ROMANIAN JOURNAL OF BIOLOGY

ZOOLOGY

VOLUME 56, N° 2 2011

CONTENTS

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° *2*, P. 113–210, BUCHAREST, 2011

AN ELUSIVE ANOSTRACAN: DISCOVERY, EXTINCTION AND REDISCOVERY OF *TANYMASTIX STAGNALIS* (LINNAEUS, 1758) IN ROMANIA

LÁSZLÓ DEMETER[*], GABRIELLA PÉTER[**]

Tanymastix stagnalis (Linnaeus, 1758) is known from a single habitat in Romania which was built on between 2004–2006, and this species has not been observed since 2005. In order to find out whether it is still present on the site, we extracted resting eggs from soil samples from four temporary ponds nearby. The egg banks of ten other temporary ponds were sampled as a control. The extraction was done by diluting tap water and filtration of the suspension and supernatant through plankton net. *T. stagnalis* eggs were found in all four habitats on the known site but larger numbers only in one of them. *T. stagnalis* eggs were not found in the egg banks of two of the other habitats. This study shows that egg banks can add precious new information to those gathered by observations on the active communities of rare Anostraca.

Key words: Anostraca, *Tanymastix stagnalis*, egg bank, extinction, resting eggs, temporary pond, Ciuc Basin, Romania.

INTRODUCTION

Anostraca typically have resting eggs that tend to accumulate in the sediment as a result of prolonged dormancy and form resting egg banks similar to the seed banks of plants (Simovich & Hathaway, 1997; Brendonck & De Meester, 2003; Müller *et al*., 2008). Resting eggs were successfully used to complete information about distribution of Anostraca (Jass & Klausmeier, 2006; Boven *et al*., 2009).

T. stagnalis is a fairy shrimp species widespread in Europe (Löffler, 1993; Vekhoff, 1993; Brtek & Thiéry, 1995). However, it has very few known habitats in several countries, for example one locality in the Balkans, Macedonia (Petkovski, 1995), seven habitats in Ireland (Grainger, 1991), three sites in Austria (Eder & Hödl, 2002), one in Romania (Demeter & Stoicescu, 2008), a dozen localities in Hungary with most data more than 50 years old (Forró, 2000). In a recent study in the Kiskunság National Park, Hungary, *T. stagnalis* was found to be one of the most frequent large branchiopod species, occurring in 13 habitats (27.1% of large branchiopod habitats) (Boven *et al*., 2009).

T. stagnalis seems to be one of the mysterious fairy shrimps the occurrence of which is often difficult to explain. According to Freiner and Grüttner (1984), over a century between 1885 and 1981, a *T. stagnalis* habitat was inundated 23 times, and the species was present only six times. The gaps between appearance

ROM. J. BIOL. – ZOOL., VOLUME **56**, N^{o} *2*, P. 115–125, BUCHAREST, 2011

reappearance ranged from 1 to 43 years, and it was not reported twice a year. In an Irish study, *T. stagnalis* disappeared by the end of the study from one of the two studied habitats because of change in the hydroperiod of the habitat, apparently caused by a switch by the local farmer from sheep to heavy cattle that compacted the pond sediment (Grainger, 1991).

The genus *Tanymastix* is represented by two species in Romania. *Tanymastix motasi* Orghidan, 1945 was described in South Romania and considered originally a Romanian endemic (Botnariuc & Orghidan, 1953), but later it was fond in Macedonia too (Petkovski, 1995). It has no recent record in Romania (Demeter & Stoicescu, 2008). *T. stagnalis* was found only recently in Romania, known from only one site and a single habitat, which has been destroyed in the past few years (Demeter & Stoicescu, 2008).

However, it is difficult to define extinction of populations which form resting eggs that are viable for decades, although there are numerous examples of Anostraca species disappearing from a certain site, especially if located within cities (Eder & Hödl, 2002). To our knowledge, no studies are available on the identification of an Anostracan species in a habitat where the active population is known to be extinct.

This study aims at identifying *T. stagnalis* egg banks at the only known site of occurrence of this species in Romania.

MATERIAL AND METHODS

The studied species

Tanymastix stagnalis is a univoltine cold-stenothermal fairy shrimp. Adults reach 2 cm length, they are greenish color, males have large second antennae with characteristic long antennal appendices, and the female has a rounded ovisac of purple color (Fig. 1). The eggs of species from the genus *Tanymastix* are disc-shaped (Thiéry & Gasc, 1991) (Fig. 2).

The study site

The study site is located on the western edge of Miercurea-Ciuc town (25.77°E, 46.37°N, 660 m above sea level), Ciuc Basin, Romania, at the cross-road between Harghita and Lunca Mare streets (Fig. 3). This part of the town was developed as an industrial quarter from the 1960s, but previously it was sparsely inhabited by farmer families. Some households still exist. The studied habitats are located in the backyards of four-five houses, on the second terrace of the main river Olt, approximately 500 m to the east of the regulated riverbed. The habitats are probably remnants of the former riverbed and/or floodplain ponds (Kristó, 1980).

Fig. 1. Male (upper) and female (lower) of *T. stagnalis* from the studied site (orig.).

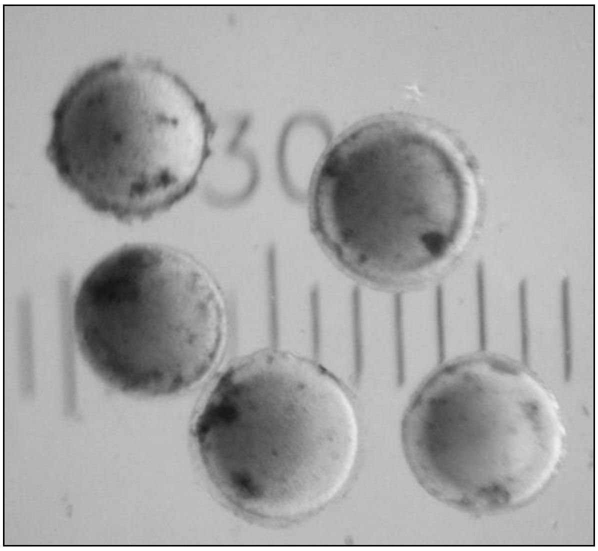


Fig. 2. Resting eggs of *T. stagnalis* extracted from pond sediment on the studied site (one increment is 0.1 mm).

According to the land owners, before 1990 some of the ponds were ploughed up from time to time. In the mid 1990s, one plot was illegally filled up with construction wastes from the local beer factory (Fig. 3, 4f). In 2005 one plot was bought by a plastic waste collection company and parts of ponds E and F were filled up. The filling, according to the neighbour land owners, increased the hydroperiod of ponds D, E and F. Between 2003 and 2005 a hotel building was started over the location of pond C (Fig. 4a-e). First the top 20 cm of the soil was removed (Fig. 4c), and in 2005 the site was filled up and drained. The construction stopped in 2005 because of lack of funds. In the meantime, the surroundings of ponds E and F were abandoned, as the owners stopped mowing the fields.

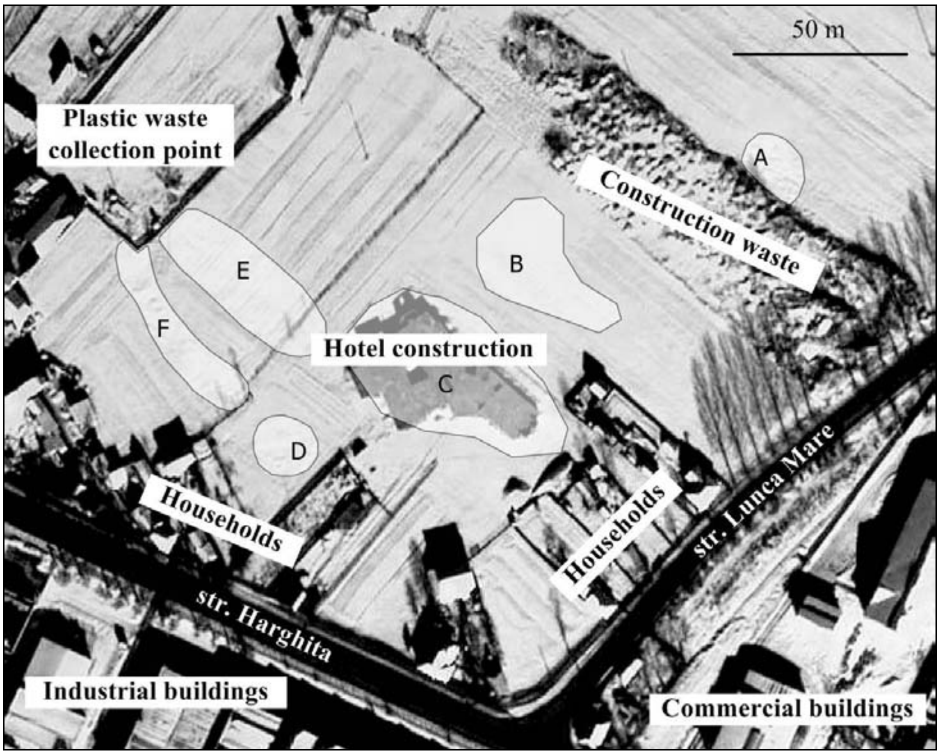


Fig. 3. The spatial setting of ponds and urban features of the studied area.
(Source of aerial photo: Google Earth; the contours of ponds are orientative).

a. Pond C before the start of the building; view from Southwest.
(10.03.2002)

b. Pond C, the top soil was removed the previous year in the dry period of the pond.
(14.04.2003)

c. Dry phase of pond C, in the background heaps of removed soil.
(14.05.2003)

d. Constructions on pond C were halted by lack of funds.
(27.05.2005)

e. The surroundings of the building are flooded;
note the filled area in the fenced corner.
(3.04.2006)

f. Pond A after snowmelt;
view from Southeast.
(3. 04.2006)

g. Pond D after being drained.
(25.03.2010)

h. Pond E, view from South; this part of the pond is mown
in the dry phase. (25.03.2010)

Fig. 4 (a-h). Habitat changes between 2002-2010 on the studied sites
(in brackets the date when the photo was made).

Sampling and egg extraction

We took sediment samples (circular pieces of sediment 5 cm deep and 10 cm diameter) from four temporary ponds from the above described site. Ten soil samples were taken from position 3 and five each from ponds 1, 2 and 4. For each soil sample, eggs were extracted from five subsamples representing 10 g of dry sediment. Dry sediment was soaked in tap water for about 24 hours, diluted to 1 l water, allowed to settle for a few seconds and the supernatant and suspension was filtered through a 80 µ plankton net. The material from the filter was transferred to a large eye glass and observed through a dissecting microscope at 15x-60x magnification. The heavy fraction of the first step was then also examined under the dissecting microscope.

The efficiency of the method was tested through two experiments. In the first experiment we released a known number of wet resting eggs and ephippia in 10 g egg-free soil, mixed them, and extracted them. In the second experiment we let the mixed eggs and sediment dry and after 24 hours we performed the extraction.

We extracted resting eggs of *T. stagnalis*, *Chirocephalus shadini*, ephippia of Cladocera: *Ceriodaphnia*, *Simocephalus* and other Cladocera. Eggs were identified using Thiéry & Gasc (1991) and our own reference material, and Cladocera to the genus level using Vandekherkove *et al*. (2004).

RESULTS

The active large branchiopod community

Lepidurus apus has been identified in three ponds, *Chirocephalus shadini, Eoleptestheria ticinensis* and *Lynceus brachyurus* in two ponds each, while *T. stagnalis* in one pond (Table 1).

Table 1

The large branchiopod species observed in the active communities of the studied habitats

Pond	***Chirocephalus shadini***	***Tanymastix stagnalis***	***Lepidurus apus***	***Eoleptestheria ticinensis***	***Lynceus brachyurus***
A	1	-	1	-	-
B	-	-	-	-	-
C	-	1	-	-	-
D	1	-	1	1	1
E	-	-	1	1	1

Phenology

T. stagnalis was identified in pond C (Fig. 1) in three consecutive years. In 2003 adults were observed between April 26 and May 6. Later in 2003 construction was started, and by September 2003 the top 20 cm of the habitat had been removed. In 2004, adults were observed between April 8 and May 8 in the habitat where the topsoil was removed. In 2005 adults were observed on April 29 and August 12.

The efficiency of egg extraction

The efficiency of egg extraction was lower in the second experiment but over 85% in all cases. For *T. stagnalis*, extraction rate efficiency was 100% in both experiments (Table 2).

Table 2

Recapture rates in the selected dormant forms

	Experiment 1		**Experiment 2**	
	N	**Recapture rate**	**N**	**Recapture rate**
Ceriodaphnia sp.	18	88.9%	15	93.3%
Simocephalus sp.	12	100.0%	8	87.5%
T. stagnalis	18	100.0%	15	100.0%
C. shadini	18	100.0%	15	93.3%

Egg bank

We identified resting eggs of *T. stagnalis* in all four sampled habitats. In habitat C sampling was not possible because the area was built in, however, in an earlier year, when the removed top soil was still on site, we extracted eggs from that soil, without quantifying it. Larger numbers of eggs were found only in one (0.17–1.6 eggs per cm^3, 102 eggs in 5 soil samples, 25 subsamples, $0.58*10^4$ per m^2). *C. shadini* eggs were found in two habitats, *Ceriodaphnia* and *Daphnia* ephippia in four, *Simocephalus* ephippia in three (Table 3).

Table 3

Average number of eggs/ephippia ± standard deviation per 10 g sediment in the studied ponds

Habitat	***C. shadini***	***T. stagnalis***	***Daphnia* sp.**	***Ceriodaphnia* sp.**	***Simocephalus* sp.**
A (n=25)	1.2 ± 1.26	0.04 ± 0.2	0	6.4 ± 1.93	0.2 ± 0.58
B (n=50)	0	0.08 ± 0.34	0.02 ± 0.14	0.42 ± 0.74	0
D (n=25)	0.28 ± 0.61	0.16 ± 0.41	0.16 ± 0.37	13.76 ± 9.63	0.04 ± 0.2
E (n=25)	0	4.08 ± 4.41	0.08 ± 0.28	10.2 ± 6.04	0.08 ± 0.28

DISCUSSION

Habitat destruction is the main threat for large branchiopods (King, 1998; Eder & Hödl, 2002). However, viable resting eggs may be present for decades in places where a habitat had been destroyed. This study demonstrates that a fast and low cost evaluation of the presence of an Anostraca species that is thought to be destroyed is possible. Many cities in Romania (Botnariuc & Orghidan, 1953) and in other parts of Europe (*e.g.* Eder *et al*., 1997) had several large branchiopod species before, but not any more. As the precise position of the habitats is not known, it is not possible to find/restore habitats, although viable resting eggs may still be there.

The original habitat where *T. stagnalis* had been observed is now completely covered by the new building, and therefore sampling was impossible. Although *T. stagnalis* eggs were found in all four studied habitats, their density was very low in three of them, so the long term viability of these populations is questionable. A large egg bank was identified next to the originally known habitat. Here the species was not observed in the active community. This is similar to the erratic occurrence of *T. stagnalis* in other cases (Freiner & Grüttner, 1984; Grainger, 1991). No *T. stagnalis* eggs were identified in sediments from an additional eight ponds in other sites where the species has not been found in the active community.

Our study site is of high nature conservation value, indicated by the presence of a number of large branchiopods, including *T. stagnalis* and protected amphibians like *Triturus cristatus* and *Rana arvalis*. Given the faunal richness of this site and its vulnerability, urgent protection measures are needed.

Acknowledgements. This study was part of a research and conservation project on temporary ponds funded by Dr. Barbara Knowles and a grant of the Sapientia Institute for Research Programmes in 2009. We would like to thank Mr. Lajos Károly for letting us access his land. Thanks go to two anonymous reviewers and Dr. Sanda Maican for useful comments on the manuscript.

REFERENCES

BOTNARIUC N., ORGHIDAN T., 1953, *Phyllopoda. Fauna R.P.R., Crustacea,* vol. IV, fasc. 2. Editura Academiei R.P.R, Bucureşti.

BOVEN L., VANSCHOENWINKEL B., DE ROECK E., HULSMANS A., BRENDONCK L., 2009, *Diversity and distribution of large branchiopods in Kiskunság (Hungary) in relation to local habitat and spatial factors: implications for their conservation*. Marine and Freshwater Research, **59**: 940–950.

BRENDONCK L., DE MEESTER L., 2003, *Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment*. Hydrobiologia, **491**: 65–84.

BRTEK J., THIÉRY A., 1995, *The geographic distribution of the European branchiopods (Anostraca, Notostraca, Spinicaudata, Laevicaudata)*. Hydrobiologia, **298**: 263–280.

DEMETER L., STOICESCU A., 2008, *A review of the distribution of large branchiopods (Branchiopoda: Anostraca, Notostraca, Spinicaudata, Laevicaudata) in Romania*. North-Western Journal of Zoology, **4** (2): 203–223.

EDER E., HÖDL W., GOTTWALD R., 1997, *Distribution and phenology of large branchiopods in Austria*. Hydrobiologia, **359**: 13–22.

EDER E., HÖDL W., 2002, *Large freshwater branchiopods in Austria: diversity, threats, and conservational status*. In: Escobar-Briones E., Alvarez F. (eds.), *Modern approaches to the study of Crustacea*. Kluwer Academic/Plenum Publishers, New York, pp. 281–289.

FORRÓ L., 2000, *Checklist, distribution maps and bibliography of large branchiopods in Hungary (Anostraca, Notostraca, Spinicaudata, Laevicaudata)*. Miscellanea Zoologica Hungarica, **13**: 47-58.

FREINER D., GRÜTTNER O., 1984, *Der eichener Kiemenfusskrebs*. Natur und Museum. Bd. **114** (10): 273–286.

GRAINGER J.N.R., 1991, *The biology of Tanymastix stagnalis (L.) and its survival in large and small temporary water bodies in Ireland*. Hydrobiologia, **212**: 77–82.

JASS J., KLAUSMEIER B., 2006, *Determining the presence of fairy shrimps (Crustacea: Anostraca) at ephemeral pond sites in Wisconsin*. Wisconsin Department Research Report **188**. Wisconsin Department of Natural Resources, Madison.

KING J.L., 1998, *Loss of diversity as a consequence of habitat destruction in California vernal pools*. In: Witham C. W., Bauder E. T., Belk D., Ferren W. R. Jr., Ornduff R. (eds.), *Ecology, conservation and management of vernal pool ecosystems – proceedings from a 1996 conference*, pp. 147–150. California Native Plant Society, Sacramento, CA.

KRISTÓ A., 1980, *The geomorphology of the surroundings of Csíkszereda*. Acta Hargitensia **1**: 273 – 296. [in Hungarian]

LÖFFLER H., 1993, *Anostraca, Notostraca, Laevicaudata and Spinicaudata of the Pannonian Region and in its Austrian area*. Hydrobiologia, **264**: 169–174.

MÜLLER S.D., BRUNETON H., SOULIÉ-MÄRSCHE I., REY T., THIÉRY A., WATERKEYN A., BRENDONCK L., SCHEVIN P., YAVERCOVSKI N., GRILLAS P., 2008, *Long-term dynamics of a mediterranean alkaline vernal pool (Rhone Delta, Southern France)*. Wetlands, **28** (4): 951–966.

PETKOVSKI S., 1995, *On the presence of the genus Tanymastix Simon, 1886 (Crustacea: Anostraca) in Macedonia*. Hydrobiologia, **298**: 307–313.

SIMOVICH M.A., HATHAWAY S., 1997, *Diversified bet-hedging as a reproductive strategy of some ephemeral pool anostracans (Branchiopoda)*. Journal of Crustacean Biology, **17** (1): 38–44.

THIÉRY A., GASC C., 1991, *Resting eggs of Anostraca, Notostraca, Spinicaudata, Laevicaudata (Crustacea, Branchiopoda) occurring in France: identification and taxonomical value*. Hydrobiologia, **212**: 245–259.

VANDEKERKHOVE J., DECLERCK S., VANHOVE M., BRENDONCK L., JEPPESEN E., CONDE PORCUNA J. M., DE MEESTER L., 2004, *Use of ephippial morphology to assess richness of anomopods: potentials and pitfalls*. Journal of Limnology, **63**: 75-84.

VEKHOFF N.V., 1993, *The fauna and zoogeography of fairy and tadpole shrimps of Russia and adjacent lands (Crustacea: Anostraca, Notostraca)*. Arthropoda Selecta, **2** (3): 11–42.

Received February 6, 2011

[*] *Sapientia University*
Department of Technical and Nature Sciences
530104 M-Ciuc, P-ța Libertății nr. 1
Romania
e-mail: domedve@gmail.com
[**] *Misgurnus Association*
530204 M-Ciuc, str. Ion Căianu nr. 67
Romania
e-mail: ptrgabriella@gmail.com

A NEW SPECIES OF THE GENUS *THERIDION* WALCKENAER, 1805 (ARANEAE: THERIDIIDAE) FROM WEST BENGAL, INDIA

SOUVIK SEN*, SUMANA SAHA**, DINENDRA RAYCHAUDHURI*

Theridion bengalensis n. sp. recorded from Jaldapara Wildlife Sanctuary, West Bengal, India is described and illustrated.

Key words: Araneae, Theridiidae, *Theridion bengalensis* n. sp., Jaldapara Wildlife Sanctuary, West Bengal, India.

INTRODUCTION

Comb footed or cob web spiders (Theridiidae) are globally represented by 2310 species with 113 genera (Platnick, 2011). These include 58 Indian species belonging to 19 genera (Sebastian & Peter, 2009). Siliwal (2009) commented that small size and lack of comprehensive literatures are the prime reasons for which Indian theridiids remained a little studied group. However, we feel less of attention to the group is the reason for our poor knowledge.

Twelve out of the 58 species are known to compose the genus *Theridion* Walckenaer, 1805 of India (Simon, 1889, 1905; Tikader, 1970, 1977; Patel, 1973; Monga & Singh, 1989; Biswas & Biswas, 1992; Majumder, 2007 and Sebastian & Peter, 2009).

During our faunistic survey (2007–2010) for the spiders of the reserve forests of Dooars, West Bengal, we came across with a *Theridion* species from Jaldapara Wildlife Sanctuary (Anonymous, 2008, www.westbengalforest.gov.in). The species after critical examination is considered as new to science and accordingly described and illustrated.

MATERIAL AND METHODS

Theridid spiders were collected and preserved following Tikader (1987) and Barrion & Litsinger (1995). The material was studied using Stereo Zoom Binocular Microscope, model Zeiss SV-11. The measurements indicated in the text are in millimeters, made with an eye piece graticule. Leg measurements are shown as: total length (femur, patella, tibia, metatarsus, tarsus).

ROM. J. BIOL. – ZOOL., VOLUME **56**, N^o *2*, P. 127–131, BUCHAREST, 2011

Abbreviations: CL = Cephalothorax length; CW = Cephalothorax width; AL = Abdominal length; AW = Abdominal width; TL = Total length; AME = Anterior median eyes; ALE = Anterior lateral eyes; PME = Posterior median eyes; PLE = Posterior lateral eyes; JWLS = Jaldapara Wildlife Sanctuary; WB = West Bengal.

TAXONOMY

Family Theridiidae Sundevall, 1833
Subfamily Theridiinae Sundevall, 1833
Genus *Theridion* Walckenaer, 1805

Theridion bengalensis n. sp.
(Figs. 1–5, 6–7)

Type material

Holotype: ♀, Sisamara, JWLS, WB, India, 03.V.2008, Coll. S. Sen; *Paratype*: 1♀, otherwise data same as holotype.

Description

Female (Holotype):

CL- 1.46, CW- 1.39, AL- 2.50, AW- 2.16, TL- 3.96.

Cephalothorax (Fig. 1) yellowish brown, cephalic region little elevated, cervical furrows distinct. Eyes 8, pearly white, basally ringed with black, arranged in 2 recurved rows, anterior row more recurved than posterior, eye diameter: AME > PME > ALE = PLE, laterals contiguous. Interocular distance: AME – AME = 0.07, ALE – AME = 0.10, ALE – ALE = 0.35, PME – PME = 0.07, PLE – PME = 0.10, PLE – PLE = 0.35, AME – PME = 0.05.

Clypeus yellowish brown, height – 0.18, width – 0.21.

Abdomen (Fig. 1) grayish brown, longer than wide, clothed with brown hairs, dorsum with few scattered white spots; venter grey, spinnerets yellowish brown, small.

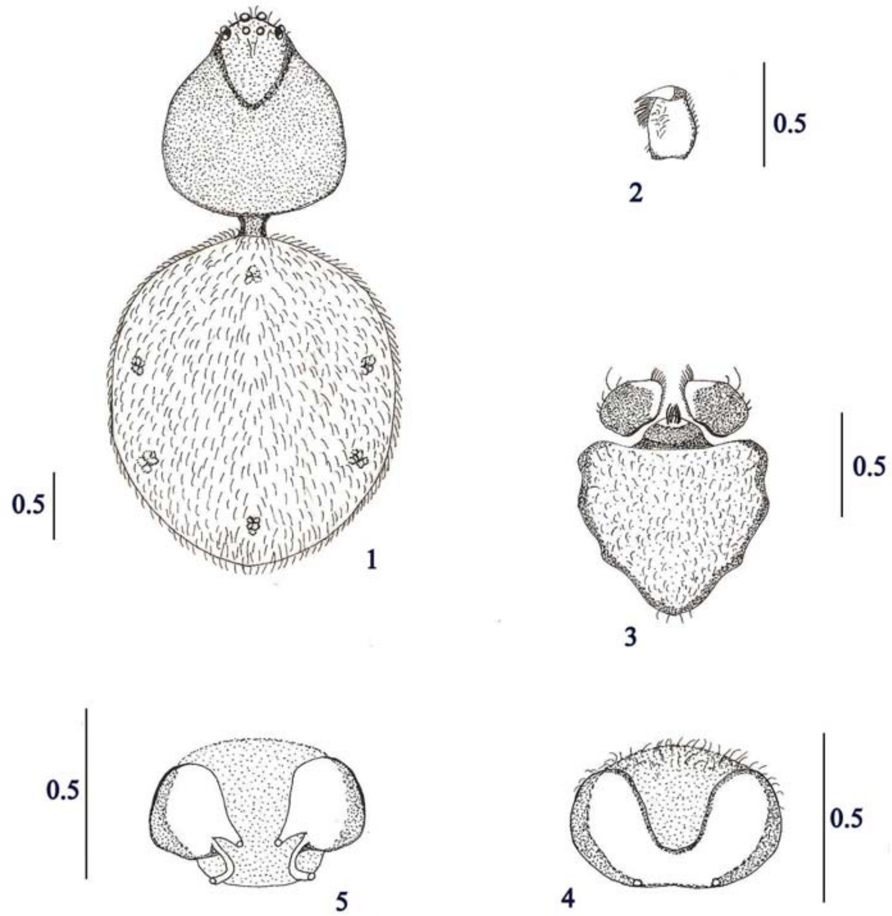
Chelicerae (Fig. 2) brown, small, margins devoid of any tooth, scopulate, fangs dark brown, small, curved.

Both *maxillae* and *labium* (Fig. 3) brown, with apices paler, scopulate, maxillae longer than wide, labium wider than long, basally broad and fused with sternum.

Sternum (Fig. 3) brown, margins dark brown, cordate, clothed with fine brown hairs.

Legs yellowish brown, long, slender, with few short spines, tarsal claw 3, toothed except inferior claw. Leg measurements: I 8.39 (2.27, 0.63, 2.27, 2.45, 0.77); II 5.61 (1.0, 0.54, 1.63, 1.81, 0.63); III 3.79 (0.63, 0.54, 0.90, 1.18, 0.54); IV 6.51 (1.63, 0.54, 1.72, 1.90, 0.72). Leg formula 1423.

Epigynum – Internal genitalia (Figs. 4–5 and 7): Spermatheca sac like, atrium nearly as long as wide, copulatory ducts outwardly curved, copulatory openings small, circular.

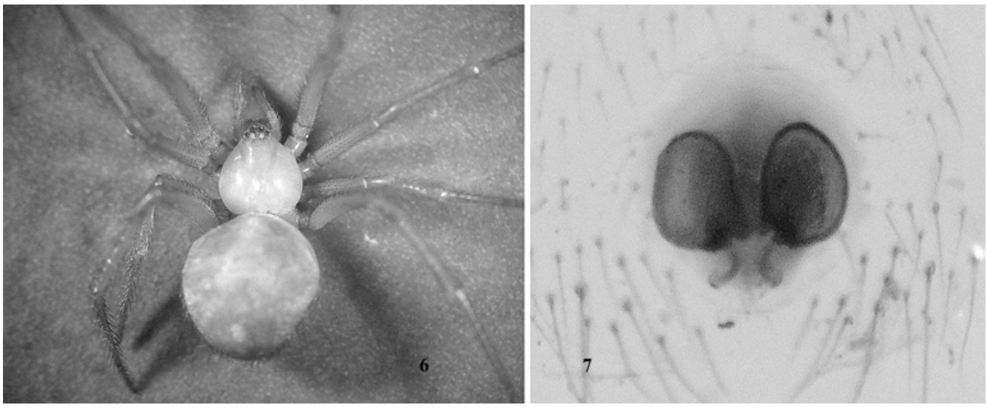


Figs. 1–5. *Theridion bengalensis* n. sp. Female (Holotype): 1. Cephalothorax and abdomen (dorsal view); 2. Chelicerae (ventral view); 3. Maxillae, labium and sternum (ventral view); 4. Epigynum (ventral view); 5. Internal genitalia (dorsal view).

Type deposition. Entomology Laboratory, Department of Zoology, University of Calcutta, registration no. EZC 0024-11.

Distribution. India, West Bengal (known until now only from the type locality).

Etymology. The specific epithet is derived from the name of the state Bengal.



Figs. 6–7. Images of *Theridion bengalensis* n. sp. Female: 6. General habitus; 7. Internal genitalia.

Remarks. None of the Indian congeners appear close to the present species chiefly because of the typical epigynum and internal genitalia. The species however, shows affinity to *Theridion necijaense* Barrion and Litsinger, 1995 but can be separated by: i) spermatheca sac like, atrium nearly as long as wide, copulatory ducts outwardly curved (spermatheca nearly rectangular, atrium longer than wide, copulatory ducts never so in *T. necijaense*); ii) cheliceral margins devoid of any tooth (promargin of chelicerae with 2 teeth in *T. necijaense*); iii) abdominal venter devoid of any band (abdominal venter with a median white band in *T. necijaense*). Such differences appear to justify the erection of a new species.

Acknowledgements. We thank Department of Biotechnology, Govt. of India (BT/PR6391/NDB/51/078/2005 dated 20.11.2006) for sponsoring the project and Directorate of Forests, Govt. of West Bengal and the Head, Department of Zoology, University of Calcutta for necessary support.

REFERENCES

ANONYMOUS, 2008, *West Bengal Forest Department.* Available from: http://www.westbengalforest.gov.in. (accessed on 3rd April, 2011).

BARRION A.T., LITSINGER J.A., 1995, *Riceland spiders of South and Southeast Asia.* CAB International, UK & IRRI, Philippines, 716 pp.

BISWAS B.K., BISWAS K., 1992, *State Fauna Series 3: Fauna of West Bengal, Araneae: Spiders, part 3.* Zoological Survey of India: 357-500.

MAJUMDER S.C., 2007, *Pictorial handbook on spiders of Sunderbans: West Bengal.* Zoological Survey of India, 138 pp.

MONGA K., SINGH J.P., 1989, *A new species of Theridion Walckenaer (Araneae: Theridiidae) from India.* Journal of Bombay Natural History Society, **86**: 81-82.

PATEL B.H., 1973, *Some interesting theridiid spiders (Araneae: Theridiidae) from Gujarat, India.* Bulletin of British Arachnological Society, **2**: 149-152.

PLATNICK N.I., 2011, *The World spider catalog, version 11.5*. American Museum of Natural History. Available from: http://research.amnh.org/entomology/spiders/catalog/index.html. (accessed on 3rd April, 2011)

SEBASTIAN P.A., PETER K.V., 2009, *Spiders of India*. Universities Press (India) Pvt. Ltd., 614 pp.

SILIWAL M., 2009, *A new species of the genus Chrysso O. P. Cambridge (Araneae: Theridiidae) from Arunachal Pradesh, India*. Biosystematica, **3**: 5-9.

SIMON E., 1889, *Arachnides de l'Himalaya, recuellis par MM. Oldham et Wood-Mason, et faisant partie des collections de l'Indian Museum. Première partie*. Journal of Asiatic Society of Bengal, **58**: 334-344.

SIMON E., 1905, *Arachnides (1re partie). In Voyage de M. Maurice Maindron dans l'Inde méridionale. 7e Mémoire*. Annals of Entomological Society of France, **74**: 160-180.

TIKADER B.K., 1970, *Spider fauna of Sikkim*. Records of Zoological Survey of India, **64**, 83 pp.

TIKADER B.K., 1977, *Studies on spider fauna of Andaman and Nicobar Islands, Indian Ocean*. Records of Zoological Survey of India, **72**: 153-212.

TIKADER B.K., 1987, *Hand book of Indian spiders*. Zoological Survey of India, 251 pp.

Received May 19, 2011

[*] *Entomology Laboratory,*
Department of Zoology,
University of Calcutta,
35, Ballygunge Circular Road, Kolkata- 700019, India.
e-mail: souvik_ind_06@yahoo.co.in;
dinendrarccu@gmail.com
[**] *Department of Zoology, Lady Brabourne College, Govt. of West Bengal,*
P- ½, Suhrawardy Avenue, Kolkata- 700017, India.
e-mail: sahasumana2010@gmail.com

XYLOPHAGOUS INSECTS ATTACK DEGREE IN WOOD PIECES FROM THE ROMANIAN PEASANT MUSEUM, BUCHAREST

AURORA MATEI, IRINA TEODORESCU*

This paper assesses the damage degree that some insect pests incur on lots of wood pieces stored in the Romanian Peasant Museum, Bucharest. A number of 1300 wood pieces (furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, handicrafts) were controlled and 368 were found to be damaged by xylophagous insect species. Seven insect species were identified, belonging to Coleoptera order: Anobiidae (*Anobium punctatum*, *Hadrobregmus pertinax*, *Ptilinus pectinicornis*, *Xestobium rufovillosum*), Lyctidae (*Trogoxylon impressum*, *Lyctus linearis*), and Cerambycidae families (*Hylotrupes bajulus*). From 20 tree species identified as the source of wood pieces, the insect attack was registered on pieces made from the wood of 13 tree species. Six categories of damage degree (heavy, medium-heavy, medium, low-medium, low and very low attack) were established. Heavy and medium-heavy attacks were registered only on pieces manufactured from *Fagus sylvatica* wood and medium-heavy attack on those made from *Abies* sp. and *Acer pseudoplatanus* wood. In all other cases dominant have low and very low attack. The heavy attack was registered to furnishings, house wares, and hank reels. Based on attack frequency and damage degree, most noxious insect pests in wood pieces stored in Romanian Peasant Museum were species of Anobiidae and Cerambycidae families.

Key words: wood destroying insects, damage degrees, wood pests, biodeterioration.

INTRODUCTION

Wood is one of the oldest materials used by people for making tools, utensils, shelter, ships, and vehicles because it can easily be worked with simple tools (Urger *et al*., 2001). It has been known recently that wood as a raw material is used in around 10,000 different areas such as building construction, furniture design and decoration, parquetry, production of musical instruments, wire poles, Palladian wood materials, timber cladding panels, plywood panels, wood wafers, wood fibreboards and production of paper and carton (Colak *et al*., 2006, cited by Unal *et al*., 2009).

As nations try to increase the yield of wood, there are some biotic factors affecting the amount and quality of wood products harvested from forest stands and used in structures. Many economically important forest pests are phloem feeders or wood borers, which are important to both pre-harvest and post-harvest

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° *2*, P. 133–145, BUCHAREST, 2011

management. The stored materials are very attractive as breeding sites and for development by wood destroying insects. There are also some structure-infesting insects that cause considerable economic damage to wood buildings and household items, such as furniture, roof frames, and other woody materials used in or outside homes (Akbulut *et al*., 2008).

Several organisms are capable of wood degradation including fungi, marine borers, insects, bacteria, mice, and rats. The principal organisms that can degrade wood are fungi, insects, and bacteria (Mustață, 1998; Highley, 1999; Ebeling, 2002; Moşneagu & Gămălie, 2006). Their attack on wood museum collections, especially in the storage areas, is a form of biodegradation.

The main degrading organisms to wood museum collections and old books (with wood covers) are the wood-feeding insect larvae (coleopterans beetles) that feed and grow within the wood, creating a network of tunnels closely packed with frass (fine dust) and cause the most damages. These larvae are difficult to detect because they never come to the wood surface, their feeding actions create voids in the wood and can severely degrade their structural integrity.

When selecting hardwood lumber for building or manufacturing purposes, any evidence of powder-post infestation should not be overlooked, because the beetles may continue to be active long after the wood is put to use. Once wood is infested, the larvae will continue to develop, even though the surface is subsequently painted, oiled, waxed, or varnished. Certain beetles may complete development and emerge several years after the wood is dry, often raising a question as to the origin of the infestation (Highley, 1999). Upon maturity, the beetle adults emerge, leaving the wood surface perforated with small, round or oval holes, named “the adults exit holes, flight holes, emergence holes”. These holes are made by adults with mandibles through the wood surface, in order to emerge and fly off.

Because the xylophagous larvae excavate burrows inside wood and in some cases live for years without breaking through the wood surface, the beetle attack can be detected only by larvae activity result (characteristic powder-like sawdust, named frass, that is pushed out of the emergence holes), by the adults’ exit holes upon the wood surface or by alive or dead adults presence.

In museums, homes or buildings the insect species pests of different wood pieces and of old books with wood covers (Antonie & Teodorescu, 2009; Matei & Teodorescu, 2011) belong to Coleoptera orders, especially Anobiidae (deathwatch beetles, furniture beetle), Cerambycidae (long-horned beetles, house borers), and Lyctidae (true powderpost beetles) families.

MATERIAL AND METHODS

Our investigations in the Romanian Peasant Museum, Bucharest were made in storage areas, and the wood attack was probably old, manifested in the original places of wood pieces (rural households from different counties) or many years ago, in these museum storage areas.

Since the attack on museum pieces was old, larvae and frass were absent and the species or genus identification was based on the alive or dead adult insects and especially on the exit holes characteristic shape and size (Leary, 2002). To assess comparatively the damage degree, we established six degrees of attacks: heavy, medium-heavy, medium, low-medium, low, and very low.

The attack categories were established upon the adult exit holes' mean density (number of exit holes/cm^2): *heavy* attack: 4.5–8 exit holes/cm^2; *medium-heavy* attack: 2.5–4.5 exit holes/cm^2; *medium* attack: 1.5–2.5 exit holes/cm^2; *low-medium* attack: 0.5–1.5 exit holes/cm^2; *low* attack: 0.1–0.5 exit holes/cm^2; *very low* attack: few exit holes (1–10) from the entire surface of piece.

RESULTS AND DISCUSSION

In the storage areas of Romanian Peasant Museum, a number of 1,300 objects (pieces) made of wood (furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, and handicrafts) were controlled and 368 (28 %) of these were damaged by xylophagous insect species.

Seven insect species, belonging to three families were detected: Anobiidae with the species *Anobium punctatum* (De Geer, 1774) (common furniture beetle, death-watch beetle, house borer), *Hadrobregmus pertinax* (L., 1758) (= *Anobium pertinax*) (obstinate death watch beetle), *Ptilinus pectinicornis* (L., 1758) (fan-bearing wood-borer), *Xestobium rufovillosum* (De Geer, 1774) (death-watch beetle), Lyctidae with the species *Trogoxylon impressum* (Comolli, 1837) = *Lyctus impressum* and *L. linearis* (Goeze 1877) and Cerambycidae with the species *Hylotrupes bajulus* (L.) (old house borer) (Matei & Teodorescu, 2011). These species are now cosmopolitan, probably native to Europe. The origin zone of insect museum pests is difficult to establish.

From 1300 controlled pieces, manufactured of tree wood, the tree species could be established for 797 (61%), and remained unknown for 503 pieces. From the 797 pieces of known origin, only 222 were attacked by xylophagous insects while 575 were intact. From the 503 pieces the attack of xylophagous insects was present on a number of 146 (Table 1). A number of 20 tree species was identified as the source of the wood pieces and the insect attack was registered on pieces manufactured from the wood of 13 tree species (Table 2). There was a high number of pieces issued from *Abies* sp. (soft-wood, easy processing), *Fagus sylvatica*, and *Acer pseudoplatanus* wood (Fig. 1). The number of attacked pieces was higher in the case of the pieces manufactured from *Fagus sylvatica, Abies* sp., *Acer pseudoplatanus*, and *Corylus avellana* wood.

Table 1

Number of pieces with or without attack of insects from established or unknown tree species

Wood pieces categories	Number of pieces
Controlled wood pieces in storage areas of Museum	1300
Wood pieces with tree species established	**797**
Wood pieces with tree species established and with attack of insects	222
Wood pieces with tree species established and without attack of insects	575
Wood pieces with tree species unknown	**503**
Wood pieces with tree species unknown and with attack of insects	146
Wood pieces with tree species unknown and without attack of insects	357
Total attacked pieces	368

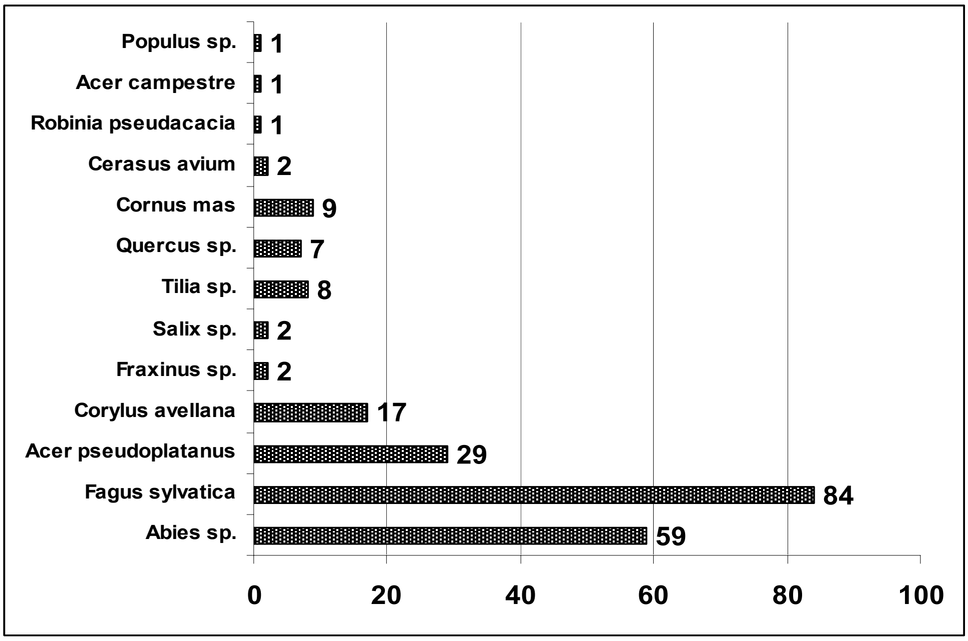


Fig. 1. Number of pieces manufactured from the wood of 13 tree species.

In the case of five tree species (*Acer campestre, Cerasus avium, Fraxinus* sp., *Populus* sp., *Robinia pseudacacia, Salix* sp.), the number of attacked pieces was very low. The attack was not registered on pieces made from wood coming from seven tree species (*Betula pendula, Carpinus betulus, Cerasus vulgaris, Juglans regia, Prunus domestica, P. cerasifera, Pyrus communis*). In the cases without attack and in those with only few attacked wood pieces it is not applicable to discuss the wood receptively/unreceptively or to assess the damage degree of attack.

In many cases an associate attack of two or three insect species was registered on the same piece, and the Anobiidae species were present in all of these associations. A high number of pieces presented Anobiidae single attack, especially those manufactured from wood of *Abies* sp., *Fagus sylvatica*, and *Acer pseudoplatanus* (Table 3). Attack frequency was high on furnishings, hank reels, distaffs, and house wares, low on musical instruments, and very low on cartman tallies (Table 4).

Table 2

Number and attack frequency of museum wood pieces with tree species wood identified

Tree species	**Number of controlled pieces**	**Attacked pieces**	
		Number	**%**
Abies sp.	262	59	22.5
Fagus sylvatica	176	84	47.7
Acer pseudoplatanus	97	29	29.9
Corylus avellana	43	17	39.5
Fraxinus sp.	39	2	5.1
Prunus domestica	37	0	0
Salix sp.	34	2	5.8
Tilia sp.	29	8	27.5
Quercus sp.	27	7	25.9
Cornus mas	17	9	52.9
Cerasus avium	15	2	13.3
Carpinus betulus	8	0	0
Betula pendula	4	0	0
Robinia pseudacacia	2	1	50.0
Cerasus vulgaris	2	0	0
Acer campestre	1	1	100.0
Populus sp.	1	1	100.0
Juglans regia	1	0	0
Pyrus communis	1	0	0
Prunus cerasifera	1	0	0
	797	222	

Comparative analysis of damage degrees on different tree species, as the source of wood pieces, revealed a strong preference for *Fagus sylvatica* wood. Indeed, about half of the *Fagus sylvatica* wood pieces registered heavy and medium-heavy attacks, and about 20% of the pieces registered very low attack (Fig. 2). In all other cases of wood essences heavy attack was not registered.

Table 3

Number of cases with simple and associate attack on different tree source of wood

Attack type	Number of attacked pieces	**Tree source of wood**												
		Abies sp.	*Fagus sylvatica*	*Acer pseudoplatanus*	*Corylus avellana*	*Fraxinus*	*Salix* sp.	*Tilia* sp.	*Quercus* sp.	*Cornus mas*	*Cerasus avium*	*Robinia pseudacacia*	*Acer campestre*	*Populus* sp.
Single atack Anobiidae	117	25	52	22	6	-	1	4	4	3	-	-	-	-
Single atack Cerambycidae	12	12	-	-	-	-	-	-	-	-	-	-	-	-
Single atac Lyctidae	38	-	18	3	5	1	1	1	1	3	2	1	1	1
Associated attack 2 Anobiidae	38	11	13	3	5	-	-	3	1	2	-	-	-	-
Associated attack 1 Anobiidae + 1 Cerambycidae	9	9	-	-	-	-	-	-	-	-	-	-	-	-
Associated attack 1Anobiidae + 1 Lyctidae	6	-	1	1	1	1	-	-	1	1	-	-	-	-
Associated attack 2 Anobiidae + 1 Cerambycidae	2	2	-	-	-	-	-	-	-	-	-	-	-	-

Table 4

Attack frequency to main categories of wood pieces

Wood pieces	**Total**	**Damaged**	**Attack frequency**
Distaffs	339	149	43.9
Tisting spindles	236	17	7.2
Musical instruments	207	9	4.3
House wares	150	42	28
Furnishings	122	94	77
Cartman tallies	119	1	0.8
Hank reels	68	47	69
Handicrafts	59	9	15.25

In the pieces manufactured from *Abies* sp. and *Acer pseudoplatanus* wood, we registered 14–15 % cases of medium-heavy attack (Figs. 3, 4). In the pieces manufactured from *Corylus avellana* wood, heavy and heavy-medium attacks were not registered (Fig. 5). Low and very low attacks were dominant on *Corylus avellana* wood pieces (76 %) and *Abies* wood pieces (61 %), very low attack being dominant on *Acer pseudoplatanus* wood pieces (55 %).

The damage degree to categories of wood pieces was analyzed comparatively. Furnishings, hank reels, and house wares pieces registered heavy attack degree. On furnishings pieces heavy and medium-heavy attacks surpassed 50 %, while heavy, medium-heavy, and medium degree of attack was registered in 72 % of the cases (Fig. 6). Heavy attack registered on hank reels reached about 20 %, and heavy, medium-heavy, and medium attacks, registered 64 % (Fig. 7). In the case of housewares objects, heavy attack surpassed 10 % and heavy, medium-heavy, and medium attack was registered in 50 % of the pieces (Fig 8). In other cases there were not registered heavy and medium-heavy attacks.

In the case of distaffs pieces, low and very low attacks surpassed 72 %, while low, very low, and low-medium attacks reached 85 % (Fig. 9). On musical instruments and handicrafts pieces only very low and low attacks were registered, dominant being the very low attacks (Fig. 10).

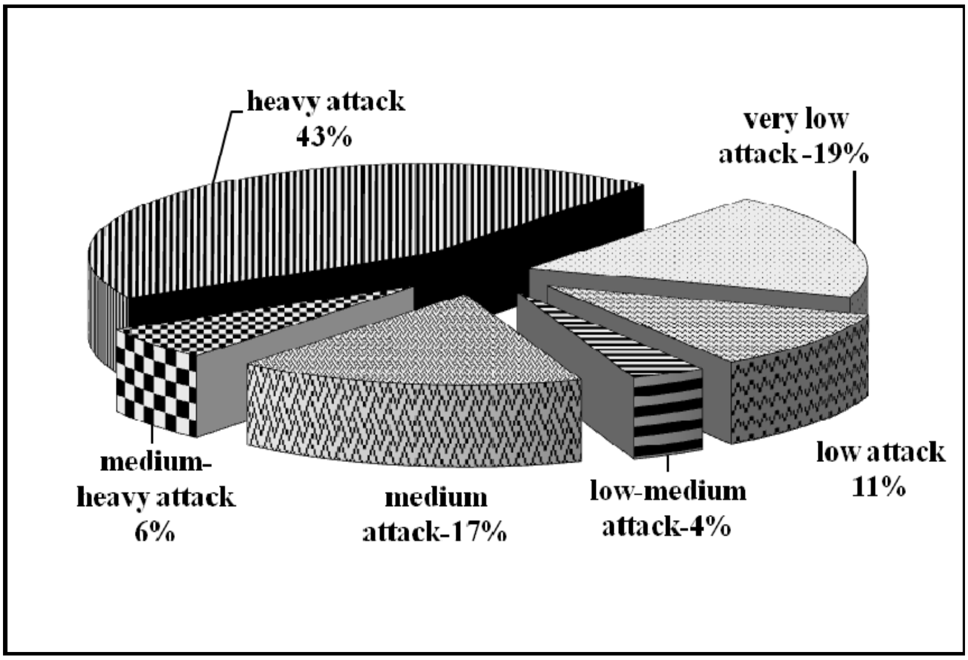


Fig. 2. Damage degree on pieces manufactured from *Fagus sylvatica* wood.

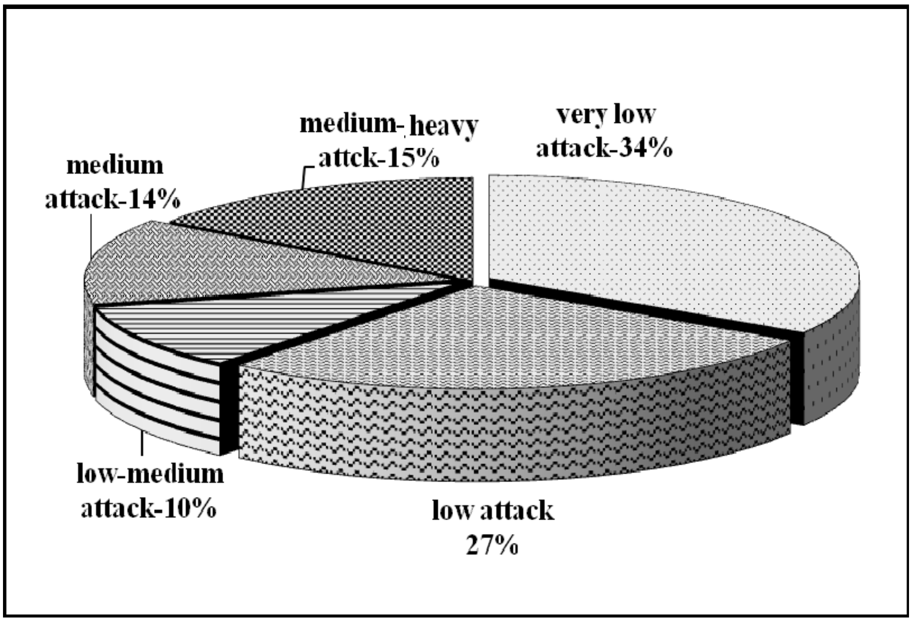


Fig. 3. Damage degree on pieces manufactured from *Abies* sp. *w*ood.

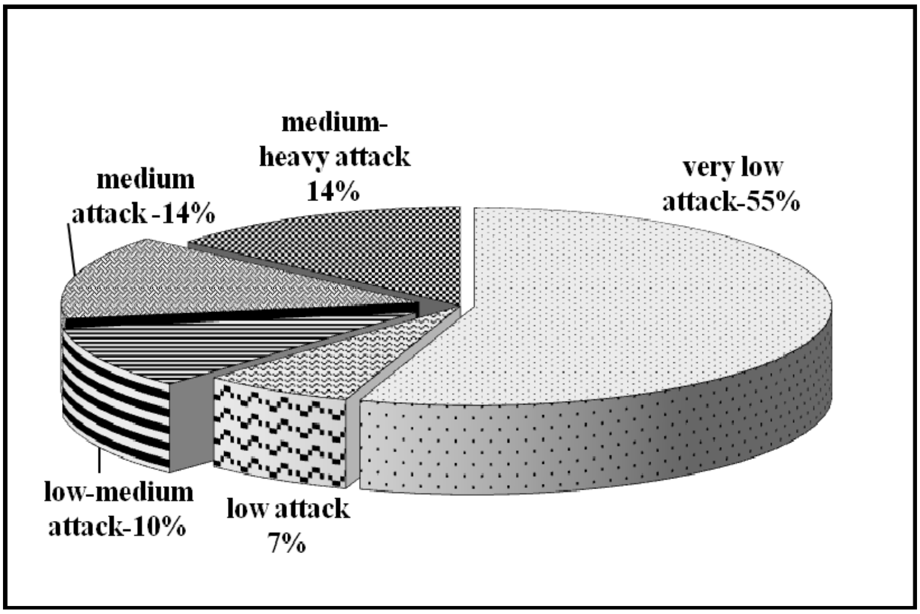


Fig. 4. Damage degree on pieces manufactured from *Acer pseudoplatanus* wood.

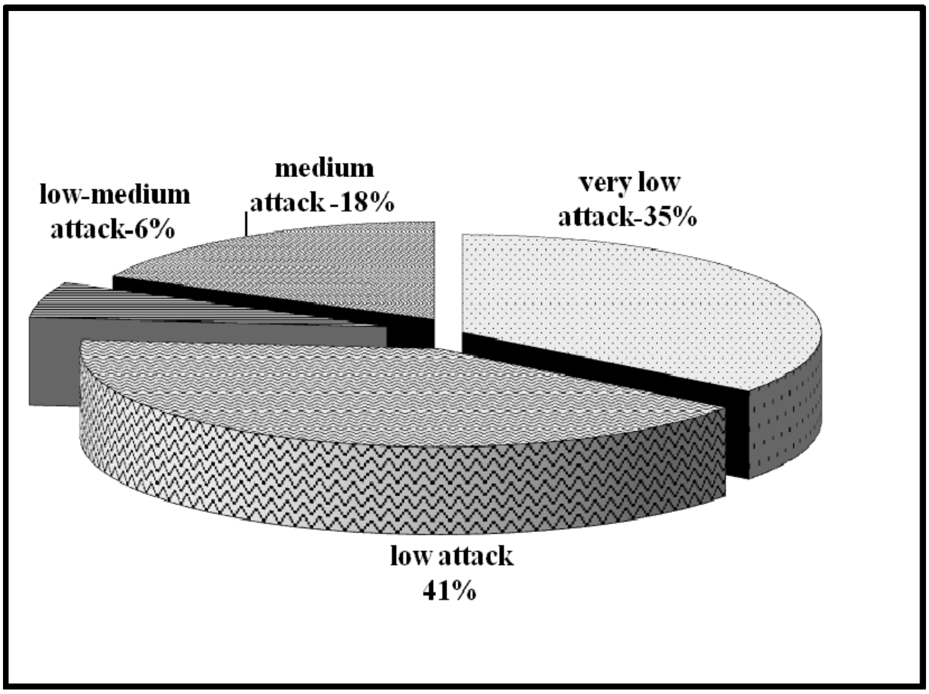


Fig. 5. Damage degree on pieces manufactured from *Corylus avellana* wood.

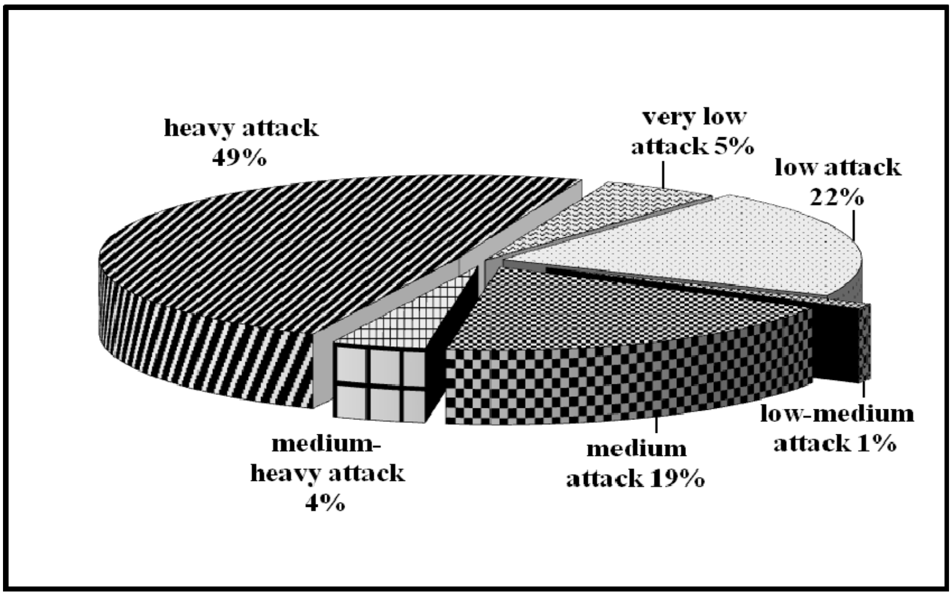


Fig. 6. Damage degree to furnishings pieces.

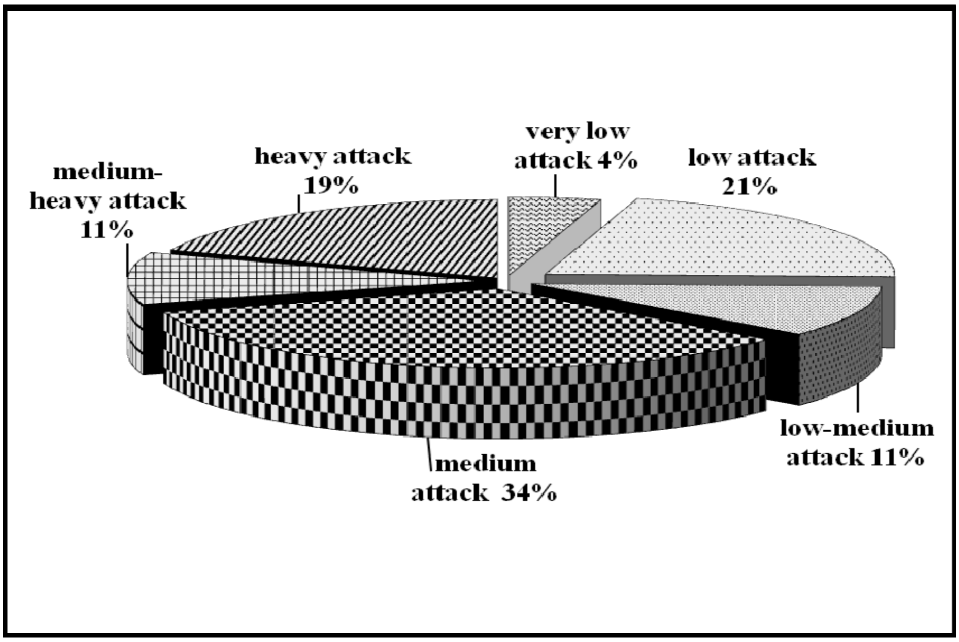


Fig. 7. Damage degree to hank reels pieces.

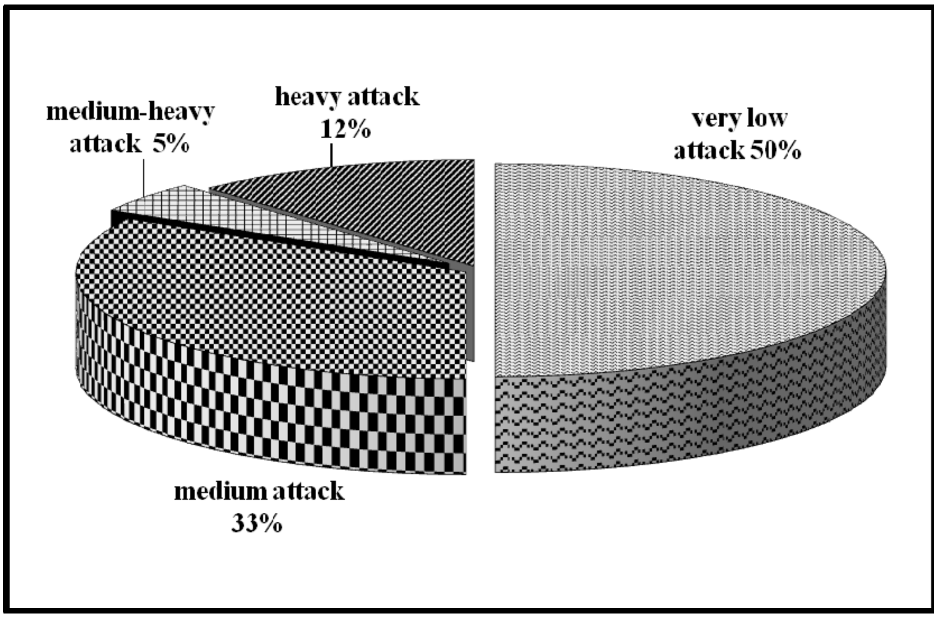


Fig. 8. Damage degree to house wares pieces.

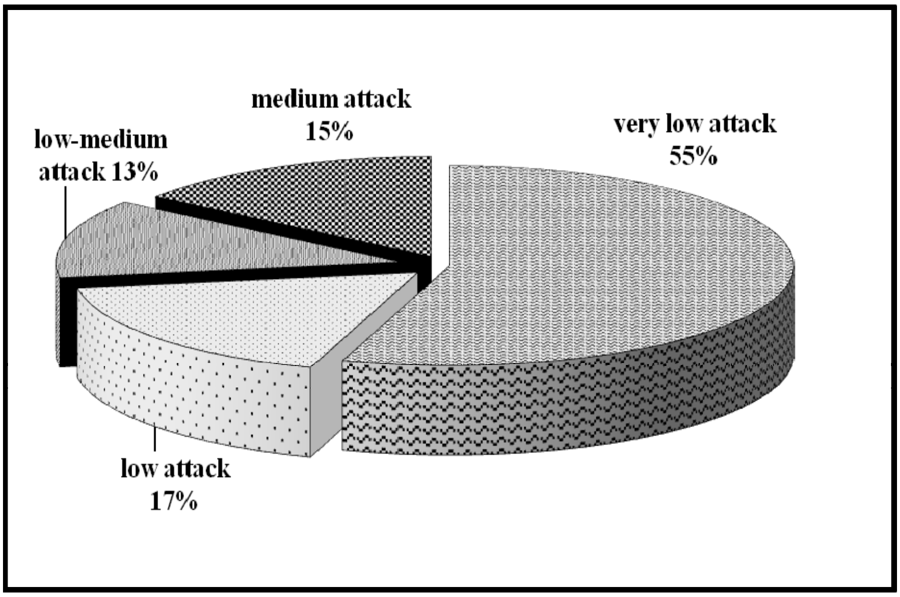


Fig. 9. Damage degree to distaffs pieces.

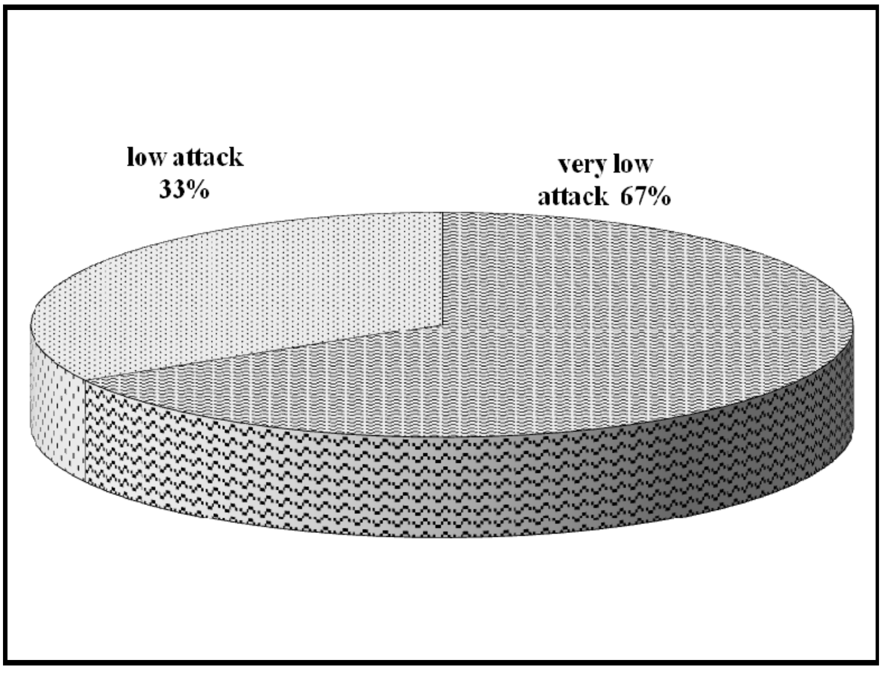


Fig. 10. Damage degree to musical instruments and handicrafts.

Very low attack was dominant to distaffs, handicrafts and musical instruments and significant medium attack was registered on hank reels, house wares, and furnishings. Minimum values of damage degree were recorded to cartman tallies (one attacked pieces, with very low attack, out of 119 controlled pieces). All attacked pieces of twisting spindles registered a very low attack.

In order to avoid insects attack in the Peasant Museum it is necessary to control for signs of attack all wood pieces arriving from rural households (and especially the old ones) and closed windows and exterior doors (to prevent the infiltration of some pests), but also to monitor the temperature and relative humidity, to regularly clean the collections and storage areas, to periodically make a visual inspection of the collections in order to detect flight holes, fine dust of wood under the wooden pieces or beetle adults, to use traps (sticky, pheromone, or light) to capture the insects, and possibly to attempt to eradicate pests by spraying with chemical substances (approximately every three months) or by using heat (high temperature). Correctly identifying museum pests is a very important step to treat infection and prevent future occurrence.

CONCLUSIONS

From 1,300 wood pieces (furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, handicrafts) controlled in the Romanian Peasant Museum stored areas, 368 were damaged by seven xylophagous coleopteran species, belonging to Anobiidae (*Anobium punctatum*, *Hadrobregmus pertinax*, *Ptilinus pectinicornis*, *Xestobium rufovillosum*), Lyctidae (*Trogoxylon impressum*, *Lyctus linearis*), and Cerambycidae families (*Hylotrupes bajulus*).

Wood pieces manufactured from 13 tree species (*Abies* sp., *Acer pseudoplatanus*, *A. campestre*, *Cerasus avium*, *Cornus mas*, *Corylus avellana*, *Fagus sylvatica*, *Fraxinus* sp., *Populus* sp., *Robinia pseudacacia, Salix* sp., *Quercus* sp., *Tilia* sp.) registered different degrees of damage.

To assess damage degrees, we established six levels of attack (very low, low, low-medium, medium, medium-heavy, and heavy). Heavy and medium-heavy attacks were registered only on wood pieces made from *Fagus sylvatica*, while in all other cases the low and very low attacks were dominant.

From the numerous categories of wood pieces (furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, handicrafts), heavy attacks were registered only on furnishings, house wares, and hank reels pieces.

Based both on the attack frequency and damage degree, most noxious pests were Anobiidae species, especially on pieces manufactured from *Fagus sylvatica* wood, furnishings, hank reels, and house wares. Most noxious insect pests were Anobiidae and Cerambycidae species.

Acknowledgements. This study was partially funded by CNCSIS (UEFISCSU) by project no. 1953/2009.

REFERENCES

AKBULUT S., KETEN A., YUKSEL B., 2008, *Wood Destroying Insects in Düzce Province*. Turkish Journal of Zoology, **32**: 343-350.

ANTONIE I., TEODORESCU I., 2009, *European and exotic insect pest species in Brukenthal museum* (*Sibiu city, Romania*). Romanian Journal of Biology-Zoology, **54** (2): 139-149.

COLAK MA., CIVELEK HS., ERDIL YZ., 2006, *Investigations on the effects of Hylotrupes bajulus (L.) and Trichoferus griseus (F.) (Coleoptera: Cerambycidae) damaged on wooden constructions on some mechanical and physical characteristics of wood***.** Turkish Journal of Entomology, **30** (4): 261-273.

EBELING W., 2002*, Wood-Destroying Insects and Fungi.* Chapter 5, part Urban Entomology, 128-167, Entomology UC Riverside mailto: leowest@mail.ucr.edu

HIGHLEY T.L., 1999, *Biodeterioration of Wood.* Chapter 13, 13,1-13.16, *In*: *Wood handbook – Wood as an engineering material*. Gen. Tech. Rep. FPL–GTR–113. Madison, WI: U.S. D. A. Forest Service, Forest Products Laboratory. 463 p., http://www.conradfp.com/ pdfs/ch13-Biodeterioration-of-Wood.pdf.

LEARY P., 2002, *The eradication of insect pest in buildings*. Building Conservation Directory www.buildingconservation.com

MATEI A., TEODORESCU I., 2011, *Xylophagous insect species, pest in wood collection from Romanian Peasant Museum*. Romanian Journal of Biology-Zoology, **56** (1): 61-74.

MOŞNEAGU M., GĂMĂLIE G., 2006, *Coleoptere dăunătoare bunurilor de patrimoniu de la Mânăstirea Agapia*. Muzeul Olteniei Craiova, Oltenia, Studii şi Comunicări, Ştiinţele Naturii, **XXII**: 184-189.

MUSTAŢĂ M., 1998, *Insecte dăunătoare bunurilor de patrimoniu*. Edit. Univ. „Al.I. Cuza", Iaşi.

UNAL S., OZCAN E., KAYGIN A.T., 2009, *Wood-destroying Coleopteran species in the historical buildings in Kastamonu, in Turkey*. African Journal of Biotechnology, **8** (10): 2349-2355.

URGER A., SCHNIEWIND A.P., UNGER W., 2001, *Conservation of Wood Artifacts*. Springer-Verlag Berlin, Heidelberg, New York, 578 pp.

* * *, http://www.nps.gov/history/museum/publications/conserveogram/cons_toc.html. Identifying Museum Insect Pest Damage, 2008, Conserve Ogram, 1-4.

* * *, http://www.kendal.bioresearch.ca.uk/domest.htm

* * *, http://www.nps.gov/history/museum/publications/conserveogram/cons_toc.html

* * *, http://zipcodezoo.com/Animals/A/Anobium_punctatum/

* * *, http://www.kendalluk.com/woodworm.htm Wood-Boring Beetles

* * *, http://frontpage.woodland-trust.org.uk/ancient-tree-forum/atfecology/invertebrate.htm

* * *, http://www.ehow.co.uk/how_7525381_identify-flying-wood-eating-insects.html

* * *, http://www.rentokil.co.za/pest-guides/insects-and-spiders/wood-damaging-beetles/index.html

Received March 12, 2011

[*] *University of Bucharest*
Department of Systems Ecology and Sustainability
Splaiul Independenţei 91-95, 050095
Bucharest, Romania
e-mail: Teodorescubiologie@yahoo.com

HABITAT REQUIREMENTS OF MOSQUITO LARVAE

ZOLTÁN KENYERES*, NORBERT BAUER**, SÁNDOR TÓTH***,
TAMÁS SÁRINGER-KENYERES****

We studied basic habitat requirements of the mosquito species occurring in Hungary. 1239 samples were taken (mainly around Lake Balaton and in the Tisza Region). Our results showed that the basic indicator variables in the colonization of different mosquito species are: (i) water surface area, (ii) presence of pondweed vegetation, (iii) depth, transparency and temperature of the breeding water. These variables related closely to the regime (permanent or temporary) of the water body. It was confirmed by quantitative ecological analyses that the most important human-biting mosquito species (*e.g. Aedes vexans, Ochlerotatus sticticus, Ochlerotatus annulipes, Ochlerotatus cantans, Aedes rossicus*) usually occur in shallow, quick-warm, regularly drying, clear water bodies with minimal cover of the water surface and without presence of hydrophyta plant species (both in shaded and lightened places).

Key words: mosquitoes, assemblages, aquatic habitat use, pH, temperature, hydrological regime, Hungary.

INTRODUCTION

Presence-absence and size of the populations (spatial and temporal) are determined mainly by the habitat-requirements of the species (Morris, 2003). Several habitat variables (*e.g.* annual temperature, mean temperature of the coldest month, pH, length of the flooded period, size, temperature and vegetation of the breeding site) partake in organization of the larval mosquito assemblages (Becker, 1989; Paradise, 2000; Schäfer, 2004; Fischer & Schweigmann, 2004; Bauer *et al.*, 2011). Besides the above mentioned factors some special requirements of mosquitoes are also known. For example, females during egg laying prefer black colour and ignore green colour of artificial containers (Williams, 1962; McDaniel *et al.*, 1976; Jones & Schreiber, 1994). Mosquito species show extreme differences in habitat-dependency (Schäfer, 2004; Tóth, 2004; Sattler *et al.*, 2005; Alfonzo *et al.*, 2005; Azari-Hamidian, 2007).

The above mentioned aspects of the organization of mosquito assemblages have not been studied in detail in Central-Europe, in spite of the fact that this is an important region from a mosquito-control point of view. In this paper we present the aquatic habitat requirements of mosquitoes in Hungary. Many potential factors which may determine the organization of the larval mosquito assemblages in this region have not been researched with quantitative studies before.

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° *2*, P. 147–162, BUCHAREST, 2011

The goal of the study was to determine the aquatic habitat variables important for the colonization of different mosquito species (potential factors are shading, pH, vegetation, length of the water supply).

MATERIAL AND METHODS

Location

We collected 1239 samples in regions (Balaton Region, Lake Tisza) of Hungary being rich in mosquito breeding sites. 591 samples were collected in the Region of Lake Tisza, altitudes of these sites were between 80 and 95 metres height above sea level. Overall extension of the sampled breeding sites around Lake Tisza was 1135 hectares. 648 samples were collected in the Region of Lake Balaton, altitudes of these sites were between 91 and 140 metres height above sea level. Overall extension of the sampled breeding sites around Lake Balaton was 1033 hectares.

Repartition of the samples among the breeding site-types was the following: (1) marshy (reed-bed and sedgy) closed vegetation (MV): 419 samples; (2) marshy (reed-bed and sedgy) closed vegetation with pondweeds (MV+): 266 s.; (3) herb vegetation (HV): 261 s.; (4) lakes (OW): 121 s.; (5) forest vegetation (FV): 146 s.; (6) artificial containers: 26 s.

Sampling methods and timing

In each sampling site 10 square metres quadrat was studied. Sampling of the mosquitoes was carried out with a 20cm-sized-circle straining net. Larvae collected by 2–3 dips were defined as one sample. It means examination of 1 litre breeding water in every site.

Samples were taken between 04 September 2003 and 06 June 2007. The time of the samplings within months was shaped to the temporal importance of the breeding sites [14 samples were taken in February; March (101); April (290); May (171); June (39); July (87); August (225); September (233); October (76); November (3)].

Part of the sampling sites was sampled repeatedly, but many of them were sampled just once only. Merging of the samples taken in the same place in different time is unreasonable, because the ecological factors of the breeding sites (they are usually temporary habitats) can be assigned to the time of the given sampling.

In laboratory 31227 larvae of 30 species were determined. The detected mosquito species and their most important life-characters are listed in Table 1. (nomenclature: Becker *et al*., 2003). After the first mentions the abbreviations of the species names (see Table 1) are used in the text.

Sampled habitat variables

In each sampling site the following habitat variables were recorded: altitude; plant association(s) in the habitat; the most typical plant species of the water and waterside; presence of pondweed vegetation (0/1); cover of the water surface (1–5: 1 = 0%, 2 = 1–20%, 3 = 20–40%, 4 = 40–60%, 5 = 80–100%); pH; temperature, depth, regime (permanent or temporary, 0/1), transparency (in scale 1–5), shading (1–5: 1 = no shaded; 2 = shaded by short grass (< 20 cm), 3 = shaded by tall grass (> 20 cm) or recessing (canals), 4 = opened forest, forest ecotone, 5 = closed forest) of the breeding sites.

Data analysis

Relations between occurrences of mosquito larvae and potential influential habitat variables were studied by Pearson-correlation, logistic regression, multiple regression and canonical correspondence analysis. Binary matrices were analysed by logistic regression, others were analysed by all the methods mentioned. Significant correlations of Pearson-analyses were supervised by Bonferroni technique (acceptance at $p < 0.001$). Database of the recorded plant species was reduced. 348 plant taxa were recorded, out of which 125 were listed in more than 10 samples. Logistic regression (simplex method) was accomplished with these species (presence-absence matrices of the plant and mosquito species were used).

Independent variables of the multiple regression were the density values of all the collected species, dependent variables were the following: altitude, water depth, water transparency, regime (permanent or temporary), presence of the pondweed vegetation, cover of the water surface, shading, temperature and pH of the water. Multicollinearity has to be taken into consideration because of several coherent variables of the regression.

Environmental variables of canonical correspondence analysis were the temperature, pH, transparency, regime (permanent or temporary), shading and depth of the water, cover of the water surface, presence of the pondweed vegetation and altitude.

For the statistical analyses STATISTICA 6.0 (Statsoft, 1995) and PAST (Hammer *et al*., 2001) statistical program packages were used.

RESULTS

Based on the confirmed (Bonferroni technique) results of the Pearson correlation analyses (Table 3) presence of *Ochlerotatus annulipes* (Meigen, 1830), *Anopheles claviger* (Meigen, 1804), and *Culiseta annulata* (Schrank, 1776) is related to breeding sites occurring in submountain regions. On the contrary, presence of *Aedes rossicus* Dolbeshkin, Goritzkaja & Mitrofanova, 1930, *Ochlerotatus flavescens* (Müller, 1764), *Anopheles maculipennis* Meigen, 1818, *Anopheles messeae* Falleroni, 1926, *Anopheles hyrcanus* (Pallas, 1771), *Culex modestus* Ficalbi, 1890, *Uranotaenia unguiculata* Edwards, 1913 is related to low altitude. Multiple regression confirmed the above mentioned relations without

Ochann, Anomes, Anohyr species and showed that *Ochlerotatus dorsalis* (Meigen, 1830) and *Culex martinii* Medschid, 1930 are related to plains (Table 6).

Pearson correlation analyses showed (confirmed by Bonferroni technique) (Table 3) that water transparency influences the density of *Ochann* and *Anocla* – densities of the mentioned species are in ordinal relation with the water transparency. Multiple regression did not confirm these relations, and showed that *Coquillettidia richiardii* (Ficalbi, 1889) is related to cloudy water and *Ochlerotatus sticticus* (Meigen, 1838) is related to clear water (Table 6).

Based on Pearson correlations (confirmed by Bonferroni technique) (Table 3) permanent regime of the breeding water influences positively the density of *Coqric, Anomac, Anocla, Anomes* and *Culmod*. On the contrary, *Aedes cinereus* Meigen, 1818, *Aedvex, Ochann* and *Ochfla* are related to temporary waters. Multiple regression did not confirme this relation in connection with *Anomes* and *Ochfla*. Furthermore, it pointed out that *Culex hortensis* Ficalbi, 1890, *Anomes, Anohyr* connect to temporary waters (Table 6).

Pearson correlation analyses showed (confirmed by Bonferroni technique) (Table 4) that the presence of the pondweed vegetation affects positively the density of *Coqric, Anomac, Culmod*, and negatively the density of *Aedvex* and *Ochfla*. Multiple regression confirmed these relations, furthermore it showed that the presence of the pondweed vegetation and the presence of *Ochlerotatus rusticus* (Rossi, 1790), *Aedros* and *Culhor* are in inverse relation, further presence of the pondweed vegetation and the presence of *Culex territans* Walker, 1856 is in ordinal relation.

Correlation analyses (confirmed by Bonferroni technique) demonstrated (Table 4) that the cover of the water surface (mainly cover of *Lemna* spp., *Spirodela*, *Wolffia*) influences the density of the species in different ways. Significant positive relations were revealed between the cover of the water surface and the density of *Coqric, Anomac* and *Culmod*, while between the cover of the water surface and the density of *Aedes vexans* (Meigen, 1830), *Aedcin, Ochann*, *Ochfla* significant negative relations were revealed. Multiple regression confirmed these results and revealed negative relations between the density of *Anohyr, Culex pipiens* Linnaeus, 1758 and the cover of the water surface (Table 6).

Pearson correlation (confirmed by Bonferroni technique) analyses showed (Table 4) that *Ochlerotatus cantans* (Meigen, 1818), *Ochrus* and *Culann* related to shaded-, half-shaded habitats. Multiple regression confirmed these relations and revealed further negative (*Aedcin, Anomac, Anopheles atroparvus* Van Thiel, 1927) and positive (*Anohyr, Ochsti*) relations between shading and density of the mosquito species (Table 6).

Pearson correlation analyses (confirmed by Bonferroni technique) (Table 4) of the densities and pH showed that *Ochlerotatus refiki* (Medschid, 1928) and *Ochann* relate to basic, *Culiseta morsitans* (Theobald, 1901) to acidic waters.

Multiple regression showed rather different results. It marked *Aedvex, Aedros, Ochlerotatus cataphylla* (Dyar, 1916), *Ochlerotatus caspius* (Pallas, 1771)*, Ochrus, Ochdor, Anomac, Culhor, Uraung* as species related to basic pH, and *Ochsti, Anocla, Culpip, Culter*, *Culann* as species related to acidic pH (Table 6). The study sites are situated in large natural habitats and the well-researched treeholes (e.g. Bradshaw & Holzapfel, 1991; Sota *et al*., 1994; Dahl & Blackmore, 2001) characterized by special water chemistry were not sampled.

Table 1

Abbreviations and significant life-strategy features of the collected mosquito species
[Legends: OS = oviposition site (WS: water surface; DS = dry surface); HS = hibernation state
(E: egg; L: larva, F: female); NG = number of generations within year
(M = multivoltine, U = univoltine)]

Species	**Abb.**	**OS**	**HS**	**NG**
Aedes vexans (Meigen, 1830)	*Aedvex*	DS	E	M
Aedes cinereus Meigen, 1818	*Aedcin*	DS	E	M
Aedes rossicus Dolbeshkin, Goritzkaja & Mitrofanova, 1930	*Aedros*	DS	E	M
Ochlerotatus excrucians (Walker, 1856)	*Ochexc*	DS	E	U
Ochlerotatus annulipes (Meigen, 1830)	*Ochann*	DS	E	U
Ochlerotatus cantans (Meigen, 1818)	*Ochcan*	DS	E	U
Ochlerotatus caspius (Pallas, 1771)	*Ochcas*	DS	E	M
Ochlerotatus cataphylla (Dyar, 1916)	*Ochcat*	DS	E	U
Ochlerotatus sticticus (Meigen, 1838)	*Ochsti*	DS	E	M
Ochlerotatus rusticus (Rossi, 1790)	*Ochrus*	DS	L	U
Ochlerotatus refiki (Medschid, 1928)	*Ochref*	DS	E	U
Ochlerotatus flavescens (Müller, 1764)	*Ochfla*	DS	E	U
Ochlerotatus nigrinus (Eckstein, 1918)	*Ochnig*	DS	E	U
Ochlerotatus dorsalis (Meigen, 1830)	*Ochdor*	DS	E	U
Coquillettidia richiardii (Ficalbi, 1889)	*Coqric*	WS	L	U
Anopheles maculipennis Meigen, 1818	*Anomac*	WS	F	M
Anopheles claviger (Meigen, 1804)	*Anocla*	WS	L	M
Anopheles messeae Falleroni, 1926	*Anomes*	WS	F	M
Anopheles hyrcanus (Pallas, 1771)	*Anohyr*	WS	F	M
Anopheles algeriensis Theobald, 1903	*Anoalg*	WS	L	M
Anopheles atroparvus Van Thiel, 1927	*Anoatr*	WS	F	M
Culex modestus Ficalbi, 1890	*Culmod*	WS	F	M
Culex pipiens Linnaeus, 1758	*Culpip*	WS	F	M
Culex territans Walker, 1856	*Culter*	WS	F	M
Culex torrentium Martini, 1924	*Cultor*	WS	F	M
Culex hortensis Ficalbi, 1890	*Culhor*	WS	F	M
Culiseta morsitans (Theobald, 1901)	*Culmor*	DS	L	U
Culex martinii Medschid, 1930	*Culmar*	WS	F	M
Culiseta annulata (Schrank, 1776)	*Culann*	WS	F	M
Uranotaenia unguiculata Edwards, 1913	*Uraung*	WS	F	M

Table 2

Number of positive samples and habitat requirements of the recorded mosquito species based on the measured data (minimum, modus and maximum values)

Species	**Nr of samples**	**Water depth (cm)**	**Water transparency (scale)**	**Water pH**	**Water temperature (°C)**	**Cover of the water surface (scale)**	**Shading (scale)**
Aedes vexans	251	3-(20)-60	1-(3)-5	6.7-(8.5)-10.2	7.8-(14.1)-28.7	1-(1)-4	1-(3)-5
Aedes cinereus	169	3-(20)-60	1-(3)-5	6.7-(8.5)-11.6	6.2-(15.5)-27.9	1-(1)-4	1-(3)-5
Aedes rossicus	20	10-(20)-40	1-(3)-4	7.5-(8.5)-9.0	8.3-(11.6)-17.2	1-(1)-3	2-(3)-4
Ochlerotatus excrucians	32	5-(15)-40	2-(3)-5	6.7-(6.9)-9.4	8.3-(14.2)-20.2	1-(1)-4	2-(3)-4
Ochlerotatus annulipes	224	3-(20)-60	1-(3)-5	6.7-(8.5)-10.9	7.8-(11.3)-26.3	1-(1)-5	1-(3)-5
Ochlerotatus cantans	62	5-(15)-40	1-(3)-5	6.8-(8.4)-10.9	9.1-(14.8)-24.6	1-(1)-5	1-(4)-5
Ochlerotatus caspius	117	3-(10)-45	1-(3)-4	6.7-(8.5)-9.5	8.3-(23.4)-30	1-(1)-4	1-(2)-4
Ochlerotatus cataphylla	34	5-(20)-40	1-(3)-5	6.7-(8.2)-10.9	9.1-(12.4)-21.3	1-(1)-3	2-(3)-4
Ochlerotatus sticticus	122	5-(20)-50	2-(3)-5	6.8-(8.5)-9.3	9.3-(22.3)-28.5	1-(1)-4	1-(3)-5
Ochlerotatus rusticus	51	3-(20)-50	1-(3)-5	6.7-(8.2)-12.1	6-(15.2)-26.3	1-(1)-5	2-(3)-5
Ochlerotatus refiki	5	3-(30)-30	3-(4)-5	8.2-10.2	6.6-(11.8)-22.3	1 (1)	3-(4)-4
Ochlerotatus flavescens	71	5-(15)-45	1-(3)-4	6.7-(8.4)-11.6	7.8-(11.3)-26.3	1-(1)-3	1-(3)-4
Ochlerotatus nigrinus	2	25-30	3-(3)	8.5-(8.5)	12.3-(12.3)	1-(1)	2-(2)
Ochlerotatus dorsalis	13	10-(20)-35	1-(2)-3	7.9-(8.1)-11.6	8.1-23.3	1-(1)-4	1-(2)-3
Coquillettidia richiardii	35	15-(20)-75	2-(3)-5	6.2-(8.2)-9.3	8.2-(13.2)-25.8	1-(4)-5	2-(3)-4
Anopheles maculipennis	464	0.2-(20)-120	1-(3)-5	6.2-(8.3)-12.1	8.2-(21.6)-30.2	1-(1)-5	1-(3)-5
Anopheles claviger	95	7-(20)-120	2-(3)-5	6.8-(8.1)-10.7	7.1-(9.6)-23.7	1-(2)-5	1-(3)-5
Anopheles messeae	52	0.2-(20)-70	2-(3)-5	6.6-(8.9)-10.4	12.3-(20.1)-28.5	1-(1)-5	1-(3)-5
Anopheles hyrcanus	16	5-(20)-60	2-(3)-4	7.2-(9.3)-10.4	8.7-26.5	1-(1)-4	2-(3)-4
Anopheles algeriensis	4	30-(30)-60	3-(3)-4	8.6-(10.4)-10.4	18.8-22.3	1-(3)-3	2-(3)-4
Anopheles atroparvus	8	10-(10)-40	2-(3)-3	8.2-(9.0)-9.0	11.8-26.3	1-(1)-3	1-(2)-2
Culex modestus	189	0.2-(20)-120	1-(3)-5	6.1-(8.2)-14.1	8.7-(21.6)-28.8	1-(1)-5	1-(3)-5
Culex pipiens	492	3-(10)-120	1-(3)-5	6.2-(8.3)-14.1	6.2-(24.1)-33.2	1-(1)-5	1-(3)-5
Culex territans	111	5-(20)-120	2-(3)-5	6.2-(7.4)-14.1	7.1-(20.7)-33.2	1-(1)-5	1-(3)-5
Culex torrentium	1	30	4	8.6	22.3	1	2
Culex hortensis	23	5-(10)-60	2-(3)-4	7.0-(8.5)-10.1	8.2-(25.8)-29.5	1-(1)-5	1-(3)-4
Culiseta morsitans	17	10-(20)-50	1-(3)-5	6.8-(7)-8.2	7.1-17.7	1-(2)-4	2-(3)-4
Culex martinii	1	40	5	8.1	13.8	3	3
Culiseta annulata	213	5-(20)-120	1-(3)-5	6.2-(8.4)-14.1	7.8-(20.1)-26.1	1-(1)-5	1-(3)-5
Uranotaenia unguiculata	61	5-(10)-80	2-(3)-5	6.2-(8)-14.1	13.2-(21.6)-30.4	1-(1)-5	1-(3)-5

Table 3

Correlations between densities of the mosquito species and the recorded habitat variables I. [Significant values of the correlation analyses are bold; significant values were confirmed by Bonferroni technique (acceptance at $p < 0.001$) are bold and marked (*)]

Species	**Altitude**		**Water depth**		**Water transparency**		**Regime (t/p)**	
Aedvex	0.0358	p=0.2080	-0.0284	p=0.3180	**0.0738**	**p=0.0090**	**-0.0965**	**p=0.0010***
Aedcin	0.0273	p=0.3380	-0.0179	p=0.5300	-0.0229	p=0.4200	**-0.1490**	**p=0.0001***
Aedros	**-0.0800**	**p=0.0050**	0.0059	p=0.8350	-0.0069	p=0.8090	-0.0484	p=0.0880
Ochexc	0.0514	p=0.0700	-0.0169	p=0.5520	0.0046	p=0.8720	**-0.0598**	**p=0.0350**
Ochann	**0.1149**	**p=0.0001***	**-0.0614**	**p=0.0310**	**0.1421**	**p=0.0001***	**-0.1535**	**p=0.0001***

Table 3

(continued)

Ochcan	0.0545	p=0.0550	-0.0422	p=0.1380	**0.0872**	**p=0.0020**	**-0.0659**	**p=0.0200**
Ochcat	**0.0754**	**p=0.0080**	-0.0303	p=0.2870	0.0397	p=0.1630	**-0.0564**	**p=0.0470**
Ochsti	**0.0715**	**p=0.0120**	-0.0397	p=0.1630	**0.0924**	**p=0.0010**	-0.0523	p=0.0660
Ochrus	**0.0775**	**p=0.0060**	**-0.0703**	**p=0.0130**	-0.0035	p=0.9020	**-0.0680**	**p=0.0170**
Ochref	0.0375	p=0.1870	0.0101	p=0.7230	0.0167	p=0.5560	-0.0328	p=0.2490
Ochfla	**-0.0571**	**p=0.0440**	**-0.0619**	**p=0.0290**	0.0375	p=0.1880	**-0.1155**	**p=0.0001***
Ochnig	-0.0441	p=0.1210	0.0144	p=0.6140	-0.0028	p=0.9200	-0.0273	p=0.3380
Ochdor	**-0.0688**	**p=0.0150**	-0.0043	p=0.8810	**-0.0718**	**p=0.0110**	-0.0505	p=0.0760
Coqric	-0.0205	p=0.4720	**0.1143**	**p=0.0001***	0.0258	p=0.3650	**0.1733**	**p=0.0001***
Anomac	**-0.1980**	**p=0.0001***	**0.0694**	**p=0.0150**	-0.0429	p=0.1320	**0.2519**	**p=0.0001***
Anocla	**0.1651**	**p=0.0001***	**0.1301**	**p=0.0001***	**0.1186**	**p=0.0001***	**0.1468**	**p=0.0001***
Anomes	**-0.0770**	**p=0.0070**	-0.0076	p=0.7880	0.0057	p=0.8410	**0.0710**	**p=0.0130**
Anohyr	**-0.1061**	**p=0.0001***	-0.0153	p=0.5910	0.0002	p=0.9930	0.0314	p=0.2700
Anoalg	-0.0217	p=0.4460	0.0419	p=0.1410	0.0276	p=0.3320	0.0446	p=0.1170
Anoatr	-0.0434	p=0.1270	-0.0157	p=0.5820	-0.0091	p=0.7490	-0.0423	p=0.1370
Culmod	**-0.1604**	**p=0.0001***	0.0299	p=0.2930	-0.0433	p=0.1270	**0.1555**	**p=0.0001***
Culpip	-0.0383	p=0.1780	-0.0509	p=0.0730	-0.0449	p=0.1140	-0.0068	p=0.8110
Culter	0.0137	p=0.6290	**0.0571**	**p=0.0450**	0.0248	p=0.3830	**0.0796**	**p=0.0050**
Cultor	0.0223	p=0.4340	0.0156	p=0.5830	0.0395	p=0.1650	-0.0193	p=0.4980
Culhor	-0.0526	p=0.0640	-0.0270	p=0.3420	0.0399	p=0.1600	-0.0477	p=0.0930
Culmor	**0.0850**	**p=0.0030**	0.0180	p=0.5260	-0.0143	p=0.6160	0.0125	p=0.6610
Culmar	0.0497	p=0.0800	0.0374	p=0.1890	**0.0810**	**p=0.0040**	0.0419	p=0.1400
Culann	**0.1377**	**p=0.0001***	-0.0380	p=0.1820	0.0443	p=0.1190	-0.0362	p=0.2030
Uraung	**-0.1131**	**p=0.0001***	-0.0276	p=0.3320	0.0160	p=0.5740	0.0077	p=0.7860

Pearson correlation analyses (confirmed by Bonferroni technique) (Table 4) between the mosquito larval densities and temperature-values showed that *Anomac, Anomes, Culmod, Culpip, Culhor, Uraung* are related to high water temperature, but *Ochann* and *Anocla* are related to low water temperature. Multiple regression also showed rather different results. It confirmed the results in connection with density of *Anomes* and *Uraung,* but showed several further dependence. Based on this *Aedcin, Ochlerotatus excrucians* (Walker, 1856), *Ochcan, Ochcas, Culter, Culmor*, *Culann* occur in warm waters, *Aedvex, Ochref, Ochcat*, *Ochdor, Coqric, Anoatr* in larval habitats characterized by low temperature (Table 6).

Logistic regression of the presence-absence matrix of the mosquitoes and plant species revealed several positive and negative relations between the occurrences (see Table 5). Mosquito species related to permanent waters (*Anopheles algeriensis* Theobald, 1903*, Anocla, Anohyr*, *Anomac, Anomes, Coqric, Culann, Culmod, Culter, Uraung*) occur usually parallel with hydrophytic marshy plant species (*Alopecurus pratensis, Butomus umbellatus, Calystegia sepium,* *Carex acutiformis, Ceratophyllum demersum, Iris pseudacorus, Typha angustifolia Lythrum virgatum*) and aquatic plants (*Hydrocharis morsus-ranae, Lemna minor, L. trisulca, Salvinia natans, Spirodela polyrhiza*). Whereas occurences of mosquito species related to temporary waters (*Aedcin, Aedvex, Ochann, Ochcan, Ochcas, Ochfla, Ochrus, Ochsti*) show negative correlations with hydrophytic plant species, but occur together with typical species of marshy meadows and grasslands (*e.g. Carex acutiformis, Alopecurus pratensis*).

Canonical correspondence analysis showed that (1) temperature of the breeding site, (2) shading, pH and transparency of the water, and (3) regime (permanent or temporary), depth, water surface cover, presence of the pondweed vegetation are three indicator variables groups which assign the species composition and structural features of the mosquito larval assemblages (Fig. 1).

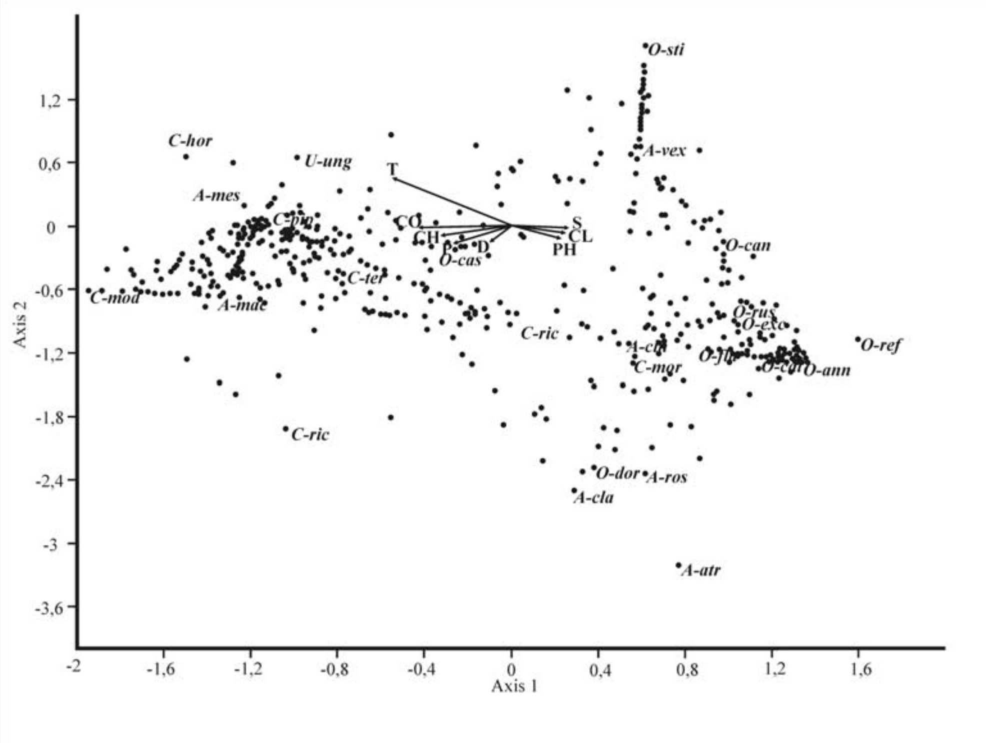


Fig. 1. Based on the results of the canonical correspondence analysis (1) temperature of the breeding site, (2) shading, pH and transparency of the water, and (3) regime, depth, cover of the water surface, presence of the pondweed vegetation are the three indicator variables groups which assign the species composition and structural features of the mosquito larval assemblages.

Table 4

Correlations between densities of the mosquito species and the recorded habitat variables II. [Significant values of the correlation analyses are bold; significant values were confirmed by Bonferroni technique (acceptance at $p < 0.001$) are bold and marked (*)]

Species	**Pondweed vegetation**		**Cover of the water surface**		**Shading**		**pH**		**Temperature**	
Aedvex	**-0.1072**	**p=0.0001***	**-0.0939**	**p=0.0010***	0.0427	p=0.1330	0.0574	p=0.1140	0.0540	p=0.1370
Aedcin	-0.0513	p=0.0710	**-0.1200**	**p=0.0001***	-0.0306	p=0.2820	**0.0864**	**p=0.0170**	-0.0377	p=0.2990
Aedros	**-0.0740**	**p=0.0090**	**-0.0573**	**p=0.0440**	-0.0209	p=0.4620	**0.0472**	**p=0.1940**	-0.0956	p=0.0080
Ochexc	-0.0341	p=0.2310	-0.0354	p=0.2130	0.0116	p=0.6830	-0.0542	p=0.1360	-0.0575	p=0.1130
Ochann	**-0.0811**	**p=0.0040**	**-0.1495**	**p=0.0001***	**0.0824**	**p=0.0040**	**0.1401**	**p=0.0001***	**-0.1713**	**p=0.0001***
Ochcan	**-0.0781**	**p=0.0060**	**-0.0701**	**p=0.0140**	**0.1049**	**p=0.0001***	**0.0714**	**p=0.0490**	-0.0191	p=0.5990
Ochcat	**-0.0718**	**p=0.0110**	-0.0495	p=0.0810	**0.0733**	**p=0.0100**	0.0165	p=0.6490	**-0.0884**	**p=0.0150**
Ochsti	**-0.0617**	**p=0.0300**	**-0.0562**	**p=0.0480**	**0.0901**	**p=0.0010**	0.0542	p=0.1360	**0.0787**	**p=0.0300**
Ochrus	**-0.0868**	**p=0.0020**	**-0.0596**	**p=0.0360**	**0.1064**	**p=0.0001***	0.0096	p=0.7910	**-0.1084**	**p=0.0030**
Ochref	-0.0409	p=0.1500	-0.0376	p=0.1870	**0.0637**	**p=0.0250**	**0.1181**	**p=0.0010**	-0.0381	p=0.2950
Ochfla	**-0.1040**	**p=0.0001***	**-0.1259**	**p=0.0001***	-0.0348	p=0.2210	**0.1079**	**p=0.0030**	**-0.0862**	**p=0.0170**
Ochnig	-0.0341	p=0.2310	-0.0313	p=0.2710	-0.0456	p=0.1090	0.0220	p=0.5440	-0.0370	p=0.3080
Ochdor	-0.0241	p=0.3970	-0.0417	p=0.1430	**-0.0630**	**p=0.0270**	0.0623	p=0.0860	-0.0582	p=0.1090
Coqric	**0.1267**	**p=0.0001***	**0.1188**	**p=0.0001***	0.0543	p=0.0560	**-0.0995**	**p=0.0060**	-0.0484	p=0.1830
Anomac	**0.1527**	**p=0.0001***	**0.1325**	**p=0.0001***	-0.0556	p=0.0510	0.0430	p=0.2370	**0.2358**	**p=0.0001***
Anocla	0.0131	p=0.6440	**0.0624**	**p=0.0280**	0.0181	p=0.5240	-0.0657	p=0.0700	**-0.1894**	**p=0.0001***
Anomes	0.0446	p=0.1170	0.0475	p=0.0950	0.0042	p=0.8820	**0.0737**	**p=0.0420**	**0.1470**	**p=0.0001***
Anohyr	0.0181	p=0.5260	-0.0156	p=0.5820	0.0355	p=0.2120	0.0641	p=0.0780	0.0565	p=0.1200
Anoalg	0.0635	p=0.0250	0.0126	p=0.6570	-0.0063	p=0.8250	**0.1056**	**p=0.0040**	0.0351	p=0.3340
Anoatr	-0.0245	p=0.3900	-0.0467	p=0.101	**-0.0721**	**p=0.0110**	0.0705	p=0.0520	-0.0484	p=0.1830
Culmod	**0.1301**	**p=0.0001***	**0.1482**	**p=0.0001***	-0.0423	p=0.1370	**0.1206**	**p=0.0010**	**0.1769**	**p=0.0001***
Culpip	-0.0161	p=0.5700	-0.0241	p=0.3960	-0.0095	p=0.7380	-0.0219	p=0.5470	**0.2516**	**p=0.0001***
Culter	0.0514	p=0.0710	**0.0568**	**p=0.0460**	0.0317	p=0.2650	-0.0017	p=0.9620	**0.0903**	**p=0.0130**
Cultor	0.0336	p=0.2380	-0.0221	p=0.4370	-0.0322	p=0.2570	0.0269	p=0.4590	0.0318	p=0.3820
Culhor	-0.0466	p=0.1010	0.0098	p=0.7290	-0.0368	p=0.1960	0.0616	p=0.0900	**0.1468**	**p=0.0001***
Culmor	-0.0325	p=0.2540	0.0298	p=0.2950	0.0106	p=0.7100	**-0.1328**	**p=0.0001***	**-0.0844**	**p=0.0200**
Culmar	-0.0241	p=0.3970	0.0259	p=0.3630	0.0039	p=0.8910	0.0027	p=0.9420	-0.0267	p=0.4620
Culann	-0.0376	p=0.1870	-0.0248	p=0.3840	**0.1142**	**p=0.0001***	-0.0225	p=0.5370	-0.0346	p=0.3410
Uraung	0.0167	p=0.5570	0.0256	p=0.3670	0.0433	p=0.1270	0.0563	p=0.1210	**0.1286**	**p=0.0001***

Table 5

Relations between the presence of the mosquito species and the presence of the plant species based on logistic regression (Simplex method, Chi-square and p values) [Abbreviations of the plant names: Bu_um = *Butomus umbellatus*; Ce_de = *Ceratophyllum demersum*; Hy_mo = *Hydrocharis morsus-ranae*; Le_mi = *Lemna minor*; Le_tr = *Lemna trisulca*; Sa_na=*Salvinia natans*; Sp_po = *Spirodela polyrhiza;* Ty_an=*Typha angustifolia*; Ty_la=*Typha latifolia* Ca_ac = *Carex acutiformis*; Ec_cr=*Echinochloa crus-galli*; Ir_ps=*Iris pseudacorus*; Al_pa = *Alopecurus pratensis*; Ca_se=*Calystegia sepium*; Ly_vi=*Lythrum virgatum*]

Taxon	**Bu_um**	**Ce_de**	**Hy_mo**	**Le_mi**	**Le_tr**	**Sa_na**	**Sp_po**	**Ty_an**	**Ty_la**	**Ca_ac**	**Ec_cr**	**Ir_ps**	**Al_pa**	**Ca_se**	**Ly_vi**
Aedvex	(-)6.0963	(-)32.7069	(-)23.4746	(-)55.0575	(-)19.6958	(-)41.0586	(-)34.3495	(-)14.4852	(-)19.0954	11.8405	(-)5.9299	16.6017			
	p=0.0135000	p=0.0000000	p=0.0000013	p=0.000000	p=0.0000091	p=0.0000000	p=0.0000000	p=0.0001415	p=0.0000125	p=0.0005803	p=0.0148910	p=0.0000462			
Aedcin		(-)15.4365	(-)9.7634	(-)4.8845		(-)26.5510	(-)22.0491			8.7938	(-)8.8025			(-)4.5183	
		p=0.0000855	p=0.0017818	p=0.0271057		p=0.0000003	p=0.0000027			p=0.0030247	p=0.0030103			p=0.0335400	
Aedros				(-)17.9461											5.7163
				p=0.0000228											p=0.0168135
Ochexc			(-)8.2377					(-)6.1311		7.4455					
			p=0.0041055					p=0.0132872		p=0.0063629					
Ochann	(-)27.2667	(-)34.9108	(-)47.6154	(-)24.7676	(-)4.6987	(-)36.1478	(-)40.1551		(-)19.9217	117.2044	(-)20.4100	18.1859	(-)4.8272	(-)22.3906	
	p=0.0000002	p=0.0000000	p=0.0000000	p=0.0000007	p=0.0301917	p=0.0000000	p=0.0000000		p=0.0000081	p=0.0000000	p=0.0000063	p=0.0000201	p=0.0280207	p=0.0000022	
Ochcan	(-)9.0572	(-)8.0753	(-)16.2172	(-)5.3664		(-)9.2768	(-)9.3653	(-)8.6260	(-)7.2598	19.1546		13.4197	(-)7.7501	(-)5.9812	(-)10.16572
	p=0.0026186	p=0.0044900	p=0.0000566	p=0.0205335		p=0.0023226	P=0.0022131	p=0.0033163	p=0.0070552	p=0.0000121		p=0.0002494	p=0.0053739	p=0.0144643	p=0.0014321
Ochcat							(-)8.4570			21.3379					
							p=0.0036388			p=0.0000039					
Ochcas	(-)17.5170	(-)11.7131		(-)26.6073	(-)5.2855	(-)11.3927	(-)13.9755		(-)14.2534	(-)17.4612	9.5858	(-)9.0197	75.2097		4.1627
	p=0.0000286	p=0.0006214		p=0.0000003	p=0.0215095	p=0.0007382	p=0.0001855		p=0.0001601	p=0.0000294	p=0.0019626	p=0.0026729	p=0.0000000		p=0.0413303
Ochsti		(-)16.3155	(-)10.0615	(-)18.6662	(-)8.6254	(-)18.7443	(-)18.9305	(-)6.6159		8.4252		6.1946			
		p=0.0000537	p=0.0015154	p=0.0000156	p=0.0033173	p=0.0000150	p=0.0000136	p=0.0101115		p=0.0037030		p=0.0128188			
Ochrus		(-)5.9259				(-)7.5796	(-)12.9721	(-)8.4653		24.7512				(-)9.5049	
		p=0.0149253				p=0.0059063	p=0.0003486	p=0.0036223		p=0.0000007				p=0.0020509	
Ochref										13.9710					
										p=0.0001860					
Ochfla	(-)10.4130	(-)9.9021	(-)12.0111	-17.8610		(-)10.6664	(-)17.9528		(-)15.7637	6.3820		13.2755	6.3498	(-)13.3632	
	p=0.0012525	p=0.0016524	p=0.0005295	p=0.0000238		p=.0010921	p=0.0000227		p=0.0000719	p=0.0115326		p=0.0002693	p=0.0117439	p=0.0002570	
Ochnig													8.1022		
													p=0.0044238		
Ochdor										(-)7.4036			42.5107		
										p=0.0065126			p=0.0000000		
Coqric		6.6864	9.6381	5.0673	22.7713	6.4938	10.2022		51.2584	(-)6.3239					
		p=0.0097190	p=0.0019074	p=0.0243869	p=0.0000018	p=0.0108292	p=0.0014041		p=0.0000000	p=0.0119167					

Table 5

(continued)

Anomac	8.9987 p=0.0027037	54.6141 p=0.0000000	39.8667 p=0.0000000			85.79544 p=0.0000000	50.5309 p=0.0000000	49.4395 p=0.0000000	8.7762 p=0.0030540	(-)98.3445 p=0.0000000	5.8504 p=0.0155782	(-)20.74967 p=0.0000053		10.10287 p=0.0014818	8.4541 p=0.0036446
Anocla	7.8702 p=0.0050285		6.9080 p=0.0085851	15.2297 p=0.0000954	13.2979 p=0.0002661			(-)5.9418 p=0.0147912	8.4850 p=0.0035833		(-)8.1538 p=0.0042997		(-)9.4170 p=0.0021515		(-)15.80606 p=0.0000703
Anomes		5.2951 p=0.0213909		4.5303 p=0.0333074	11.0570 p=0.0008845	8.8465 p=0.0029386	9.7121 p=0.0018232	6.5400 p=0.0105520	10.9502 p=0.0009370	(-)13.8844 p=0.0001947		(-)4.987010 p=0.255452			
Anohyr	5.2501 p=0.0219519	5.1998 p=0.0225958	6.9957 p=0.0081742			12.4545 p=0.0004176		13.0621 p=0.0003018							
Anoalg	5.4917 p=0.0191129	3.9350 p=0.0472980		8.2112 p=0.0041659	11.5364 p=0.0006833			6.6301 p=0.0100310							
Anoatr													22.8075 p=0.0000018		
Culmod	6.9152 p=0.0085503	47.9084 p=0.0000000	62.2562 p=0.0000000	44.2330 p=0.0000000	10.3757 p=0.0012781	73.7738 p=0.0000000	53.5835 p=0.0000000	37.9657 p=0.0000000	23.7387 p=0.0000011	(-)44.6800 p=0.0000000		(-)15.21104 p=0.0000963	(-)9.0701 p=0.0026002	12.2682 p=0.0004614	
Culpip				7.4189 p=0.0064575						(-)27.5562 p=0.0000002	52.6489 p=0.0000000	(-)14.9850 p=0.0001086		15.0357 p=0.0001057	6.9796 p=0.0082482
Culter	15.1416 p=0.0000999	17.2257 p=0.0000333	23.3352 p=0.0000014	18.4784 p=0.0000172		14.4759 p=0.0001422	11.9327 p=0.0005523		5.7293 p=0.0166891				(-)4.468527 p=0.0345325		(-)5.4402 p=0.0196837
Cultor					5.2750 p=0.0216394										
Culhor								12.2031 p=0.0004777			9.8531 p=0.0016969				10.55501 p=0.0011599
Culmor					4.5461 p=0.0330013				9.6124 p=0.0019344						
Culann	5.5486 p=0.0185014			14.5132 p=0.0001394						8.3626 p=0.0038327	(-)8.6260 p=0.0033162	3.9072 p=0.0480865	(-)36.3026 p=0.0000000	14.5833 p=0.0001343	(-)23.9973 p=0.0000010
Uraung						4.6211 p=0.0315877		9.9543 p=0.0016062		(-)27.8091 p=0.0000001	6.3735 p=0.0115879	(-)14.3112 p=0.0001552		9.6211 p=0.0019251	12.0682 p=0.0005136

DISCUSSION

Our results confirmed some former statements about habitat requirements of the mosquito larvae and revealed the most important indicator variables.

Effect of the shading

Our results about affinity to shaded or opened habitats also fit into the former knowledge. It is known that the light requirement characterizes each mosquito species individually. Two species-groups can be separated: group of sunny-preferent taxa and group of shady-preferent taxa (Joy & Clay, 2002; Tóth, 2007). In our samples the sunny-preferent taxa were typical in habitat-types of herb vegetation (HV) and marshy closed vegetation (MV), shady-preferent taxa were typical in habitat-type of forest vegetation (FV).

Effect of the temperature of the breeding water

Our results showed that the univoltine species related to low water temperature. These species hatch usually in early spring, influence of the low temperature is determinant both in their phaenology and longer breeding time (Becker *et al.*, 2003). Further, same species breeds slowly in low temperature and fast in high temperature (Bayoh & Lindsay, 2003; Barton & Aberton, 2005; Tóth, 2007).

Vegetation of the breeding site as a habitat variable

We stated that occurrences of the mosquito species are related to plant species with similar habitat requirements. Based on this, typical mosquito larvae of permanent waters accompanied by pondweeds and other hydrophytic plant species, whereas typical mosquito larvae of temporary waters occur parallel with plant species typical in humid grasslands, or meso-xerophytic meadows.

Cover of the water surface as a limiting factor

Our results showed that the full-covered (mostly by vegetation, *e.g.* pondweeds, lemnas) water surfaces and the large, deep, billowy water bodies are unsuitable for the majority of the mosquito larvae (mainly for the human-biting species). It caused by the biology of mosquitoes: (1) mosquito larvae accommodated to water-life consequentially, so they get oxygen from the air with their siphon (Becker *et al.*, 2003); (2) mosquito larvae are sensible to water movement, because that balks their respiration and kills them (Tóth, 2007).

Effect of water cover

According to our results the period of the water cover (permanent or temporary) plays the most important role in organization of the mosquito assemblages (Table 2). Separation of the species related to this habitat variable based on the relation to dissolved oxygen and the feeding preferences connecting to that. Hatching of *Aedes* and *Ochlerotatus* species takes place at low dissolved oxygen level (Horsfall *et al.*, 1958; Judson, 1960; Becker, 1989). Whereas, hatching of *Anopheles* species related to high level of dissolved oxygen (Surendran & Ramasamy, 2005; Opoku *et al*., 2007). Caused by these differences *Aedes* and *Ochlerotatus* species related to temporary, *Anopheles* species related to permanent, continuously refreshed breeding waters. The water cover period is also important from the egg laying point of view (Schäfer, 2004). Species related to permanent waters are usually multivoltine, which lay their eggs on the water surface. *Aedes* and *Ochlerotatus* species lay their eggs on dry surfaces (usually on wet soil), breeding of these species related to regularly drying zones of marshy vegetation paches and pits of meteoric water.

Determination of the most influential habitat variable

Results of our study marked the period of the water cover as a basic indicator variable of mosquito larval assemblages (Fig. 2). It based on presumably that the period of water cover determines several other habitat variables, like water transparency, presence/absence of pondweed vegetation and percental cover of the water surface. Influence of these parameters was also confirmed by statistical analyses.

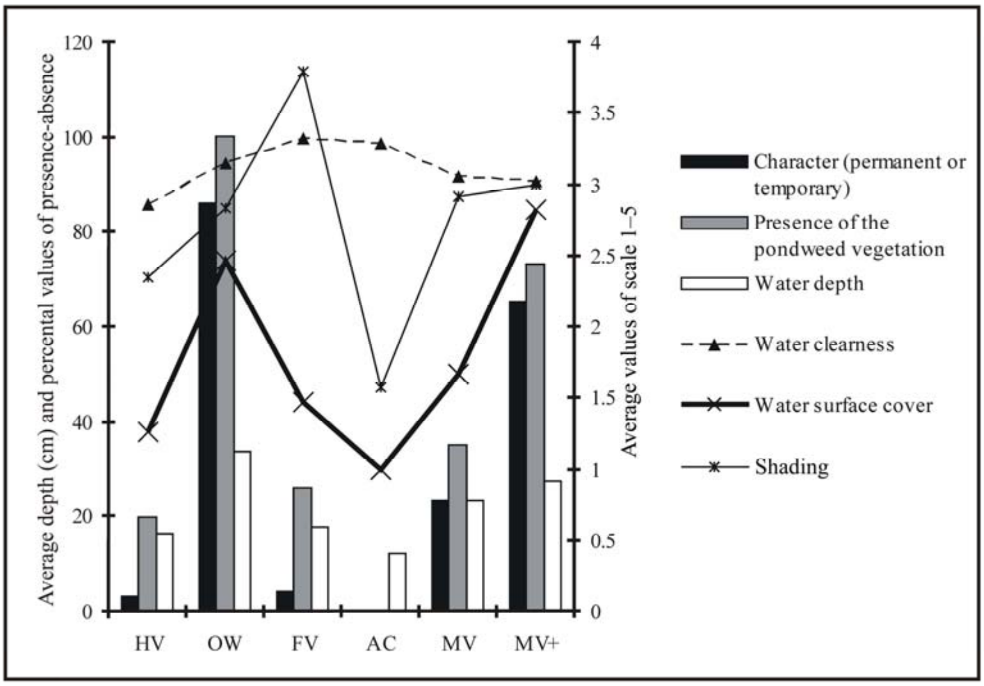


Fig. 2. Basic indicator variables in distribution of the mosquitoe species are related to the regime (permanent or temporary) of the water body [HV: herb vegetation; OW: open water surface; FV: forest vegetation; AC: artificial containers; MV: marshy (reed-bed and sedgy) closed vegetation; MV+: marshy (reed-bed and sedgy) closed vegetation with pondweeds].

Table 6

Significant betas of the multiple regression between densities of the mosquito species and the recorded habitat variables

Species	Altitude	Water depth	Water transparency	Regime (0/1)	Pondweed vegetation	Water surface cover	Shading	Temperature	pH
Aedvex				-0.1046	-0.1172	-0.1012		-0.0043	0.0523
Aedcin			0.0626	-0.0965		-0.0780	-0.0570	0.0041	0.0523
Aedros	-0.0705				-0.0627				0.0519
Ochexc								0.0066	
Ochann	-0.0705		0.1531	-0.0860		-0.1055	0.0864		
Ochcan			0.0935				0.0796	0.0220	0.0601
Ochcat								-0.0637	0.0378
Ochcas	-0.1027			-0.0649	-0.0792	-0.0679		0.0294	0.0653
Ochsti	0.1215		0.0876				0.1108		-0.0126
Ochrus		-0.0704		-0.0538	-0.0713		0.0905		0.0215
Ochref							0.0571	-0.0307	
Ochfla	-0.0801					-0.0688			
Ochnig									
Ochdor	-0.0703						-0.0583	-0.0447	0.0672
Coqric		0.1134	-0.0682	0.1713	0.1236	0.1117		-0.0366	
Anomac	-0.1792	0.0817		0.2380	0.1287	0.0900	-0.0724		0.0239
Anocla	0.1793	0.1336	0.1153	0.1375					-0.0309
Anomes				-0.0837				0.0061	0.0443
Anohyr				-0.0651		-0.0765	0.0586		
Anoalg									
Anoatr							-0.0637	-0.0346	
Culmod	-0.0889			0.0967	0.0949	0.1266			
Culpip						-0.0565			-0.0322
Culter		0.0590		0.0716	0.0600			0.0432	-0.0073
Cultor									
Culhor				-0.0477	-0.0711				0.0677
Culmor	0.0530							-0.0408	
Culmar	-0.0632								
Culann	0.1181						0.1075	0.0079	-0.0071
Uraung	-0.0931							0.0314	0.0293

CONCLUSIONS

Colonization of the mosquito larvae is influenced by the period of the water cover basically. Multicollinearity with the period of the water cover was revealed in connection with some other influental habitat variables: mosquito species related to ephemeral waters are usually related to clear waters, absence of pondweed vegetation and minimal cover of the water surface. On the contrary, mosquito species related to permanent waters are usually related to less water transparency, presence of pondweed vegetation and increased cover of the water surface. Dominat human-biting mosquito species (e.g. *Aedvex, Ochsti, Ochann, Ochcan, Aedros*) usually breed in shallow, quick-warm, regularly drying, clear water bodies with minimal cover of the water surface and without presence of hydrophyta plant species. These habitats occur both in shaded and lightened places.

Our results show that larval habitats of the human-biting mosquitoes separate unequivocally from the habitats irrelevant from biological control point of view. It gives good basis for the more precise mapping and detection of the target areas, and for the environmentally friendly, economically rational BTI-treatments.

Acknowledgements. The research was supported by the National Development Agency (Hungary) (INNO-7-2006-0002 OMFB-01516/2006). We are grateful to Mrs. Lena Liebermann for the revision of the English translation.

REFERENCES

ALFONZO D., GRILLET M.E., LIRIA J., NAVARRO J-C., WEAVER S.C. & BARRERA R., 2005, *Ecological Characterization of the Aquatic Habitats of Mosquitoes (Diptera: Culicidae) in Enzootic Foci of Venezuelan Equine Encephalitis Virus in Western Venezuela*. Journal of Medical Entomology, **42** (3): 278–284.

AZARI-HAMIDIAN S., 2007, *Larval Habitat Characteristics of Mosquitoes of the Genus Culex (Diptera: Culicidae) in Guilan Province, Iran*. Iranian Journal of Arthropoda-Borne Diseases, **1** (1): 9–20.

BARTON P.S. & ABERTON J.G., 2005, *Larval Development and Autogeny in Ochlerotatus camptorhynchus (Thomson) (Diptera: Culicidae) from Southern Victoria*. Proceedings of the Linnean Society of New South Wales, **126**: 261–267.

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

BAYOH M.N. & LINDSAY S.W., 2003, *Effect of temperature on the development of the aquatic stages of Anopheles gambiae sensu stricto (Diptera: Culicidae)*. Bulletin of Entomological Research, **93**: 375–381.

BECKER N., 1989, *Life strategies of mosquitoes as an adaptation to their habitats*. Bulletin of the Society of Vector Ecology, **14**: 6–25.

BECKER N., PETRIC D., ZGOMBA M., BOASE C., DAHL C., LANE J. & KAISER A., 2003, *Mosquitoes and their control*. Kluwer Academic/Plenum Publishers, New York, 498 pp.

BRADSHAW W.E. & HOLZAPFEL C.M., 1991, *Fitness and habitat segregation of British tree-hole mosquitoes*. Ecological Entomology, **16**(2): 133–144.

DAHL C. & BLACKMORE M.S., 2001, *The distribution and status of Ochlerotatus geniculatus (Olivier) in Fennoscandia*. European Mosquito Bulletin, **9**: 12–16.

FISCHER S. & SCHWEIGMANN N., 2004, *Culex mosquitoes in temporary urban rain pools: Seasonal dynamics and relation to environmental variables*. Journal of Vector Ecology, **29** (2): 365–373.

HAMMER Ø., HARPER D.A.T. & RYAN P.D., 2001, *PAST: Paleontological Statistics Software Package for Education and Data Analysis*. Palaeontologia Electronica, **4** (1): 1–9.

HORSFALL W.R., LUM P. & HENDERSON L., 1958, *Eggs of floodwater mosquitoes. V. Effect of oxygen on hatching of insect eggs*. Annales of the Entomological Society of America, **51**.

JONES C.J. & SCHREIBER E.T., 1994, *Color and height affects oviposition site preferences of Toxorhynchites splendens and Toxorhynchites rutilus rutilus (Diptera: Culicidae) in the laboratory*. Environmental Entomology, **23**: 130–135.

JOY J.E. & CLAY J.T., 2002, *Habitat Use by Larval Mosquitoes in West Virginia*. American Midland Naturalist, **148** (2): 363–375.

JUDSON C.L., 1960, *The physiology of hatching of aedine mosquito eggs: Hatching stimulus*. Annales of the Entomological Society of America, **53**.

McDANIEL I.N., BENTLEY M.D., LEE H.-P. & YATAGAI M., 1976, *Effects of color and larval-produced oviposition attractants on oviposition of Aedes triseriatus*. Environmental Entomology, **5**: 553–556.

MORRIS D.W., 2003, *Toward an ecological synthesis: a case for habitat selection*. Oecologia, **136**: 1–13.

OPOKU A.A., ANSA-ASARE O.D. & AMOAKO J., 2007, *The Occurrences and Habitat Characteristics of Mosquitoes in Accra, Ghana, West Africa*. Journal of Applied Ecolology, **11**: 81–86.

PARADISE C.J., 2000, *Effects of pH and resources on a processing chain interaction in simulated treeholes*. Journal of Animal Ecology, **69**: 651–658.

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**: 4–19.

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**.

SOTA T., MOGI M. & HAYAMIZU E., 1994, *Habitat stability and the larval mosquito community in treeholes and other containers on a temperate Island*. Researches on Population Ecology, **36** (1): 3–104.

STATSOFT INC., 1995, *STATISTICA for Windows (Computer program manual)*. StatSoft, Inc., 2325 East 13th Street, Tulsa.

SURENDRAN S.N. & RAMASAMY R., 2005, *Some characteristics of the larval breeding sites of Anopheles culicifacies species B and E in Sri Lanka*. Journal of Vector Borne Diseases, **42**: 39–44.

TÓTH S., 2004, *Mosquito Fauna of Hungary (Diptera: Culicidae)*. Natura Somogyiensis 6, Somogy Megyei Múzeumok Igazgatósága, Kaposvár (In Hungarian), 327 pp.

TÓTH S., 2007, *Identification keys to Mosquitoes I. (Larvae)*. Pannónia Füzetek 1, Pannónia Központ Kft., Keszthely (In Hungarian), 96 pp.

WILLIAMS R.E., 1962, *Effect of coloring oviposition media with regard to the mosquito Aedes triseriatus (Say)*. Journal of Parasitology, **48**: 919–925.

Received July 2, 2011

[*] *Acrida Conservational Research L.P., H-8300 Tapolca,*
Deák F. u. 7., Hungary,
e-mail: kenyeres.zol@gmail.com
[**] *Department of Botany, Hungarian Natural History Museum,*
H-1087 Budapest, Könyves K. krt. 40., Hungary.
e-mail: bauer@bot.nhmus.hu
[***] *H-8420 Zirc, Széchenyi u. 2., Hungary,*
e-mail: flycatcher@vnet.hu
[****] *St-Art Chem L.P., H-8360 Keszthely, Vak Bottyán u. 37.,*
Hungary,
e-mail: tamas@saringer.hu

POPULATION CHARACTERISTICS OF *PORCELLIUM COLLICOLA* (VERHOEFF, 1907) AND *TRACHELIPUS ARCUATUS* (BUDDE-LUND, 1885) (ISOPODA: ONISCIDEA) INHABITING THE LEAF-LITTER OF SOME OAK FORESTS FROM SOUTHERN ROMANIA

FINICA MARIANA IVANOV

The population characteristics of two terrestrial isopod species – *Porcellium collicola* and *Trachelipus arcuatus*, inhabiting the leaf-litter of two oak forest from Vlăsia plain (Southern Romania) are presented. The structure of isopod populations was analyzed based on the types of individuals identified in population (males, non-reproductive females, ovigerous females, post-ovigerous females and post-marsupial mancas) and the sex ratio. The percentage of different types of individuals in studied populations was correlated to the breeding period and the recruitment of the new individuals in population. The ratio of males to females fluctuated throughout the sampling period. The overall sex ratio was in favour of females in almost all studied populations.

Key words: *Porcellium collicola*, *Trachelipus arcuatus*, population structure, sex ratio, oak forests, Southern Romania.

INTRODUCTION

Terrestrial isopods (Isopoda: Oniscidea) are the only group of crustaceans fully adapted to terrestrial life. They are important macro-detritivores in many terrestrial ecosystems, contributing directly and indirectly to decomposition processes (Zimmer, 2004). Population characteristics were studied in different species of terrestrial isopods, as *Armadillidium vulgare* (Paris & Pitelka, 1962), *Protracheoniscus politus* (Radu & Tomescu, 1972), *Armadillo officinalis* (AlJetlawi & Nair, 1994), *Porcellionides pruinosus* (Achouri *et al*., 2003), *Armadillidium pelagicum* (Hamaied & Charfi-Cheikhrouha, 2004; Hamaied *et al*. 2004) and *Atlantoscia floridana* (Araujo & Bond-Buckup, 2005).

Terrestrial isopod species from Romania fauna were studied primary from a taxonomical standpoint. Ecological studies were performed in the second half of the 20th century and at the beginning of the 21st century, most of them as research themes in PhD programs that finalized with the publication of their results (Hotea *et al*., 2003). There are relatively few studies on population characteristics of terrestrial isopod species from Romania, most of them referring to the sex ratio (Accola *et al*., 1993; Dolnițchi-Olariu & Tomescu, 1997; Mureşan *et al*., 2003; Tomescu *et al*., 2000, 2001, 2008). A complex study was performed in a

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° *2*, P. 163–178, BUCHAREST, 2011

Protracheoniscus politus population from a deciduous forest in Western Romania (Radu & Tomescu, 1972).

The present study was carried out in two oak forests from Vlăsia plain (Southern Romania), in which there were identified two terrestrial isopod species from Family Trachelipodidae: *Porcellium collicola* (Verhoeff, 1907) and *Trachelipus arcuatus* (Budde-Lund, 1885). This paper presents the population structure of *Porcellium collicola* and *Trachelipus arcuatus* in the study zone based on the types of individuals identified in population and the sex ratio.

MATERIAL AND METHODS

The two studied forests, named Brânzeasca and Moara Vlăsiei, are located near Bucharest (Fig. 1), in forest steppe. Brânzeasca is a *Quercus frainetto* wood, while Moara Vlăsiei is a *Quercus robur* – *Q. pedunculiflora* forest (Doniţă *et al.*, 1990). The study area is located in medium flat plain, at an elevation of 90 meters. The climate is continental, with July average temperature 23 °C and January average temperature – 2.9 °C. The mean annual precipitation is 550 mm and the average monthly precipitations are minimal in February-March and October-November and maximal in June. In Brânzeasca the soil is clay chernozem, while in Moara Vlăsiei the soil is cambic chernozem.

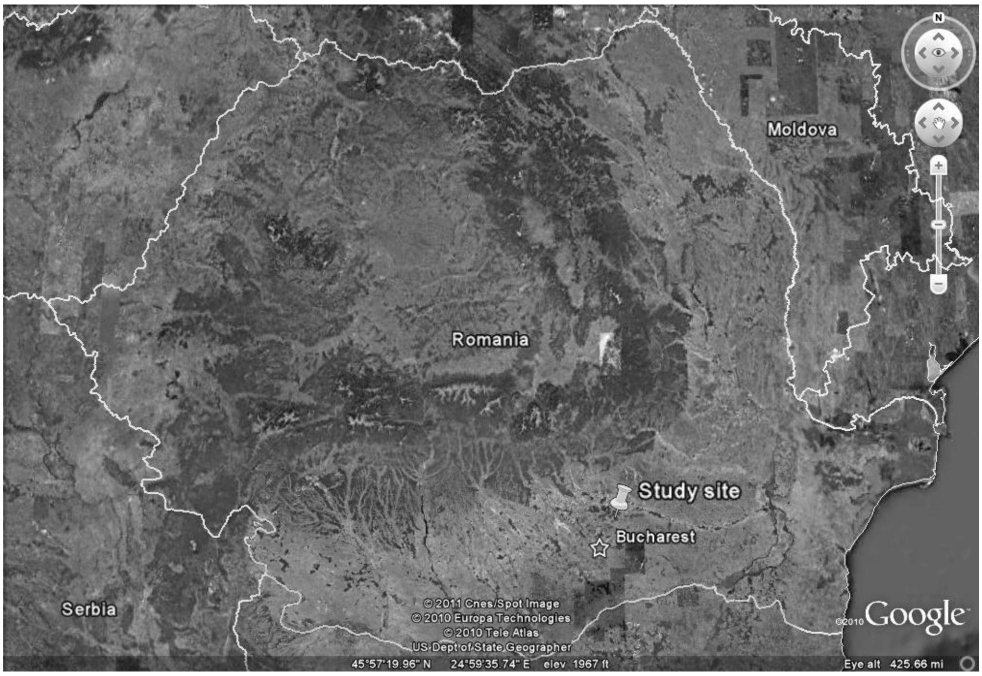


Fig. 1. Location of the study site in Romania (modified from www.earth.google.com).

In the two studied forests there were selected four stands and within them there were randomly chosen five perimeters of 10 square meters: two in Brânzeasca, named stations 1 (44°39’09.1”N, 26°15’35.7”E) and 2 (44°38’52.7”N, 26°15’18.7”E), and three in Moara Vlăsiei, named stations 3 (44°37’28.4”N, 26°13’24.9”E), 4 (44°37’29.2”N, 26°13’18.6”E) and 5 (44°37’21.3”N, 26°13’09.3”E) (Fig. 2).

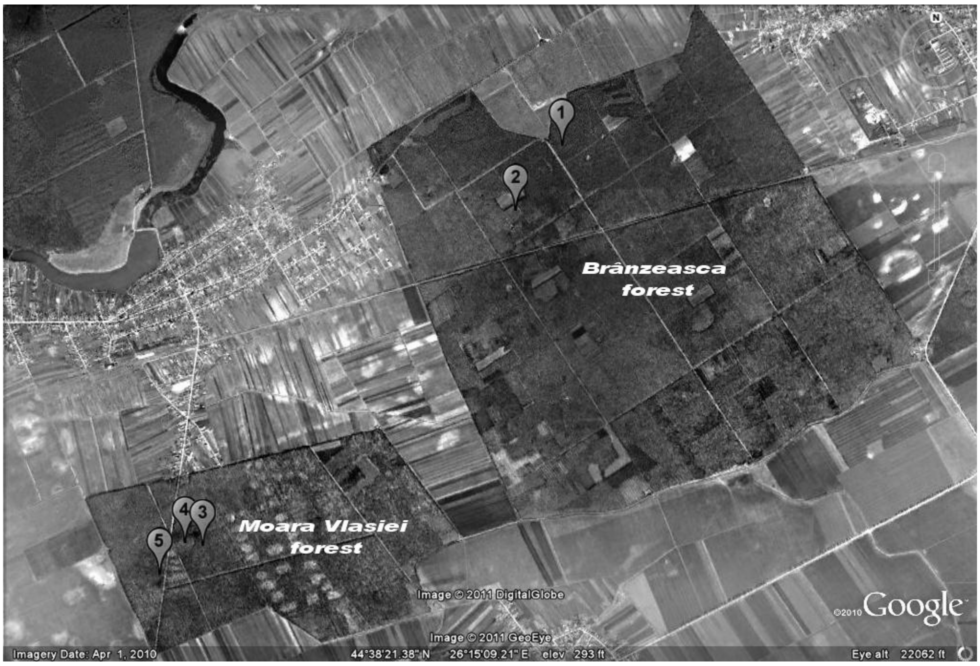


Fig. 2. Location of the stations in the studied forests (modified from www.earth.google.com).

In station 1, located in a 63 years native stand, the tree layer is composed of *Quercus frainetto*, *Q. pedunculiflora* and *Acer platanoides*, the shrubs layer consists of *Crataegus monogyna* and *Cornus sanguinea*, and the herbaceous layer is composed of *Galium aparine*, *Urtica dioica*, *Lamium album*, *Alliaria officinalis* and *Polygonatum officinale*. Station 2 was located in a 30 years plantation of *Quercus pedunculiflora* and *Acer platanoides*, with the shrubs layer consisted of *Cornus sanguinea*, and the herbaceous layer composed of *Geum urbanum*, *Bromus sterilis* and *Lysimachia vulgaris*.

In station 3, located in an 81 years native stand, the tree layer is composed of *Quercus pedunculiflora*, *Q. robur* and different species of hard wood (maple, hornbeam, etc.), the shrubs layer consists of *Crataegus monogyna* and *Cornus sanguinea*, and the herbaceous layer is composed of *Galium aparine*, *Lamium album* and *Polygonatum officinale*. Station 4 was placed in the stand corresponding to station 3, in an area subjected for almost 10 years to progressive cutting of *Quercus robur*. This station was distanced at more than 50 meters from station 3 to ensure statistical independence (Magura *et al.*, 2000). In station 5, located in a 65 years native stand, the tree layer is composed of *Quercus pedunculiflora*, *Q. robur* and *Q. frainetto*, the shrubs layer consists of *Crataegus monogyna* and *Cornus sanguinea*, and the herbaceous layer is composed of *Galium aparine*, *Geum urbanum* and *Erodium cicutarium*. The distance between the sampling areas from the two studied forests was approximately 4 km (Fig. 2).

Terrestrial isopods were collected using two methods: pitfall traps and isopods extraction from leaf-litter samples using Berlese-Tullgren devices. In each station were placed ten pitfall traps, arranged in a grid, with a 1.5 meters distance between the lined traps. The containers were filled with a mixture of ¾ 40% ethylene glycol and ¼ 4% formalin. The content of the traps was removed monthly, from 20th of April 2008 to 7th of May 2009 (except for December 2008 and January 2009). From each studied natural stand (stations 1 and 3) were also collected monthly 10 leaf-litter samples, from May till October 2008. Each sample was obtained by random application, inside and outside the perimeter of each station, of a 40x40 centimetres frame. This represents the recommended area in terrestrial isopods studies (Radu & Tomescu, 1972; Paoletti & Hassall, 1999). In laboratory, the leaf-litter samples were placed in Berlese-Tullgren devices, where they were maintained for five days.

Isopods from all samples were identified using Radu (1985) and Schmidt (1997), counted and sexed. The material consisted of 6.320 specimens from pitfall traps and 228 specimens from leaf-litter samples. Five types of individuals were identified, mainly based on secondary sexual characters: (1) males (recognised by the presence of genital apophyses and copulative stylets), (2) non-reproductive females (without genital apophyses and brood pouch), (3) ovigerous females (with brood pouch containing eggs, embryos or larvae), (4) post-ovigerous females (with empty brood pouch) and (5) post-marsupial mancas (recognized by the small size and the absence of secondary sexual characters) (Achouri *et al.*, 2003; Hamaied & Charfi-Cheikhrouha, 2004; Araujo & Bond-Buckup, 2005), the first developmental stage of terrestrial isopods out of marsupial pouch (Tomescu & Crăciun, 1987).

In case of small specimens (juveniles) of *Porcellium collicola*, it was hard to differentiate between non-reproductive females and post-marsupial mancas, because the presence of secondary sexual characters in immature individuals is evident only in males. The male sexual characters were visible only in individuals of minimum 2.0 mm in length, so that the specimens without secondary sexual characters and more than 2.0 mm in length were considered non-reproductive females, and the specimens of less than 2.0 mm in length were classified as post-marsupial mancas.

The sex ratio was estimated by the ratio of males to females for the entire period of the study and for each sampling period. The observed and expected values were compared using Chi-square (χ^2) test (Aljetlawi & Nair, 1994; Paoletti & Cantarino, 2002; Hamaied & Charfi-Cheikhrouha, 2004; Araujo & Bond-Buckup, 2005; Achouri *et al.*, 2008). This test allows the identification of a significant deviation from the theoretic 1:1 ratio. The results were considered statistically significant if the calculated value of the probability to reject the null hypothesis was less than 0.05. The analyses were implemented using the Microsoft Excel program.

RESULTS

The characteristics of terrestrial isopod populations from the leaf-litter of the studied forests were established based on data obtained from pitfall traps. Although the extraction of isopods from leaf-litter samples allows a more exact characterisation of isopod populations, only pitfall traps method provided a sufficient number of individuals for population analysis. Thus, the density of studied isopod populations could not be estimated.

Population structure on types of individuals

The structure of isopod populations was analysed only for the stations from where a large number of specimens was collected: station 1, 3, 4 and 5 for *Porcellium collicola* and station 2 for *Trachelipus arcuatus*.

Population structure of *Porcellium collicola* was similar for all the analysed stations (Figs. 3-6). The males had the highest percentage in population in April-May and September-October. Non-reproductive females were well represented in population in April-May and from September to November. A gradual reduction of the percentage of non-reproductive females from April to July was observed.

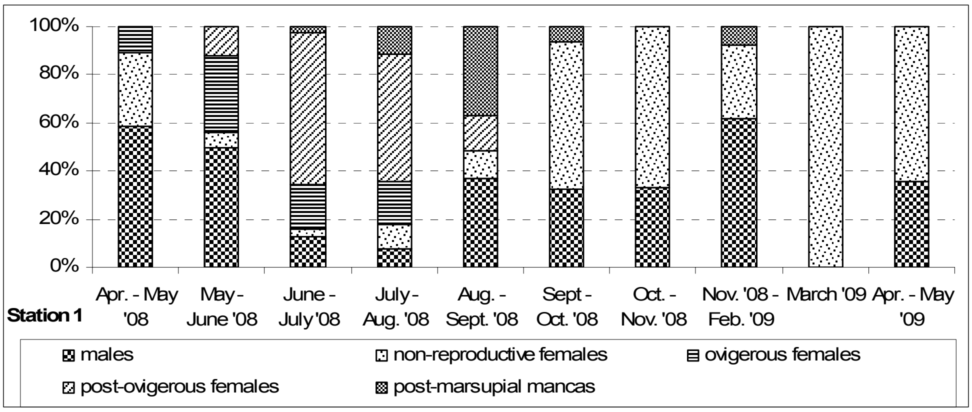


Fig. 3. The structure of *Porcellium collicola* population in station 1.

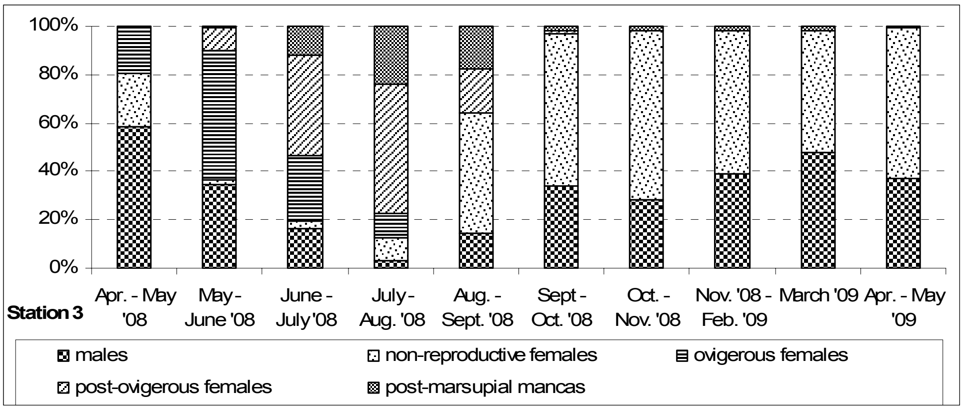


Fig. 4. The structure of *Porcellium collicola* population in station 3.

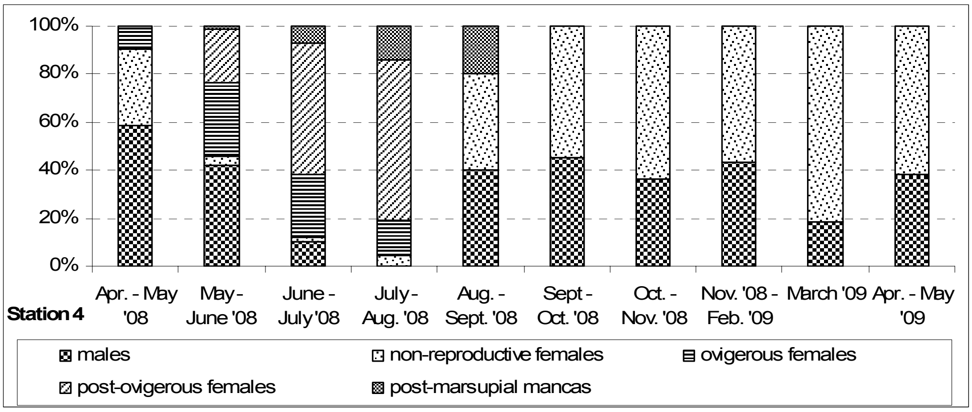


Fig. 5. The structure of *Porcellium collicola* population in station 4.

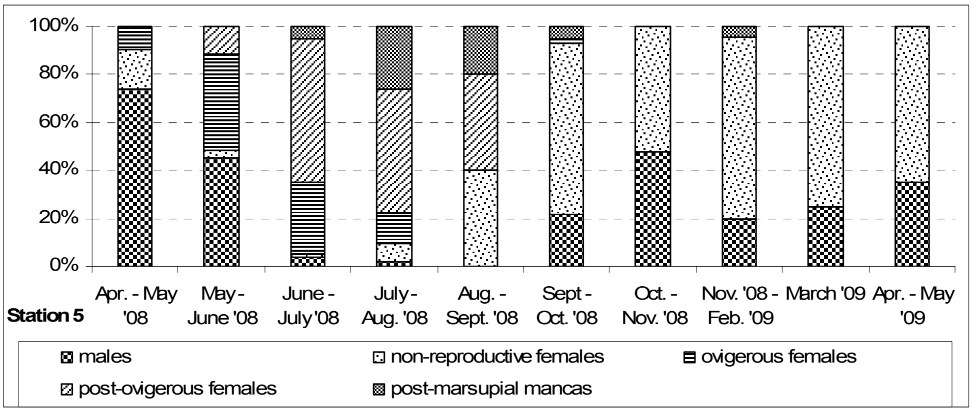


Fig. 6. The structure of *Porcellium collicola* population in station 5.

Ovigerous females of *P. collicola* were present in population from April-May to July-August, except for station 5, from where one ovigerous female was captured in September-October. The highest percentage of the ovigerous females in population was recorded in May-June, a month after their appearance in captures. Post-ovigerous females were first collected a month after the appearance of ovigerous females in captures (May-June) and had the highest percentage in population during the summer period (from June to August). These were the last reproductive females that were present in a population. The data obtained by extracting the isopods from leaf-litter samples collected in station 1 and 3 showed the presence of the post-ovigerous females in a population beginning with 20th of May. The absence of the post-ovigerous females from pitfall traps in April-May could be explained by the reduced mobility of females after brood release.

Post-marsupial mancas of *P. collicola* appeared in captures in the same period with post-ovigerous females (May-June) in stations 3 and 4, and a month after the capture of the first females with empty brood pouch (June-July) in stations 1 and 5. The highest percentage of post-marsupial mancas in captures was recorded from July to September.

During the autumn and winter periods, as well as at the beginning of the next spring, the females were dominated in *P. collicola* populations. In April-May 2009 the ovigerous females were absent in captures from station 1, 4 and 5, while in station 3 were collected only two such specimens.

The population structure of *Trachelipus arcuatus* (Fig. 7) was analysed using only four types of individuals; post-marsupial mancas were not collected. The highest number of *T. arcuatus* specimens in captures was recorded from May to August, when in population were present all the identified types of individuals: males had a percentage that varied between 20% and 36%; non-reproductive females were present during the entire period, being more abundant in May-June; ovigerous and post-ovigerous females had the highest percentage in June-July (30%). Post-ovigerous females appeared in captures simultaneous with ovigerous females (May-June), and were present in population until the beginning of fall (September-October).

Sex ratio

Sex ratio is the number of males to females and varies between 0 (no males) to a large number (many males per females). In station 1, the sex ratio of *Porcellium collicola* varied between 0.10 and 2.00. It was in favour of males in April-May, August-September and November-February and in favour of females in the other sampling periods. The sex ratio deviated significantly from 1:1 proportion in April-May 2008 and 2009, June-July and July-August (Fig. 8). The overall ratio of males to females over the entire period of study was female-biased (0.81) and was statistically significant ($\chi^2 = 19.11$, $p < 0.0001$).

In station 2, from where was collected the smallest number of *P. collicola* specimens (26), the sex ratio was male-biased in April-May in both studied years, and female-biased in June-July. It deviated significantly from the expected values only in April-May 2008 (Fig. 9). The overall sex ratio over the entire period of study was in favour of males (3.33) and was statistically significant (χ^2 = 7.58, p = 0.0059).

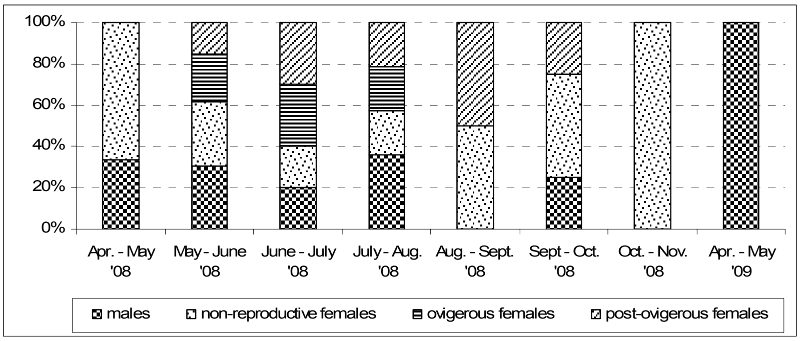


Fig. 7. The structure of *Trachelipus arcuatus* population in station 2.

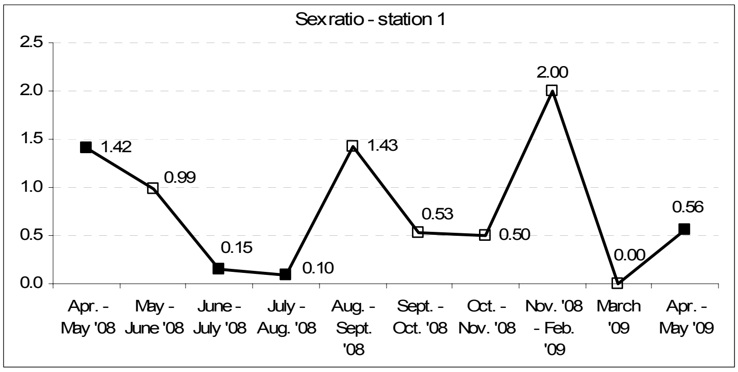


Fig. 8. The variation of sex ratio in *Porcellium collicola* population from station 1. The empty squares indicate significant deviations from 1:1 proportion.

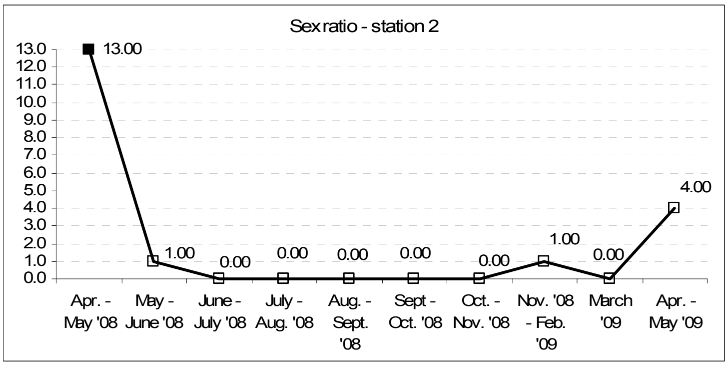


Fig. 9. The variation of sex ratio in *Porcellium collicola* population from station 2. The empty squares indicate significant deviations from 1:1 proportion.

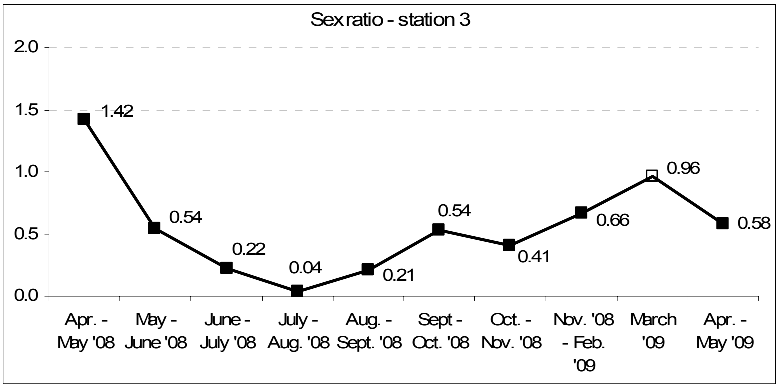


Fig. 10. The variation of sex ratio in *Porcellium collicola* population from station 3. The empty squares indicate significant deviations from 1:1 proportion.

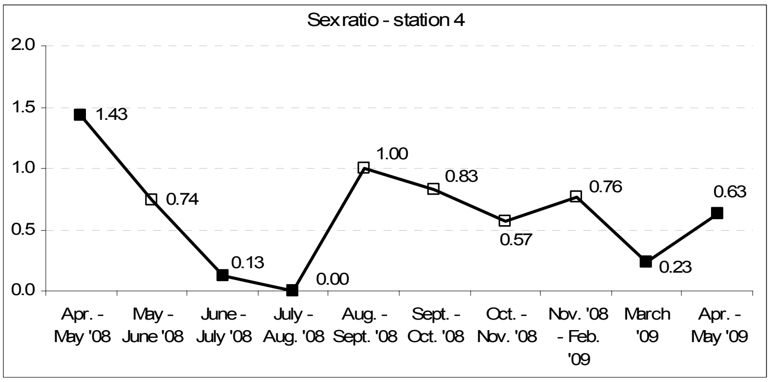


Fig. 11. The variation of sex ratio in *Porcellium collicola* population from station 4. The empty squares indicate significant deviations from 1:1 proportion.

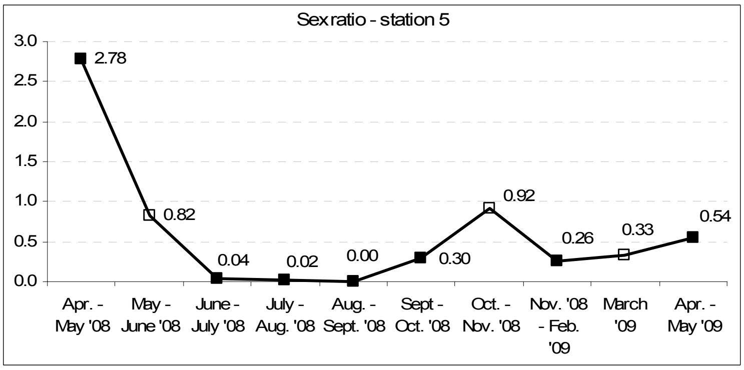


Fig. 12. The variation of sex ratio in *Porcellium collicola* population from station 5. The empty squares indicate significant deviations from 1:1 proportion.

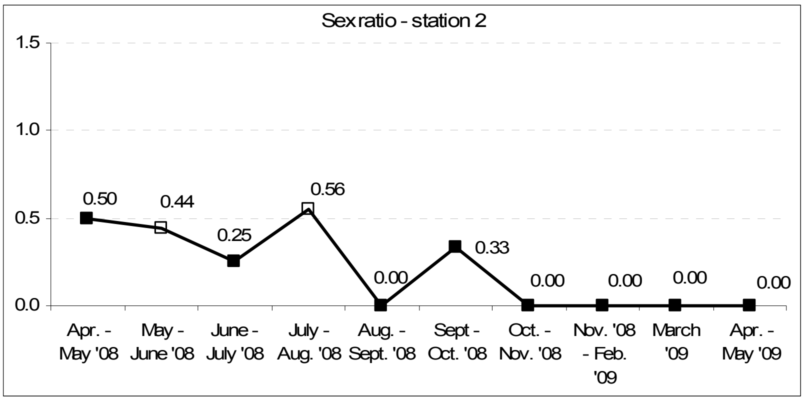


Fig. 13. The variation of sex ratio in *Trachelipus arcuatus* population from station 2. The empty squares indicate significant deviations from 1:1 proportion.

In station 3, from where was captured the highest number of *P. collicola* individuals, the sex ratio fluctuated between 0.04 and 1.42 and it was in favour of males only in April-May 2008. Females outnumbered the males in the other sampling periods, except for March 2009 when the sex ratio was very close to 1:1. The sex ratio deviated significantly from 1:1 proportion in all sampling periods, except for March 2009 (Fig. 10). The overall ratio of males to females over the entire period of study was female-biased (0.55) and was statistically significant ($\chi^2 = 167.08$, $p < 0.0001$).

In station 4, the sex ratio varied between 0.13 and 1.43. The males outnumbered the females in April-May 2008, while females predominate over males in the other sampling periods, except from July to September 2008 when the sex ratio was 1:1. The χ^2 test showed significant differences between males and females in population during April-May 2008 and 2009, June-July and July-August (Fig. 11). The overall sex ratio over the entire period of study was in favour of females (0.83) and was statistically significant ($\chi^2 = 7.19$, $p = 0.0073$).

In station 5, the ratio of males to females fluctuated between 0.02 and 2.78. The sex ratio was male-biased in April-May 2008. After that period the percentage of males reduced gradually, so that from the end of May to the next spring the sex ratio was in favour of females. The sex ratio deviated significantly from the expected values throughout the sampling period, excepting May-June and October-November 2008 and March 2009 (Fig. 12). The overall ratio of males to females over the entire period of study was females-biased (0.64) and was statistically significant ($\chi^2 = 23.01$, $p < 0.0001$).

In *Trachelipus arcuatus* population from station 2 the sex ratio varied between 0.25 and 0.56. In August-September and October-November 2008 were captured only females. The activity of this species was interrupted during winter period and at the beginning of the next spring. The sex ratio was in favour of females over the entire activity period of the species. The χ^2 test showed significant differences between males and females in population during almost all sampling periods, excepting May-June and July-August (Fig. 13). The overall sex ratio over the entire period of study was in favour of females (0.38) and was statistically significant ($\chi^2 = 48.13$, $p = 0.0001$).

DISCUSSION

In *Porcellium collicola* populations from the study zone, the high percentage of males in April-May was associated with the beginning of the reproductive season, when sexually mature individuals participate in copulation, and the high percentage of males in September-October was correlated to the presence of individuals from the new generation that begin to differentiate the secondary sexual characters. Secondly, the high percentage of non-reproductive females in April-May was determined by the presence of immature females that did not reach the minimum size to participate to reproduction, while the high percentage of this type of females during the autumn months was determined mainly by the presence of the immature females from the new generation. The gradual reduction of the percentage of non-reproductive females from April to July, that corresponded to the first part of the reproductive season, suggests that females participate to reproduction gradually, as they reach the minimum size for reproduction. In terrestrial isopods, the participation of females to reproduction depends on reaching a minimum body size (Zimmer, 2004).

The highest percentage of *P. collicola* ovigerous females in studied populations corresponded to the first part of the reproductive season. The absence of the ovigerous females from captures in April-May 2009 was probably determined by the reduced mobility of females after the eggs deposition in the brood pouch, that take place shortly after the pre-reproductive moult (Johnson, 1985; Warburg, 1994).

The highest percentage of *P. collicola* post-marsupial mancas in captures corresponded to the second part of the breeding season. The young stages of terrestrial isopods have a low mobility (Dolnițchi-Olariu & Tomescu, 1997; Zimmer, 2004), so that the entering of the new individuals in population cannot be exactly established based on the presence of post-marsupial mancas in pitfall traps, which are activity traps. The capturing rate of pitfall traps depends on activity/mobility of the animals (Southwood & Henderson, 2000; Thomas *et al.*, 2006) and the way in which their mobility/activity is influenced by the habitat characteristics, as temperature, humidity, water content of the soil, plant coverage etc. (Hornung, 1989; Tomescu *et al.*, 1995; Santos *et al.*, 2007).

The highest number of *Trachelipus arcuatus* specimens was recorded in a period that corresponded with the reproductive season. In April-May 2008 and from August 2008 to May 2009 the number of *T. arcuatus* specimens collected was small, so that the percentage of the types of individuals in captures indicated rather the activity pattern of the species than the population structure.

The study of sex ratio in bisexual species provides information regarding their reproductive potential. In case of the species where the males mates only once or a few times, the reproductive potential is dependent on the numerical equilibrium of the two sexes in population (Accola *et al.*, 1993). In the present study, the sex ratio in *Porcellium collicola* and *Trachelipus arcuatus* populations varied greatly along the annual activity period.

These results suggest that the sex ratio studies should consider the temporal variability of this parameter and not rely upon punctual sampling to obtain a realistic picture, a fact also remarked for *Armadillidium vulgare* and *Trachelipus rathkei* (Paoletti & Cantarino, 2002).

The overall sex ratio over the entire study period indicated the predominance of females in populations, excepting station 2, from where was collected the smallest number of *Porcellium collicola* specimens. In almost all studied terrestrial isopod species the females outnumber males (Paris & Pitelka, 1962; Hamaied & Charfi-Cheikhrouha, 2004; Achouri *et al.*, 2003, 2008). The numerical dominance of females may represent an advantage by increasing the reproductive potential of the species (Dolnițchi-Olariu & Tomescu, 1997; Tomescu *et al.*, 1992, 2000), being known that in terrestrial isopods the mortality rate is very high (over 98%) in juvenile stages (Tomescu *et al.*, 2001).

Predominance of females has been observed in other *Porcellium collicola* populations (Tomescu *et al.*, 1992, 2008) and other terrestrial isopod species from Romanian fauna (Dolnițchi-Olariu & Tomescu, 1997; Tomescu *et al.*, 1992, 2000, 2001; Mureşan *et al.*, 2003), including species inhabiting leaf-litter, as *Protracheoniscus politus* (Radu & Tomescu, 1972; Tomescu *et al.*, 2008).

In this study, the variation of the sex ratio in *Porcellium collicola* populations indicated that throughout the year the females outnumbered males, excepting April-May that corresponded to the peak of the reproductive activity. Similar results were obtained for *Protracheoniscus politus* (Radu & Tomescu, 1972) and *Ligidium hypnorum* populations (Tomescu, 1973; Tomescu *et al.*, 1992).

The genetically determined sex ratio is 1:1 (males: females). In populations of very young terrestrial isopod of various species the sexes are approximately equal, even though later in life one of the sexes may become predominant (Paris & Pitelka, 1962; Radu & Tomescu, 1972; Aljetlawi & Nair, 1994; Achouri *et al.*, 2008). There are terrestrial isopod species, such as *Schizidium tiberianum*, where the percentage of adult males is less than 10% in natural population even though the sex ratio of the juvenile brood is 1:1, and species where a sex ratio of 1:1 is maintained in the natural populations even at old ages (Warburg, 1994).

Although the number of females in terrestrial isopod populations is generally greater than the number of males (Paris & Pitelka, 1962; Radu & Tomescu, 1972;

Aljetlawi & Nair, 1994; Hamaied & Charfi-Cheikhrouha, 2004; Achouri *et al*., 2003, 2008), the sex ratio may change seasonally, possibly owing to the different mortality rate of males and females during the life cycle (Paris & Pitelka, 1962; Dolnițchi-Olariu & Tomescu, 1997; Achouri *et al*., 2008). The mortality rate of the two sexes is influenced by the behavioural differences between males and females during mating season, by the physiological state after reproduction (Tomescu *et al*., 1992, 2008) or the age of the individuals (Aljetlawi & Nair, 1994; Tomescu *et al*., 2008). Thus, the ovigerous females of *Ligidium hypnorum* feed very little and remain hidden during gestation, while the males are more mobile in the breeding period and thus more exposed to predators (Tomescu *et al*., 1992). The gravid females of *Armadillidium vulgare* exhibit a considerably reduced feeding rate and often may not come to the soil surface thus reducing their exposure to predation and desiccation (Lawlor, 1976). In *Protracheoniscus politus*, Radu & Tomescu (1972) observed that a large number of males died shortly after they participate to reproduction and only a small number of them remain in population. In some species, as *Armadillidium vulgare* (Paris & Pitelka, 1962), *Porcellionides sexfasciatus* and *P. pruinosus* (Achouri *et al*., 2008), the sex ratio may modify as a consequence of increasing females mortality after the breeding period, either immediately after brood release or during the post-parturial moult (Paris & Pitelka, 1962).

In the present study, the increased percentage of males in *Porcellium collicola* populations in the first part of the reproductive season may be determined by the increased mortality of the ovigerous females during pre-reproductive moult. An animal in moult, being immobilised for several hours, becomes more susceptible to unfavourable changes of microclimate conditions and therefore more exposed to desiccation (Paris & Pitelka, 1962; Accola *et al*., 1993). In this period mortality rate is higher, especially in unfavourable climatic conditions (Accola *et al*., 1993).

The predominance of males in *Porcellium collicola* populations in April-May, which corresponded to the beginning of the breeding season, may also be the result of the sampling method that depends on animal's mobility. During mate period, males have a greater mobility due to the active female-searching behaviour (Paoletti & Cantarino, 2002). After mating and eggs deposition in marsupial pouch, ovigerous females are less active, remaining generally hidden and eating less (Accola *et al*., 1993). The behavioural difference between males and females in the first part of the reproductive season may result in collecting a greater number of males and a smaller number of females in pitfall traps. After mating, a reduction in the presence or activity of males and an increase in ovigerous female's mobility in search for food and appropriate breeding sites (Paoletti & Cantarino, 2002) may result in increasing the number of females in captures.

In *Trachelipus arcuatus* population from the studied forest plantation the sex ratio was in favour of females during the entire annual activity period. A tendency towards female-biased sex ratio has been repeatedly documented in natural populations of *Trachelipus rathkei* from places affected by human disturbance (Paoletti & Cantarino, 2002).

CONCLUSIONS

In *Porcellium collicola* populations from the studied oak forests the males had the highest percentage at the beginning of the reproductive period, when they were represented mainly by adult individuals that participate in copulation, and at the end of this period, when in population were present immature males from the new generation. Non-reproductive females were present in population during the entire annual activity period, being represented at the beginning of the breeding season and throughout this period by immature females that did not reach the minimum size to participate to reproduction and by females that participated to reproduction, and during autumn months mainly by immature females from the new generation. Ovigerous females were well represented in population in the first part of the breeding season, while post-ovigerous females and post-marsupial mancas had the highest percentage in population during summer period that corresponded to the second part of the reproductive season.

The percentages of the types of *Trachelipus arcuatus* individuals in captures from the forest plantation indicated rather the annual activity pattern of the species than the population structure.

The sex ratio underwent large fluctuations throughout the annual activity period in all studied terrestrial isopod populations. The overall annual sex ratio over the entire sampling period indicated the predominance of females, excepting the *Porcellium collicola* population from the forest plantation. In *Porcellium collicola* populations the sex ratio was male-biased during the period of maximum reproductive activity and female-biased over the rest of annual activity period. In *Trachelipus arcuatus* population from the forest plantation the ratio of males to females was female-biased during the entire annual activity period of the species.

REFERENCES

ACCOLA S., TOMESCU N., BERCIU C., RUSU C., 1993, *The reproduction of Trachelipus difficilis rotundatus Radu, 1950 (Crustacea: Isopoda)*. Revue Roumaine de Biologie, Série de Biologie Animale, **38**: 115-121.

ACHOURI M.S., CHARFI-CHEIKHROUHA F., MARQUES J.C., 2003, *Biology, population structure, and field-growth rates of Porcellionides pruinosus (Brandt, 1833) (Isopoda, Oniscidea) at Garat Nâam (Kasserine, Tunisia)*. Crustaceana, **75** (10): 1241-1262.

ACHOURI M.S., CHARFI-CHEIKHROUHA F., ZIMMER M., 2008, *Reproductive patterns in syntopic terrestrial isopod species (Crustacea, Isopoda, Oniscidea) from Morocco*. Pedobiologia, **52**: 127-137.

ALJETLAWI A.A., NAIR G.A., 1994, *Breeding and population biology of Armadillo officinalis (Isopoda: Oniscidea) in Benghazi, Libya*. Journal of Arid Environments, **27**: 141-255.

ARAUJO P.B., BOND-BACKUP G.B., 2005, *Population structure and reproductive biology of Atlantoscia floridana (van Name, 1940) (Crustacea, Isopoda, Oniscidea) in southern Brazil*. Acta Oecologica, **28**: 289-298.

DOLNIŢCHI-OLARIU L., TOMESCU N., 1997, *Aspects ecologiques de la population d'isopodes terrestres de la zone des Dornas*. Anuarul Muzeului Naţional al Bucovinei, **1997**: 81-92.

DONIŢĂ N., CHIRIŢĂ C., STĂNESCU V. (eds.), 1990, *Tipuri de ecosisteme forestiere din Romania [Types of forest ecosystems in Romania]*. pp. 283-285, 308-309. Institutul de Cercetări şi Amenajări Silvice, Seria a-II-a, Bucureşti.

HAMAIED S., CHARFI-CHEIKHROUHA F., 2004, *Life cycle and population dynamics of Armadillidium pelagicum Arcangeli, 1955 (Isopoda, Oniscidea) at Aouina*. Comptes Rendus Biologies, **327**: 343-352.

HAMAIED S., NASRI-AMMAR K., CHARFI-CHEIKHROUHA F., 2004, *Phénologie de la reproduction d'un population naturelle de Armadillidium pelagicum Arcangeli, 1955 (Isopoda, Oniscidea)*. Comptes Rendus Biologies, **327**: 335-342.

HORNUNG E., 1989, *Population dynamics and spatial distribution of Trachelipus nodulosus (C. L. Koch, 1838) (Crustacea: Isopoda) in a sandy grassland*. Monitore Zoologico Italiano, **4**: 399-409.

HOTEA R., TOMESCU N., MUREŞAN D., HOTEA M., 2003, *Cercetări faunistice şi ecologice asupra izopodelor terestre de pe versantul sudic al Munţilor Gutâi [Faunistical and ecological researches on terrestrial isopods from the south slope of Gutâi Mountains]*. Analele Universităţii Oradea, Fascicula Biologie, **X**: 33-37.

JOHNSON C., 1985, *Mating behavior of the terrestrial isopod, Venezillo evergladensis (Oniscoidea, Armadillida)*. American Midland Naturalist, **114**: 216-224.

LAWLOR L.R., 1976, *Moulting, growth and reproductive strategies in the terrestrial isopod, Armadillidium vulgare*. Ecology, **57**: 1179-1194.

MAGURA T., TÓTHMÉRÉSZ B., BORDÁN ZS., 2000, *Effects of nature management practice on carabid assemblages (Coleoptera: Carabidae) in a non-native plantation*. Biological Conservation, **93**: 95-102.

MUREŞAN D., TOMESCU N., OLARIU L., HOTEA R., 2003, *Cercetări faunistice şi ecologice asupra izopodelor terestre din sectorul mijlociu al bazinului Arieşului [Faunistical and ecological researches on terrestrial isopods from the middle sector of Arieş River Basin]*. Analele Universităţii Oradea, Fascicula de Biologie, **X**: 27-32.

PAOLETTI M.G., CANTARINO C..M., 2002, *Sex ratio alterations in terrestrial woodlice populations (Isopoda: Oniscidea) from agroecosystems subjected to different agricultural practices in Italy*. Applied Soil Ecology, **19**: 113-120.

PAOLETTI M.G., HASSALL M., 1999, *Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators*. Agriculture, Ecosystems and Environment, **74**: 157-165.

PARIS O.H., PITELKA F.R., 1962, *Population characteristics of the terrestrial isopod Armadillidium vulgare in California grassland*. Ecology, **43** (2): 229-248.

RADU V. GH., 1985, *Fauna Republicii Socialiste România, Crustacea, Volumul IV, Fascicula 14, Ordinul Isopoda, Subordinul Oniscoidea, Crinochaeta [Fauna of Romania, Crustacea, Volume IV, Fascicle 14, Order Isopoda, Suborder Oniscoidea, Crinochaeta]*. Editura Academiei R.S.R., Bucureşti, 158 pp.

RADU V.GH., TOMESCU N., 1972, *Studiul populaţiei de Protracheoniscus politus–Koch (Crustacea-Izopode) dintr-o pădure de foioase [The study of a Protracheoniscus politus–Koch (Crustacea-Isopoda) population from a deciduous forest]*. Studia Universitatis Babeş-Bolyai, Biologia, **17** (1): 75-82.

SANTOS S.A.P., CABANAS J.E., PEREIRA J.A., 2007, *Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type*. European Journal of Soil Biology, **43**: 77-83.

SCHMIDT C., 1997, *Revision of the European species of the genus Trachelipus Budde-Lund, 1908 (Crustacea: Isopoda: Oniscidea)*. Zoological Journal of the Linnaean Society, **121**: 199-244.

SOUTHWOOD T.R.E., HENDERSON P.A., 2000, *Ecological Methods.* Third Edition. Blackwell Science Ltd.: 276-278.

THOMAS C.F.G., BROWN N.J., KENDALL D.A., 2006, *Carabid movement and vegetation density: Implications for interpreting pitfall trap data from split-field trials*. Agriculture, Ecosystems and Environment, **113**: 51-61.

TOMESCU N., 1973, *Reproduction and postembryonic ontogenetic development in Ligidium hypnorum (Cuvier) and Trichoniscus pusillus (Brandt, 1833) (Crustacea, Isopoda)*. Revue Roumaine de Biologie, Série de Biologie Animale, **18**: 403-413.

TOMESCU N., ACCOLA S., BERCIU C., 1992, *Reproduction of terrestrial isopods of Cheile Turzii Natural Reservation (Romania)*. Studia Universitatis Babeş-Bolyai, Biologia, **37**: 39-45.

TOMESCU N., ACCOLA S., PAŞCA C., 1995, *Ecology of the populations of terrestrial isopods (Crustacea: Isopoda) in Cheile Turzii*. Studia Universitatis Babeş-Bolyai, Biologia, **40**: 78-94.

TOMESCU N., ARDELEAN G., MUREŞAN D., POPA V., 2000, *Ecology of terrestrial isopods in the Nature Reserve Scăriţa-Belioara, România*. Studia Universitatis Babeş-Bolyai, Biologia, **45** (1): 57-64.

TOMESCU N., BOGDAN H., PETER V.I., COVACIU-MARCOV S.D., SAS I., 2008, *Terrestrial isopods from western and north-western Romania*. Studia Universitatis Babeş-Bolyai, Biologia, **53** (2): 3-15.

TOMESCU N., CRĂCIUN C., 1987, *Postembryonic ontogenetic development in Porcellio scaber (Crustacea: Isopoda)*. Pedobiologia, **30**: 345-350.

TOMESCU N., MUREŞAN D., POPA V., 2001, *The terrestrial isopod fauna in the superior basin area of the Someşul Cald River*. Studia Universitatis Babeş-Bolyai, Biologia, **46** (2): 43-47.

WARBURG M.R., 1994, *Review of recent studies on reproduction in terrestrial isopods*. Invertebrate Reproduction and Development, **26** (1): 45-62.

ZIMMER M., 2004, *Effects of temperature and precipitation on a flood plain isopod community: a field study*. European Journal of Soil Biology, **40**: 139-146.

Received July 16, 2011

Ecological University of Bucharest,
Faculty of Ecology and Environmental Protection,
1G Vasile Milea Blvd., 061341 Bucharest, Romania
e-mail: finica_ivanov@yahoo.com

ON THE ASYMMETRY OF SOME MORPHOLOGICAL CHARACTERS OF *CARANGOIDES CAERULEOPINNATUS* (RUPPELL, 1830) (FAMILY CARANGIDAE) COLLECTED FROM THE SEA OF OMAN

LAITH JAWAD[*], JUMA MOHAMED AL-MAMRY[*], SUAD MOHAMED AL-BIMANI[**],
FATEN KHAMIS AL-GHAFARI[**], DAWOOD AL-MAMARY[*]

Asymmetry analysis has been carried out for some bilateral characters of *Carangoides caeruleopinnatus* (Family Carangidae) collected from the Oman Sea. The results showed that eye diameter demonstrated the highest incidence of asymmetry among the characters studied. The lowest asymmetry value was for the head length. An increasing trend in the asymmetry value with fish length is also obtained for preorbital length, number of pectoral fins. The possible causes of asymmetry in this species are herein discussed in relation to different pollutants and their presence in the area.

Key words: *Carangoides caeruleopinnatus*, asymmetry, morphology, the Sea of Oman, Oman.

INTRODUCTION

In natural population, profound bilateral asymmetries in phenotype are a widespread phenomenon and comprise an emerging but controversial direction in evolutionary studies. These subtle asymmetries, usually known as "fluctuating asymmetry" (FA) (Van Vallen, 1962; Palmer & Strobeck, 1986; Leary & Allendorf, 1989), generally originate from developmental instability (Waddington, 1942); these have received significant attention due to negative correlations with fitness, including associations with sexual selection (Møller & Hoglund, 1991; Watson & Thornbill, 1994) and trait function (Mather, 1953; Moodie, 1977; Palmer & Strobeck, 1986; Balmford *et al.*, 1993; Gummer & Brigham,1995; Clarke, 1998). Especially asymmetry has detrimental effects on the biomechanics of the principle function of the organism (Moodie & Reimchen, 1976; Reimchen, 1983; Bergstrom & Reimchen, 2003). Nonetheless, the consequences of asymmetry in such effects vary among habitats (Bergstrom & Reimchen, 2003), suggesting a complex and site-dependent relationship with fitness.

Fluctuating asymmetry results from the inability of individuals to undergo identical development of a trait on both sides of the body. It is also suggested that fluctuating asymmetry represents a measure of the sensitivity of development to environmental stress (Møller & Pomiankowski, 1993).

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° 2, P. 179–188, BUCHAREST, 2011

Members of the Family Carangidae are among the most important commercial fish in the Oman Sea generally and in the Sultanate of Oman in particular. Several species have been observed in the area and along the coasts of Oman (Randall, 1995). Meristic and metric characters are highly sensitive to environmental factors and show a significant defect once they became exposed to environmental stress (Fowler, 1970).

This work studied the bilateral asymmetry phenomenon in selected morphological characters of the carangid species*, Carangoides caeruleopinnatus* collected from the Oman Sea and designed to determine: (1) if bilateral asymmetry occurs in the chosen characters; (2) the extent of asymmetries; (3) the direction of the asymmetrical development, as shown by one side tending to have a larger number of elements; (4) the possible usefulness of the information in future taxonomic studies of *Carangoides caeruleopinnatus*.

MATERIAL AND METHODS

Two hundreds and fifty five specimens of *Carangoides caeruleopinnatus* were collected from the Oman Sea on March 3rd, 2010. This carangid species is pelagic and it is found in large numbers in the coastal areas of the Oman Sea. The five bilateral characters used to compare asymmetry were as follows: (1) length of the pre-orbital distance (mm), measured from the mouth to the anterior edge of the orbit; (2) length of the post-orbital distance (mm), measured from the posterior edge of the eye to the posterior edge of the operculum; (3) orbital diameter (mm), measured from the anterior to the posterior edges of the eye; (4) head length (mm), measured from the mouth to the edge of the preoperculum; (5) number of pectoral fin rays, a count of the total number of pectoral fin rays, including the uppermost ray.

Most characters were counted and measured under a binocular dissecting microscope. For specimens too large to fit under a microscope, a magnifying glass was used. In the statistical analysis, the square coefficient of asymmetry variation (CV2a) for meristic and metric characters was calculated according to Valentine *et al*. (1973) as:

$$\mathrm{CV2a} = (\mathrm{S}\ r + l\ \mathrm{X}\ 100/\mathrm{X}\ r + l)$$

where S r + l is the standard deviation of the signed difference, X r + l is the mean of the character, which is calculated by adding the absolute scores for both sides and dividing by the sample size.

To eliminate scaling problems associated with growth in morphometric characters (non-discrete, measurable), each measurement was divided by a conventional standardizing measurement (*e.g.*, head length was used in the present study). Every metric measurement was treated in a similar manner and the squared coefficient of asymmetry was determined as before.

RESULTS

The results of the asymmetry data analysis of the previously listed characters of *C. caeruleopinnatus* collected from Oman Sea are shown in Table 1. The highest value was recorded for the orbital diameter and the lowest value for the head length. The percentage of individuals showing asymmetry in eye diameter was the highest among the percentages recorded for asymmetry of the five characters (65.82 % of the total fish studied) and the lowest percentage was for individuals with asymmetry in the head length (21.64 % of the total fish studied).

Table 1

Squared coefficient asymmetry (CV2a) values and character means (X r + l) of *Carangoides caeruleopinnatus*

Character	**CV2a**	**N**	**Character mean**	**% of individuals with asymmetry**
Preorbital length	5.65	55	2.86	43.64
Postorbital length	5.04	55	4.78	43.46
Orbital diameter	18.29	55	1.97	65.82
Number of Pectoral Fin ray	10.04	55	18.89	25.46
Head length	2.87	55	6.86	21.64

The result of the asymmetry direction as whether individuals are left handed or right handed have shown that all the characters studied are dextral, where the right side showed higher value over the left side, except for pectoral fin ray count where shown to be sinistral, where count on the left side is larger than that of the right side. For pectoral fin ray count, bilateral asymmetry occurred in 25.46% of the total of 55 *C. caeruleopinnatus* examined. Of specimens exhibiting asymmetry, 48.2% had larger left side counts. As to the preorbital, postorbital lengths, orbital diameter and head length, the bilateral asymmetry exhibited was 43.6%, 43.5%, 65.8.6% and 21.6% respectively. Of these asymmetric specimens, 368.5%, 479.5%, 55.7% and 48.9% are right sided respectively.

Individuals of *C. caeruleopinnatus* were grouped into length classes (Table 2). A trend of increase in the asymmetry values was noticed in the preorbital length and pectoral fin ray count.

Table 2

Squared coefficient of asymmetry and character means (X_{r+1})
by size class of *Carangoides caeruleopinnatus*

Size Class	**n**	**CV^2_a**	**Character mean X_{r+1}**	**% of individuals with asymmetry**
Preorbital length				
20.1-21.0	10	0	3.7	0
21.1-22.0	26	3.43	2.96	50.00
22.1-23.0	26	5.23	3.29	66.67
23.1-24.0	20	6.88	3.21	40.00
24.1-25.0	22	6.99	3.02	58.33
25.1-26.0	25	7.00	2.04	0
26.1-27.0	26	7.24	2.35	33.33
27.1-28.0	21	8.04	4.05	100.00
28.1-29.0	22	9.66	2.28	50.00
29.1-30.0	23	9.82	2.28	33.33
30.1-31.0	21	21.98	2.50	0
31.1-32.0	12	41.32	2.20	50.00
Total	255			
Postorbital length				
20.1-21.0	10	0	5.85	100
21.1-22.0	26	11.66	4.69	66.67
22.1-23.0	26	4.91	5.28	66.67
23.1-24.0	20	6.87	5.15	70.00
24.1-25.0	22	2.83	4.96	66.67
25.1-26.0	25	2.00	3.87	60.00
26.1-27.0	26	5.59	4.37	50.00
27.1-28.0	21	0	6.25	100.00
28.1-29.0	22	3.09	4.03	50.00
29.1-30.0	23	1.56	4.62	33.33
30.1-31.0	21	0	4.35	100.00
31.1-32.0	12	12.82	3.95	100.00
Total	255			
Orbital diameter				
20.1-21.0	10	0	2.3	100
21.1-22.0	26	4.66	1.89	16.67
22.1-23.0	26	27.21	2.10	50.00
23.1-24.0	20	55.96	2.09	40.00
24.1-25.0	22	9.38	2.03	16.67
25.1-26.0	25	0	1.72	0
26.1-27.0	26	0	1.82	0
27.1-28.0	21	0	2.45	100.00
28.1-29.0	22	0	1.75	0
29.1-30.0	23	0	1.93	0
30.1-31.0	21	0	1.80	0
31.1-32.0	12	0	1.75	0
Total	255			

Table 2

(continued)

Size Class	**n**	**CV^2_a**	**Character mean X_{r+l}**	**% of individuals with asymmetry**
Head length				
20.1-21.0	10	0	8.00	0
21.1-22.0	26	1.22	6.81	50.00
22.1-23.0	26	1.22	7.39	50.00
23.1-24.0	20	1.19	7.24	10.00
24.1-25.0	22	8.45	7.05	75.00
25.1-26.0	25	0	5.96	0
26.1-27.0	26	4.10	6.32	33.33
27.1-28.0	21	0	8.95	100.00
28.1-29.0	22	0	5.90	0
29.1-30.0	23	8.57	6.83	66.67
30.1-31.0	21	0	6.45	100.0
31.1-32.0	12	5.75	5.90	100.00
Total	255			
Number of pectoral fin ray				
20.1-21.0	10	0	19.00	100.00
21.1-22.0	26	4.58	19.08	16.67
22.1-23.0	26	5.67	18.83	0
23.1-24.0	20	6.88	19.05	20.00
24.1-25.0	22	6.99	19.08	16.67
25.1-26.0	25	7.97	18.30	20.00
26.1-27.0	26	8.26	19.17	33.33
27.1-28.0	21	12.34	18.50	100.00
28.1-29.0	22	14.22	18.75	50.00
29.1-30.0	23	68.88	18.50	66.67
30.1-31.0	21	68.90	18.00	0
31.1-32.0	12	69.98	18.50	0
Total	255			

DISCUSSION

It is obvious from the living habits of *C. caeruleopinnatus* that there is a relationship between asymmetry and environmental stress. Such conclusion is based on the feeding behavior and the high metabolic rate of this fish. During feeding, this species usually swims together in a school and moves around the water. The pattern that individuals of *C. caeruleopinnatus* move around in water may provide them more opportunity to be exposed to stress. Alternatively, greater energy is in need for such an active fish. The energy is needed to compensate for the energy loss during their development and towards manipulating environmental stress. Some energy is needed to be allocated for maintaining homeostasis (Mitton, 1994). Abnormal development may become evident if available energy is not sufficient to buffer the stress effects homeostasis (Mitton, 1994; Somarakis *et al.*, 1997). Consequently, higher levels of asymmetry have resulted in *C. caeruleopinnatus*.

There is some variation in the asymmetry values among the five morphological characters studied in *C. caeruleopinnatus*. Based on previous studies in this field, it is possible to conclude that there is a direct correlation between environmental stress due to pollution and asymmetry in this species. Such environmental factors are present in the waters of the coastal area of the Sea of Oman. However, character like eye diameter showed higher asymmetry values than those of the other characters studied. High asymmetry values for this character have also been recorded in several freshwater and marine fish species (Al-Hassan *et al.*, 1990; Al-Hassan & Hassan, 1994; Jawad 2001, 2003; Jawad *et al.*, 2010). Such agreements in results of asymmetry might indicate the vulnerability of those two characters to immediate changes in the environment. It is impossible at this stage to confirm that these levels of asymmetry correlate to various types of environmental pollution, and the morphology of the fish species in question is not available. However, based on previous studies, it is possible to conclude that there is a direct correlation between environmental stress, due to pollution, and asymmetry in this species. Such environmental factors are present in the waters of the Sea of Oman.

On the other hand, the low asymmetry values obtained for the other four characters might be explained on the basis that these characters are designated with high functional importance and are highly canalized during ontogeny thus giving low level of FA (Palmer & Strobeck, 1986; Møller & Pomiankowski, 1993).

The size of the fins is among the characters that have functional importance in fishes and seem to be under detrimental effect of asymmetry. As they play an important role in locomotion, the pectoral fins have functional value and thus the efficiency of predator evasions probably depends on their functionality (Gonçalves *et al.*, 2002). Pectoral fins are of use during parental care as their advantage becomes clear in the process of egg oxygenation, and recently, Künzler & Bakker (2000) have demonstrated that the area of the pectoral fins correlates with paternal quality in sticklebacks *Gasterosteus aculeatus* L. With the moderately high asymmetry value recorded for pectoral fin ray count of *C. caeruleopinnatus*, it is quite possible for such asymmetry to hinder the basic functions of the pectoral fin.

Several authors have recorded and discussed the effects of the asymmetry on body proportions (Moodie & Reichmen, 1976; Reichmen, 1983; Bergstrom & Reimchen, 2003). The effect of asymmetry on the orbital diameter, pre and postorbital length might increase or decrease the preorbital and postorbital areas in the fish skull. Such changes might lead to differential enlargement which is restricted to the nasal pole of the eye. If such enlargement happened, retinal expansion will take place (Zygar *et al.*, 1999). Moreover, by having more space in the naso-temporal region this might affect fish visual acuity (Cameron, 1995; Zygar *et al.*, 1999).

The individuals of *C. caeruleopinnatus* appeared to be right handed for the pectoral fin ray count and right handed for the remaining four metric characters. It is not possible at this stage to judge whether left handed or right handed individuals are the naturally successful individuals as such dextrality and senistrality in these characters might interfere with important biotic functions of the fish (Zygar *et al.*, 1999).

In taxonomic and racial studies involving pectoral fin ray count, the orbital diameter, pre and postorbital lengths, interchanging counts and differences in dimensions from left and right sides of *C. caeruleopinnatus* introduce an additional source of variation to taxonomists who depend on these characters in separating this species or its populations. These characters are regularly used by taxonomists to set up the unique morphological features of the species in question. Bilateral asymmetry has shown to create problems to fish taxonomists and taxonomists of animal groups other than fish such as owl (Norberg, 1977).

Pollution of sea water and sediments by hydrocarbons, heavy metals, pesticides and organic matter are considered the main cause of environmental stress. Pollution is not unusual for Oman Sea, as reports of adverse conditions in this part of the world continue alerting people to local ecological disasters (De Mora *et al.*, 2004, 2005; Al-Darwish *et al.*, 2005; Tolosa *et al.*, 2005; Abdel Gawad *et al.*, 2008; Khan, 2008).

The environmental causes might be natural events. Several factors are known to produce nutritional deficiencies such as various pathogens and various population phenomena (Bengtsson & Hindberg, 1985), and it is highly possible that these factors may be in action in the Oman Sea, since they seem to be common in the aquatic environment.

Several authors have shown a relationship between the coefficient of asymmetry and fish length (Al-Hassan *et al.*, 1990; Al-Hassan & Hassan, 1994; Al-Hassan & Shwafi, 1997; Jawad, 2001; Jawad *et al.*, 2010), where there was a trend of increase in the asymmetry value with the increase in fish length. The results show a trend of increasing asymmetry with fish length in for preorbital length and pectoral in ray count. This trend is probably the result of incomplete development; character means are always the lowest in smaller size classes (Valentine *et al.*, 1973). The same results were obtained by Valentine *et al.* (1973) in selected fish species collected from California, U.S.A. They suggested two possible hypotheses that may account for such a trend; these are the ontogenetic changes which are an increase in asymmetry with size (age) and the possible historical process which is a secular increase in asymmetry.

CONCLUSIONS

An asymmetry in the bilateral characters of carangid fish *Carangoides caeruleopinnatus* (Family Carangidae) was observed. The eye lens diameter showed the highest asymmetry value and the head length showed lowest value. It is also observed that fish length is increasing with the value of the asymmetry for preorbital length, number of pectoral fins. The possible causes of different pollutants and their presence in the area might be the cause behind the asymmetry obtained in the bilateral characters of the species in question.

Acknowledgements. We would like to thank the Ministry of Fisheries Wealth, the Agriculture and Fisheries Development Fund and the Marine Science and Fisheries Centre for giving us the opportunity to work on the fish samples within the qualitative and quantitative distribution of marine organisms in the Sultanate of Oman and to provide the appropriate financial support.

REFERENCES

ABDEL GAWAD E.A., AL-AZAB M., LOTFY M.M., 2008, *Assessment of organic pollutants in coastal sediments*. UAE. Environmental Geology, **54**: 1091-1102.

AL-DARWISH H.A., ABD EL-GAWAD E.A., MOHAMMED F.H., LOTFY M.M., 2005, *Assessment of organic pollutants in the offshore sediments of Dubai, United Arab Emirates*. Environmental Geology, **48**: 531-542.

AL-HASSAN L.A.J., HASSAN S.S., 1994, *Asymmetry study in Mystus pelusius collected from Shatt al-Arab River, Basrah, Iraq*. Pakistan Journal of Zoology, **26**: 276-278.

AL-HASSAN L.A.J., SHWAFI N.A.A., 1997*, Asymmetry analysis in two marine teleost fishes collected from the Red Sea coast of Yemen*. Pakistan Journal of Zoology, **29**: 23-25.

AL-HASSAN L.A.J., A.Y. AL-DOUBAIKEL A.Y., WAHAB N.K., AL-DAHAM N.K., 1990, *Asymmetry analysis in the catfish, Heteropneustes fossilis collected from Shatt al-Arab River, Basrah, Iraq*. Rivesta di Idrobiologia, **29**: 775-780.

BALMFORD L., I.L. JONES I.L., THOMAS A.L.R., 1993, *On avian asymmetry: evidence of natural selection for symmetrical tails and wings in birds*. Proceedings of the Royal Society of London, B **252**: 245-251.

BENGTSSON B.E., HINDBERG M., 1985, *Fish deformities and pollution in some Swedish waters*. Ambio, **14**: 32-35.

BERGSTROM C.A., REIMCHEN T.E., 2003, *Asymmetry in structural defenses: insights into selective predation in the wild*. Evolution, **57**: 2128-2138.

CAMERON D.A., 1995, *Asymmetric retinal growth in the adult teleost green sunfish (Lepomis cyanellus)*. Visual Neuroscience, **12**: 95–102.

CLARKE G.M., 1998, *Developmental stability and fitness: the evidence is not quite so clear*. American Naturalist, **152**: 762-766.

DE MORA S., FOWLER S.W., WYSE E., AZEMARD S., 2004, *Distribution of heavy metals in marine bivalve, fish and coastal sediments in the Gulf and Gulf of Oman*. Marine Pollution Bulletin, **49**: 410-424.

DE MORA S., FOWLER S.W., TOLOSA I., VILLENEUVEILL J.-P., C. CATTINI C., 2005, *Chlorinated hydrocarbons in marine biota and coastal sediments from the Gulf and Gulf of Oman*. Marine Pollution Bulletin, **50**: 835-849.

FOWLER J.A., 1970, *Control of vertebral number in teleosts-an embryological problem*. Quarterly Review of Biology, **45**: 148-167.

GONÇALVES D.M., SIMÕES P.C., CHUMBINHO A.C., CORREIA M.J., FAGUNDES T., OLIVEIRAl R.F., 2002, *Fluctuating asymmetries and reproductive success in the peacock blenny*. Journal of Fish Biology, **60**: 810-820.

GUMMER D.L., BRIGHAM R.M., 1995, *Does fluctuating asymmetry reflect the importance of traits in little brown bats (Myotis lucifugus)?* Canadian Journal of Zoology, **73**: 398-413.

JAWAD L.A., 2001, *Preliminary asymmetry analysis of some morphological characters of Tilapia zilli (Pisces: Cichlidae) collected from three localities in Libya*. Bollettino del Museo Regionale di Scienze Naturali di Torino, **18**: 251-257.

JAWAD L.A., 2003, *Asymmetry in some morphological characters of four sparid fishes from Benghazi, Libya*. Oceanological and Hydrobiological Studies, **32**: 83-88.

JAWAD L.A., TAHER M.M.A., NADJI H.M.H., 2001, *Age and asymmetry studies on the Indian mackerel, Rastrelliger kanagurta (Osteichthyes: Scombridae) collected from the Red Sea coast of Yemen.* Indian Journal of Marine Science, **30**: 180-182.

JAWAD L.A., J.M. AL-MAMRY J.M., A.A. AL-KHARUSI A.A., & S.H. AL-HABSI, 2010. *Asymmetry in certain morphological characters of the carangid species Decapterus russelli, collected from Lemah coastal area, on the northern part of Oman Sea*. Oceanological and Hydrobiological Studies, **39**: 55-62.

KHAN N.Y., 2008, *Integrated management of pollution stress in the Gulf.* In: *Protecting the Gulf's Marine Ecosystems from Pollution*, pp. 57-92, Abuzinada A.H., H-J. Barth, F. Krupp, B. Boer and Z. Al-Abdessalaam (Eds.), Birkhauser, Basel.

KÜNZLER R., BAKKER T.C.M., 2000, *Pectoral fins and paternal quality in sticklebacks*. Proceedings of the Royal Society of London B, **267**: 999-1004.

LEARY A., ALLENDROF F.W., 1989, *Fluctuating asymmetry as an indicator of stress: implications for conservation biology.* Trend in Evolution, **4**: 214-217.

MATHER K., 1953, *Genetical control of stability in development*. Heredity, **7**: 297–336.

MITTON J.B., 1994, *Enzyme Heterozygosity, Metabolism, and Developmental Stability*: In: *Developmental Instability and Evolutionary Implications*, pp. 49-68, Markow T.A. (Ed.), Kluwer Academic Publishers, The Netherlands.

MOODIE G.E.E., 1977, *Meristic variation, asymmetry, and aspects of the habitat of Culea inconstans (Kirtland), the brook stickleback, in Manitoba*. Canadian Journal of Zoology, **55**: 398-404.

MOODIE G.E.E., REIMCHEN T.E., 1976, *Phenetic variation and habitat differences in Gasterosteus populations of the Queen Charlotte Islands*. Canadian Field Naturalist, **90**: 471-474.

MØLLER A.P., HOGLUND J., 1991, *Pattern of fluctuating asymmetry in avian feather ornaments: implications for models of sexual selection.* Proceedings of the Royal Society of London B, **245**: 1-5.

MØLLER A.P., POMIANKOWSKI A., 1993, *Punctuated equilibria or gradual evolution: fluctuating asymmetry and variation in the rate of evolution*. Journal of Theoretical Biology, **161**: 359-367.

NORBERG R.A., 1977, *Occurrence and independent evolution of bilateral ear asymmetry in owls and implications on owl taxonomy.* Philosophical Transaction of the Royal Society of London Series B, **280**: 375-408.

PALMER A.R., STROBECK C., 1986, *Fluctuating asymmetry: measurements, analysis and pattern*. Annual Review of Ecology and Systematic, **17**: 391-421.

PARENTI L.R., 1986, *Bilateral asymmetry in phallostethid fishes (Atherinomorpha) with description of a new species from Sarawak*. Proceedings of California Academy of Science, **44**: 225-236.

RANDALL J.E., 1995, *Coastal fishes of Oman*, Hawaii University Press, Hawaii, U.S.A., 439 pp.

REIMCHEN T.E., 1983, *Structural relationships between spines and lateral plates in three spine stickleback (Gasterosteus aculeatus*). Evolution, **37**: 931–946.

SOMARAKIS S., KOSTIKAS I., TSIMENIDES N., 1997, *Fluctuating asymmetry in the otoliths of Lawa fish as an indicator of condition conceptual and methodological aspects*. Journal of Fish Biology, **51**: 30-38.

TOLOSA I., DE MORA S.J., FOWLER S.W., VILLENEUVEILL J.-P., BARTOCCI J., CATTINI C., 2005, *Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman*. Marine Pollution Bulletin, **50**: 1619-1633

VALENTINE D.W., SOULE M.E., SAMOLLOW P., 1973, *Asymmetry in fishes: a possible statistical indicator of environmental stress.* Fishery Bulletin, **71**: 357-370.

VAN VALLEN L., 1962, *A study of fluctuating asymmetry*. Evolution, **16**: 125-142.

WADDINGTON C.H., 1942, *Canalization of development and the inheritance of acquired characters.* Nature, 1**50**:563–565.

WATSON P.J., THORNBILL R., 1994, *Fluctuating asymmetry and sexual selection.* Trends in Ecology and Evolution, **9**: 21-25.

ZYGAR C.A., RUSSELL M.J., FERNALD D., 1999, *Nasotemporal asymmetry during teleost retinal growth: preserving an area of specialization*. Journal of Neurobiology, **41**: 435-442.

Received April 24, 2011

[*] *Marine Science and Fisheries Centre, Ministry of Fisheries Wealth,*
Sultanate of Oman, P.O. Box 427, Postal Code 100 Muscat.
laith_jawad@hotmail.com
[**] *High Technical College, Muscat, Sultanate of Oman*

RELATIONSHIP BETWEEN FISH SIZE AND OTOLITH SIZE AND WEIGHT IN THE PELAGIC SPECIES *RHYNCHORHAMPHUS GEORGI* (VALENCIENNES, 1846) (FAMILY HEMIRAMPHIDAE) COLLECTED FROM THE SEA OF OMAN

LAITH JAWAD*, JUMA AL-MAMRY*, HAJER AL-MAMARI**,
MANAL AL-YARUBI**, HAITHEM AL-BUSAIDI*

The regressions between otolith size (length and width), otolith weight and fish length of the pelagic species *Rhynchorhamphus georgi* living the coasts of the Sea of Oman were provided. No differences between right and left otolith sizes and weight were detected by t-test, so a single linear regression was plotted against standard length (SL) for otolith length (OL), otolith width (OW) and otolith weight (OWE). Data fitted well to the regression model for both OL and OWE to TL (R^2 0.82-0.89). These relationships provide a reliable tool in feeding studies and also provide support to palaeontologists in their research on fish fossils.

Key words: *Rhynchorhamphus georgi*, pelagic fish, otolith weight, fish-otolith sizes, Sea of Oman, Oman.

INTRODUCTION

Among the important elements of marine ecosystem are the small pelagic fishes due to their significant contribution in the food web as they notably contributing to canalize the energy connecting the lower and upper trophic levels (Palomera *et al*., 2007). Fluctuations in the populations of these fishes due to fishing or to environmental factors can contribute to modify the structure and functioning of marine ecosystems (Cury *et al*., 2000).

Studies on feeding behavior have shown the pelagic species to be among the primary trophic source for commercially important benthic fishes (Martin & Christiansen, 1997; Bergstad *et al*., 2003; McIntyre *et al*., 2006) and for large pelagic fish species (Anonymous, 2005; Hussaini, 2006). Moreover, several other predators, such as marine mammals might rely on these food resources (Hassani *et al*., 1997; Pauly *et al*., 1998).

There is not much information about predation on small pelagic fish species including *Rhynchorhamphus georgi* in the Sea of Oman area. In his study on the food and feeding habits of some piscivorous fish species in the Sea of Oman, Hussaini (2006) showed that the four pelagic fish species, *Sardinella gibbosa, S. longiceps, Decapterus russelli* and *Rhynchorhamphus georgii* contributed

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° *2*, P. 189–200, BUCHAREST, 2011

between 37% and 86% of the total fish biomass of the Sea of Oman and Persian Gulf in June 2004. This author also named the piscivorous fish that feed upon the small pelagic fish species mentioned above as kingfish, *Scomberomorus commerson* and the tuna fish species *Thunnus* (*T. albacares, T. obesus* and *T. tonggol*) and they were sufficiently abundant in the Sea of Oman to have an impact on the pelagic fish prey resource. Ali (2007) studied the diet of some large pelagic fish species living in the Sea of Oman and estimated the percentage of small pelagic fish, including *R. georgii*, in their diet as over 60% of the total diet in their stomach. The percentage by weight of fish in the diets of these piscivorous species increased with increasing predator length (Hussaini, 2006).

Moreover, Anonymous (2005) studied the predation of small pelagic fish species in the Sea of Oman by benthopelagic fish at different where the pelagic fishes occur during the daytime and suggested that the occurrence of these pelagic species at the benthic boundary layer is primarily through truncation of their pelagic vertical distributions rather than horizontal impingement.

The identification and quantification of the pelagic fish preys are frequently a difficult task in the feeding studies (Battaglia *et al*., 2010). In general, the prey specimens are already partially or totally digested and the hard remains in stomachs, intestines, faeces and scats are the only diagnostic features that can be considered. Among those hard remains, otoliths are quite resistant to the digestion and they considered the important tool for prey classification in several dietary studies (Montevecchi & Myers, 1995; Greenstreet *et al*., 1998). The examination of sagittae from faeces of marine mammals and sea birds usually required to examine the regurgitated digestive pellets in order to identify the preys (Duffy & Laurenson, 1983; Johnstone *et al*., 1990; Pierce & Boyle, 1991).

For the above reasons and for their high interspecific variability, number of keys and identification guides on fish otolith morphology has been published (Smale *et al*., 1995; Campana, 2004; Lombarte *et al*., 2006; Tuset *et al*., 2008). The importance of estimation of the biomass of the otolith and their numerical abundance are fundamental to understand fish prey's energy contribution to predator diet. The relationship between fish length and otolith size and weight is usually used in several fish species to build the body size and prey biomass. In such a practice, the otolith measurement has been used (Wyllie, 1987; Gamboa 1991; Granadeiro & Silva, 2000; Harvey *et al*., 2000; Waessle *et al*., 2003; Lychakov *et al*., 2006; Battaglia *et al*., 2010).

In paleontological studies, fossil otoliths have proved to be a good taxonomic tool to add knowledge to the taxonomic status of the ancient fauna of the planet when they compared those to the recent reference collections (Nolf, 1985, 1995; Girone *et al*., 2006). Thus, otolith data are widely used in the fields of paleoecology, paleobathymetry, paleoclimatology, paleobiogeography and biostratigraphy (Nolf, 1995).

The aim of the present study is to provide data about the relationship between otolith size (length and width) and weight and fish length of the pelagic teleost fish, *Rhynchorhamphus georgi* (Valenciennes, 1846) collected from the Sea of Oman. The data offer a helpful tool for feeding studies and also give support to palaeontologists in their investigation on fossils.

MATERIAL AND METHODS

Fish specimens were collected during the period 2009–2010 throughout the coasts of the Sea of Oman using cast nets. The specimens were identified following Randall (1995). Total length (TL; most anterior point to the posterior tip of the caudal fin) was considered and measured to the nearest millimeter. Sagittae were (total of 240 individuals, *i.e.*, 480 otoliths) removed through a cut in the cranium to expose them then cleaned and stored dry in glass vials and the left and right otolith were considered separately. Specimens with obvious evidence of calcite crystallization (Strong *et al.*, 1986) or other aberrant formations were rejected. Each sagittae, systematically placed with the sulcus acusticus oriented through the observer and its length was determined using hand-held vernier callipers and defined as the longest dimension between the rostrum and postrostrum axis (nomenclature of Smale *et al.*, 1995) and width as the dimensions from the dorsal to ventral edge taken at right angles to the length through the focus of the otolith. Individual otolith weight (in milligram) was determined using an electronic balance. The relationship between otolith size (length, width) and weight and fish size (TL) were determined using least squares linear regression for the following parameters: otolith length (OL)-fish length (TL), otolith width (OW)-fish length (TL) and otolith weight (OWE)-fish length (TL). These equations were first calculated for both left and right otoliths and ANCOVA test (Fowler & Cohen, 1992) was used to check any differences between regressions. The regression coefficients were compared and when significant differences ($P < 0.05$) were not found, the H_0 hypothesis ($b_{right} = b_{left}$) was accepted. When the equations did not differ statistically, a single linear regression was reported for each parameter (OL; OW; OWE).

RESULTS

The range of the standard length of the specimens used in this study is 100–400 mm with a mean of 213 mm. The fish lengths available for the species in question were those observed in commercial fisheries and research surveys but the extremes of length ranges were under sampled.

The different relationships between fish length and otolith length, width and weight are shown in Tables 1–3. The maximum otolith length and width recorded were 6 and 13.5 m respectively while the maximum otolith weight was 0.96 g. The range in observed values for otolith length, width, and weight of the species in question are 1.5–6.0, 3.5–13.5 mm, and 0.001–0.96 g respectively.

In the analysis of morphometric parametrs (Otolith length and width) and weight against total length, no considerable differences between right and left otoliths were detected by ANCOVA test ($P > 0.05$) (Figs. 1–6; Tables 1–2). Thus, single linear regression was plotted for each parameter (Figs. 7–9; Table 3). Data fitted well to the regression model for three parameters to TL as demonstrated by the high values of the coefficient of determination (Table 3).

Table 1

Results of regression analysis between left otolith dimension and weight on fish length

Parameter	Intercept	Slope	Correlation
Length	0.6221	19.572	0.1351
Width	6.8995	8.4099	0.7812
Weight	38.204	3.088	0.913

Table 2

Results of regression analysis between right otolith dimension and weight on fish length

Parameter	Intercept	Slope	Correlation
Length	6.7549	2.6897	0.8735
Width	6.7341	7.8147	0.756
Weight	37.243	3.441	0.8687

Table 3

Results of the regression analysis of fish length on otolith dimension

Parameter	Intercept	Slope	Correlation
Length	6.6201	3.5097	0.8952
Width	2.2694	10.102	0.8352
Weight	19.631	18.2	0.8225

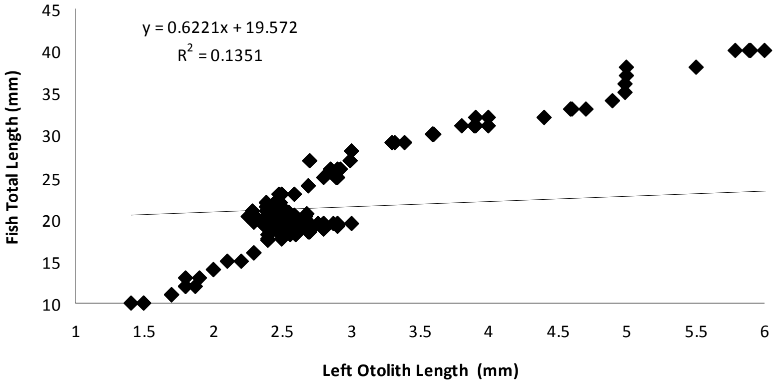


Fig. 1. Total length-left otolith length relationship.

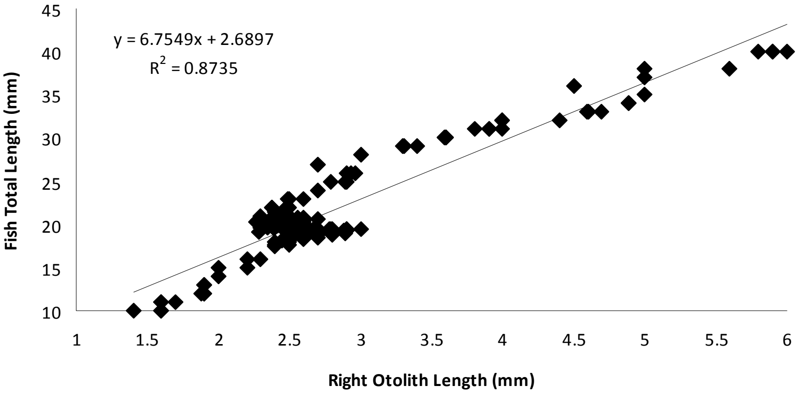


Fig. 2. Total length-right otolith length relationship.

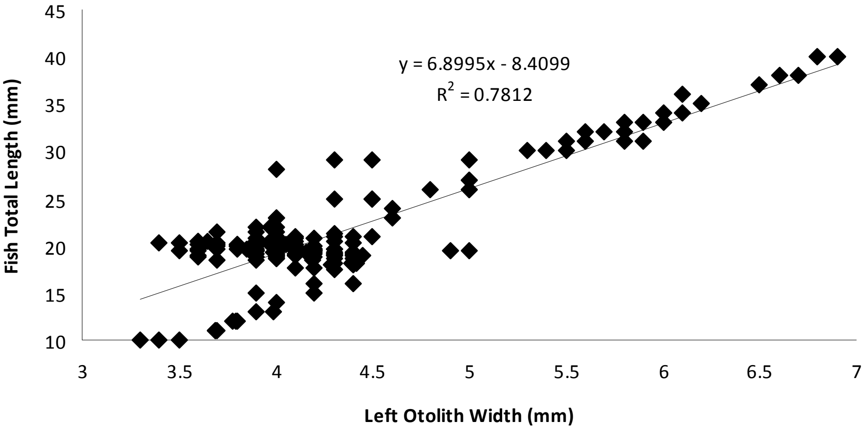


Fig. 3. Total length-left otolith width relationship.

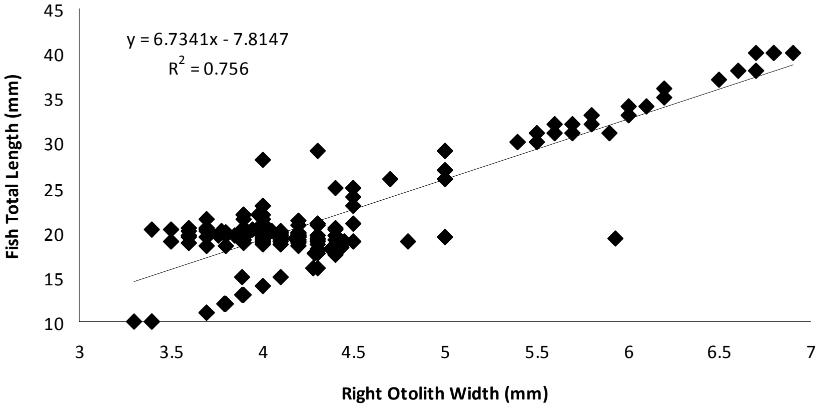


Fig. 4. Total length-right otolith width relationship.

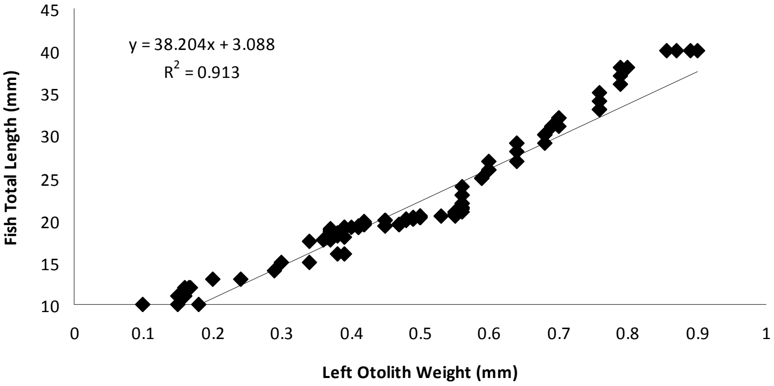


Fig. 5. Total length-left otolith weight relationship.

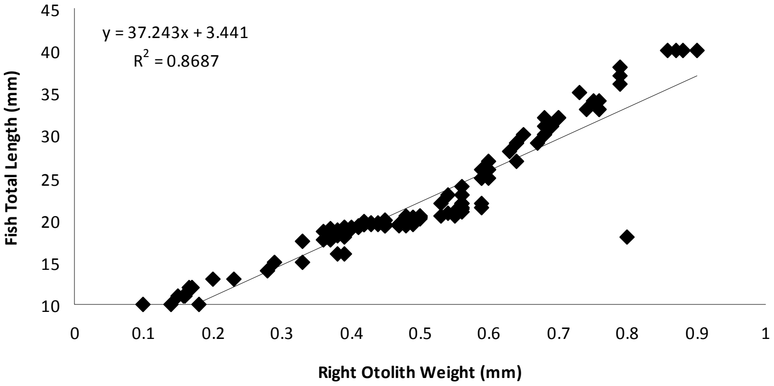


Fig. 6. Total length-right otolith weight relationship.

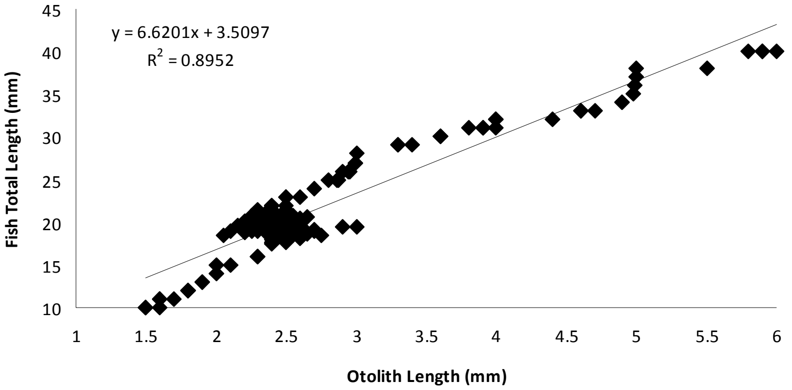


Fig. 7. Total length-mean otolith length relationship.

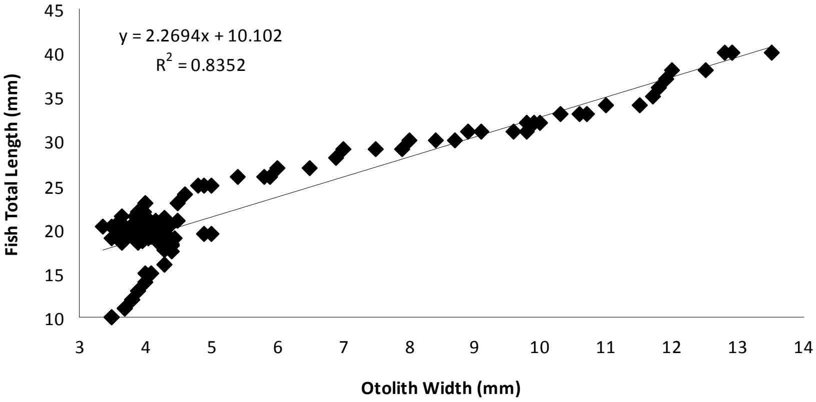


Fig. 8. Total length-mean otolith width relationship.

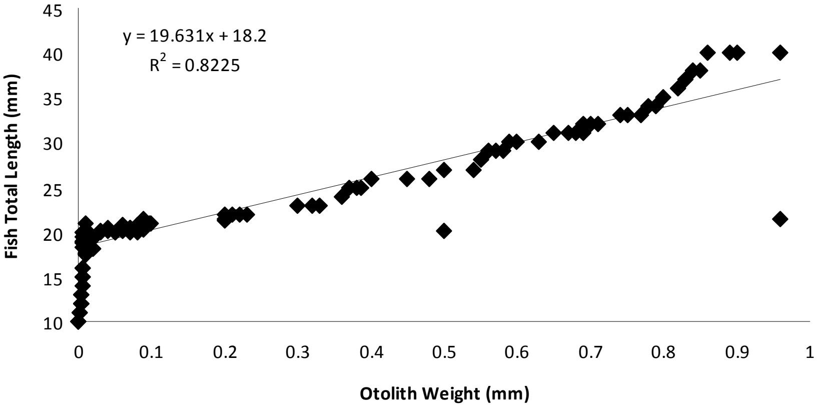


Fig. 9. Total length-mean otolith weight relationship.

DISCUSSION

Due to their high inter-specific variability in shape, otoliths reconsidered as a profound taxonomic tool in fish species identification (Battaglia *et al.*, 2010). Therefore the identification of fish preys is supported by some reference works (Smale *et al.*, 1995; Campana, 2004; Lombarte *et al.*, 2006; Tuset *et al.*, 2008), however, only certain geographical areas are covered and the access to reference material remains requisite (Santos *et al.*, 2001). Thus, an essential objective of researchers studying the marine predators' feeding habits is to add more information to what is already available of the fish otolith morphology and on the estimation of specific equations, which is useful to calculate the size and mass of preys.

The results of the present paper address to this need, providing TL-OL, TL-OW and TL-OWE relationships for the pelagic fish species, *Rhynchorhamphus georgi*. Despite the important role of this species in top predators' diet, its biology and ecology had not been well investigated until today in Oman at least.

The work at hand presents a preliminary useful tool for better understanding the trophic relationships in the coasts of the Sea of Oman food web. As pointed out by number of feeding studies in several fish groups (Castriota *et al.*, 2007; Falautano *et al.*, 2007; Consoli *et al.*, 2008; Karakulak *et al.*, 2009) the rebuilding of prey biomass from otolith size and weight may of benefit to the pelagic fishes as to show their role in the surface of the sea and benthic environment trophic structure. In the Sea of Oman, there are only limited studies that took into consideration the appropriate quantification of the prey biomass and classification to the species level the food items of different piscivorous fish species. Al-Alawi (2002) and Mohamed (2003) have identified and estimated the percentage of *R. georgi* in the diet of a number of piscivorous fish species living in the Sea of Oman such as *Scomberomorus commerson*. This percentage is exceeding 45% of the piscivorous diet and at the same time indicates the importance of the species in question in the diet of this predator and other predators such as several members of the genus. Consequently, the large pelagic fish *Scomberomorus commerson* is considered to be a serious predator of pelagic fish in the Sea of Oman when 58% of *Scomberomorus commerson* biomass could be considered to be mainly piscivorous.

It is important at this stage to draw the attention to some limitations to the use of biomass reconstruction from otolith size and weight. The growth of individuals belonging to the same species may show some variations for different areas and stock (Campana & Casselman, 1993; Reichenbacher *et al.*, 2009) or between sexes (Wyllie, 1987). Such correlations between otolith growth and variation in areas and stock or between sexes have been noticed in otolith of a large number of fish species obtained from the Sea of Oman (personal observation). Furthermore, otolith size might become underestimation due to the exposure of the otoliths to chemical and mechanical abrasion in the digestive track of predators (Jobling &

Breiby, 1986; Granadeiro & Silva, 2000). Similar results were obtained for the otolith of some fish species obtained from the stock of fishes collected from the Sea of Oman. In these otoliths, the ornaments usually found on the mesial side of the otolith have been severely abrassed and the anterior and posterior edges of the otolith have been deteriorated and the size of the otolith looks much smaller than that of the otolith recovered directly from the fish body of the same species (Sulaiman *et al*., 2007; Saad, 2005; Khalid, 2007).

On contrary of the previous studies on the relationship between fish and sagitta sizes and weight (Wyllie, 1987; Gamboa, 1991; Granadeiro & Silva, 2000; Harvey *et al*., 2000; Waessle *et al*., 2003; Battaglia *et al*., 2010), this paper supplies additional information by considering both the otolith length (OL), otolith width (OW) and otolith weight (OWE). It is more suitable to calculate more than two equations (TL-OL, TL-OW and TL-OWE) since the tip of the otolith rostrum or the dorsal or ventral edges of the otolith may be damaged, making it impossible to measure the OL or OW. Moreover, the coefficient of determination of the three regression equations attained a high value (Table 2).

In contrast with the findings of (Harvey *et al*., 2000; Waessle *et al*., 2003) the otolith of the species in question did not show significant differences in size between left and right sagittae. This finding is in agreement with the results of Battaglia *et al*. (2010).

In spite of all data fitted well with the linear regressions obtained in the present study, it is advisable to use these equations within the fish size range limit reported for this species in the results section. Authors who studied a wide range of fish length and include larvae in their sample, have supplied two different TL-OL regressions, one for the small sized fish and another for the adult specimens (Nishimura & Yamada, 1988; Linkowski, 1991). Such situation is not applicable to the results obtained in the present study as no larvae were present in the sample as the individuals of *R. georgi* collected in the present paper belong to the 100–400 mm TL range, the regressions TL-OL, TL- OW and SL- OWE calculated here in can be accepted.

CONCLUSIONS

Our study has shown a strong correlation between fish length and otolith dimensions and weight. Such a correlation indicates the usefulness of the otolith proportions and weight in back calculation of the length of the fish in biological and paleontological studies. No significant differences have been observed between right and left otolith size and weight.

Acknowledgements. Our sincere thank goes to the Ministry of Agriculture and Fisheries Wealth, Marine Science and Fisheries Centre and the directorate of Agriculture and Fisheries Developmental Fund for giving us the opportunity to work on the fish samples within the qualitative and quantitative distribution of marine organisms in Sultanate of Oman and to provide the appropriate financial support.

REFERENCES

ALI M.M., 2007, *Food of four large pelagic fish species in the Gulf of Oman*. Report to the Government of Oman, 234 pp.

AL-ALAWI Y.R., 2002, *Food and feeding habits of some fish species in the Sea of Oman*. Report to the Ministry of Agriculture, Iran, 125 pp.

ANONYMOUS, 2005, *The significance of pelagic prey to the benthopelagic fish in the benthic layer of the Sea of Oman.* Report to the Government of United Arab Emirates, 45 pp.

BATTAGLIA P., MALARA D., ROMEO T., ANDALORO F., 2010, *Relationship between otolith size and fish size in some mesopelagic and bathypelagic species from the Mediterranean Sea (Strait of Messina), Italy*. Marine Science, **74**: 605-612.

BERGSTAD O.A., WIK Å.D., HILDRE Ø., 2003, *Predator-Prey relationship and food sources of the Sagerrak deep-water fish assemblage*. Journal of Northwest Atlantic Fisheries Science, **31**: 165-180.

CAMPANA S.E., CASSELMAN J.M., 1993, *Stock discrimination using otolith shape analysis*. Canadian Journal of Fisheries and Aquatic Sciences, **50**: 1062-1083.

CAMPANA S.E., 2004, *Photographic atlas of fish otoliths of the Northwest Atlantic Ocean*, pp. 284. Ottawa, Ontario: NRC Research Press.

CASTRIOTA L., PIPITONE C., CAMPAGNUOLO S., ROMANELLI M., POTOSCHI A., ANDALORO F., 2007, *Diet of Coryphaena hippurus (Coryphaenidae) associated with FADs in the Ionian and Southern Tyrrhenian Seas*. Cybium, **31**: 435-441.

CONSOLI P., ROMEO T., BATTAGLIA P., CASTRIOTA L., ESPOSITO V., ANDALORO F., 2008, *Feeding habits of the albacore tuna Thunnus alalunga (Perciformes, Scombridae) from central Mediterranean Sea*. Marine Biology, **155**: 113-120.

CURY P., BAKUN A., CRAWFORD R.J.M., JARRE A., SHANNON L.J., VERHEYE H. M., 2000, *Small pelagic in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems*. ICES Journal of Marine Science, **57**: 603-618.

DUFFY D.C., LAURENSON L.J.B., 1983, *Pellets of cape cormorants as indicators of diet*. Condor, **85**: 305-307.

FALAUTANO M., CASTRIOTA L., FINOIA M.G., ANDALORO F., 2007, *Feeling ecology of little tunny Euthynnus alletteratus in the central Mediterranean Sea*. Journal of Marine Biological Association of U. K., **87**: 999-1005.

FOWLER J., COHEN L., 1992, *Practical statistics for field biology*. John Wiley and Sons, Chichester, New York, Brisbane, Toronto, 227 pp.

GAMBOA D.A., 1991, *Otolith Size versus Weight and Body-Length Relationships for Eleven Fish Species of Baja California, Mexico.* Fisheries Bulletin U.S., **89**: 701-706.

GIRONE A., NOLF D., CAPPETTA H., 2006, *Pleistocene fish otoliths from the Mediterranean Basin: a synthesis*. Geobios, **39**: 651-671.

GRANADEIRO J.P., SILVA M. A., 2000, *The use of otoliths and vertebrae in the identification and size-estimation of fish in predator-prey studies*. Cybium, **24**: 383-393.

GREENSTREET S.P.R., MCMILLAN J.A., ARMSTRONG E., 1998, *Seasonal variation in the importance of pelagic fish in the diet of piscivorous fish in the Moray Firth, NE Scotland: a response to variation in prey abundance?* ICES Journal of Marine Science, **55**: 121–133.

HARVEY J.T., LOUGHLIN T.R., PEREZ M.A., OXMAN D.S., 2000, *Relationship between fish size and otolith length for 6kiss3 species of fishes from the eastern North Pacific Ocean*. NOAA Technical Report NMFS, **150**: 35.

HASSANI S., ANTOINE L., RIDOUX V., 1997, *Diets of albacore, Thunnus alalunga, and dolphins, Delphinus delphis and Stenella coerulaeoalba, caught in the northeast Atlantic albacore drift-net fishery: A progress report*. Journal of Northwest Atlantic Fisheries Science, **22**: 119-123.

HUSSAINI M.M., 2006, *Pelagic fish as important food items in the piscivorous fish in the Sea of Oman and the Persian Gulf.* Report to the Department of Environment, Tehran, Iran, 98 pp.

JOBLING M., BREIBY A., 1986, *The use and abuse of fish otoliths in studies of feeding habits of marine piscivores*. Sarsia, **71**: 265-274.

JOHNSTONE I.G., HARRIS M.P., WANLESS S., GRAVES J.A., 1990, *The usefulness of pellets for assessing the diet of adult shags Phalacrocorax aristotelis*. Bird Study, **37**: 5-11.

KARAKULAK F.S., SALMAN A., ORAY I.K., 2009, *Diet composition of bluefin tuna (Thunnus thynnus L. 1758) in the Eastern Mediterranean Sea, Turkey*. Journal of Applied Ichthyology, **25**: 647-651.

KHALID M.H., 2007, *Food items of some piscivorous fishes obtained from the Sea of Oman*. Quarterly Report, Ministry of Environment, Iran, 345 pp.

LINKOWSKI T.B., 1991, *Otolith microstructure and growth patterns during the early life history of lanternfishes (Family Myctophidae)*. Canadian Journal of Zoology, **69**: 1777-1792.

LOMBARTE A., CHIC Ò., PARISI-BARADAD V., OLIVELLAl R., PIERA J., GARCÍA-LADONA E., 2006, *A web-based environment from shape analysis of fish otoliths*. The AFORO database (http://www.cmima.csic.es/aforo/). Scientia Marina, **70**: 147-152.

LYCHAKOV D.V., REBANE Y.T., LOMBSARTE A., FUIMAN L.A., TAKABAYASHI A., 2006, *Fish otolith asymmetry: morphometry and modelling*. Hearing Research, **219**: 1-11.

MARTIN B., CHRISTIANSEN B., 1997, *Diets and standing stocks of benthopelagic 19 fishes at two bathymetrically different midoceanic localities in the northeast 20 Atlantic*. Deep-Sea Research, **44**: 541-558.

MCINTYRE J.K., BESAUCHAMP D.A., MAZUR M.M., OVERMAN N.C., 2006, *Ontogenetic trophic interactions and benthopelagic coupling in lake Washington: evidence from stable isotopes and diet analysis*. Transaction of the American Fisheries Society, **135**: 1312-1328.

MOHAMED G.O., 2003, *Food and feeding habits of some piscivorous fishes collected from the Sea of Oman.* Annual Report of the Ministry of Environment, Iran, 256 pp.

MONTEVECCHI W.A., MYERS R.A., 1995, *Prey harvests of seabirds reflect pelagic fish and squid abundance on multiple spatial and temporal scales*. Marine Ecology Progress Series, **117**: 1- 9.

NISHIMURA A., YAMADA J., 1988, *Geographical differences in early growth of walleye Pollock Theragra chalcogramma, estimated by back-calculation of otolith daily growth increments*. Marine Biology, **97**: 459-465.

NOLF D., 1985, *Handbook of Paleoichthyology. Otolithi piscium*. 10, pp. 141. Stuttgart, Springer Verlag.

NOLF D., 1995, *Studies on fossil otoliths – the state of the art*. In: D.H. Secor, J.M. Dean and S.E. Campana (Eds.). *Recent developments in fish otolith research*, pp. 513-544. University of South Carolina Press.

PALOMERA I., MARTA C., TUDELA S., 2007, *Modelling the ecological role of small pelagic fish in the Catalan Sea (NW Mediterranean)*. Rapports Commission Internationale Exploration Scientifique Mere, **38**.

PAULY D., TRITES A.W., CAPULI E., CHRISTENSEN V., 1998, *Diet composition and trophic levels of marine mammals*. ICES Journal of Marine Science, **55**: 467-481.

PIERCE G.J., BOYLE P.R., 1991, *A review of methods for diet analysis in piscivorous marine mammals*. Marine Biology Annual Review (London), **29**: 409-486.

RANDALL J.E., 1995, *Coastal fishes of Oman*. Bathurst, Australia: Crawford House Publishing Pty Ltd., 439 pp.

REICHENBACHER B., KAMRANI E., ESMAEILI H.R., TEIMORI A., 2009, *The endangered cyprinodont Aphanius ginaonis (Holly, 1929) from southern Iran is a valid species: evidence from otolith morphology*. Environmental Biology of Fishes, **86**: 504-521.

SAAD S.Y., 2005, *On the food items of some piscivorous fishes collected from the Sea of Oman*. Annual Report, Ministry of Environment, 456 pp.

SANTOS M.B., CLARKEl M.R., PIERCE G.J., 2001, *Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions*. Fisheries Research, **2**: 121-129.

SMALE M.J., WATSON G., HECHT T., 1995, *Otolith atlas of southern African marine fishes*. Ichthyological Monographs, JLB Smith Institute of Ichthyology, **1**: 253.

STRONG M.B., NEILSON J.D., HUNT J., 1986, *Aberrant crystallization of pollock (Pollachius virens) otoliths*. Canadian Journal of Fisheries and Aquatic Sciences, **43**: 1457-1463.

SULAIMAN D.F., HAMEEDI N.B., ILAHIl U.K., 2007, *Food and feeding habits of some piscivorous fishes collected from the Sea of Oman*. Scientific Report No. 5, Ministry of Fisheries, Iran, 98 pp.

TUSET V.M., LOMBARTE A., ASSIS C.A., 2008, *Otolith atlas for the western Mediterranean, north and central eastern Atlantic*. Scientia Marina, **72S1**: 7-198.

WAESSLE J.A., LASTA C.A., BAVERO M., 2003, *Otolith morphology and body size relationships for juvenile Sciaenidae in the Río de la Plata estuary (35–36°S)*. Scientia Marina, **67**: 233-240.

WYLLIE E.T., 1987, *Relationship of otolith length to total length in rockfishes from northern and central California*. Fishery Bulletin, **85**: 383-387.

Received April 28, 2011

[*] *Marine Science and Fisheries Centre, Ministry of Fisheries Wealth,*
P.O. Box 427,
Postal Code 100 Muscat, Sultanate of Oman
laith_jawad@hotmail.com
[**] *High Technical College, Muscat, Sultanate of Oman*

EFFECTS OF OIL POLLUTION
ON *EURYGLOSSA ORIENTALIS* AND *PSETTODES ERUMEI*
IN THE PERSIAN GULF

ZAHRA KHOSHNOOD [*], REZA KHOSHNOOD [**], MAJID AFKHAMI [***], AIDA KHAZAALI [****]

The studied species (*Euryglossa orientalis* and *Psettodes erumei*) were collected from two areas of North coast of the Persian Gulf: Bandar Abbass and Bandar Lengeh. Concentrations of Ni and V in the liver of both species were higher in Bandar Abbass than in Bandar Lengeh. These quantities were higher in *P. erumei*, in both sampling areas. Cellular alterations including: degeneration, necrosis and tissue disruption were observed in the liver of both species, and these alterations were severe in *P. erumei*. The results showed that Bandar Abbass was more polluted than Bandar Lengeh, and because Ni and V were oil pollution indicators, and the studied fish were benthic, they can receive a considerable amount of oil pollution through their biological activities like feeding.

Key words: heavy metals, flatfish, oil pollution, Persian Gulf.

INTRODUCTION

Marine pollution is a global environmental problem. Different human activities on land, in the water and in the air contribute to the contamination of seawater, sediments and organisms with potentially toxic substances. After discharge into the sea, contaminants can stay in the water in dissolved form or they can be removed from the water column through sedimentation to the bottom sediments (Oliveira Ribeiro *et al*., 2002).

Contamination with heavy metals on different scales have been intensively studied in recent years, due to the fact that metals are persistent, toxic, tend to bioaccumulate, and they pose a risk to humans and ecosystems (Szefer, 2002; Rainbow, 2002) The main reason for this is the increasing metal input to the coastal zone from both rivers and non-point sources, especially in developing countries. Metal contamination can have adverse effects on marine organisms only after metal uptake and accumulation (Cajaraville *et al*., 2000; Funes *et al*., 2006).

Histopathological alterations can be used as indicators for the effects of various pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the action of a few several xenobiotics could initiate the

ROM. J. BIOL. – ZOOL., VOLUME **56**, N° *2*, P. 201–209, BUCHAREST, 2011

formation of a specific enzyme that causes changes in the metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, *i.e.* histopathological biomarkers on a tissue level (Bailey *et al.*, 1996).

Previous studies reported that the exposure of fish to pollutants (agricultural, industrial and/or sewage) resulted in several pathological alterations in different tissues of different fish species. Histopathological changes were observed in the muscle of fish as a result of exposure to different toxicants (Braunbeck *et al.*, 1998). The liver, the major metabolic organ, comes in close contact with xenobiotics absorbed from the environment and liver lesions are often associated with aquatic pollution.

Vinodhini & Narayanan (2008) studied bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (common carp) in 2008. In this study they determined the bioaccumulation of heavy metals in various organs of the fresh water fish exposed to heavy metal contaminated water system. The experimental fish were exposed to Cr, Ni, Cd and Pb at sublethal concentrations for periods of 32 days. The order of heavy metal accumulation in the gills and liver was Cd > Pb > Ni > Cr and Pb > Cd > Ni > Cr. Similarly, in case of kidney and flesh tissues, the order was Pb > Cd > Cr > Ni and Pb > Cr > Cd > Ni. In all heavy metals, the bioaccumulation of lead and cadmium proportion was significantly increased in the tissues of *Cyprinus carpio* (Vinodhini & Narayanan, 2008).

Pollution studies in the Persian Gulf area, collectively known as ROPME Sea Area (RSA), are very important. The Persian Gulf comprises a relatively shallow, semi-enclosed water body with very high evaporation rates and poor flushing characteristic, and so much pollution discharge into the sea, having more limitations for dilution and slower dispersion rather than what occurs in other open marine systems (De Mora *et al.*, 2004). Two heavy metals were approached in the present study: Ni and V, which are also the indicators of oil pollution (a very common contamination in the Persian Gulf).

Flat fish in near shore waters are benthic organisms because of their proximity to the sediments so they are more susceptible to come into contact with pollutants; flat fish can be indicators for pollutants, and are also important for human health through fisheries consumption.

MATERIAL AND METHODS

Fish samples were collected along two coastal areas of Hormoz strait, North of the Persian Gulf, on the coastal lines of Iran. A total of 24 samples of fish species, including *Euryglossa orientalis* and *Psettodes erumei,* have been caught from April to June 2006. These two species were collected from two fishing regions, including Bandar Abbas and Bandar Lengeh (Fig. 1).The fish species were randomly collected from commercial catches landed at local fishing ports.

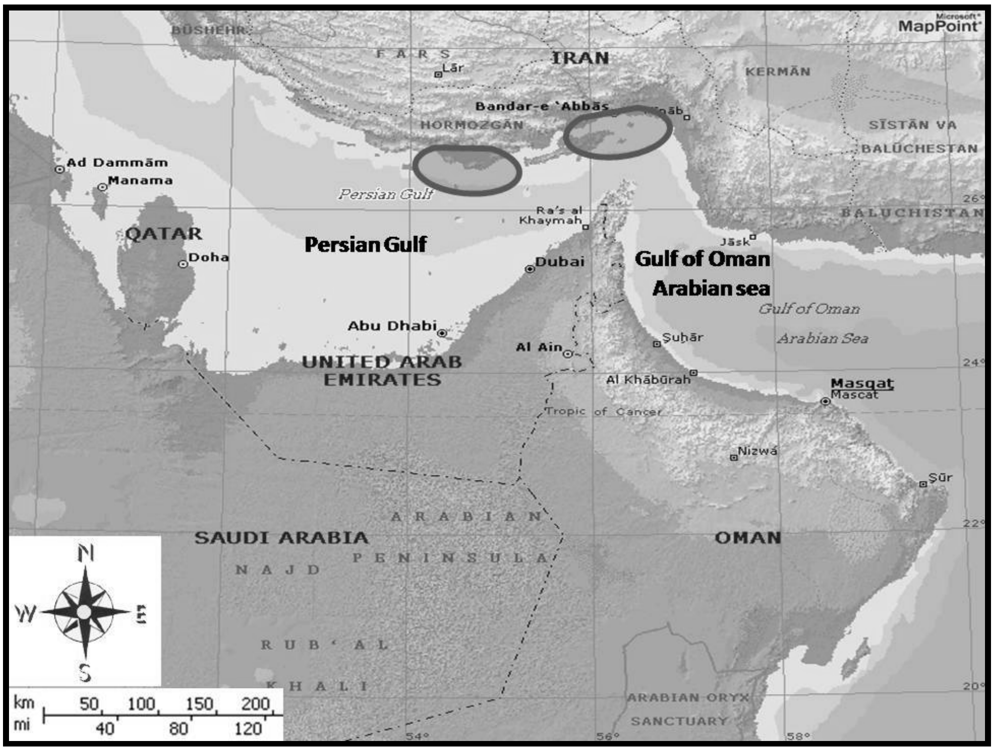


Fig. 1. Map showing the sampling area.

Immediately after the biological material collection, the fish samples were stored on ice in an isolated box (Eaton *et al.*, 1995) and transferred to the laboratory. Body weight and length of fish were measured and the gender was determined. Then male fish were selected and a part of dorsal muscle from each was dissected as a sample. The fish liver tissue was also removed and prepared for processing. All of the samples were dried at 60º C for 48h in the oven (Gregory *et al.*, 2005).

All pieces of glassware were cleaned by soaking in 10% v/v HNO3 for 12 h and then rinsing with ultra-pure water. Between 0.2 and 0.4 g of dried sample material were weighed and then digested in acid-cleaned Teflon microwave vessels with 5 ml of ultra-pure nitric acid (65% v/v). A typical microwave digester was operated for 30-40 min at a target digestion temperature at 200ºC after allowing at least 1 h for cooling. The digested samples were transferred to a graduated plastic test tube and brought up to volume (50 ml) with Mili-Q-water (Gregory *et al.*, 2005).

Liver samples were fixed in Bouin‘s solution for a 24 hours period, dehydrated in a graded series of ethanol, and embedded in Paraplast (Merck).

Three-micrometer-thick sections were finally obtained and stained in hematoxylin/fuchsin for examination by light microscopy (Martoja & Martoja-Pierson, 1967).

In this study the statistical analysis was done using the SPSS software (Version 11.5). The data were tested to check normality using the Kolmogorov-Smirnov test, which showed the fact that they have a normal distribution. The Pearson's correlation test was used to assess any significant relationship of heavy metals concentration in tissue with sampled fish length and weight in the two studied regions (level of significance, $p < 0.05$). Also in addition, the paired sample t-test was used to compare tissue heavy metals concentrations between the studied stations.

RESULTS AND DISCUSSION

The maximum length of *E. orientalis* recorded in Bandar Lengeh (station 2) was 41 cm and weight of this sample was 1010 g, maximum weight for weight of *E. orentalis*. *P. erumei'*s maximum length measurement was recorded in station 1 (Bandar Abbas), 58 cm, and the weight of this sample was 2,650 g (Table 1).

Table 1

Biometry results for fish samples

Species	**Station**	**Length**		**Weight**		**No. of samples**
		Range	Mean	Range	Mean	
E. orientalis	St1	24.5-36.5	32.3	240-710	585	6
	St2	24-41	32.1	209-1010	604	6
P. erumei	St1	39-58	48.1	754-2650	1672	6
	St2	31.5-51	43.0	497-2286	1309	6

Tables 2 and 3 show the concentration of Ni and V in liver of two fish in the study areas. The concentration of Ni, V in *P. erumei* was higher than in *E. orientalis*, this maybe because of feed habitat and position of *P. erumei* in food chain and/or pyramid, because *P. erumei* is a predator feeding on small fish.

Tables 4 to 7 show the correlation between length/weight and metal concentration in *P. erumei* in two stations. Figures 2 and 3 show concentration of Ni and V in two flatfishes.

Table 2

Mean and Range of concentration of Ni in liver of species (ppm)

Station	Species	Range	Mean	± SD
Bandar Abbass	*P. erumei*	1.02-2.20	1.28	0.21
	E. orientalis	1.04-1.01	0.89	0.36
Bandar Lengeh	*P. erumei*	1.02-2.51	1.12	0.42
	E. orientalis	0.54-1.23	0.88	0.28

Table 3

Mean and range of concentration of V in liver of species (ppm)

Station	Species	Range	Mean	± SD
Bandar Abbass	*P. erumei*	1.89-2.63	2.43	1.89-2.63
	E. orientalis	1.72-2.23	1.89	1.72-2.23
Bandar Lengeh	*P. erumei*	2.11-2.42	2.22	2.11-2.42
	E. orientalis	1.07-1.94	1.54	1.07-1.94

Table 4

Correlation between length/weight and metal concentration in *P. erumei* in Bandar Abbass

Metals in tissue	V	Ni
Length **Pierson correlation index**	0.988	0.968
Sig (2-tailed)	0.000	0.002
Weight **Pierson correlation index**	0.996	0.988
Sig (2-tailed)	0.000	0.000

Table 5

Correlation between length/weight and metal concentration in *P. erumei* in Bandar Lengeh

Species	Station	Length		Weight		No. of samples
		Range	Mean	Range	Mean	
E.orientalis	St1	24.5-36.5	32.3	240-710	585	6
	St2	24-41	32.1	209-1010	604	6
P. erumei	St1	39-58	48.1	754-2650	1672	6
	St2	31.5-51	43.0	497-2286	1309	6

Table 6

Correlation between length/weight and metal concentration in *E. orientalis* in Bandar Abbass

Metals in tissue	V	Ni
Length	0.952	0.941
Pierson correlation index	0.003	0.005
Sig (2-tailed)		
Weight	0.996	0.945
Pierson correlation index	0.000	0.004
Sig (2-tailed)		

Table 7

Correlation between length/weight and metal concentration in *E. orientalis* in Bandar Lengeh

Metals in tissue	V	Ni
Length	0.993	0.967
Pierson correlation index	0.000	0.002
Sig (2-tailed)		
Weight	0.996	0.960
Pierson correlation index	0.000	0.002
Sig (2-tailed)		

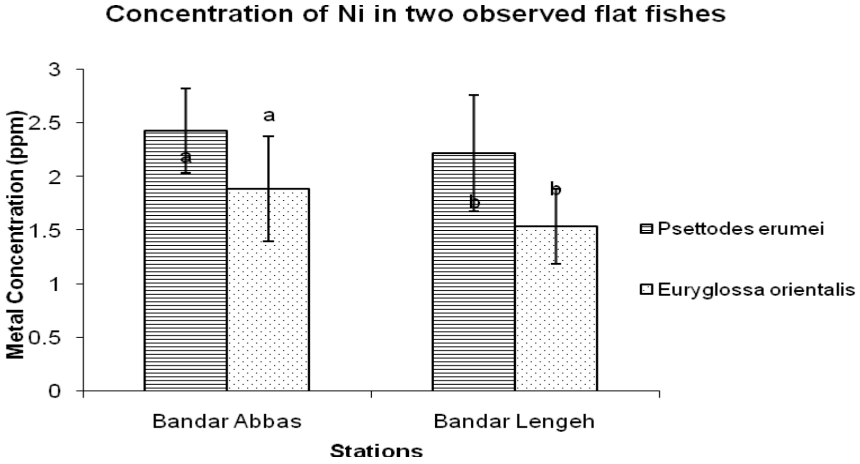


Fig. 2. Concentration of Ni in two flat fishes (*E. orientalis* and *P. erumei*).

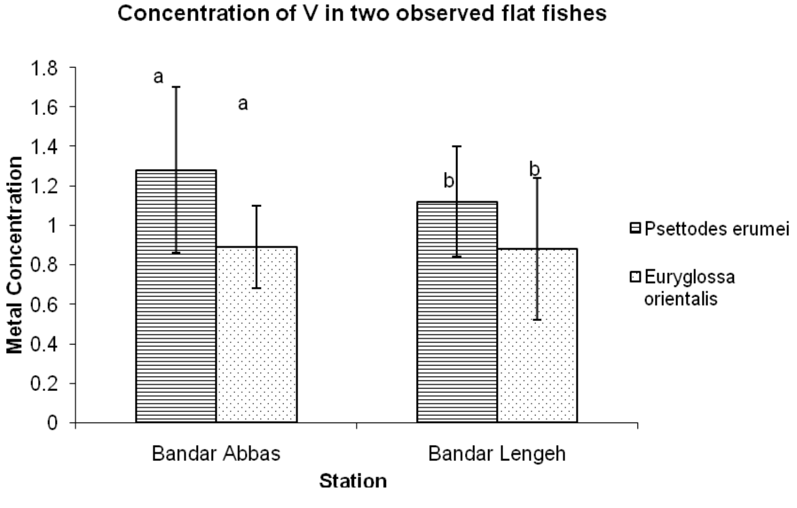


Fig. 3. Concentration of V in two flat fish (*E. orientalis* and *P. erumei*).

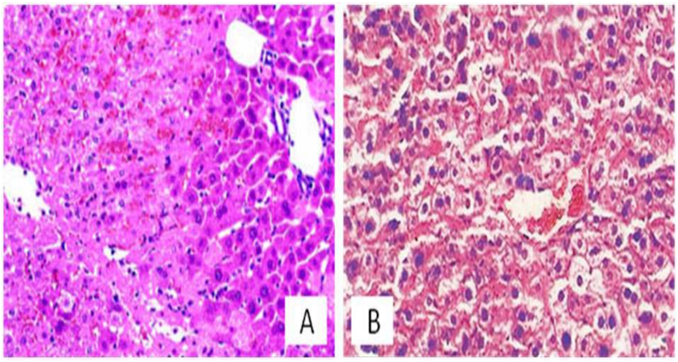


Fig. 4. Liver histopathology of *P. erumei* in Bandar Abbass (A) and Bandar Lengeh (B). Hemorrhagia, cell necrosis and degeneration were the most histopathological alterations observed in liver of *P. erumei* like the alterations observed in *E. orientalis* but milder.

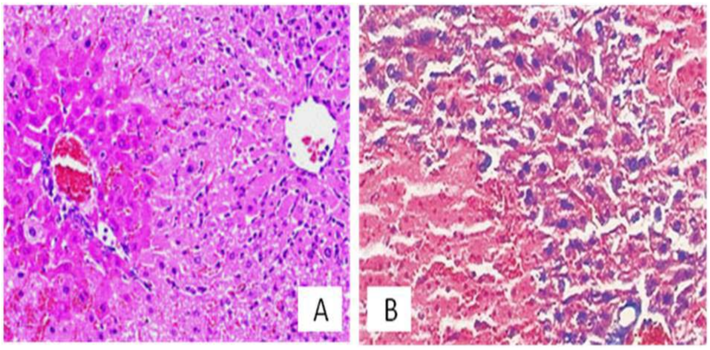


Fig. 5. Liver histopathology of *E. orientalis* in Bandar Abbass (A) and Bandar Lengeh (B). Hemorrhagia, cell necrosis and degeneration were the most frequent histopathological alterations observed in liver of *E. orientalis*. Hepatocytes alterations were more severe in Bandar Abbass sample than in Bandar Lengeh sample.

Fig. 4 (A and B) showed histopathological alterations in liver of *P. erumei* in Bandar Abbass and Bandar Lengeh respectively; these alterations were degeneration and necrosis of hepatocytes and tissue disruption and hemorrhagia.

Fig. 5 (A and B) showed histopathological alterations in the liver of *E. orientalis* in Bandar Abbas and Bandar Lengeh; cellular alterations were the same as the *P. erumei*, but they were more severe. In both species cellular alterations were more severe in Bandar Abbass than in Bandar Lengeh.

CONCLUSIONS

Results of the concentrations of the Ni and V in two studied species, P. *erumei* and *E. orientalis* that have the same living conditions (benthic) showed that fishing grounds of Bandar Abbass were more polluted than Bandar Lengeh. As the fact that Ni and V were indicators of oil pollution, it shows that transportation of ships and freighters, especially those carrying oil and fuels were the most likely sources of pollution in the area. Also the results showed that the concentrations of these two heavy metals were significantly related with length and weight of the fishes, this result confirming the principals of heavy metals bioaccumulation in organisms.

No significant differences in heavy metals concentration between two studied species (*P. erumei* and *E. orientalis*) in two sampling areas (Bandar Abbass and Bandar Lengeh), perhaps because of the same living conditions for both species.

Vanadium and nickel are present as porphyrins in crude oils. Data concerning V in seafood are scarce, but bioaccumulation of V in mollusks has been suggested (Edel & Sabbioni, 1993). Ni can cause toxicity if its levels exceed the regulated values in food. The WHO (1992) recommends 100–300 µg of Ni for daily intake. The lowest and highest V contents were found as 0.82 µg/g in hake (*Merluccius* sp) and 5.14 µg/g in cockle (Cardiidae). For Ni, the minimum content is found in mussel (*Mytilus* sp) (2.94 µg/g) and the maximum in cockle (46 µg/g) (WHO, 1992).

The biological and ecological responses to certain pollutants (organic and inorganic) may vary from changes at the population/community level, to organ/tissue, and even to molecular level (Bailey *et al*., 1996). Since histological and histopathological changes produced by pollutants in organs and tissues can occur before they produce irreversible effects on the biota, histological methods can be used in conjunction with other parameters and/or ecotoxicological bioindicators as an early warning system, for the survival of the species, as well as for environmental protection.

Acknowledgements. We express our special thanks to Hossein Pasha, who had kindly assisted us in data analysis. We are also grateful to M. Afkhami, M. Ehsanpour, and S. Mosafer for their valuable help in fish sampling and laboratory analysis. This work was funded by the Islamic Azad University Science and Research Branch of Ahwaz.

REFERENCES

BAILEY G.S., WILLIAMS D.E., HENDRICKS J.D., 1996, *Fish models for environmental carcinogenesis: the rainbow trout.* Environmental Health Perspectives, **104**: 5-21.

BRAUNBECK T., STREIT B., HINTON D.E., 1998, *Cytological alterations in fish hepatocytes following in vivo and in vitro sublethal exposure to xenobiotics–structural biomarkers of environmental contamination. In:* Fish Ecotoxicology Birkhauser Verlag , Switzerland , pp. 61-140.

CAJARAVILLE M.P., BEBIANNO M.J., BLASCO J., PORTE C., SARASQUETE C., VIARENGO A., 2000, *The use of biomarkers to assess the impact of pollution in coastal environment of the Iberian Peninsula: a practical approach*. Science of Total Environment, **247**: 295-311.

DE MORA S., FOWLER S.W., WYSE E., AZEMARD S., 2004, *Distribution of heavy metals in marine bivalves, fish and coastal sediments in Persian Gulf and Gulf of Oman.* Marine Pollution Bulletin, **49**: 410-424.

EDEL J., SABBIONI E., 1993, *Accumulation, distribution and form of vanadate in the tissues and organelles of the mussel Mytilus edulis and the goldfish Carassius auratus.* Science of Total Environment, **133**: 139-151.

EATON A.D., CLESCEND L.S., GREENBERG A.E., FRANSON M.A.H., 1995, *Standard methods for examination of water and waste water.* 19th Ed. American Pub. Assoc. (APHA), Washington.

FUNES V., ALHAMA J., NAVAS J.I., LOPEZ-BAREA J., PEINADO J., 2006, *Ecotoxicological effects of metal pollution in two mollusc species from the Spanish South Atlantic littoral.* Environmental Pollution, **139**: 214-223.

GREGORY G.P., RAJOTTE J.W., COUTURE P., 2005, *Effects of industrial metals on wild fish populations along a metal contamination gradient.* Ecotoxicology and Environmental Safety, **61**: 287-312.

MARTOJA R., MARTOJA-PIERSON M., 1967, *Initiation aux techniques de l' histologie animale.* Éd. Masson et Cie CRC Press Paris, France, 345 pp.

OLIVEIRA RIBEIRO C.A., BELGER L., PELLETIER É., ROULEAU C., 2002, *Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (Salvenilus alpinus).* Environmental Research, **90**: 217-225.

RAINBOW P.S., 2002, *Trace metal concentrations in aquatic invertebrates: why and so what?* Environmental Pollution, **120**: 497-507.

SZEFER P., 2002, *Metals, Metalloids and Radionuclides in the Baltic Sea Ecosystem.* Elsevier Science, Amsterdam, 752 pp.

VINODHINI R., NARAYANAN,M., 2008, *Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp).* International Journal of Environmental Science and Technology, **5** (2): 179-182.

WHO, 1992, Technical report series no. 505, Evalution of certain food addition & the contamination mercury, lead & cadmium.

Received August 2, 2011

* *Islamic Azad University, Dezful Branch, Dezful,Iran,*
e-mail: zkhoshnood@gmail.com
** *Islamic Azad University, Tehran Central Branch, Tehran, Iran,*
e-mail: RezaKhoshnood@gmail.com
****Yung Researchers Club, Islamic Azad University,*
Bandar Abbas Branch, Bandar Abbas, P.O. Box: 79159-1311, Iran,
e-mail: m_afkhami82@yahoo.com
****Yung Researchers Club, Islamic Azad University,*
Bandar Abbas Branch, Bandar Abbas, P.O. Box: 79159-1311, Iran,
e-mail: Aida.khazaali@yahoo.com