 70°, the mites per individual counted, and the attachment site (head, thorax, abdomen, wings or legs) was noted. Mites were cleared by lactic acid then identified under microscope.

Influence of local or meteorological conditions: under Mediterranean climatic conditions trapping yield depends on rainfalls, on temperature and on wind strength: during the favourable period for culicids (spring to autumn), the rainfalls (spring and autumn) favour the number and the volume of temporary ponds (enhancing the number of emerging mosquitoes: *O. caspius*), whereas the temperature from spring to summer (with maximal rising 30°C.) favours the drying of the larval habitats but shortens the larval development (from 20 to 4 days in summer conditions) in temporary habitats. The number of windy days moves the populations away from larval sites (RIOUX 1958), or limits the flight (70 days per year with wind speed > 60 km/h, about 10 in summer). To limit a too large local effect, results of the different traps are summed. To limit the immediate climatic effect, the total capture of two consecutive periods are summed. Both years 2000 and 2001 were characterized by heavy drought (6 months with P. mm<2*T. °C) (FIG. 1). In 2001, rainfalls cut sampling period in July and September, and flooded the larval sites of culicids.

Host and parasite densities. the frequency index (**IF**) points out the maximal abundances: the ratio $\mathbf{IF=p_i/(\Sigma(p_i)/n}$ (with $\mathbf{p_i}$ frequency in the site and **n** = total number of sampled sites) is > 1 when the number of collected individuals is larger than expected. The infestation rate may be higher when 1) the parasite shares few potential hosts (stenoxenic) and when 2) the individuals of the host are rare.

Distribution of larval mites will tend to be aggregate when the hosts are few, the distribution tends to be regular or randomly distributed if hosts are abundant. The simplest method is to calculate the patchiness index (LLOYD, 1967). The patchiness index is the ratio between the mean crowding **m*** (function of the number of neighbours on the culicid) and the theoretical mean **m =** n. mites/n. mosquitoes per sample. In each sample, $\mathbf{m^*= \Sigma N^*(N-1)}$, **N** = number of mites bore by the mosquito. **m*** estimates the coefficient of dispersion **CD** (SD^2/m) by the relation **m*=m + (CD-1)** and, when **CD**=1, **m*=m** (LLOYD 1967). The patchiness index **m*/m** is >1 for patchy distribution.

RESULTS

General results: More than 35000 females of mosquitoes were collected with 18170 and 20560 in 2000 and 2001 in respect. Eight species were identified belonging to the genera *Culex*, *Ochlerotatus, Culiseta*, *Aedes*, *Anopheles* and *Coquilletidia*. The larval habitats of these species are continental water with salinity <10/1000, ponds peopled by *Phragmites australis* (Cav.) (*Coquilletidia*, *Culex*), anthropic zones (*Culex, Anopheles*) or brackish waters and salt marshes (*Ochlerotatus*). Only 91 and 69 were found with mites in 2000 and 2001 respectively (TABLE 1 & 2).

The parasites: Thyas barbigera Viets 1908 was collected in Camargue near the sampling sites associated

Sampling period	Mean number of *O. caspius* per night	Infested /Total	Number of Thyas.	Mean load in parasite per infested individual	Infestation rate
18-June 03 July	16	1/242	1	1.0	0.4
04-27 July	1.5	1/40	1	1.0	2.5
28-July-21August	2.9	1/69	1	1.0	1.4
21-August 29 September	4.4	2/169	7	3.5	2.0
30-September 5 October	41.9	2/293	2	1.0	0.03

TABLE 3 : Infested *Ochlerotatus caspius* in 2001.

Sampling period	Mean number of *C. pipiens* per night	Infested /Total	*Arrenurus* sp.	Parasites per infested individual	Infestation rate %
01-16 May	10	6/150	25	4.2	4
17-May 1 June	25.5	7/383	23	3.3	2
02–9 June	8.4	1/59	1	1.0	2
10-17-June	73	12/516	30	2.5	2
18 June 3 July	21.5	10/322	18	1.8	3
04-11 July	62	3/434	13	4.3	0.1
12-27 July	12.6	1/190	4	4.0	0. 5
28-July 4 August	51.7	9/362	72	8.0	2
05-20 August	33.5	4/503	10	2.5	0.8
21-28 August	3.7	2/26	4	2.0	7
29-August 05 September	2.2	1/16	1	1.0	6
06 21 September	7.4	2/111	3	1.5	2
22-29 September	1.2	1/16	1	1.0	6
30-September 5 October	0.7	1/5	1	1.0	20

TABLE 4: Infested *Culex pipiens* during two consecutive weeks in 2001.

with *Eylais sp*. Larvae were found solely on *Ochlerotatus caspius* (43 insects: mean infestation rate: 0.2%). Arrenurus were found parasite on 134 *Culex spp.* (both *C. pipiens* and *C. modestus* Ficalbi, 1889), and on only 6 *C. modestus* (0.3%) and 128 on *C. pipiens* (0.9%) (TABLE 2).

Remarks: (1) In literature, *Thyas* were often found parasitic on *Ochlerotatus* (syn. *Aedes*) (MULLEN 1974, 1975a) and the data confirm the link between *T. barbigera* and *Ochlerotatus* in Southern France. The larval habitats of the culicid are non-permanent and brackish water. The mite is known as jumping on the mosquitoes when the female is on the water surface or near it (MULLEN, 1983), notably for egg laying. When *O. caspius* was found active, *T. barbigera* was found (TABLE 3). (2) Both *C. pipiens* and *C. modestus* were found with *Arrenurus* spp. That several hosts were shared is the rule among Arrenuridae (BOHONAK *et al*. 2004). The aquatic larva seeks out adequate host, attacks both the winged adult after emergence, or egg-laying females: the host can be both virgin or copulated females. (3) The mean infestation rate was less than 1%: this rate is consistent with infestation rates published in literature when large survey were made on water mites (ZAWAL 2002),

Seasonal active mosquitoes and seasonal infestation

(2001 trapping: 20 sites sampled weekly May October, one night per week). Results were obtained by cumulating two consecutive weeks on the 11 sites where mosquitoes were found with mites.

a) *T. barbigera* on *O. caspius* (TABLE 3): Few infested individuals were collected. Maximal infestation was 6 larvae per mosquito. When the host was found active, the parasite was found.

b) *Arrenurus* ssp. on *C. pipiens* (TABLE 4): Higher infestation rate in late September when few *Culex* were caught. The number of infested individuals was not correlated to the number of mosquitoes (TABLE 4). The number of larvae per individual varies from 1 to 8. The mites were attached on the thorax, behind and around the head, rarely on other places.

First day of the sampling periods *	Samples with parasitized Culex: Total number of *C. pipiens*	Local infestation rate (%) per 15 days	Mean number of mites/ *C. pipiens*
18/04	33	12.1	4.75
3/05	117	1.7	5
17/05	383	1.8	3.14
31/05	130	1.3	1.7
14/06	767	2.6	2.4
28/06	434	0.5	6.5
12/07	190	1.1	2
26/07	834	1.5	1.31
10/08	26	7.7	2
24/08	16	6.3	1
7/09	111	1.8	1
21/09	21	9.5	1

TABLE 5: Infested sites (2001); *Culex* infestation rate.

	IF infested pipiens	IF active pipiens
IF active pipiens	-0.565	
p=	0.035	
IF Acari	-0.267	0.546
p=	0.356	0.044

TABLE 6: Correlation of preferences (Pearson's correlation, Minitab 13 ®) (IF : frequency index)

Seasonal infestation rate on C. pipiens

The infestation rate varies from 0 to 1.9 % considering the total of captures. Excluding the traps where no mite was collected (whether no mites were present in the larval habitat, whether the density was too low and the probability to collect a infested mosquito was about 0), we observed that the local infestation rate reached higher values (12.1 %: end of April; 9.5 %: end of September; 6 to 8 %: end of July and August). At least two successive peaks of infestation occurred during the observed period. (TABLE 3, FIG. 2): early spring to mid May, and late summer to the early autumn. The first peak corresponds to the presence of adult *Arrenurus* spp. in inland waters (deutonymphs collected from January to March). The second peak could be due to the second and third cohorts of mite larvae. The mean number of mites per *Culex* varied independently (with delayed peaks in regard of the number of active *Culex* : FIG. 2).

Aggregate distribution?

Considering each sample in the 11 sites, distribution of infested Culex is aggregate: (m*/m)>1.8 (m*=2.9; m=2) (2978 *Culex,* 61 with parasites). This aggregate distribution is more evident by gathering the samples by sampling week: (m*=8.3, m=2.6). The most favourable periods to find infested *Culex* were May-June, July and early August. The maximal number of infested individuals did not correspond with the maximal number of active *Culex* but with the maximal number of mites (FIG. 3).

A simple correlation calculation showed: 1) number (estimated by trapped number) of active *C. pipiens* is negatively correlated to the infestation rate, and 2) the number of water mite larvae is negatively correlated to the number of infested *C. pipiens* (TABLE 6).

Three periods may be observed: 1) the periods with abundant mosquitoes but with few individuals found with parasites; and the periods with high infestation rate with 2) few active mosquitoes, or 3) with significant abundance of mosquitoes. The most important results may be that highest infestation rate was observed mid August and from late September to early October when fewer *C. pipiens* were collected. Whereas *C. pipiens* multiplied with overlapping cohorts, cohorts of mites are non overlapped (20 days to six weeks after the heavy unexpected rainfalls in July and late August that got "active again" the larval culicid's biotopes after the summer drought).

DISCUSSION AND CONCLUSION

Culicids survive during cold season by the wintering stage: the fertilized female i.e. *Culex pipiens*, or the egg *Ochlerotatus caspius*. *C. pipiens* inhabits fresh water ponds, whereas *A. caspius* lives in salt marshes, egg-lays on mud, hatching being determined by humidity after rainfalls.

1) By the use of attractive traps, only the active female culicids are collected. It is not obvious that the heavily infested mosquitoes were sampled if the highest infestation induced a large loss of fitness.

2) The hypothesis that parasite and host cycles are chronologically congruent and that high prevalence of parasitism induced a loss of fitness of the host was tested in permanent ponds (ROLFF 2000; LANCIANI &

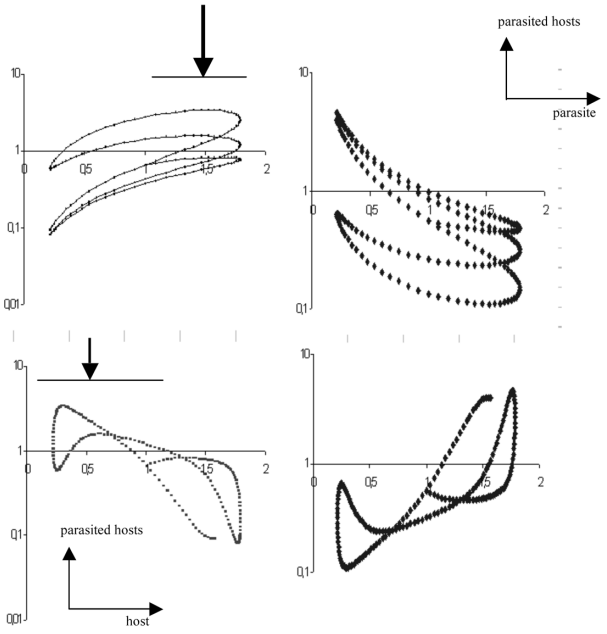


FIG. 3 : Ratio Parasite/Host : Hypothesis for modelling : non overlappig cohorts, delay between successive cohorts is longer for the parasite, (Left) or for the host (Right). Estimated maximal parasitism rate (in each cohort, % of parasited females depends on parasite densities). Two factors are tested: the density of host (bottom) or density of potential parasites. (Ratio between the H and P periods is 1/3 in both cases). Aggregate distribution is expected when number of parasites is high and hosts are rarer (Left, arrow) when parasite's development is the longest. Maximal values of the ratio parasite/host depends on host number when host's development is longer (Right).

Boyett 1980; SMITH 1983). Here the conditions differed greatly.

3) We trapped parous *Ochlerotatus* female with parasites. *Arrenurus* (which attacks virgin or parous females) on *C. pipiens* can be found on both "young" and "old" females. We can hypothesize that the infestation rate (*Culex* spp.) depends closely on the relative abundance of mites and culicid larvae in the mosquito's larval habitat.

4) We cannot hypothesize that the habitats shared by mites and mosquitoes were uniform or randomly distributed, nor that that some habitats might be "preferred". In this hypothesis, if the ecological niche of the free stages of the parasite is narrower than the wide niche of the host, the fluctuation of infestation rate reveals asynchronous calendars for host and parasite, or vagaries of the mite colonization by a flying vector.

5) The hypothesis that the "infestation rate" is not correlated with the "availability of hosts" seemed consistent (TABLE 5). Larvae were found sharing the two species of *Culex* genus. The mean rate of infestation is about 1%. Highest values are 12.1 % for *Arrenurus* spp. on *C. pipiens* in April-May 2001, 7.7% in August 2001 and 6.3% in September 2001. Culicids are known to be occasionally infested by water mite larvae (MULLEN 1974; SMITH 1983) with some peaks registered locally, and the prevalence showed a negative binomial distribution (LANCIANI & BOYETT 1980). Our observation agreed with these data. The demography (more than one hundred eggs per female) suggests high larval mortality (MITCHELL 1998)**.** Higher parasitism rate was found when few hosts are active: these data agree the theory based on simulated models (FIG. 3): *Culex pipiens* might be the preferential host -at least in this biotope-, but the damages are limited because only tardy females (in the cohorts) are heavily attacked. Did this "sacrifice" result from environmental constraints? This pattern differs greatly observations on **specialized** arrenurid and damselfly (ROLFF *et al.* 2001), characterized by host and parasite synchronism, or when the host is available all over the key season. Asynchronous cycles could reveal opportunistic behaviour.

REFERENCES

BOHONAK A.J., SMITH B.P., THORNTON M., 2004. – Distributional, morphological and genetic consequences of dispersal for temporary pond water mites.– Freshwater Biology **49**: 170-180.

BÖTTGER K. & MARTIN P., 2003. – On the morphology and parasitism of *Arrenurus globator* (O.F. Müller, 1776) (Hydrachnidia, Acari) — a water mite with an unusual extensive host spectrum.– Acarologia **43**: 49-57.

CASSAGNE-MÉJEAN F., 1966. – Contribution à l'étude des Arrenuridae de France.– Acarologia, 8: 1-186.

LANCIANI C.A. & BOYETT J.M., 1980. – Demonstrating parasitic water mite-induced mortality in natural host populations.– Parasitology, **81**: 465-475.

LANCIANI C.A., 1979. – Water mite induced mortality in a natural population of the mosquito *Anopheles crucians* (Diptera: Culicidae).– J. Med. Entomology. **15**: 529-532.

LANCIANI C.A., 1983. – Overview of the effects of water mite parasitism on aquatic insects.– In Hoy A. Cunnin-

gham G.L. and Knutson L. (Eds), Biological control of pests by mites. U.S.A.: 86-90.

Lloyd M, 1967. – Mean crowding.– J. Anim. Ecol. **36**: 1-30.

Mitchell R., 1998. – The behavior of *Arrenurus* larvae (Acari : Hydrachnidea) parasitizing Diptera.– Acarologia, **39**: 49-55.

Mullen G.R., 1974. – Acarine parasites of mosquitoes. II Illustrated larval key to families and genera of mites reportedly parasitic on Mosquitoes.– Mosquitoes News, **34**: 183-195.

Mullen G.R., 1975a. – Acarines parasites of mosquitoes I. A critical review of all known records of mosquitoes parasitized by mites.– J. Med. Ent.: **12**: 27-36.

Mullen G.R., 1975b. – Parity determinations in adult mosquitoes parasitized by aquatic mites (Acari: Hydrachnellae).– In 62nd Annual Meeting New Jersey Mosquito Extermination Association. Atlantic City, USA: 90-94.

Rioux J-A., 1958. – Les Culicides du "Midi" Méditerranéen. Paul Lechevalier Ed. Paris. 303 pp.

Rolff J. Vogel C. Poethke H.J., 2001. – Co-evolution between ectoparasites and their insect host: a simulation study of a damselfly-water mite interaction.– Ecological Entomology, **26**: 638-645.

Rolff J., 2000. –Water mite parasitism in damselflies during emergence: two hosts, one pattern.– Ecography, **23**: 273-282**.**

Schaffner E., Angel G., Geoffroy B., Hervy J.-P., Rhaiem A. & Brunhes J., 2001.– Les moustiques d'Europe, IRD & EID Eds. Montpellier France. (CD-Rom).

Smit H. & H. van der Hammen, 2000.– Atlas van de Nederlandse Watermijten (Acari: Hydrachnidia).– Nederlandse Faunistische Mededelingen **13**: 3-272.

Smith B.P. & McIver S.B., 1984. – The patterns of mosquito emergence (Diptera: Culicidae; *Aedes* spp.): their influence on host selection by parasitic mites (Acari: Arrenuridae; Arrenurus ssp.).– Can. J. Zoology, **62**: 1106-1113

Smith B.P., 1983 – The potential of mites as biological control agents of mosquitoes.– In Hoy A. Cunningham G.L. and Knutson L. (Eds), Biological control of pests by mites. U.S.A. 79-85.

Smith BP & McIver SB., 1984.– The impact of *Arrenurus danbyensis* Mullen (Acari: Prostigmata; Arrenuridae) on a population of *Coquillettidia perturbans* (Walker) (Diptera: Culicidae).– Canadian Journal of Zoology **62**: 1121-1134.

Smith IM & Oliver DR., 1986.– Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts.– Canadian Entomologist **118**: 407-472.

Stechmann D.H., 1977. – Zur Morphologie mitteleuropaïsher *Arrenurus*-larven (Hydrachnellae, Acari).– Acarologia, 18:503-518.

Stechmann D.H., 1978. – Eiblage, parasitismus und postparasitische Entwicklung von *Arrenurus* Arten (Hydrachnellae, Acari).– Zeitschrift für angewandte Entomologie, **82**: 349-355.

Walter D. & Proctor H., 1999. – Mites Ecology, Evolution and Behaviour. CABI Publishing, UK.: 322p.

Zawal A., 2002. – Parasitism of water mite larvae (Hydrachnellae) of the genus *Hydrachna* on water beetles in Poland.– Acarologia, **42**: 361-370.