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Effect of Chlorpyrifos on the Activities of Non-specific Esterases in *Musca domestica* L.

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Abstract.- The present study was undertaken to compare the activities of non-specific esterases (*i.e.*, α-esterases and β-esterases) in chlorpyrifos-treated and control groups of *Musca domestica.* Higher activities of esterases were found in chlorpyrifos-treated flies, both for males and females as compared to the control flies. Higher activities of non-specific esterases are reliable indicators for the future development of chlorpyrifos resistance in *M. domestica* in the area.

Keywords: *M. domestica*, esterases, insecticides.

Insecticides are the most important components in the control of insects worldwide. However, their repetitive sequential use leads to the dissemination of insecticide resistance all over the world (Montella *et al*., 2012). There are many physiological mechanisms for insecticide resistance but increased metabolic detoxification and decreased target site sensitivity are considered important (Ranson, 2011). In metabolic resistance, an increase in the expression or activity of esterases (EST), glutathione-S-transferases and the cytochrome P450 superfamily of enzymes has long been documented (Li *et al*., 2007; Russell *et al*., 2011). Among these enzymes, esterases are of great attention as they can be involved in resistance to the leading chemicals that are extensively used in insect pest-control efforts worldwide (Li *et al*., 2007; Nauen, 2007). These are mainly involved in detoxification of carbamates and organophosphates and to a limited extent in metabolism of pyrethroid (Hemingway and Ranson, 2000), as these insecticides contain carboxylester and phosphotriester bonds which are subject to attack by esterase enzymes.

Musca domestica is one of the major public health pests (Cetin *et al*., 2006). In *M. domestica* insecticide resistance has been reported worldwide (Marcon *et al*., 2003; Shono and Scott, 2003; White *et al*., 2007). Measuring the activity of detoxifying enzymes in natural populations is an important step in monitoring insecticide resistance mechanisms worldwide. The present study was designed with the aim to determine the activity of non-specific esterases in chlorpyrifos treated and control groups of *M. domestica.*

Materials and methods

Flies were divided into two groups: control and treated. Each group contained 20 flies. The experimental flies were exposed to a sub-lethal dose of insecticide for one hour and then transferred to clean jars. After 24 hours of exposure, survivors were frozen at -20°C for 30 minutes in order to immobilize them. Afterwards, their legs, wings and abdomens were removed and the remainder of the body of each fly was separately homogenized in 600 µl of phosphate buffer (100 mM, PH 7.0) containing 0.01% (w/v) of Triton X-100. This crude homogenate was centrifuged at 13000rpm for five minutes at 4°C. The supernatant (crude extract) was collected and was used as the enzyme source for biochemical estimation of non-specific esterases (α and β esterases).

To measure the activities of non-specific esterases, the method of Van Asperen (1962) was followed. Alpha naphthyl acetate (Substrate A) and beta naphthyl acetate (Substrate B) were used as substrates. Optical densities (O.Ds) were recorded at a wavelength of 620 nm for alpha naphthyl acetate and 545 nm for beta naphthyl acetate using a spectrophotometer (UV-1700). The optical density of the reference was subtracted from the optical density of the solution containing the supernatant. The resulting ODs were compared with standard curves to convert the absorbance to product concentrations. The enzyme activities were expressed as nmol of product formed/min/mg of protein. Based on the previous studies we predicted higher esterase activities in females compared to males.

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Two sample T-test was applied to compare enzyme activities among the control and insecticide-treated groups. Minitab 16 was used for the statistical analysis.

Results

The comparison of the activities of non-specific esterases, in control and insecticide-treated female and male flies is depicted in Table I. Significantly higher activities of α and β esterases were recorded in chlorpyrifos-treated flies in comparison with the control for both males and females. Esterase activity was higher in females compared to males as predicted (Table I).

Table I.- Activity of non-specific esterases in control and insecticide-treated groups of female and male houseflies.

House fly	Enzyme activity (mM/min/mg of protein)	Control (n=20)	Chlorpyrifos treated (n= 20)	P-value
Female	α esterases	37.3	53±4.7	0.333
	β esterases	83.66	117±9.2	0.009
Male	α esterases	44.18	69.14±7.7	0.027
	β esterases	65.2	92.5±4	0.017

Discussion

Our results revealed higher activities of esterases in chlorpyrifos-treated houseflies. These results correspond with Villani *et al*. (1983) who recorded high esterase activity in chlorpyrifos-resistant mosquitoes and Zhang *et al.* (2012) who found increased activity of esterases in whitefly, *Bemisia tabaci* in China. Yaqoob *et al.* (2013) reported higher activities of esterases in Trichlorofon, Malathion and λ-cyhalothrin exposed fruit fly (*Bactocera zonata*). Rodríguez *et al*. (1999) has also reported the involvement of esterases in chlorpyrifos resistant *Aedes aegypti*. Prabhakaran (1995) has documented the involvement of esterases in chlorpyrifos-resistant German cockroaches. Different authors reported involvement of esterases in OP-resistant insects (Qiao *et al*., 1999; Takahashi and Yasutomi, 1987).

We concluded from our study that although the tested population was not resistant to chlorpyrifos but higher activities of non-specific esterases are reliable indicators for the future development of chlorpyrifos resistance in *M. domestica* in the area.

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Effect of Weather Conditions on Dynamics of the Black-headed Gull, *Chroicocephalus ridibundus*, Wintering on Jakuševec, Zagreb City Rubbish Dump

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Abstract.- Temperature and precipitation are known to have an effect on the onset of bird migration, but studies were mostly taken on Passeriformes. Between 2001 and 2006 we studied the wintering numbers and dynamics of migration of a short-distance migrant, black-headed gull, *Chroicocephalus ridibundus*, on Zagreb city rubbish dump, Jakuševec. Mean monthly numbers showed significant negative correlation with the local winter temperature, with higher numbers in the coldest months. Arrival of black-headed gulls correlated with North Atlantic Oscillation (NAO) index for the period from September to November. Precipitation didn't have an effect on the timing of migration or maximum numbers. Our results indicate that arrival time for black-headed gull depended on the conditions in breeding areas, while departure time did not respond to NAO or local temperature.

Key words: Black-headed gull, NAO index, onset of migration.

The effect of climate on bird migration has been intensively studied during the last few decades. Temperature and precipitation are known to have an effect on the onset of bird migration (Hüppop and Hüppop, 2003; Hubalek, 2004). The North Atlantic Oscillation (NAO) index is defined as the difference of normalized atmospheric pressure at sea level between Island and Azores. Negative winter NAO index indicates colder and dryer winters in Europe, while positive indicates warmer winter with more precipitation. It is frequently used as the weather indicator for western and central Europe and near-coastal areas (Hüppop and Hüppop, 2003; Zalakevicius *et al*., 2006). It has been shown that changes in the timing of arrival are greater among short-distance migrants than long-distance migrants (Hubalek, 2004; Rainio *et al*., 2006). However, not all authors observed such differences, especially in eastern Europe where the NAO index has a weaker impact (Zalakevicius *et al*., 2006). Data from many more localities are needed, especially for non-passerine birds that are less represented in studies of bird–climate relationship (Tryjanowski *et al.*, 2002; Zalakevicius *et al.*, 2006; Kralj and Dolenec, 2008), before a general conclusion about the timing of migration over wider areas can be reached.

Phenological studies in Croatia were up to now restricted to the spring arrival of breeding passerines (Dolenec, 2003; Kralj and Dolenec, 2008; Dolenec *et al*., 2009). We chose a short-distance migrant black-headed gull wintering in Croatia to see whether the weather conditions had an influence on their wintering numbers and dynamics of migration.

Materials and methods

Black-headed gull is one of the most common gull species in the Western Palaearctic (Cramp and Simmons, 1983). The Croatian breeding population is around 1500 pairs, however the population is greatly increased during migration and in winter (Kralj, 1997). Black-headed gulls that breed more northerly migrate and winter more southerly than those breeding in more southern areas (Cramp and Simmons, 1983; Olsen and Larrson, 2004).

Zagreb, the largest city in the country, is situated in the north-western part of Croatia. Having around one million inhabitants it has the biggest rubbish tip and is the place with the highest concentration of wintering gulls in the country. During the winter months more than 15,000 gulls feed at the Zagreb city rubbish dump, Jakuševec (Jurinović and Kralj, 2012).

Recoveries of black-headed gulls ringed in winter in Zagreb and observed during breeding season as well as the recoveries of black-headed gulls ringed as chicks and found wintering in Zagreb

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showed that they mostly originated from eastern Europe (Fig. 1).

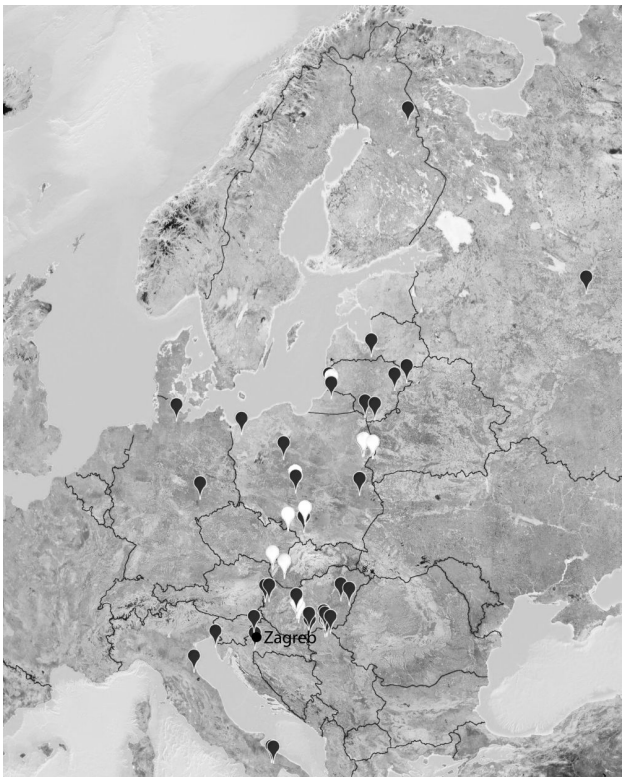

Fig. 1. The origin of the black-headed gulls wintering on Jakuševec, Zagreb. White spots show ringing sites for chicks recorded on Jakuševec during winter. Black sports are observation sites between April and June for black-headed gulls ringed in winter on Jakuševec.

Gulls were counted once a week, two hours after sunrise, during the whole period from January 4th 2001 till December 26th 2006. We used a method of estimation counts to assess the number of birds present at the rubbish tip (Bibby *et al*., 1992). Mean monthly numbers were calculated for each month.

We compared the arrival and departure dates with climate conditions on both breeding and wintering grounds. Small flocks of black-headed gulls or individual birds can, however, be seen throughout the year. Therefore, instead of analysing the first arrival dates and departure dates, we analysed the first or last occurrence of flocks over 1000 black-headed gulls. We used NAO index (NAO index data for investigated period was downloaded from URL http://www.cru.uea.ac.uk/cru/data/nao.htm on 9th March 2013) to represent weather conditions at the breeding grounds. The mean NAO winter indices for September-November were calculated, according to Hüppop and Hüppop (2003) and Marra *et al.* (2005). To describe the weather at the wintering grounds, the mean monthly temperature and monthly precipitation in Zagreb for six year period (provided by the Meteorological Office in Zagreb) were used. Calendar days of the arrival and departure of flocks were converted into Julian dates (1 for 1st July) with correction for leap-years. Julian dates were converted back to conventional format when reporting results. Pearson correlation ($P<0.05$) was used to examine the relationships between the gull data (mean monthly numbers and first and last occurrence of flocks over 1000 black-headed gulls) and average temperature and precipitation in Zagreb, NAO index and Julian dates.

Results

Black-headed gull is the most numerous gull species on Jakuševec with maximum of up to 13,500 birds recorded daily. They are present at Jakuševec from October till April (Fig. 2). During the rest of the year they are sporadic and present in small numbers.

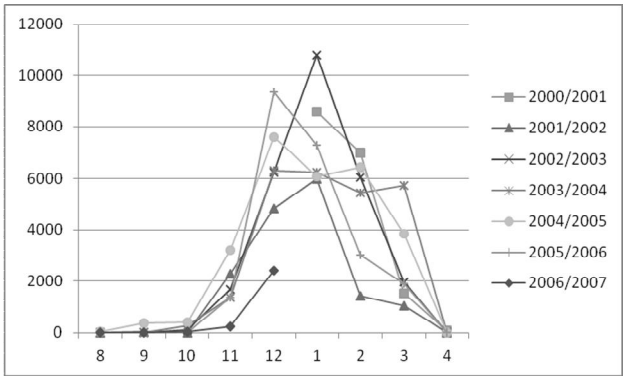

Fig. 2. Mean monthly number of black-headed gulls found on Jakuševec during the months of the year starting with August.

Mean monthly numbers showed significant negative correlation with the local winter temperature (November to March), showing higher

Table I.- The first and the last occurrence of flocks over 1000 black-headed gulls at Zagreb city rubbish dump and NAO and climate data for the study period.

Winter	Arrival date	Departure date	NAO index (Sept-Nov)	Temperature (ºC)						Precipitation (mm)					
				Sept	Oct	Nov	Dec	Jan	Feb	Sept	Oct	Nov	Dec	Jan	Feb
2001/02	10.11.	17.3.	-0,98	14.4	14.0	3.1	-2.4	-0.1	5.9	211.6	7.2	114.7	36.7	91.4	18.2
2002/03	8.11.	9.3.	-1,78	15.2	11.2	9.7	1.9	-1.8	-2.7	98.6	99.1	83.0	79.7	19.7	53.7
2003/04	13.11.	7.3.	-1,01	15.5	9.2	7.8	1.4	-0.6	2.5	114.9	118.1	57.8	30.7	76.2	37.5
2004/05	15.11.	17.3.	-0,07	16.0	13.0	6.6	1.6	-0.7	-2.2	79.1	138.6	46.0	54.2	68.6	69.0
2005/06	15.11.	17.3.	-0,32	16.7	11.6	4.9	1.0	-1.7	1.2	87.3	30.7	73.5	135.2	27.7	73.9
2006/07	8.12.	19.3.	-0,43	17.5	12.9	8.4	3.6	5.6	6.6	64.9	4.8	60.2	35.9	53.8	41.2
Median	**14.11.**	**17.3.**													

numbers in the coldest months (r = -0.724, *P* <0.001). Arrival of black-headed gulls (first occurrence of flocks over 1000 birds) was correlated with NAO index for the period from September to November (r = 0.927, *P* <0.05), while the departure was not correlated with NAO index or with the local weather. We did not find any significant correlation between local precipitation and gull numbers or timing of their movements (Table I).

Individual black-headed gulls are present on Jakuševec for almost the whole year, their number increases in November. The number higher than 100 varied between 30th August to 3rd November, whereas the numbers over 1000 showed a much more uniform pattern from 8th November to 8th December which was shown to be positively correlated with autumn (September to November) NAO index.

Discussion

During the last 15 years many studies have shown the migratory movements correlated with weather conditions. These studies were mostly done on migratory passerine birds (Tryjanowski *et al.*, 2002; Zalakevicius *et al*., 2006, Kralj and Dolenec, 2008). Our study showed that black-headed gulls wintering in Croatia harmonize the autumn migration (measured through the timing of their arrival to wintering grounds) according to the weather conditions on breeding grounds. NAO index did not have any effect on the departure from wintering ground in Croatia (situated far away from the northern coasts), which confirmed its decreasing effect in the eastern Europe away from the northern sea coast (Zalakevicius *et al*., 2006). Precipitation did not show an effect on the timing of migration or maximum numbers. Precipitation has been proven to have an effect on migratory birds, but mostly through food availability (Studds and Marra, 2011) that has the great impact on the selection of wintering grounds (Chamberlain *et al*., 2007). Due to the daily disposal of garbage on the landfill sites, the availability of food usually did not change with time or local weather conditions. Even the high snow cover does not have any effect on the food availability.

Lack of correlation between the local climate factor or NAO index and departure date of the black-headed gulls indicates that the departure time in this population is either intrinsic or triggered by some other extrinsic factor (such as length of the day). This finding is contrary to Hubalek's (2004), who found that during a period of more than 100 years black-headed gull showed negative correlation with NAO index for their spring arrival in Czech Republic. Rainio *et al*. (2006) also showed that the spring migration of Finnish black-headed gulls breeding in the boreal and arctic zone responded to NAO index. However, study of the spring migration phenology is usually restricted to arrival dates, while birds are known to adjust their migration *en route* (Tøttrup *et al*., 2008), hence the departure from wintering grounds and arrival to breeding sites, must not necessarily be correlated with the same factors. Such adjustments are known for long-distance migrants, but might also be present, to a lesser extent, in short-distance migrants.

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Seasonal Dynamics of *Fasciola hepatica* Burdens in Sheep at a Diagnostic Laboratory in HeBei Province, China

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Abstract.- The seasonal dynamics of *Fasciola hepatica* burdens in sheep at a diagnostic laboratory in HeBei Province, China was determined. In this study, 106711 fecal samples were examined during 01.06.2009-31.05.2010. The overall prevalence of *F. hepatica* was 0.9%. It was significantly ($p<0.05$) high (4.51%) during mid July to the end of August, while the prevalence (%) was 0.59%, 0.22%, 0.12% and 2.01% in August, September, October and November, respectively. No parasites were observed during December-February and May. In March and April, the prevalence was 0.18% and 0.41%, respectively. Thus it can be concluded that the *F. hepatica* prevalence in sheep is highest in most hot days of the year, thus the meat of sheep during these days should not be eaten.

Keywords: Fecal samples, prevalence, Flotation techniques, sedimentation techniques.

In developing countries, fascioliasis is one of the most prevalent diseases of domesticated animals (Dreyfuss *et al*., 2007) and has highly been described as the most important single helminthes infection of domestic animals (Novobilsky *et al*., 2007) resulting in great economic losses to the animal industry (Ménard *et al*., 2001). This infection

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prevails most widespread in extremely endemic regions of East, West and Central Africa (Papadopoulos *et al*., 2003; Oliveira *et al*., 2008; Dorchies, 2007). Previously, many studies have been conducted regarding the evaluation and prevalence studies of *F. hepatica*. To clarify the role of the nutria *Myocastor coypus* in the epidemiology of domestic fasciolosis in Loire-Atlantique (department of western France), experimentation has been conducted previously (Ménard *et al*., 2001). Ovine fascioliasis takes part in major constraints to small ruminant production in Ethiopia. To assess the prevalence of *Fasciola* species infections of sheep in Middle Awash River Basin, and to compare *Fasciola* species, the fecal samples have been tested using the ethyl-acetate centrifugation technique to identify eggs of *Fasciola* species (Oliveira *et al*., 2008). The fasciolosis can be easily controlled by understanding genetic structure and status of genetic variation of the *F. hepatica* populations. A group of researchers has genetically evaluated *F. hepatica* isolates from various hosts, employing sequence analysis of ribosomal ITS1 and RAPD-PCR (Rokni *et al*., 2010). Another group of researchers determined the efficiency of treatment against *F. hepatica* on a sheep and goat farm during an eruption of fasciolosis, in which a high proportion of deaths occurred (Ahmed *et al*., 2007). Immunological testing of bulk tank milk has also been used to assess fasciolosis by employing ELISA (Mascoma, 2004). Meat scrutiny documentation in an abattoir located in Ahwaz, Iran, from 20.03.1999 to 19.03.2008 has been used to assess the prevalence and enduring drift of liver fluke disease in sheep, goats and cattle in the constituency (Ahmadi and Meshkehkar, 2010). Using the indirect ELISA and Benedeck's sedimentation tests, the prevalence of *F. hepatica* in cattle, goats and sheep from the municipalities of Guaymas and Cajeme, Sonora State, Mexico has been estimated (Munguía-Xochihua *et al*., 2007). Tasawar *et al*. (2007) have also determined prevalence of *F. hepatica* in goats and relationship between body weight, age, breed of the host and also effect of parasite on the litter size of the host in Pakistan.

Even though substantial worldwide studies have been, literature survey showed no publication from HeBei Province, one of the larger districts of China. So keeping in view, the importance of parasite the project was designed to collect information on the seasonal dynamics of *F. hepatica* burdens in sheep at a diagnostic laboratory in HeBei Province, China using microscopic technique.

Materials and methods

This study was carried out during 01.06.2009-31.05.2010 in a diagnostic laboratory in HeBei Province, China under the supervision of a veterinary physician. In order to collect information on the seasonal dynamics of *F. hepatica* burdens in sheep, the collected fecal samples were stored in bottles containing 5% formaline and examined using flotation and sedimentation techniques.

In flotation technique, 2 g of fecal sample was mixed with 15-30 ml of flotation solution (normal saline having 8.5 g sodium chloride/1000 ml distilled water) to make a homogeneous mixture. A drop of this mixture is placed on a glass slide, covered with a cover slip and then examined the prepared slide using a microscope for the examination of liver fluke eggs.

In sedimentation technique, 1 g of fecal sample was mixed with 30 ml of tap water in beaker. The mixture was strained through the strainer into another beaker and was allowed to remain undisturbed for 20-30 min. The liquid in the top of the beaker was poured off without disturbing the sediment at the bottom. A drop from the sediment was placed on the slide, covered with cover slip and examined under the microscope.

Data analysis

Using SPSS version 17.0, the collected data was analyzed with a probability level set at $P < 0.05$.

Results

During the study of seasonal dynamics of *F. hepatica* burdens in sheep, 106711 fecal samples of sheep were examined during 01.06.2009-31.05.2010 in a diagnostic laboratory, HeBei Province, China. The parameters studied were the overall prevalence (prevalence in a year) and monthly variation in the prevalence of this parasite. According to these results, the overall prevalence of *F. hepatica* in sheep was 0.9%. Based on results, *F. hepatica*

prevalence was significantly ($p<0.05$) high (4.51%) during mid July to the end of August, while the prevalence (%) was 0.59%, 0.22%, 0.12% and 2.01% in August, September, October and November. No parasites were observed during December-February and May. In March and April, the prevalence was 0.18% and 0.41%.

Discussion

According to these results, the overall prevalence of *F. hepatica* in sheep was 0.9%. Based on results, *F. hepatica* prevalence was significantly ($p<0.05$) high (4.51%) during mid July to the end of August, while the prevalence was 0.59%, 0.22%, 0.12% and 2.01% in August, September, October and November. Figure 1 shows hyperplasia of the bile duct in a sheep liver infected with Fasciola hepatica in the first week of August. No parasites were observed during December-February and May. In March and April, the prevalence was 0.18% and 0.41%.

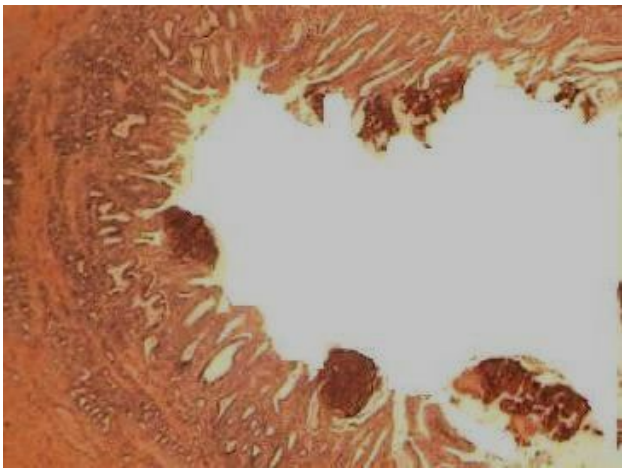

Fig. 1. Hyperplasia of the bile duct in a sheep liver infected with *Fasciola hepatica* in the first week of August.

There is an important role of ovine fascioliasis from the major constraints to small ruminant production in Ethiopia. Oliveira *et al*., (2008) assessed the prevalence of fascioliasis in sheep in Middle Awash River Basin, Ethiopia. They tested 3,697 fecal samples using the ethyl-acetate centrifugation method to recognize eggs of *Fasciola* species. The overall prevalence of fascioliasis was found to be 13.2%. The results exhibited that *Fasciola* species illness was higher among Afar (13.5%) than blackhead breed (9.1%). Regarding the seasonal aspects, the highest infection rate was found during the cool period (6.9%), while lowest infection rate was observed during the rainy time (0.8%) (Oliveira *et al*., 2008).

It has been observed that liver fluke infections in herbivores are widespread worldwide, including Iran. Ahmadi and Meshkehkar (2010) used meat examination documentations in an abattoir located in Ahwaz (Iran), from 20.03.1999 to 19.03.2008 to elaborate the prevalence and enduring drift of liver fluke disease in sheep, goats and cattle in this area. The authors collected data of 3186755 livestock including 2490742 sheep, 400695 goats and 295318 cattle 20.03.1999 to 19.03.2008 and overall 144495 (4.53%) livers were observed affected including 35.01% fascioliasis. The prevalence of liver condemnations due to fasciolosis decreased each year during 1999-2000 and 2007-2008 for cattle, sheep and goats. In some animals, there were statistically non-significant ($p>0.05$) differences with respect to season. More prevalent liver damage due to fasciolosis was observed in slaughtered cattles during summer. This study may act as a useful baseline data for the future scrutiny of these potentially vital parasitic infections in this area.

Ghazani *et al*. (2008) determined the prevalence of liver fluke infection in sheep in the northwest area of Iran. It is reported that the prevalence rate of *F. hepatica* was 8.57% in livers of 140 sheep that were collected by systematic random sampling.

Munguía-Xochihua *et al*. (2007) proposed this study to assess the prevalence of *F. hepatica* in cattle, goats and sheep from the municipalities of Guaymas and Cajeme, Sonora State, Mexico employing the indirect ELISA and Benedeck's sedimentation tests. A total of 2,936 serum and fecal samples from 1,346 bovines, 1,199 goats and 381 sheep were tested. It was observed from the results that the prevalence for fasciolosis was moderate in these municipalities. In bovines, a prevalence of 11.4±0.9% was observed employing the sedimentation test and 24.4±1.2% for the indirect ELISA method. In goats, a prevalence of 24.5±1.2%

was observed for the fecal analysis and 43±1.5% for the indirect ELISA test. In sheep, the prevalence for sedimentation and indirect ELISA tests was 19.4±2.0% and 30.6±2.7%, respectively.

Tasawar *et al*. (2007) collected bimonthly the faecal Samples of 80 goats belonging to Nachi and Teddy breeds from neighboring areas of Multan, Pakistan. The overall prevalence of *Fasciola hepatica* was observed o be 28.75%. More significant ($p<0.05$) rate of parasite infection was observed in Teddy (42.10%) than in Nachi goats (16.67%). Highest prevalence (35.71%) of parasite was observed in age group of 13-24 months, while lowest (18.18%) in age group of greater than 36 months. Significantly ($P<0.05$) different prevalence was observed in different age groups.

Conclusion

It can be concluded that the *Fasciola hepatica* prevalence in sheep is highest in most hot days of the year, thus the meat of sheep during these days should be avoided.

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Conflict of interest declaration

There is no conflict of interest among the authors over the contents of this study.

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Some Population Parameters of The Lessepsian Suez Puffer (*Lagocephalus suezensis*) From Iskenderun Bay, Northeastern Mediterranean, Turkey

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Abstract.- In this study the length-weight relationships (LWRs), length-length relationships (LLRs) and relative condition factor were determined for *Lagocephalus suezensis* captured in the Gulf of Iskenderun, northeastern Mediterranean Sea. Sampling was carried out by commercial bottom trawls using at 24 and 50 meters depths during August and December 2012. The population of Suez puffer fish showed the expected ratio of 1:1. The exponent *b* values were 2.6764±0.0025 for male and 2.9144±0.0019 for female and 2.7946±0.0014 for sexes combined of *L. suezensis*. The coefficient of determination (R^2) was significant for all individuals. The types of growth for both sexes were negative allometric growth for *L. suezensis*. The LLRs between the three length measurements (total length, fork length, standard length) were highly correlated (R^2>0.9527). Relative condition factor (K_{rel}) ranged from 1.165±0.009 (for male) to 1.169±0.009 (for female). This study presented

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the first reference on LLRs and condition factor for Lessepsian puffer from NE Mediterranean coast of Turkey.

Key words: *Lagocephalus suezensis*, Lessepsian puffer, length-weight relationships, length-length relationships, condition factor, North-eastern Mediterranean Sea

Many lessepsian fishes have migrated to the eastern Mediterranean Sea, by the opening of Suez Canal in 1869 (Başusta and Erdem, 2000; Başusta *et al*., 2002, 2013). The lessepsian puffer (*Lagocephalus suezensis* Clark and Gohar, 1953) is one of the lessepsian fish species inhabiting eastern Mediterranean Sea and originally endemic to the Red Sea. This species entered the Mediterranean from the Red Sea via the Suez Canal. It feeds on small benthic invertebrates, spawns in the summer season, eggs and larvae are planktonic, when threatened capable of inflating the body by rapidly engulfing water or air (Golani *et al*., 2006). The first record of the lessepsian puffer was in 1977 from the Lebanon coast (Mouneimne, 1977). Its distrubution expanded to other areas of the Mediterranean (Israeli coast: Golani, 1996, Turkish coast: Bilecenoglu *et al*., 2002, Syrian coast: Saad 2005, Rhodes Island: Corsini *et al*., 2005, Libyan coast: Ben-Abdallah, 2011).

Length-weight relationships of fishes are useful in determining the weight when only length measurement are available and permit comparisons of species between different habitats or areas, and are required in management and conservations of fish stocks. Length-length relationships are very important for the comparative growth studies (Moutopulos and Stergiou, 2002; Erguden *et al*., 2011; Oliveira *et al*., 2012)

There is very limited information about the suez puffer at the present time, except for the systematic and zoogeography in Mediterranean Sea. This study is to determine sex ratio, the length-weight relationships (LWRs), length-length relationships (LLRs) and relative condition factor of *L. suezensis* from the Gulf of Iskenderun, northeastern Mediterranean Sea.

Materials and methods

The suez puffer were collected by commercial bottom trawlers at 24 to 50 meter depths in the Gulf of Iskenderun, northeastern Mediterranean Sea (36°37’ 830” E, 35°38’ 520” N; 36° 33’717” E, 35° 34’872” N; 36° 33’360” E, 35° 34’154” N; 36° 30’946” E, 35° 21’385” N) between August and December 2012. The trawler was equipped with 44 mm stretched mesh size nets at the cod-end. Trawling lasted 2 hours and the trawling speed was 2.5 knots. All samples were transported to the laboratory where the total weights (TW) and total length (TL), fork length (FL) and standard length (SL) were recorded to within 0.01g and to 0.1mm. Fish specimens were identified by Golani *et al.* (2006).

The sex ratio was given as males: females (M: F) calculated using the formula: total number of male/total number of females (Oliveira *et al.*, 2012).

All total lengths and weights were fitted to the length-weight equation: $W=aL^b$, by using least square methods with Statistica software. In the length-weight equation *a* and *b* are intercept and the slope (=exponent) of the length-weight curve, respectively (King, 1995; Can *et al.*, 2002; Başusta and Ciçek, 2006; Başusta *et al*., 2012; Turker-Cakir *et al*., 2008; Koc *et al*., 2008; Daliri *et al*., 2012; Raeisi *et al*., 2012). The *b* value for this species was tested by a *t*-test at the 0.05 significance level to verify if it was significantly different from 3. LWRs for suez puffer were calculated separately according to the sex. LLRs were established using linear regression analysis: TL-SL, SL-FL and FL-TL (Erguden *et al*., 2011).

Results and discussion

A total of 485 females and 494 males of *L. suezensis* were collected during the study period (Table I). The sex ratio for this species was 1:1 not departing from the expected sex ratio. Information on sex ratio is important for understanding the relationship between individuals, the environment and the state of population (Oliveira *et al*., 2012). The estimated parameters of the length-weight relationships and length characteristics (number of fish (n), size range and weight range), the coefficient of determination (R^2) and type of growth are presented in Table I. The results showed that the exponent *b* values were 2.6764 for males and 2.9144 for females (Fig. 1A,B) and 2.7946 for sexes

Table I.- Length-weight relationship of *Lagocephalus suezensis*, Iskenderun Bay, Turkey.

Sex	n	TL (cm) min-max	Mean TL (±SD)	W (g) min-max	Mean W (±SD)	*a*	*b* (±SE)	R^2	*Growth type*	*References*
F	485	7.1-17.1	13.843±1.316	6.0-60.1	31.63 ±8.903	0.0145	2.9144±0.0019	0.883	A(-)	In this study
M	494	6.5-16.7	13.944±1.296	6.0-52.0	32.017±8.510	0.027	2.6764±0.0025	0.832	A(-)	In this study
AI	979	6.5-17.1	13.890 ±1.306	6.0-60.1	31.82 ±8.704	0.0198	2.7946±0.0014	0.858	A(-)	In this study
AI	86	10.20-16.70	12.900 ±1.570	12.50-54.88	27.83 ±9.90	0.0236	2.749±0.063	0.957	A(-)	Ergüden *et al.* (2009)

F (Female), M (Male), AI (All Individuals), Sample size, total length (TL), weight (W) and equation parameters for *a* and *b*, and R^2 values (n= sample size; a, intercept of the regression; b, slope or regression coefficient; R^2 coefficient of determination); SD Standard Deviation; SE Standard Error; A(-), Negative Allometri

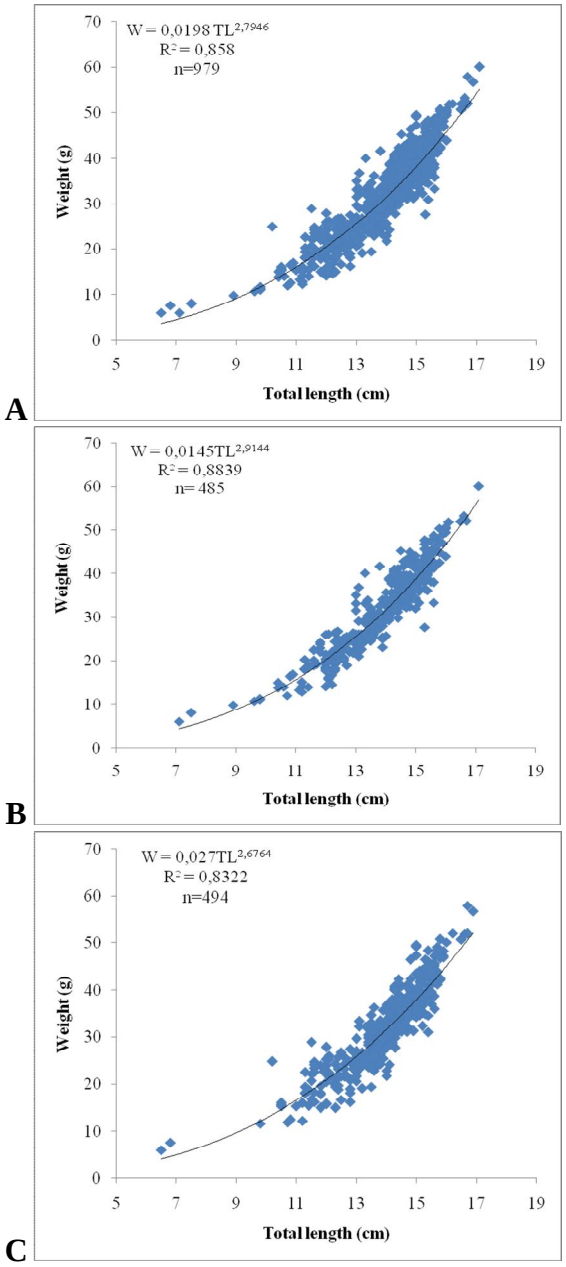

Fig. 1. Length-weight relationships of *Lagocephalus suezensis* for all individuals (A), females (B) and males (C)

combined of *L. suezensis* (Fig. 1C). The type of growth for both sexes was negative allometric growth (b<3) for this species, no significant differences (P<0.05) were found between males and females of *L. suezensis*, thus negative allometric growth indicates a slimmer body. Erguden *et al.*, (2009) calculated *a* and *b* values for this species and

Table II.- Estimated parameters for the conversion between the length measurements (TL, FL and SL in cm) for *Lagocephalus suezensis from* Iskenderun Bay, Turkey.

Sex	n	Equation	Constant a	Slope b	SE (b)	R^2
Female	485	SL = a + bFL	0.024	0.901	0.058	0.958
		TL = a + bSL	3.471	0.887	0.059	0.741
		FL = a + bTL	0.014	0.933	0.062	0.784
		SL = a + bFL	-0.1851	0.917	0.060	0.948
Male	494	TL = a + bSL	4.1169	0.837	0.058	0.743
		FL = a + bTL	-0.601	0.975	0.063	0.795
		SL = a + bFL	-0.083	0.909	0.041	0.952
Both	979	TL = a + bSL	3.804	0.861	0.041	0.742
		FL = a + bTL	-0.283	0.953	0.044	0.790

TL, total length; FL, fork length; SL, standart length

this values were similar in our study. In this work, the data did not represent a total year, thus, these calculated parameters should be considered to represent only a particular season or time of the year. Maximum total length and maximum weight of *L. suezensis* were 17.1cm and 60.1g respectively in this study (Table I).

Relative condition factor (K_{rel}) did not differ significantly between sexes ($P > 0.05$) and ranged from 1.165±0.009 (for male) to 1.169±0.009 (for female). Conversions among length measurements are given in Table II.

There were no data available on LLRs of suez puffer. In conclusion, the present study provides basic information on length-weight and length-length relationships, relative condition factor and sex ratio for the suez puffer that will be useful for fishery management in future.

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