

Short Communications

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New Records for Longhorned Beetles Fauna of Iraq (Coleoptera: Cerambycidae)

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Abstract.- The paper gives seven new records of species for Iraqi longhorned beetles fauna as *Aegosoma scabricorne* (Scopoli, 1763) for the subfamily Prioninae; *Cerambyx* (s.str.) *welensii* (Küster, 1845), *Osphranteria coerulescens* Redtenbacher, 1850, *Plagionotus* (*Echinocerus*) *floralis* (Pallas, 1773), *Plagionotus* (*Neoplacionotus*) *bobelayei* (Brullé, 1832), *Chlorophorus sartor* (O. F. Müller, 1766) and *Stenopterus rufus* (Linnaeus, 1767) for the subfamily Cerambycinae.

Keywords: Cerambycidae, longhorned beetle.

Unfortunately, Iraqi fauna of longhorned beetles has not been known well, although fauna of neighboring areas is rather well known. The only comprehensive known work is the unpublished thesis of Ismail (1983).

Piecemeal information is known from other works. For example Palaeartic catalogue of Löbl and Smetana (2010) includes a total of 46 species of 25 genera viz., 3 genera and 3 species of subfamily Prioninae, 2 genera and 4 species of subfamily Lepturinae, 1 genus and 1 species of subfamily Spondylidinae, 12 genera and 18 species of subfamily Cerambycinae and 7 genera and 20 species of subfamily Lamiinae. Ismail (1983) provided a total of 47 species of 26 genera viz., 3 genera and 5 species of subfamily Prioninae, 3 genera and 5 species of subfamily Lepturinae, 11 genera and 18 species of subfamily Cerambycinae, and 9 genera and 19 species of subfamily Lamiinae.

Özdikmen and Ali (2012) described Iraqi fauna-which comprised-more-taxa-than-the-ones-mentioned-in-the-catalogue. However, they

determined many misidentified taxa in Ismail's work without seeing his specimens. Consequently, the real status of 21 additional species that were given for the fauna of Iraq only on the basis of Ismail's work are still under discussion until their future confirmation. Özdikmen *et al.* (2013) have described a new species from Iraq as *Dorcadion mosulense*. From this point of view, we accept that Iraqi fauna includes a total of 47 species of 25 genera according to the Palaeartic catalogue of Löbl and Smetana (2010) and Özdikmen *et al.* (2013).

Materials and methods

Some specimens were collected in Iraq between 2012 and 2013. Besides, we have found some longhorned beetles in the museums (Museum of Erbil and Museum of Abu Ghraib in Baghdad). Currently, all specimens are preserved in Gazi University in Ankara (Turkey).

Identification of chorotypes is based on the chorotype classification of the Anatolian fauna proposed by Vigna Taglianti *et al.* (1999).

The data of distribution are given on basis of Löbl and Smetana (2010, 2011), Danilevsky (2010, 2012a,b, 2013), Özdikmen (2011) and Miroshnikov (2011).

Results

Family Cerambycidae Latreille, 1802: 211

Subfamily Prioninae Latreille, 1802: 212

Tribe Aegosomatini Thomson, 1861: 308

Genus *AEGOSOMA* Audinet-Serville, 1832: 162

A. scabricorne (Scopoli, 1763: 54)

Material examined

One male from Sulaymaniyah prov.: Biyara, VI.1973 in Museum of Erbil.

Chorotype

The species has a Euro-Iranian chorotype. It is distributed in the most parts of Europe and Asian Turkey, Caucasus, Transcaucasia (Armenia, Azerbaijan), Iran and Middle East (Syria and Lebanon).

Remarks

The species is a new record for Iraq. It was

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not recorded for Iraq in the catalogue of Löbl and Smetana (2010).

Genus *CERAMBYX* Linnaeus, 1758: 388
Subgenus *CERAMBYX* Linnaeus, 1758: 388
C. welensii (Küster, 1845: 44)

Material examined

One female from Forestry Department of Agriculture Faculty of Salahaddin University, Erbil. Erbil prov.: Safeen Mt., VIII.2007, with a light trap, on oak trees (*Quercus* sp.); 2 males from Biology Department of Education Faculty of Salahaddin University, Erbil.

Chorotype

The species has a West Mediterranean-Iranian chorotype. It is distributed in Southern Europe, European and Asian Turkey, Transcaucasia (Azerbaijan, Georgia), Cyprus, Syria, Israel, Jordan, Lebanon and Iran (Özdikmen, 2011).

Remarks

The species is a new record for Iraq. It was not recorded for Iraq in the catalogue of Löbl and Smetana (2010).

Tribe Callichromatini Swainson & Shuckard, 1840: 293

Genus *OSPHRANTERIA* Redtenbacher, 1850: 50
O. coerulescens Redtenbacher, 1850: 50

Material examined

Two females from Museum of Erbil. Erbil prov.: Salahaddin, V.2012; 2 males from Duhok prov.: Amadiyah, IV-VII.2013.

Chorotype

The species has been known only from Turkey, Iran and Pakistan until now (Bentanachs, 2012; Danilevsky, 2013). So, it has a SW-Asiatic chorotype with the present record.

Remarks

The species is a new record for Iraq. It was not recorded for Iraq in the catalogue of Löbl & Smetana (2010).

Tribe Stenopterini Gistel, 1848: [9]
Genus *STENOPTERUS* Illiger, 1804: 120
S. rufus (Linnaeus, 1767: 642)
Subspecies *S. rufus syriacus* Pic, 1892: 22

Material examined

One male from Museum of Erbil.

Chorotype

The subspecies has a Syrian chorotype. It is distributed only in S Turkey, Syria, Israel and Lebanon.

Remarks

The species is a new record for Iraq. It was not recorded for Iraq in the catalogue of Löbl & Smetana (2010).

Tribe Clytini Mulsant, 1839: 70
Genus *PLAGIONOTUS* Mulsant, 1842: 1
Subgenus *ECHINOCERUS* Mulsant, 1862: 143
P. floralis (Pallas, 1773: 724)

Material examined

One male, one female from Erbil prov.: Topzawa, 2012; 2 females from Erbil prov.: Choman, Hasarost Mt., VII.2013.

Chorotype

The species has a Euro-Siberian + Central Asian chorotype. It is distributed in S, E Europe, European and Asian Turkey, Israel, Jordan, Iran, Caucasus, Transcaucasia (Armenia, Azerbaijan, and Georgia), Kazakhstan, Kirgizia, Tajikistan, Turkmenistan, Uzbekistan, Siberia and NE China.

Remarks

The species is a new record for Iraq. It was not recorded for Iraq in the catalogue of Löbl and Smetana (2010).

Subgenus *NEOPLAGIONOTUS* Kasatkin, 2005: 51
P. bobelayei (Brullé, 1832: 253)

Material examined

One male from Mosul prov.: Hamam Al-Alil, IV.2012; 1 male from Erbil prov.: Topzawa, 08.VI.2002.

Table I.- An updated list of all known Iraqi Cerambycidae.

FAMILY CERAMBYCIDAE Latreille, 1802: 211

SUBFAMILY PRIONINAE Latreille, 1802: 212

Ergates faber (Linnaeus, 1760: 187)

Aegosoma scabricorne (Scopoli, 1763: 54) (*)

Mesoprionus persicus (Redtenbacher, 1850: 49)

Pogonarthron semenowi (Lameere, 1912: 224)

SUBFAMILY LEPTURINAE Latreille, 1802: 218

Stictoleptura cordigera cordigera (Füssli, 1775: 14)

Stictoleptura rufa dimidiata (Daniel & Daniel, 1891: 11)

Stictoleptura tripartita (Heyden, 1889: 329)

Stenurella bifasciata bifasciata (O. F. Müller, 1776: 93)

SUBFAMILY SPONDYLIDINAE Audinet-Serville, 1832: 123

Alocerus moesiacus (Frivaldszky, 1837: 177)

SUBFAMILY CERAMBYCINAE Latreille, 1802: 211

Hesperophanes sericeus (Fabricius, 1787: 152)

Trichoferus fasciculatus fasciculatus (Faldermann, 1837: 266)

Trichoferus fissitarsis Sama, Fallahzadeh & Rapuzzi, 2005: 125

Trichoferus griseus (Fabricius, 1792: 325)

Stromatium unicolor (Olivier, 1795: 58)

Jebusaea hammerschmidti Reiche, 1878: CLIV

Cerambyx apiceplicatus Pic, 1941: 2

Cerambyx cerdo cerdo Linnaeus, 1758: 392

Cerambyx welensii (Küster, 1845: 44) (*)

Purpuricenus apicalis Pic, 1905: 163

Purpuricenus mesopotamicus Al-Ali & İsmail, 1987: 536, 543

Purpuricenus wachanrui Levrat, 1858: 261

Calchaenesthes diversicollis Holzschuh, 1977: 129

Aromia moschata ambrosiaca (Steven, 1809: 40)

Osphrantheria coeruleascens Redtenbacher, 1850: 50 (*)

Certallum ebulinum (Linnaeus, 1767: 637)

Certallum thoracicum (Sharp, 1880: 247)

Stenopterus rufus syriacus Pic, 1892: 22 (*)

Phymatodes testaceus (Linnaeus, 1758: 396)

Plagionotus floralis (Pallas, 1773: 724) (*)

Plagionotus bobelayei (Brullé, 1832: 253) (*)

Chlorophorus varius damascenus (Chevrolat, 1854: 483)

Chlorophorus sartor (O. F. Müller, 1766: 188) (*)

SUBFAMILY LAMIINAE Latreille, 1825: 401

Dorcadion hellmanni Ganglbauer, 1884: 486

Dorcadion irakense Al-Ali & İsmail, 1987: 1

Dorcadion mesopotamicum Breuning, 1944: 12

Dorcadion mosulense Özdikmen, Al-Hamadani, Ali, 2013: 1

Apomecyna lameerei (Pic, 1895: 77)

Oberea oculata (Linnaeus, 1758: 394)

Oxyilia argentata languida (Ménétriés, 1839: 42)

Mallosia mirabilis mirabilis (Faldermann, 1837: 283)

Phytoecia humeralis humeralis (Waltl, 1838: 471)

Phytoecia pretiosa Faldermann, 1837: 298

Phytoecia kurdistan Ganglbauer, 1884: 572

Phytoecia puncticollis puncticollis Faldermann, 1837: 291

Phytoecia balcanica (Frivaldszky, 1835: 268)

Phytoecia mesopotamica Breuning, 1948: 91

Phytoecia croceipes Reiche & Saulcy, 1858: 17 [RN]

Phytoecia drurei Pic, 1909: 153

Phytoecia geniculata geniculata Mulsant, 1862: 420

Phytoecia coeruleascens coeruleascens (Scopoli, 1763: 49)

Phytoecia irakensis Breuning, 1967: 435

Agapanthia suturalis (Fabricius, 1787: 149)

Agapanthia frivaldszkyi Ganglbauer, 1884: 546

The updated list includes 54 species from Iraq with the present seven new records. The new records are marked with an asterisk (*).

Chorotype

The species has a Turano-Mediterranean chorotype. It is distributed in SE Europe, European and Asian Turkey, Syria, Israel, Jordan, Iran, Caucasus, Transcaucasia (Armenia, Azerbaijan, Georgia), and Turkmenistan.

Remarks

The species is a new record for Iraq. It was not recorded for Iraq in the catalogue of Löbl and Smetana (2010).

Genus *CHLOROPHORUS* Chevrolat, 186: 290

Subgenus *CHLOROPHORUS* Chevrolat, 1863: 290

C. sartor (O. F. Müller, 1766: 188)

Material examined

One male from Duhok prov.: Kani Masi, 27.VI.1975 in Museum of Erbil.

Chorotype

The species has Euro-Siberian chorotype. It is distributed in C, S and E Europe, Siberia, Kazakhstan, Turkmenistan, Asian Turkey, Caucasus, Transcaucasia (Armenia, Azerbaijan, and Georgia), Iran, Turkey and Middle East (Syria, Jordan, Israel and Lebanon).

Remarks

The species is a new record for Iraq. It was not recorded for Iraq in the catalogue of Löbl and Smetana (2010).

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Prevalence of Antibodies to Hepatitis C Virus Among the Population of Lahore City, Pakistan

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Abstract.- The present study was conducted with an objective to determine the prevalence of anti-HCV antibody in four major blood transfusion centers of Lahore city. A total of 15,898 healthy voluntary blood donors and professional blood donors were subjected to anti-HCV testing by enzyme linked immunosorbent assay (ELISA) of which 249 were found to be reactive for anti-HCV antibody, yielding an overall prevalence of 1.57%. The anti-HCV reactivity showed a maximum prevalence rate of 4.23 % in the age group of 30-40 years.

Key words: Anti-HCV antibody, Parenteral injections.

The prevalence of hepatitis C and other infections is increasing in urban areas of developing countries (Mujeeb *et al.*, 2000). Infection with hepatitis C virus (HCV) is a major cause of transfusion associated hepatitis, cirrhosis and hepatocellular carcinoma. Risk factors for HCV seropositivity were lower education level, non-sterilized injection needles and blood transfusion occupations, smoking, and age > 50 years (Wang *et al.*, 2002). In studies of risk factors among patients presenting with acute and chronic hepatitis C, a history of intravenous drug use is the most common finding, accounting for 40% or more of subjects (Wang *et al.*, 2002). Intravenous drug use by non-sterilized needles is the most common cause for

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HCV cases in the developing world (Kane *et al.*, 2003). The prevalence of anti-hepatitis C virus antibodies among intravenous drug users is considered highest among high risk groups. Transmission via sexual contact, however, remains controversial. It has been estimated that on the average each person in the developing world receives 1.5 injections per year (Othman and Monem, 2003).

American Red Cross estimated the risk of transfusion-transmitted hepatitis C virus is 1:1,935,000 (Pomper *et al.*, 2003). The study carried out in Lahore indicated high seroprevalence (17.78 %) of HCV (Akhtar *et al.*, 2013). High seroprevalence of anti-HCV antibodies in the healthy voluntary blood donors in India has been reported (Jain *et al.*, 2003). In southern Italy HCV seroprevalence was found to be 45% (Proietti *et al.*, 2003). The current research study was carried out in order to determine the seroprevalence of anti-HCV antibodies in local population of Lahore, Pakistan to determine the disease statistics in this area for better control of disease in future.

Materials and methods

The present study was conducted with an objective to evaluate the prevalence of anti-HCV antibodies in 4 major blood transfusion centers (Pakistan Institute of Cardiology, Fatmid, Red Cross Society and Sir Ganga Ram Hospital) of Lahore, screening a large number of healthy voluntary blood donors and professional blood donors. Study period was from June 2001 to June 2004. Sample of all the donors who attended the above mentioned centers were taken and subjected to anti-HCV testing. A total of 15,898 healthy voluntary blood donors and professional blood donors were subjected to anti-HCV testing using a commercially available anti-HCV ELISA kit Biocare Diagnostics Ltd., China.

Results

It was found that 249 samples were reactive for anti-HCV antibody, yielding an overall prevalence of 1.57%. The age distribution of anti-HCV reactivity showed a maximum prevalence rate of 4.23 % in the age group of 30-40 years. In addition, there was a clear trend of decreasing positivity for anti-HCV with increasing age and this

trend was statistically significant (Table I).

Table I.- Prevalence of HCV in blood donors.

Parameter		Positive cases	Prevalence (%)	Total prevalence (%)
Blood donors (n=15,898)	Male (n=10,000)	170	1.70	1.57
	Female (n=5898)	79	1.33	
Age	1-19 (n=7000)	96	1.37	
	20-29 (n=8000)	115	1.43	
	30-40 (n=898)	38	4.23	

Discussion

The results of the present study show that the prevalence of anti-HCV antibodies in the healthy voluntary blood donors attending blood transfusion centers of Lahore. These healthy voluntary blood donors were silent carrier of hepatitis C virus. In majority of the industrialized nations, prevalence of hepatitis C virus antibodies has been reported to be 60.5% among intravenous drug abusers, 1.96% among the prostitutes, and 0.95% among blood donors (Othman and Monem, 2003). Prevalence of anti-HCV in southern of Italy was higher in blood donors compared to general population (Proietti *et al.*, 2003). In Saudi Arabia the HCV prevalence was the lowest among the blood donors and general population and the highest among the higher risk group (Daw *et al.*, 2003). The model study of WHO showed that about 2.3-4.7 million HCV infections may result from unsafe injections each year in developing countries (Kane *et al.*, 2003). In the case-control study, from Hafizabad, Pakistan, persons who received more therapeutic injections were more likely to be infected with HCV compared to persons averaging zero injections per year (Khan *et al.*, 2006).

According to a study of Saudi Arabia, Egyptian donors showed a prevalence of 27.2% of anti-HCV followed by Yemen (5.2%), Bangladeshis (2.0%), Pakistanis (1.9%) and other nationalities (0.5%) (Ahmad *et al.*, 1995).

Another study reported that the overall

prevalence was 2.24% in Saudi Arabia. It is lower than that reported in Spain (7.3%) but higher than reports from the United Kingdom (0.5 to 1%), Italy (0.87%), or Germany (0.24-79%) (Al-Mofarreh *et al.*, 2003). It was noted by a group of workers that patients who received more injections were more likely to be infected with hepatitis C. If oral and injected medications were equally effective, 44% of patients preferred injected medication.

Conclusion

It is concluded that anti-HCV antibodies are present in 1.57% population of blood donors of Lahore and high prevalence rate (4.23%) was observed in persons of age 30-40 years. The HCV prevalence rate can be reduced by proper blood testing of donors before transfusions.

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Determination of Multiple Drug Resistance Against Field Isolates of *Pasteurella multocida* from Bovines in District Naseerabad, Baluchistan, Pakistan

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Abstract.- The present study was conducted in District Naseerabad, Balochistan to determine the multiple drug resistance against *Pasteurella multocida* isolates from bovines. For isolation and identification of bacteria forty nasal swab samples (n=20 buffalo; n=20 cattle) were collected aseptically and cultured on blood agar. Sixteen samples (n=9 buffalo; n=7 cattle) were found positive microbiologically for *P. multocida*. Disc diffusion test to determine *in-vitro* antibiotic sensitivity of *P. multocida* showed all buffalo and cattle isolates 100% sensitivity to chloramphenicol, ciprofloxacin and norfloxacin, while 100% resistance was observed against colistin, streptomycin and trimethoprim for both species. So, it is concluded that chloramphenicol, ciprofloxacin and norfloxacin are the most effective antibiotics against *P. multocida* for bovines.

Key words: Haemorrhagic septicaemia, antibiotics, drug resistance, bovines.

In most countries of Asia and sub-Saharan Africa, haemorrhagic septicaemia (HS) is an endemic disease. It is widely spread in tropical countries. In Asia, HS epidemics may occur as an

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overwhelming disease in bovines and threatening the economic return of the animal to a dangerous level (Benkirane and De-Alwis, 2002). This disease is caused by *Pasteurella multocida* serotype B: 2 and E: 2 in Asia and Africa, respectively (Nawaz *et al.*, 2006). Buffaloes are more prone to HS than cattle. The shared name of HS is “*galhotu*” in most parts of Nepal and India (Tabatabaei *et al.*, 2002). Drastic changes in weather, including the advent of monsoon, hindrance caused by seasonal level of low nutrition and work pressure are some of the predisposing factors which result in occurrence of this disease in Pakistan (Farooq *et al.*, 2007). Clinical symptoms include edema, swelling of neck and head, later on swelling of lymph nodes have more often been observed in cattle and buffaloes (Ashraf *et al.*, 2009). Pakistan has a cattle population of 34.3 million and buffalo population of 30.8 million heads (Anonymous, 2009-10), while Baluchistan has a cattle population of 2.25 million and buffalo population of 0.32 million heads and most of this population is in district Naseerabad and Jaffaraabad (Anonymous, 2005-06). The disease has also been observed in vaccinated cattle and buffalo (Munir *et al.*, 2006), causing gigantic economic losses worth more than 1.887 billion rupees annually to the country (Ashraf *et al.*, 2009). *P. multocida* infection can be eliminated successfully by antimicrobial therapy with broad and narrow spectrum antibiotics. Many forms of antibiotics are available in market, being used routinely in different animals against *P. multocida* but their excessive and prolonged usage has resulted in resistance of various strains of *P. multocida* against that drug (Kumar *et al.*, 2009). Antimicrobial susceptibility testing has been conducted worldwide as rational therapies during outbreaks (Yoshimura *et al.*, 2001; Demissie, 2011). In many parts of Balochistan, antibiotic resistance in animals has been observed due to irrational use of antibiotics to treat cases of *P. multocida*. But no recorded antibiotic resistant pattern data is available for the current situation. The present study was, therefore, designed to determine multiple drug resistance against *P. multocida* isolates from bovines in District Naseerabad, Balochistan to help veterinarians and livestock farmers in handling and taking care of their livestock in a better way against HS by

providing them data regarding drug resistance against *P. multocida* in that area.

Materials and methods

Nasal swab samples from 40 bovines (n=20 buffalo; n=20 cattle) suspected for HS and showing the signs of edema, swelling of neck and head, swelling of lymph nodes were collected aseptically using 90% alcohol on nostril externally with sterilized cotton swab. The smears of nasal swabs were prepared and stained by Gram's staining for demonstration of bipolar organisms as described by Onat *et al.* (2010). Blood agar (BA) and MacConkey agar (MCA) were used as primary culture media for preliminary isolation of organisms from the samples as described by Cappuccino and Sherman (2005). A loop full of nasal swabs were streaked on blood agar and left overnight at 37°C for incubation. Next day non-haemolytic single colony from BA was transferred to MCA and incubated at 37°C for 48 h. The isolates which failed to grow on MCA were taken as *P. multocida*. Single non-haemolytic colony of the isolates was transferred from primary culture and streaked on fresh BA plate and incubated at 37°C for 24 h to obtain single colony of pure culture. *P. multocida* isolates were identified by following biochemical tests, oxidase, catalase, indole production, citrate utilization, nitrate reduction, urease production and also fermentation of sugar (glucose, maltose, arabinose, sucrose and sorbitole) tests (OIE, 2008). *P. multocida* field isolates were tested for antibiotic sensitivity by disc diffusion method (Bauer *et al.*, 1966). Diameters of the zones developed were measured and interpreted according to chart provided by the manufacturer and the zones were interpreted according to size as sensitive, intermediate and resistant.

Results and discussion

Data regarding biochemical characteristics of *P. multocida* and antibiotic sensitivity test in buffalo and cattle is shown in Table I. The total forty samples were taken after the calculation for the formula of sample size and it produced statistically significant results. Of 40 suspected bovines for *P. multocida*, 16 (buffalo=9; cattle=7) were found positive for *P. multocida* in Tehsil Dera Murad and

Table I.- Antibiotic sensitivity pattern of *P. multocida* isolates from cattle.

Antimicrobial agent	Number of positive buffalo=9			Number of positive cattle =7		
	Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
Amoxicillin	4 (44.4)	4 (44.4)	1 (11.1)	1 (14.3)	-	6 (85.7)
Ceftriaxone	-	7 (77.8)	2 (22.2)	1(14.3)	4 (57.1)	2 (28.6)
Cephaloridine	4 (44.4)	5 (55.6)	-	2 (28.6)	5 (71.4)	-
Chloramphenicol	9 (100)	-	-	7 (100)	0	-
Cinoxacin	5 (55.6)	4 (44.44)	-	5 (71.4)	2 (28.6)	-
Ciprofloxacin	9 (100)	-	-	7 (100)	-	-
Colistin	-	-	9 (100)	-	-	7 (100)
Norfloxacin	9 (100)	-	-	7 (100)	-	-
Streptomycin	-	-	9 (100)	-	-	7 (100)
Trimethoprim	-	-	9 (100)	-	-	7 (100)

Manjhoo Shori, Balochistan province of Pakistan.

In the present study, nine isolates from buffaloes and seven from cattle were 100% sensitive to chloramphenicol which is in accordance with the findings of Shayegh *et al.* (2009) and Shiferaw *et al.* (2009). However, Kumar *et al.* (2009) showed that 93% isolates were sensitive to chloramphenicol. Norfloxacin and Ciprofloxacin was 100% effective for all the isolates from cattle and buffalo as also reported by Shayegh *et al.* (2009). Prabhakar *et al.* (2010) have reported 67% sensitivity to norfloxacin and Patel (2004) found 90% of isolates sensitive to ciprofloxacin. Similar type of results for ciprofloxacin were reported by Khan *et al.* (2011). Contrary to this Kumar *et al.* (2009) found 84% isolates resistant to ciprofloxacin. In the present findings, 55.6% of the isolates from buffalo and 71.4% from cattle were found sensitive to cinoxacin. Gupta *et al.* (1996) found 72% isolates sensitive to cinoxacin which is congruent to this study. In this study 44.4% of the isolates from buffalo and 28.6% from cattle were found sensitive to cephaloridine, whereas Patel *et al.* (2004) and Verma *et al.* (2004) found that all the isolates were 100% sensitive to cephaloridin. Isolates showed 44.4% sensitivity in buffalo and 14.3% sensitivity in cattle. Mohammadi *et al.* (2006) observed 7.50% while Aye *et al.* (2001) and Jonas *et al.* (2001) observed 100% sensitivity to amoxicillin. All the isolates from cattle and buffalo showed 100% resistance against colistin, trimethoprim and streptomycin. Patel (2004) and Demissie (2011)

reported similar results for colistin and trimethoprim respectively, while Onate *et al.* (2010) only showed 80% sensitivity to trimethoprim. Zahoor and Siddique (2006) reported 97.14% sensitivity. It is concluded that there was a very minor difference among resistance and sensitivity pattern between cattle and buffaloes isolates for most of the antibiotics while chloramphenicol, norfloxacin and ciprofloxacin are the most effective antibiotics against *P. multocida* for bovines in Pakistan.

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Susceptibility of *Culex quinquefasciatus* (Diptera: Culicidae) to Deltamethrin in District Sargodha

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Abstract.- Present study was conducted to determine the resistance status of *Culex quinquefasciatus* to deltamethrin (0.5%) in district Sargodha. Only blood fed female mosquitoes were sampled for the study and subjected to residual bioassay test. The mortality rate was 100% after 24 hours of deltamethrin (0.5%) exposure. Results of residual bioassays revealed that population of *Culex quinquefasciatus* in study area is susceptible to recommended dose of deltamethrin (0.5%). Calculated LT₅₀ was 6.38 hours. Non-significant difference was observed in the activities of estrases, monooxygenases and glutathione-S-transferases (GSTs) in control and treated groups of *C. quinquefasciatus*. It is concluded that mosquito population in the study area is not resistant to the recommended dose of deltamethrin.

Keywords: *Culex quinquefasciatus*, Susceptibility and deltamethrin.

Mosquitoes (Diptera: Culicidae) are medically important group of insects (Roth *et al.*, 2010; Weaver and Reisen, 2010) that transmit many diseases in animals and humans (Roth *et al.*, 2010; Snow *et al.*, 1999; Weaver and Reisen, 2010). Insecticides are cornerstone of the efforts to manage mosquito populations (Hemingway and Ranson, 2000). Pyrethroids have been most widely used insecticides for this purpose because of their safe, cheap, effective and long-lasting nature (Bulter, 2011). However, strong dependence on insecticides

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for mosquito control has led to insecticide resistance in mosquitoes (Koffi *et al.*, 2012).

Three important groups of enzymes involved in insecticide detoxification are esterases, cytochrome P450 (CYTP450) and glutathione-S-transferases (GSTs) (Hemingway *et al.*, 2004). Elevated activities of these enzymes have been recorded among resistant mosquitoes (Sajida *et al.*, 2013; Vaughan and Hemingway, 1995; Hemingway *et al.*, 2004; Kasai *et al.*, 2000; Prapanthadhara *et al.*, 2002). Esterases are elevated in the insect body through gene amplification (Vaughan and Hemingway, 1995). GSTs are a major family of detoxification enzymes which possess a wide range of substrate specificity. Mosquito GSTs have received considerable attention in the last 20 years because of their direct relationship with the insecticide resistance in insects (Che-Mensosa *et al.*, 2009). Elevated levels of GST activity have been reported in organophosphate, organochlorine and pyrethroid resistant mosquitoes (Wei *et al.*, 2001; Tang and Tu, 1994; Vontas *et al.*, 2001, 2002). The over-expression of P450 monooxygenases in pyrethroid-resistant insects and their possible role in enhanced metabolic detoxification of pyrethroid insecticides is well documented (Rodpradit *et al.*, 2005).

Monitoring insecticide resistance in mosquito populations is very important in order to ensure the sustainability of vector control programs. In the present study we evaluated the susceptibility of *C. quinquefasciatus* against (0.5%) deltamethrin. We also studied the possible role of esterases, GSTs and monooxygenases in insecticide resistance in *C. quinquefasciatus*.

Materials and methods

Sampling

Adult blood fed female *C. quinquefasciatus* were collected from Sargodha city using aspirator. Collected specimens were released in glass jars (12 cm long and 10 cm wide and mouth of each glass jar was covered with mesh cloth).

Bioassay

To test the susceptibility of *C. quinquefasciatus* to deltamethrin (0.5%) mosquitoes were divided into control and experimental groups

(n=30 in each group). Experimental group was exposed to insecticide impregnated filter paper for one hour and then transferred to clean jar. Control group (n=30) was exposed to water impregnated filter paper. Mortality was observed at discrete intervals for 24 hours. The experiment was replicated thrice.

Enzymes estimation

Mosquitoes were exposed to the sub-lethal dose (1/20th of WHO recommended dose) of deltamethrin for one hour and then shifted to clean jars. After 24 hours of exposure, survivors were frozen at -20°C for 15-20 minutes to immobilize them, and then their wings and legs were removed. Rest of the body was homogenized in 400µl of chilled phosphate buffer (0.1 M, pH 7.0) containing 0.01% (w/v) of triton X-100. The crude homogenate was centrifuged at 14,000 rpm for 5 minutes at 4°C. The supernatant was collected and used for the estimation of activities of non-specific esterases (β -esterases), according to Baker *et al.* (1998), glutathione-S-transferases according to the method of Habig *et al.* (1974), and monooxygenases according to the method described by Vulule *et al.* (1999).

Statistical analyses

Two sample t-test was applied to analyze differences in activities of enzymes among control and treated groups. Probit analysis was used to calculate LT₅₀ value of *C. quinquefasciatus* against 0.5% deltamethrin. Software Minitab (Version 16) was consulted for statistical analyses.

Results

C. quinquefasciatus were found susceptible against 0.5% deltamethrin. There was 90% mortality in the deltamethrin treated group after 12 hours of exposure, which increased up to 100% after 24 hours (Fig. 1). There was no difference in the activities of β -esterases and GSTs between the control and treated group (Table I). Although the activity of monooxygenases in treated group was higher than control group but the difference was statistically non-significant (Table I). Results of Probit analysis indicated that 50% population of *C. quinquefasciatus* died after 6.38 hours of deltamethrin exposure.

Discussion

Results of residual bioassay test showed that all treated female mosquitoes were susceptible to the recommended dose of deltamethrin (0.5%). These results are in accordance with Tahir *et al.* (2009), who reported susceptibility of *C. quinquefasciatus* against deltamethrin in Lahore district. Jahan and Mumtaz (2010) also reported susceptibility of *A. aegypti* against deltamethrin in field population from Lahore, Pakistan. These results are contrary with Kumar *et al.* (2011), who reported that *C. quinquefasciatus* is incipient resistant to deltamethrin in India.

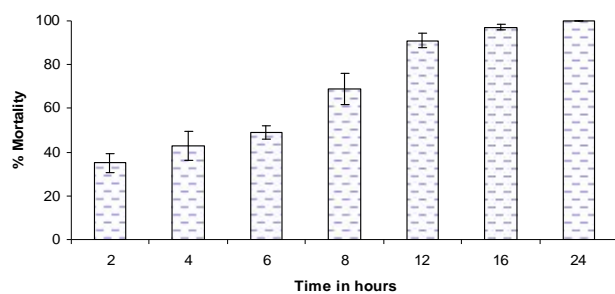


Fig. 1. Percent mortality of *C. quinquefasciatus* at discrete time intervals after exposure with 0.5% deltamethrin.

Table I.- Activities of insecticide (Mean±S.E) detoxifying enzymes in control and deltamethrin treated *C. quinquefasciatus*.

Treatment	B esterases (mM/min/mg of protein)	Glutathione-S-transferases (O.D)	Monoxygenases (µg/min/mg of protein)
Control	445±75	0.369±0.052	14.13±2.3
Deltamethrin treated	442±72	0.394±0.067	19±1.7

The insecticide treatment does not affect the enzyme significantly.

Elevated activities of three types of enzymes is thought to be involved in insecticide resistance i.e., monoxygenases, transferases and esterases (Hemingway *et al.*, 2004). Pyrethroid resistance based on metabolic detoxification is mainly due to an increase in the activity of mono-oxygenases and secondarily to an increase in the activity of esterases

(Djouaka *et al.*, 2008; Hemingway and Karunaratne, 1998).

Present study didn't show any difference in activity of β -esterase between control and treated mosquitoes. The results are in accordance with Hemingway *et al.* (1989), who found that pyrethroid resistance in Puerto Rican strain of *A. aegypti* was not associated with a quantitative change in esterase activity. However, these results are contrary with the findings of Selvi *et al.* (2006) who observed that esterases are involved in causing resistance in *C. quinquefasciatus* against pyrethroids.

Results of present study didn't show significant increase in the activity of monoxygenases in deltamethrin treated group. These results are contrary with the results of Kumar *et al.* (1991) and Ganesh *et al.* (2002) who observed the involvement of monoxygenases in deltamethrin-resistant mosquitoes. Yaicharoen *et al.* (2005) has also reported the involvement of monoxygenases in pyrethroid resistance in *A. aegypti*. The involvement of monoxygenases in causing pyrethroid resistance in *C. quinquefasciatus* has also been reported by Hardstone *et al.* (2007).

Results of present study didn't show increased activity of GST in treated group. These results are similar to findings of Bisset *et al.* (2013) who reported that GST activity is not associated with deltamethrin resistance in *A. aegypti*. However, Grant and Matsumura (1989) and Vontas *et al.* (2001) observed involvement of GSTs in pyrethroid resistance in *A. aegypti*, *Anopheles gambia*, and *Nilaparvata lugens*, respectively.

Lesser activities of insecticide detoxifying enzymes in the treated mosquitoes may be explained on account of the facts that the populations were exposed with insecticide for little time that may not be enough to activate the insect's detoxifying machinery and more time is required for the activation of detoxification process. Involvement of other detoxifying mechanisms like altered target site sensitivity (Walsh *et al.*, 2001), mutations in genes driving detoxifying machinery (Hawks *et al.*, 2005), and behavioral modifications to escape contact to toxins (Sathantriphop *et al.*, 2006) are such phenomenon which are not considered in present study but documented by many scientists.

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Effect of Fructose in Extender on Fertility of Buffalo Semen

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Abstract.- This study was carried out to determine the effect of fructose in extender on fertility of cooled buffalo semen after fixed-time insemination. Two consecutive ejaculates were collected from Nili-Ravi buffalo bull of known fertility by using artificial vagina (42°C). The pooled ejaculates were split-sampled and diluted with skimmed milk extender (37°C; 10x10⁶ motile spermatozoa/ml) SM (control) or skimmed milk with 10mM fructose (SMF). Liquid semen was stored at 5°C and used for artificial insemination after 28 hours. Cyclic buffaloes (n=40) were randomly divided into two groups. Synchronization of estrous was accomplished by two injections of 5ml prostaglandin F₂ α containing 25mg dinoprost administered 11 days apart. Each buffalo group was inseminated with either SM or SMF after 72 hours of second injection of prostaglandin F₂ α. Fertility rate (%) assessed by rectal palpation 90-days after insemination was determined for two experimental extenders. The fertility rates for SM vs. SMF were 25% vs. 40%. SMF significantly (P<0.05) improved the fertility rate in buffaloes after artificial induction of heat. In conclusion, 10mM fructose in skimmed milk extender improved the fertility of chilled buffalo bull semen after artificial induction of heat.

Keywords: Fructose, fertility, liquid semen, buffalo, oestrus synchronization

Fructose is a component of the buffalo semen that is required as energy source for

metabolism of spermatozoa. Depletion of fructose and glucose were observed after dilution with semen extender and during storage period (Sansone *et al.*, 2000). For liquid storage of buffalo semen at 5°C, among milk based extenders, skimmed milk was found superior compared to buffalo, cow, camel and homogenized milk based extenders (Akhter, 2006).

Higher post-thaw sperm motility was reported in milk based extender supplemented with fructose (Kumar *et al.*, 1994a). At refrigerated preservation (5°C) of buffalo semen, partially skimmed cow milk diluter (without egg yolk and glycerol) with fructose and sucrose conserved better motility up to 24 hours. By supplementing the appropriate concentration of sugar of choice possibly fructose, sucrose and raffinose in milk and tris based buffers, buffalo spermatozoa could be efficiently stored upto 24 hours without adding yolk and/or glycerol (Kumar *et al.*, 1994b). Similarly, in recent studies, it was observed that addition of fructose and/or glucose in skimmed milk extender improved the sperm motility, plasma membrane and acrosomal integrity of buffalo bull spermatozoa stored at 5°C (Akhter *et al.*, 2010, 2011).

Control breeding is a method through which timings of parturition can be manipulated by using hormonal therapies according to food availability and economics to fulfill the farmer's demand. Information on the effect of fructose in skimmed milk extender after fixed time inseminations in control breeding is not available. Therefore, considering the importance of the fructose as energy source for the buffalo spermatozoa this experiment was designed to study the effect of fructose on the fertility of buffalo bull spermatozoa after artificial induction of heat.

Materials and methods

The experiment was conducted during breeding season (October to December) of buffaloes. Nili-Ravi buffaloes (n = 40) maintained under standard management conditions at Livestock Experimental Station, Chak Katora, Hasilpur (Distt. Bahawalpur), Pakistan were used for artificial insemination. Semen was collected and processed at Semen Production Unit, Qadirabad, District Sahiwal, Punjab, Pakistan.

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Preparation of extenders

Powdered skimmed milk (SKIMZ®; CANDIA) 10% (w/v) was used for preparation of extender. For the preparation of control extender, skim milk (SM 10% w/v) was added with streptomycin (PDH) available as streptomycin sulphate at the concentration of 1000µg/ml and benzyl penicillin (Hubei Provincial Medicine & Health Product I/E Corp. China) at a concentration of 1000 IU/ml. Fructose 10 mM was added to SM extender to make SMF extender (Akhter *et al.*, 2010).

Semen collection, initial evaluation and processing

Semen was collected from one Nili-Ravi buffalo bull of known fertility at Semen Production Unit, Qadirabad, Sahiwal. Semen ejaculates possessing > 80 % motility, > 1 ml volume and > 0.5 million sperm /ml were used in the study. Semen sample was split into 2 aliquots and was diluted with SM or SMF extenders (37°C; 10 million spermatozoa/ ml). Extended semen was cooled slowly (approximately 2h) to 5°C and stored in cold cabinet at 5°C. Processed semen was transported at 5°C to livestock experimental stations in an ice box and was used 28 h after dilution.

Oestrus synchronization and artificial insemination

Cyclic buffaloes (n=40) were randomly divided into two groups each of 20 animals. Synchronization of estrous was accomplished by administering two injections of 5 ml prostaglandin F₂ α containing 25mg dinoprost (Lutalyse, Pharmacia NV/SA-Puurs Belgium) administered 11 days apart. Each buffalo group was inseminated with separate semen extender after 72 hours of second injection of prostaglandin F₂ α.

Pregnancy diagnosis

Buffaloes of two groups were checked for pregnancy through rectal palpation 90-days after insemination.

Statistical analysis

Chi-square test was applied to analyze the data on conception rate.

Results and discussion

The fertility rate in buffaloes with frozen semen under field conditions is poor owing to freeze thaw damage of sperm. Chilled semen, on the other hand maintains better semen quality and yielded better conception rate in buffaloes (Anzar *et al.*, 2003). Chilled buffalo semen can be efficiently stored by supplementing the extender with appropriate concentration of sugar of choice, without adding yolk and or glycerol (Kumar *et al.*, 1994b). Sugars not only serve as an energy source for the spermatozoa (glucose or fructose), but also prevent structural and sub-structural damage of sperm during preservation (Najfi *et al.*, 2013). It has been reported that sugars protect the spermatozoa from cold shock during refrigerated preservation (Fernandez-Santos *et al.*, 2007). Our previous study reported improvement in motility, *in vitro* longevity and plasma membrane integrity of buffalo bull spermatozoa with the addition of 5- 10mM fructose in skim milk extender and storage at 5°C up to seven days (Akhter *et al.*, 2010). The present study evaluated the fertility rate in buffaloes inseminated after artificial induction of heat using semen preserved in extender with or without fructose (Table I). The oestrus was synchronized using prostaglandin F₂α 11 days apart. It is pertinent to

Table I.- Conception rate in buffaloes inseminated with semen extended (5°C) in skimmed milk extender with or without fructose after artificial induction of heat.

Extenders	Total insemination	Pregnancies achieved	Conception rate (%)	Chi-square
SM	20	5	25.00	1.03
SMF	20	8	40.00*	

* Significant (P<0.05)

SM; Skimmed milk extender without fructose

SMF; Skimmed milk extender with fructose (10 mM)

mention that synchronization of oestrus in cows after the second injection of prostaglandin F₂α was reported to be more precise than after the first or single injection (Cooper, 1974). The results of the present study indicated higher conception rate in buffaloes inseminated with chilled semen preserved in skimmed milk extender with 10 mM fructose compared to control after artificial induction of heat.

The better conception rate with fructose may be attributed to its higher availability as a source of energy for sperm, or to protection during cold preservation through membrane stabilization (Najfi *et al.*, 2013). In vivo fertility rate was also recorded higher ($P < 0.05$) in buffaloes inseminated under field conditions with chilled semen stored in skimmed milk extender supplemented with 10mM fructose (Akhter, 2006). It is to believe that higher semen quality conserved during liquid storage of buffalo semen in skimmed milk supplemented with fructose is responsible for higher fertility rates (Akhter, 2006; Akhter *et al.*, 2010). It is concluded from the present study that fructose in skimmed milk extender improves the conception rate in buffaloes after fixed time insemination, inducing synchronization of oestrus in cycling buffaloes with prostaglandin F2 α 11 days apart. By improving the fertility rate in buffalo we can improve the acceptability of artificial insemination among the livestock owners that will enhance the production potential of the dairy buffalo.

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New Records of Sharks from Southern Coastal Waters of Oman in the Arabian Sea

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Abstract.- The first records of the bluntnose sixgill shark *Hexanchus griseus* and the bramble shark *Echinorhinus brucus* from southern Oman are reported. Descriptions, illustrations and morphometric data are presented. This represents the first record of *H. griseus* from Oman and extends its northernmost distribution in the Indian Ocean.

Keywords: Shark, *Hexanchus griseus*, *Echinorhinus brucus*.

Oman possesses a highly diverse ichthyofauna, due to its coastline bordering a variety of marine habitats. A total of 36 shark species have been confirmed from Omani waters (Henderson and Reeve, 2011). This includes records of *Echinorhinus brucus* from the Sea of Oman (Gulf of Oman) (Henderson *et al.*, 2007), but there are no previous reports of *Hexanchus griseus*.

Material and methods

On the 4th May 2013, a bluntnose sixgill shark, *H. griseus* and a bramble shark, *E. brucus* were caught by a local fisherman, using a deep longline tackle and a dolphinfish (*Coryphaena hippurus*) as bait at a depth of approximately 200 m. The catches were made off Dhalkut between Rakhut and Alhouta near the border with Yemen (16°42' N, 53°30' E). The specimens were identified using criteria from Compagno (1984). Morphometric measurements were taken directly from fresh (one day) frozen specimens following Compagno (1984)

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with some additions. Proportional measurements are expressed as a percentage of total length (TL).

Results and discussion

Order Hexanchiformes de Buen, 1926
Family Hexanchidae Gray, 1851
Genus *HEXANCHUS* Rafinesque, 1810
Hexanchus griseus (Bonnaterre, 1788)

Original description

Squalus griseus Bonnaterre, 1788, Tabl. encyclop. method. trois reg. Nat., Ichthyol., Paris, 9. Holotype: Unknown. Type Locality: Mediterranean Sea.

Material examined

Adult female; 188 cm TL, 31 kg; collected by a local fisherman with a longline; depth ~200 m; southern Oman coast off Dhalkut, Arabian Sea, 16°42' N, 53°30' E; 4th May 2013 (Fig. 1).

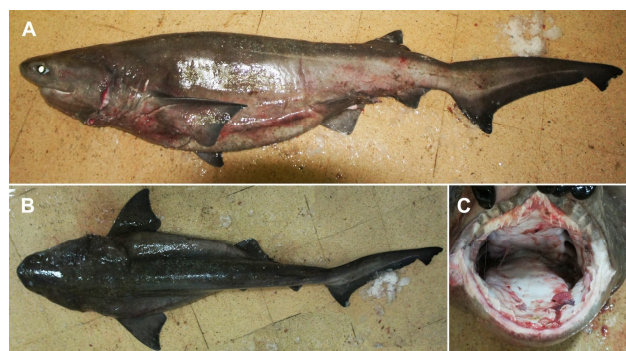


Fig. 1. *Hexanchus griseus*, adult female, 188 cm TL, Dhalkut, Oman, Arabian Sea, 4 May 2013: (A) common view, (B) dorsal view, (C) mouth.

Diagnosis

Body rather stout; head broad and rounded with six gill openings; eyes small; snout short, acutely to bluntly pointed; lower jaw with large, comblike teeth; upper jaw with narrow long-cusped teeth; only one dorsal fin, placed far posterior on back; anal fin is located behind the dorsal fin; caudal peduncle short and stout; caudal fin strongly asymmetrical, has long dorsal lobe with a subterminal notch and very short ventral lobe; body colour dark-gray above, paler below.

Morphometrics

Morphometric details of *H. griseus* from the present study are shown in Table I. While our results are in the same range as the morphometric data from two specimens measured from photographs by Palacio in FishBase (Froese and Pauly, 2013), they are more similar with direct measurement data of Akhilesh *et al.* (2010).

Table I.- Morphometric measurements of *Hexanchus griseus* and *Echinorhinus brucus* from the coastal waters of Oman.

Measurements	<i>Hexanchus griseus</i>		<i>Echinorhinus brucus</i>	
	cm	% TL	cm	% TL
Total length	188	100	193	100
Fork length	148	78.7	180	93.3
Pre-caudal length	134	71.1	162	84.1
Head length	40	21.3	49	25.3
Pre-branchial length	31	16.5	39	20.2
Pre-orbital length	10	5.3	16.5	8.5
Eye length	3.5	1.9	4	2.1
Mouth width	24	12.8	24	12.4
Pre-first dorsal length	108	57.4	127	65.8
Pre-second dorsal length	-	-	143	74.1
First dorsal fin base	9.7	5.2	12	6.2
Second dorsal fin base	-	-	11	5.7
Pre-pectoral length	44.5	23.7	54	28.0
Pectoral fin length	27	14.4	22	11.4
Pre-pelvic length	91	48.3	121	62.7
Pelvic fin length	23	12.0	23	11.9
Pre-anal length	112	59.6	-	-
Anal fin base	9	5.0	-	-
Anal-caudal length	11	5.9	-	-
Head width at 1st gill slit	19	10.1	13	6.7
Caudal peduncle height	8.5	4.5	15	7.8

Remarks

The bluntnose sixgill shark, *H. griseus* is widely distributed in tropical and temperate waters of the Atlantic, Indian and Pacific Oceans. Within the Indian Ocean it is mostly known from the southwestern part off South Africa, southern Mozambique, Madagascar, Aldabra Island group and the Comores Islands (Compagno, 1984). In 2008 one shark was found in Cochin Fisheries Harbour, on the southwest coast of India (about 10°N), amongst the deep-sea hooks & line landings from the Arabian Sea (Akhilesh *et al.*, 2010). Perhaps, because *H. griseus* inhabits waters off the

continental shelf and rarely visits the coastal waters of Oman, it does not occur in catches of local fishermen and therefore has not been recorded by researchers. Our finding of *H. griseus* in Omani waters is therefore the northernmost point of the species distribution in the Indian Ocean recorded so far.

Order Squaliformes Goodrich, 1909
 Family Echinorhinidae Gill, 1862
 Genus *ECHINORHINUS* Blainville, 1816
Echinorhinus brucus (Bonnaterre, 1788)

Original description

Squalus brucus Bonnaterre, 1788, Tabl. encyclop. method. trois reg. Nat., Ichthyol., Paris, 11. Holotype: lost. Type Locality: "L'Océan" (eastern North Atlantic).

Material examined

Adult female; 193 cm TL, 54 kg; collected by a local fisherman using deep longline; depth ~200 m; southern Oman coast off Dhalkut, Arabian Sea, 16°42' N, 53°30' E; 4th May 2013 (Fig. 2).

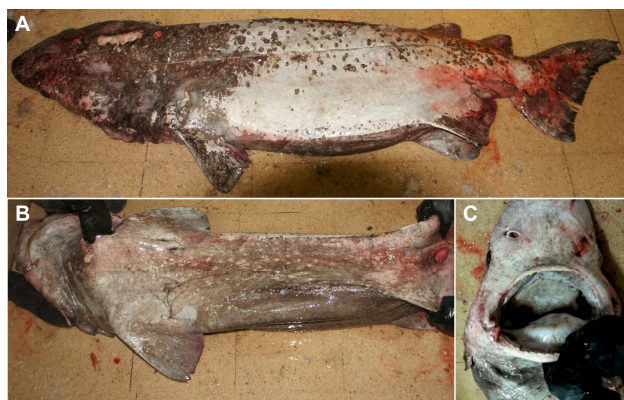


Fig. 2. *Echinorhinus brucus*, adult female, 193 cm TL, Dhalkut, Oman, Arabian Sea, 4 May 2013: (A) common view, (B) dorsal view, (C) mouth.

Diagnosis

Two small dorsal fins without spines located far back, behind the pelvic fin; anal fin absent; pectoral fins are short and angular; caudal fin without subterminal notch; body cylindrical and head moderately flattened; 5 pairs of gill slits; teeth

compressed and bladelike; body covered by irregularly scattered thornlike denticles, varying from small to large, some fused in groups and may form large plates; body colour was brown above and paler below with red blotches.

Morphometrics

Morphometric measurements of *E. brucus* are presented in Table 1. Obtained proportional lengths were notably larger than from measurements made by Palacio in FishBase (Froese and Pauly, 2013).

Remarks

The bramble shark, *E. brucus* is found in tropical and temperate waters worldwide, but it is not commonly encountered, because this deep-water species usually swims close to the bottom, typically at depths of 350–900 m, though it may enter much shallower water (Castro *et al.*, 1999; Compagno, 1984). The present record of the bramble shark, *E. brucus* in the southern coastal waters of Oman in the Arabian Sea is not as surprising as the previous species, because several specimens were caught in the northern Omani waters off Muscat in the Sea of Oman (Henderson *et al.*, 2007); it was also found in waters off Yemen (Al-Sakaff and Esseen, 1999), India (Kapoor *et al.*, 2002) and was recently recorded in the Iranian waters of the Sea of Oman (Javadzadeh *et al.*, 2011). Apparently, the bramble shark is more common than *H. griseus* and is distributed mainly along the continental shelf from South Africa to Iran.

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Diving Behavior of Scaly-sided Merganser, *Mergus squamatus* in Poyang Lake watershed, China

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Abstract. - The diving behavior of scaly-sided mergansers (*Mergus squamatus*) was studied in the Yihuang section of the Poyang Lake watershed, from December 2012 to March 2013. Mean dive duration was 23.6 ± 6.3 s ($N = 1164$) while mean time on the surface was 11.6 ± 6.6 s ($N = 1164$). Mean dive duration and mean pause duration varied with time of day being shorter in the morning than during the rest of the day. Dive efficiency, the ratio of dive duration to pause duration, during daytime hours varied from 1.9 to 2.2. Surface duration was more strongly positively related to subsequent dive durations ($r = 0.211$, $P < 0.001$), which may indicate that the animal uses the time spent on the surface to prepare for the next one.

Key words: *Mergus squamatus*, diving behavior, diving duration, diving pause duration.

The scaly-sided merganser, *Mergus squamatus*, also called the Chinese merganser, is an endemic species restricted to Asia (Wang and Xie, 2009). It breeds primarily in SE Siberia, the far east of Russia and the NE part of China and spends the winter in areas to the south of the Yangtze River. The number of *Mergus squamatus* in the world population is currently estimated at 2400 individuals (Fang *et al.*, 2009) and the species is listed in the first category of National Key Protected Wildlife Species in China (Wang and Xie, 2009). Studies have addressed issues of population distribution and size (He *et al.*, 2006; Shao *et al.*, 2012a), foraging behavior (Zhao and Pao, 1998), time budgets (Yi *et al.*, 2010), and group characteristics (Shao *et al.*, 2012b). The above studies indicated that Jiangxi province provides the main wintering habitat for scaly-sided mergansers and about 200 individuals winters in this area (Wang *et al.*, 2010; Shao *et al.*, 2012a). Scaly-sided mergansers usually feed in loose groups of 2–9 individuals (Shao *et al.*, 2012b). The birds capture prey mainly by diving. However, no data on the diving behaviors of scaly-sided merganser have been published. In this paper we present information on the diving behaviors of this species in the wintering areas to provide basic data on its foraging ecology.

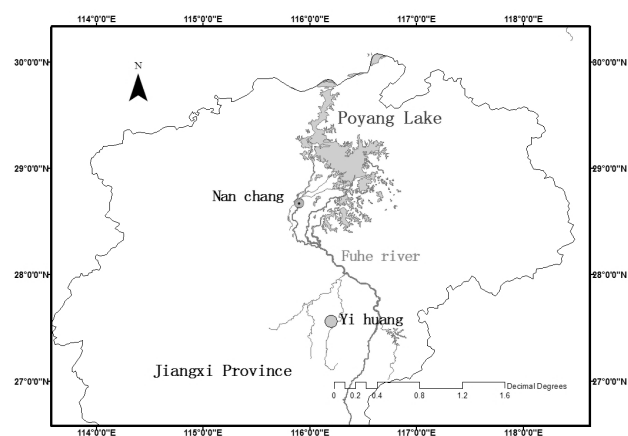


Fig. 1. Location of study sites in the Yihuang section of Poyang Lake watershed in the Jiangxi Province.

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Study area and methods

Study area

The Yihuang River (27° 03' - 29° 43'N, 116° 01' - 116° 28'E) is located in the upper reaches of the River Fu in the northern part of the Jiangxi province (Fig.1). The river is shallow and we often found some little egrets *Egretta garzetta*, feeding together with scaly-sided mergansers. The river bed is sandy and water speed is about 0.2 m/s (unpublished data). The area constitutes a transitional climate zone between southern and central China (Tao *et al.*, 2009). The Yihuang River has a humid subtropical climate with an annual average temperature between 16 and 18 °C. The average annual precipitation is 1749 mm (Huang *et al.*, 2003). The vegetation is dominated by broad-leaved deciduous forest and broad-leaved evergreen mountain forest. Scattered farmland and villages are distributed in this area.

Data collection

The size of the studied population of scaly-sided mergansers was about 25 individuals. The main activity range of these birds focused on about 3 km of river. They also dispersed to other river sections or exchanged with birds in other areas of the river. The birds fed mainly in loose groups. Focal sampling was used to quantify the diving activities of scaly-sided mergansers. To reduce the influence of pseudo-replication, we pooled the data to estimate a mean value (Hurlbert, 1984; Gonzales *et al.*, 2009). We also tried to collect data in different months and from different observation points to decrease the possibility that the same individual was monitored. Circadian differences in diving behavior were tested by comparing data divided into five two- or three-hour blocks, from 07:00 to 18:00. Data were collected by two people, one observing the birds with binoculars (30×) or a spotting scope (20-60×) while the other took notes and checked the stopwatch. Data recorded were the number of dives, dive duration and pause time between consecutive dives. Dive efficiency, defined as the dive/pause (d/p) ratio, was calculated from the mean dive and pause values (Frere *et al.*, 2002). Values presented are means \pm SD and *P*-values < 0.05 are considered significant. Observations were not separated by sex because sub-adults have similar

plumage to females. We compared mean dive duration and mean pause duration among different periods of the day using Kruskal-Wallis tests because data did not meet the requirements of parametric tests. Spearman rank correlation was used to analyze the relationship between dive durations and subsequent or previous pause durations.

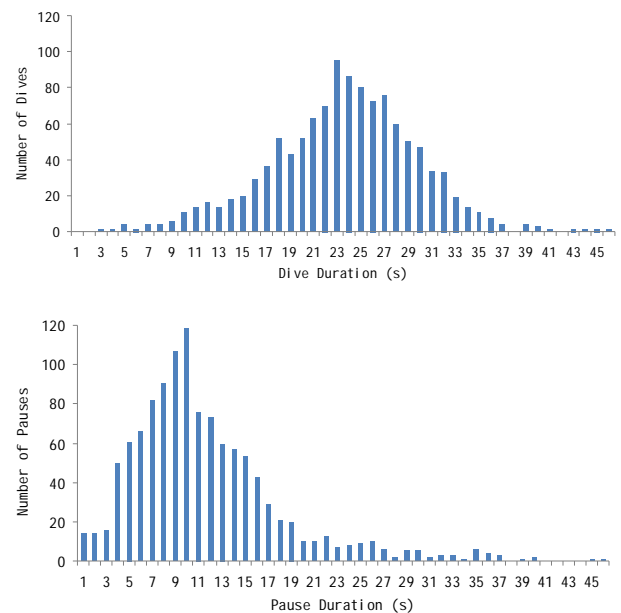


Fig. 2. Dive durations (upper graph) and pause durations (lower graph) time of scaly-sided mergansers, *Mergus squamatus* (n=1164 dives) in the Yihuang section of the Poyang Lake watershed, China.

Results

During feeding, scaly-sided mergansers remained submerged for 3 – 46 s and paused on the surface between dives for 1 – 46 s. Mean dive duration was 23.6 ± 6.3 s (N = 1164) versus a mean pause duration of 11.6 ± 6.6 s (N = 1164) (Table I). Dive duration peaked at 23 s with 72.8% of dives within 17 to 30s, while pause duration peaked at 10 s with 75.4% within 5 to 18 s (Fig. 2). Both mean dive duration ($Z = 124.707$, $df = 4$, $P < 0.001$) and mean pause duration ($Z = 76.460$, $df = 4$, $P < 0.001$) varied with time of day. Dive efficiency, the ratio of dive duration to pause duration, was 2.04 and varied during daytime hours from 1.88 to 2.21. Both dive

Table I.- Diving parameters of scaly-sided mergansers in Yihuang at different times of day.

Time	Number of dives (N)	Mean dive duration (s)	Mean pause duration (s)	Dive efficiency (mean dive duration/mean pause duration)
7:00-9:00	210	20.53±5.82 ^a	9.68±5.94 ^a	2.12
9:01-11:00	273	22.26±5.83 ^b	10.74±6.53 ^{ab}	2.07
11:01-13:00	217	23.94±6.73 ^c	12.24±6.72 ^c	1.96
13:01-15:00	240	25.91±5.47 ^d	13.79±6.56 ^d	1.88
15:01-18:00	224	25.19±6.01 ^d	11.39±6.18 ^{bc}	2.21
7:00-18:00	1164	23.58±6.27	11.58±6.59	2.04

Values with the same letter indicated no significant difference ($P>0.05$), different letters indicated significant differences ($P<0.05$)

durations and pause durations were shorter in the morning than during the rest of the day. Time spent on the surface was related positively to the previous dive duration ($r = 0.070$, $P<0.01$) and also to the duration of the subsequent dive ($r = 0.211$, $P<0.001$).

Discussion

Diving theory predicts that large birds with a potentially large capacity for oxygen storage and low metabolic rate, should be able to stay submerged longer than smaller ones (Cooper, 1986). Within a species, however, it also depends on age (Polak and Ciach, 2007). Dive durations of scaly-sided mergansers (23.6 s) were similar to values reported for Harlequin ducks *Histrionicus histrionicus* (22.7 s, Goudie, 1999), Barrow's goldeneye *Bucephala islandica* (17.5-24.3 s, Bourget *et al.*, 2007), and common goldeneye *Bucephala clangula* (19.7-23.9 s, Bourget *et al.*, 2007) and were significantly shorter than those reported for large diving birds such as rock shags *Phalacrocorax magellanicus* (47.2 s, Frere *et al.*, 2002) and imperial cormorants *Phalacrocorax atriceps* (94.8 s, Quintana *et al.*, 2004). These differences may be related to their body mass (scaly-sided merganser: 0.8-1.2 kg, Zhao, 2001; Harlequin ducks: 0.5-0.7 kg, Zhao, 2001; Barrow's goldeneye: 0.8-1.1 kg, del Hoyo *et al.*, 2011; common goldeneyes: 0.5-1.0 kg, Zhao, 2001; rock shags: 1.4 kg, Quintana, 1999; imperial cormorants: 2.5 kg, Croxall *et al.*, 1991). Black-throated divers *Gavia arctica* (2.0-3.8 kg) are larger than red-throated divers *Gavia stellata* (1.3-2.5 kg). However, dive duration of adult black-throated divers (20.6 s) was shorter than that of red-throated divers (23.3 s) (Polak and Ciach, 2007). This result

contrasts with those of our study and may be related to local water depth or habitats (sea waters vs. fresh waters) during foraging (Mori, 1997; Nocera and Burgess, 2002). Pause durations (9.7-13.8s) of scaly-sided mergansers were similar or slightly shorter than those reported for Barrow's goldeneyes (12.4-14.0s, Bourget *et al.*, 2007), common goldeneyes (10.4-12.8 s, Bourget *et al.*, 2007) and Harlequin ducks (13.5-16.8 s, Goudie, 1999). The absence of information on diving depths in those studies prevents further comparisons.

Dive efficiency of scaly-sided mergansers (1.88-2.21) was similar to that of Harlequin ducks (1.8-2.0, Goudie, 1999) and slightly greater than values reported for Barrow's goldeneyes 1.25-1.97, Bourget *et al.*, 2007 and common goldeneyes (1.55-2.34, Bourget *et al.*, 2007). The similarity in dive efficiency between scaly-sided mergansers and Harlequin ducks may be related to their similar diets (fish, gastropods and aquatic insects) (Shao *et al.*, 2010), whereas Barrow's goldeneyes and common goldeneyes feed mainly on gastropods and polychaetes with relative high population densities. Goldeneyes may need less dive time to search for prey, which could explain their lower dive efficiency relative to mergansers.

Sea ducks are generally believed to be visual feeders for which foraging is limited by light conditions, with dive durations being longer at lower light intensities (Owen, 1990). Our study challenges this general assumption. The shortest mean dive duration of scaly-sided mergansers was at 7:00-9:00 when light intensity was low. Scaly-sided mergansers prey on fish and the shorter dive duration in low light conditions may be related to prey activity; alternatively, the light intensity in the

morning was enough for the mergansers to catch their prey. The short dive duration may be also related to higher foraging intensity in the morning.

Surface pause duration for many species such as Antarctic shag *Phalacrocorax bransfieldensis* (Casaux *et al.*, 2004) and red-legged cormorant *Phalacrocorax gaimardi* (Frere *et al.*, 2002), was related positively to both the duration of the preceding and the following dive. This fact may indicate that the time spent on the surface is a period that the animal uses to both recover from the previous dive and to prepare for the next one (Gonzales *et al.*, 2009). Surface duration was more strongly positively related to the duration of the subsequent dive, indicating that these birds may anticipate the length of their next dive (Casaux, 2004).

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Susceptibility of *Cimex lectularius* L. (Heteroptera: Cimicidae) to Deltamethrin

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Abstract. In the present study we tested the susceptibility of the bed bug, *Cimex lectularius* L., against deltamethrin (0.03%), recorded its biting activity and evaluated the effect of low temperature treatment on biting activity. We observed 84% and 100% mortality 12 h post insecticide treatment in blood fed and blood unfed bugs, respectively. Highest biting activity was observed between 2–4 AM. The biting activity was significantly affected with low temperature treatment. It is concluded that the deltamethrin is very effective against bed bugs. Moreover, low temperature prevent bed bug bites in infested rooms.

Keywords: *Cimexlectularius*, bed bugs, infestation, deltamethrin

Cimex lectularius L. (bed bug) is an obligate hematophagous insect, which is gaining interest worldwide due to its recent resurgence (Goddard and deShazo, 2009). Its infestations are increasing rapidly in many parts of the world including United States (Romero *et al.*, 2007). These insects are nocturnal feeding at night (Usinger, 1966) and they have been disrupting the sleep of humans for at least for the past 3,500 years (Panagiotakopulu and Buckland, 1999). They are difficult to control as they hide in minute cracks and crevices during daylight hours and can survive several weeks to months without food depending on the life stage (Barile *et al.*, 2008).

We (two authors) are living in Brooklyn, New York since April 2013. During our stay we have learned from the local people that infestations

of bed bugs are very common here in family dwellings, apartments and hotels. The local people use different insecticides extensively for bed bug control, especially pyrethroids. Keeping in view the reports of infestation from the local people, the present study was planned. Aims of this study were: to record the resistance status of *Cimex lectularius* L. against deltamethrin (0.03%) in Brooklyn, to study their biting activities, and to evaluate the effect of low temperature treatment on their biting activity. The findings of this study will be helpful for pest control professionals and local people for the management of bed bugs in the area.

Material and methods

Susceptibility test

For this study, adult bed bugs (both blood fed and blood unfed) were collected from infested houses of Brooklyn, New York. Blood fed and blood unfed bugs were separated into two groups. Each group (n =15 for each group) was exposed to deltamethrin (0.03%) impregnated filter paper in a petri plate (9 cm wide and 2.5 cm high) for one hour. Control groups (n=15) were exposed to water treated filter papers. The mouth of each petri plate was covered with a mesh cloth. After one hour bugs were taken out from petri plates and placed in separate clean petri plates. Mortality was assessed after every three hours for 24 hours. The experiment was repeated thrice. Bugs were considered dead if they did not move their body parts when touched with a needle.

Biting activity of bed bugs

To record the biting activity of bed bugs, two volunteers spent five nights in an infested house. They remained on the beds but did not sleep from 10:00 PM to 6:00 AM. Lights were switched off during this period. The number and timing of bites were recorded by each person. The temperature of the room was between 25–28°C during the study period.

Biting activity at low temperature

To evaluate the effect of low temperature on biting activity of bed bugs, a temperature of 17°C was maintained in an infested house from 10:00 PM to 4:00PM for five days using Green Eco-friendly

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Coolant (ARC-12S). Volunteers spent five nights at this temperature in the infected room to record the data as in the above experiment. Number of bites at low temperature treatment and normal room temperature were compared using two sample t-tests.

Results and discussion

We observed 100% mortality 12 h post deltamethrin insecticide treatment in blood unfed bed bugs. However, mortality in blood fed bed bugs was 84% (Fig. 1). Deltamethrin is extremely lipophilic insecticide that can easily penetrate the cuticles of insects. It acts on the nervous system and interrupts transmission of nerve impulse (<http://www.butox-info.com>). In the present study, we observed higher mortality among unfed bed bugs compared to blood fed bugs. This might be due to more stress on unfed bugs due to the combined effect of hunger and insecticide. No mortality was observed in water treated groups (control). Pyrethroid insecticides are used on a large scale against bed bugs as they are effective, safe and cheap (Zhu *et al.*, 2013). Romero *et al.* (2007) reported a higher level of resistance in bed bug populations of Kentucky and Ohio States against deltamethrin and lambda cyhalothrin. They further reported that pyrethroid resistance generally confers cross-resistance to other insecticides, which is a major hindrance in controlling bed bugs.

According to Potter (2008) at least three insecticide treatments are required to control bed bugs in cluttered environments. Even if the insecticide is effective, bed bugs are hard to control as they hide in cracks, crevices, mattresses, bed frames and furniture (Barile *et al.*, 2008; Reinhardt and Siva-Jothy, 2007; Delaunay *et al.*, 2011). In our study although we did not observe a high level of resistance in bed bug populations in Brooklyn, 16% resistance strongly suggests the development of resistance in the future.

Maximum numbers of bites/person/day were observed between 2–4 AM (Fig. 2). The maximum bites at this time may be due to least disturbance at this time as almost all members of the family are in deep sleep at this time. Number of bites may depend on the level of infestation. Kinnear (1948) and Studdiford *et al.* (2012) noted highest feeding rate

of bed bugs just before dawn. Miller and Polanco (2011) observed that bed bugs are active at night, between midnight and 5:00 AM. During our study, persons who recorded biting activity did not sleep during the night as a sleeping person is unable to detect bed bug feeding activity due to the anesthetic, vasodilatory, and anticoagulant chemicals in the saliva of bed bugs (Ter Poorten and Prose, 2005).

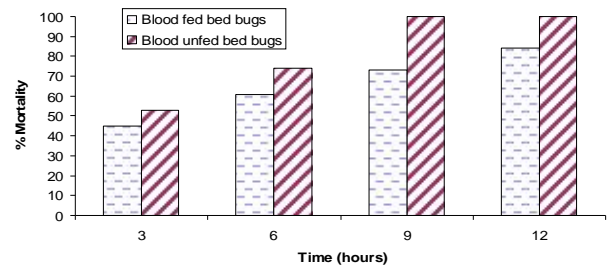


Fig. 1. Mortality of bed bugs after exposure with 0.03% deltamethrin.

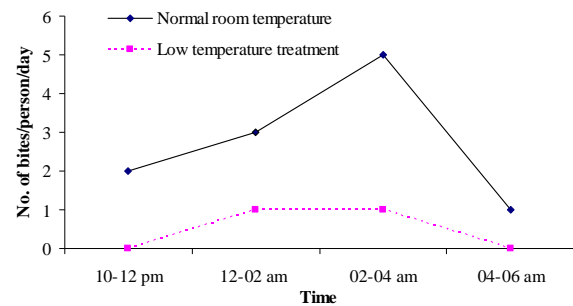


Fig. 2. Average number of bites/person/day by bed bugs in an infested house at normal temperature (26-28°C) and low temperature (17°C).

We observed a significant decline in the number of bites immediately with low temperature (T-value = 2.83; P = 0.047, Fig. 2). This might be due to less activity of bed bugs at low temperature. According to Quarles (2007), the optimal temperature for bed bugs is 27°C. He also reported that activity of bed bugs ceases at 12°C. Inactivity of bed bugs has also been reported by Ridge (2010) between 13–15°C. In our study, the activity did not cease completely as we did not maintain the room temperature as low as that maintained by Quarles

(2007) and Ridge (2010).

We conclude that deltamethrin is effective against bed bug populations in Brooklyn. The activity of bed bugs is highest during late hours of the night, and low temperature reduces the biting ability of the bugs.

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