ISSN 1302-0250

Journal of the Entomological Research Society

Volume: 21 Part: 1 2019

JOURNAL OF THE ENTOMOLOGICAL RESEARCH SOCIETY

Published by the Gazi Entomological Research Society

Subscription information

Published by GERS in single volumes three times (March, July, November) per year. The Journal is distributed to members only. Non-members are able to obtain the journal upon giving a donation to GERS.

Papers in *J. Entomol. Res. Soc.* are indexed and abstracted in Biological Abstract, Zoological Record, Entomology Abstracts, CAB Abstracts, Field Crop Abstracts, Organic Research Database, Wheat, Barley and Triticale Abstracts, Review of Medical and Veterinary Entomology, Veterinary Bulletin, Review of Agricultural Entomology, Forestry Abstracts, Agroforestry Abstracts, EBSCO Databases, Scopus and in the Science Citation Index Expanded.

Publication date: March 30, 2019

Printed by Hassoy Ofset
Tel:+90 3123415994 www.hassoy.com.tr

J. Entomol. Res. Soc., 21(1): 01-09, 2019
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

Could Plant Hormones Provide a Reliable Tool for Early Detection of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) Infested Palms?

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ABSTRACT

Rhynchophorus ferrugineus (Olivier) (Coleoptera: Curculionidae) is one of the most important pest of palms worldwide. A serious management problem is the difficulty of detecting early infestation stages, which is critical for successful control. Our main objective has been to characterize the metabolic response of *Phoenix canariensis* hort. ex Chabaud to *R. ferrugineus* injury to identify candidate biomarkers for early detection. Mechanical wounding and *R. ferrugineus* infestation resulted in different patterns of plant hormone and secondary metabolite production: SA and caffeic acid concentrations increased by several orders of magnitude following *R. ferrugineus* development within the palm 7 days after infestation. These compounds did not change in mechanically wounded palms. Therefore, these substances could be further exploited as early warning signs of infestation.

Key words: Red palm weevil, Canary islands date palm, phythormones, plant response.

Dembilio, Ó., Agut, B., Ibáñez-Gual, M.V., Flors, V.,& Jaques, J.A. (2019). Could plant hormones provide a reliable tool for early detection of *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) infested palms? *Journal of the Entomological Research Society,* 21(1), 01-09.

INTRODUCTION

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is a palm borer native to South Asia. Due to the unintended movement of infested material, this species has nowadays a broad distribution covering almost all palm growing areas worldwide (EPPO, 2008, 2009; Rugman-Jones, Hoddle, Hoddle & Stouthamer, 2013; Dembilio & Jaques, 2015; Milosavljević et al, 2018). As a result, this weevil has become one of the most destructive palm pests and is threatening palms worldwide (Dembilio, Riba, Gamón, & Jacas, 2014; Dembilio & Jaques, 2015; Jaques et al, 2017). In the European Union, *R. ferrugineus* is the major pest of palms, mostly *Phoenix canariensis* hort. ex Chabaud, an endemic palm to the Canary Islands, commonly used as ornamental in the northern shores of the Mediterranean basin and elsewhere (Dembilio & Jacas, 2011). Plant damage starts when adult females lay their eggs at the base of the fronds in holes made with their rostra. Neonate larvae bore into the palm core making channels and feeding on its inner contents. As larvae molt, grubs progressively tend to feed on the soft tissues surrounding the apical meristem until they complete development. Then, mature larvae migrate to the periphery of the stem to pupate. This cycle can take from 40 to 160 days depending on temperature and palm species (Dembilio & Jacas, 2011). A new generation emerges and these adults may remain within the same palm and reproduce until the apical meristem is destroyed resulting in the palm death (Dembilio & Jacas, 2011).

Due to these cryptic habits, a serious problem for the management of *R. ferrugineus* is the difficulty of detecting the early stages of infestation (Dembilio & Jaques, 2015; Dembilio, Jacas, & Llácer, 2009) which is key for its successful control. Different approaches have been explored so far including visual, acoustic, thermal, and olfactory sensing (Dembilio & Jaques, 2015; Jaques et al, 2017). However, their success is limited and nowadays visual detection is the most commonly used system. Yet, when first visual symptoms are detected it is often too late for the palm to recover (Dembilio & Jacas, 2011). Identifying how palms respond and deal with defense activation upon *R. ferrugineus* attack remains poorly known (Cangelosi et al, 2015; Giovino et al, 2015; Rasool et al, 2015) although this information could lead to novel approaches for early detection of this pest.

Arthropod herbivory activates in plants different responses. Once the plant has identified the attack, it can respond through activation of diverse defense genes controlled by phytohormones such as abscisic, salicylic and jasmonic acids (ABA, SA and JA, respectively) and ethylene (ET) (Glazebrook, 2001; Flors, Ton, Jakab & Mauch-Mani, 2005; Erb, Meldau, & Howe, 2012; Agut, Gamir, Jacas, Hurtado, & Flors, 2014). Indeed, Giovino et al (2015) identified key *R. ferrugineus*-modulated genes involved in *P. canariensis* innate response belonging to auxin, JA and SA pathways. These activations can result in the production of antibiotic and antixenotic compounds that can exert a negative effect on the herbivore fitness (Bennett & Wallsgrove, 1994; Chen, Gonzales-Vigil, Wilkerson, & Howe, (2007). Cangelosi et al (2015), for instance, identified filiferol, a chalconoid analogue which may be involved in antibiosis to *R. ferrugineus*

in *Washingtonia filifera* Wendl. (Dembilio et al, 2009). Similarly, Rasool et al (2015) identified several differentially accumulated peptides in infested date palms, *Phoenix dactylifera* L. In the case of *R. ferrugineus* infested *P. canariensis*, Giovino et al (2015) identified some upregulated genes involved in the biosynthesis of some S-containing compounds with well-known defensive properties. However, this activation ocurred too late to promptly counteract *R. ferrugineus* attack. Interestingly, though, these compounds and those implicated in their synthesis activation could become a precious tool to detect *R. ferrugineus* feeding/oviposition activity. If they could be unambiguously identified early enough, they could allow for a timely management of this pest.

Therefore, the main objective of the present study has been to characterize the metabolic response of *P. canariensis* to early *R. ferrugineus* injuries relative to control and mechanically injured plants, with the purpose of identifying candidate biomarkers for *R. ferrugineus* early detection.

MATERIAL AND METHODS

Plant material

Commercial pesticide-free healthy 7-year-old potted *P. canariensis* palms were used. The stipe of these palms was around 40 cm high and 35 cm wide. They were planted in 50-L containers and were watered every other day. These palms were enclosed together in groups of four in nine separate cages (36 palms in total) in a mesh house starting two months prior to the onset of the assay. This time was fixed to ensure that any possible previous infestation would be detected beforehand.

Experimental insects

Adult weevils collected in the province of Valencia in traps baited with ferrugineol (*R. ferrugineus* aggregation pheromone), ethyl acetate and pieces of palm fronds were used to directly infest palms.

Infestation

Palms in six out of the nine cages included in this assay were infested at the beginning of the assay (day 0) by releasing four adult weevils (three females and one male) per palm for one week. The remaining three cages constituted the control group. On day 0, different pinnae from the 12 control palms were removed with scissors. In total 100 g per palm were collected (time 0). Likewise, a second sample was obtained from the same palms seven days later (i.e., 7 days mechanically injured palms treatment). On that day, a similar sample was obtained from 12 palms enclosed in three of the *R. ferrugineus* infested cages (i.e., 7 days post infestation, dpi, treatment). One week later, the nine *R. ferrugineus*-infested palms in the remaining three cages were similarly sampled (i.e., 14 dpi treatment). At this time, all palms were dissected to assess their infestation status. In all cases, pinnae samples were frozen immediately after removal at -80ºC for further processing.

Sample processing

Frozen samples were first ground using a refrigerated crusher and lyophilized. Subsequently, a mixture of internal standards containing d6ABA, d4SA, d6IAA and dhJA at 100 mg kg^{-1} each was added to each sample. Dry tissue (0.05 g) was homogenized to a fine powder. Subsequently 2 ml of extraction solution (H2O:MeOH 90:10 containing 0.01% HCOOH) was added to 50 mg of frozen dried samples. After polytron homogenization on ice, samples were centrifuged for 35 min at 4.000 × g at 4ºC and the supernatant was recovered and adjusted to pH 2.8 with 6 % acetic acid and subsequently partitioned twice against an equal volume of diethyl ether and centrifuged for 3 min at 4.000 × g. Then, the organic phases were combined and evaporated using a Speed-Vac (Eppendorf®) at room temperature. The solid residue was re-suspended in 1 ml of a methanol/water (10:90, vol:vol) solution and filtered through a 0.22-lm cellulose acetate filter (13 mm pk/100 TR-200430; Olimpeak Teknokroma, Barcelona, Spain). A 20-ul aliquot of this solution was then directly injected into the HPLC system. Analyses were carried out using a Waters Alliance 2690 HPLC system (Waters®) with a Kromasil reversed phase column (100 2 mm i.d.; 5 lm; Scharlabl®). The chromatographic system was interfaced with a Quatro LC (quadrupole-hexapole-quadrupole) mass spectrometer (Micromass®). MASSLYNX NT software version 4.1 (Micromass®) was used to process the quantitative data from calibration standards and the plant samples. The calibration curves were obtained by following the protocol of Flors, Ton, Van Doorn, Jakab, García-Agustín, & Mauch-Mani (2008) and Durgbanshi et al (2005).

Statistical analyses

Hormone and secondary metabolite concentrations in control (time 0) and 7-day mechanically injured palms were compared using a dependent *t*-test for paired samples. Subsequently, both treatments were separately compared to 7 and 14-dpi treatments using 1-way ANOVA. When necessary, Tukey post-hoc test was used to separate means. In the case of JA-isoleucine (JA-Ile), as this compound remained undetectable in control palms, concentrations in infested plants were compared using a *t*-test.

RESULTS

No differences for the concentrations of SA, Indole-3-acetic acid (IAA), Caffeic, Ferulic, Chlorogenic and Cinnamic acids were found between control and mechanically injured palms (Tables 1, 2). However, when mean concentrations of both treatments were compared to infested palms, significant differences were observed in all cases except for chlorogenic acid (Tables 1, 2). Cinnamic acid became undetectable (< 5 10^{-3} µg/ l) in infested palms. Contrarily, caffeic acid concentration increased more than 10-fold in infested palms within the time frame considered (14 days). SA also increased in infested palms and there were significant differences between dates with a 5- and 8-fold increase relative to control 7 and 14 dpi, respectively. Both IAA and ferulic acid concentrations increased 7 dpi but decreased seven days later and in the case of ferulic, differences between mechanically injured palms and those infested even disappeared 14 dpi.

ABA and ABA-glucoside (ABA Glu) concentrations were higher in mechanically injured palms relative to control ones (Tables 1, 2). These concentrations were even higher in *R. ferrugineus* infested palms (about 3 times) and no differences between 7 and 14 dpi were observed. Contrarily, Glucosyl Salicylate (SAG) and JA were higher in control relative to mechanically injured palms and their concentrations were even higher in infested plants, especially SAG which presented a 20-fold increase 14 dpi compared to control. Finally, JA-Ile, the active form of JA, which was not detectable in control palms, could be detected in both mechanically injured and infested palms, especially 14 dpi.

Palms used at 7 and 14 dpi had a mean of 12.08 ± 0.80 and 11.17 ± 0.54 (max. 16 and min. 8) larvae per palm, respectively. As expected, presumed healthy palms were pest-free.

DISCUSSION

As expected, hormone-related defense pathways in *P. canariensis* were differentially affected by mechanical wounding (pruning) and infestation. Seven days after mechanical injury, palms showed increased concentrations of compounds related to the oxylipin and ABA defensive pathways. These two pathways are known to exhibit a positive cross-talk which usually antagonizes the onset of SA-dependent defenses (Flors et al*,* 2008; Glazebrook, 2005; Del Pozo, López-Matas, Ramírez-Parra, & Gutiérrez, 2005; Pieterse, Leon-Reyes, Vander Ent, Van Wees, 2009). Indeed, SA did not change in mechanically damaged palms relative to control and SAG even decreased in those palms. ABA and JA pathways have been typically associated to plant defense against wounding (Robert-Seilaniantz, Grant, & Jones, 2011) but also against insects with chewing mouthparts (Erb et al, 2012) as *R. ferrugineus*. Therefore, it is not surprising that 14 days after infestation, infested palms also showed enhanced levels of substances related to the oxylipin and ABA pathways. However, in this case the well-documented negative cross-talk of JA with SA-defensive pathways (Thaler*,* Humphrey & Whiteman, 2012) found by Giovino et al (2015) in presumably heavily infested *P. canariensis* was not observed. Indeed *R. ferrugineus* infested palms in our assay showed high increases of SA and SAG compared to control and mechanically-injured plants. Whether these differences should be attributed to the age of the palms or to the actual infestation density (15-20 years and unknown, respectively, for palms used by Giovino et al, 2015) deserves further investigations. Interestingly, the apparent deregulation of the negative SA-JA crosstalk documented in *P. canariensis* for *R. ferrugineus* has been reported in other cases of herbivory (Kant, Ament, Sabelis, Haring, & Schuurink, 2004; Kawazu et al, 2012; Agut et al, 2014).

Table 1. Concentrations (mean ± SE, ng/mg DW) of different plant hormones and secondary metabolites in control (C, time 0), mechanically injured (MI: control palms 7 days later after pruning), and *R. ferrugineus*-infested palms 7 and 14 days after infestation (7 dpi RPW and 14 dpi RPW, respectively). C and MI values were compared with a dependent t-test for paired samples (df = 11 in all cases, see table 2). Each of these values was subsequently compared with 7 and 14 dpi RPW with 1-way ANOVA (df = 2, 35 in all cases, see Table 2) and Tukey post-hoc test was used for mean separation.

	C	MI	C versus MI	7 dpi RPW	14 dpi RPW	RPW versus	
						C	MI
SA	385.02 ± 79.06	297.30 ± 48.43	C = MI	1608.91 ± 167.94	2471.83 ± 462.72	C < 7 = 14 dpi	MI < 7 = 14 dpi
IAA	166.22 ± 27.93	272.15 ± 77.99	C = MI	2308.98 ± 238.60	1115.14 ± 175.72	C < 14 < 7 dpi	MI < 14 < 7 dpi
CA (µg/ g DW)	100.61 ± 33.84	74.53 ± 17.31	C = MI	1009.70 ± 281.06	1016.34 ± 1917.01	C < 7 = 14 dpi	MI < 7 = 14 dpi
FA (µg/ g DW)	3.64 ± 1.00	7.44 ± 1.73	C = MI	16.09 ± 2.05	9.40 ± 1.02	C < 14 < 7 dpi	MI = 14 < 7 dpi
ChA	229.28 ± 111.69	221.48 ± 213.28	C = MI	464.05 ± 237.15	251.82 ± 263.26	C = 7 = 14 dpi	MI = 7 = 14 dpi
Ci (µg/ g DW)	1.66 ± 0.58	1.98 ± 0.47	C = MI	UD	UD	C > 7 = 14 dpi	MI > 7 = 14 dpi
ABA	221.92 ± 57.56	334.61 ± 52.41	C < MI	927.22 ± 132.93	984.36 ± 112.10	C < 7 = 14 dpi	MI < 7 = 14 dpi
ABAGlu	225.41 ± 57.87	314.14 ± 53.31	C < MI	992.63 ± 123.53	1048.06 ± 106.38	C < 7 = 14 dpi	MI < 7 = 14 dpi
SAG	297.33 ± 80.49	79.23 ± 36.91	C > MI	1883.00 ± 312.80	6008.52 ± 898.98	C = 7 > 14 dpi	MI = 7 > 14 dpi
JA	296.81 ± 90.04	80.58 ± 8.52	C > MI	1047.10 ± 326.27	717.18 ± 113.37	C ≤ 14 ≤ 7 dpi	MI ≤ 14 ≤ 7 dpi
JA-Ile	UD	1571.14 ± 363.94	C < MI	9.66 ± 6.84	1576.02 ± 363.21	7 < 14 dpi*	MI = 14 > 7 dpi

SA: Salicylic Acid; IAA: Indole-3-acetic Acid; CA: Caffeic Acid; FA; Ferulic Acid; ChA: Chlorogenic Acid; CiA: Cinnamic Aid; ABA: Abscisic Acid; ABAGlu: ABA-glucoside; SAG: Gulcosyl-salicylate;JA: Jasmonic Acid; JA-Ile: JA-isoleucine; UD: undetectable; NA: not applicable.

*Means compared using a t-test (df = 11).

Table 2. Results of the 1-way ANOVA (df = 2, 35 in all cases used to compare different phytophormone concentrations in control (C) and mechanically injured palms 7 days after injury (MI) with 7 and 14 dpi *R. ferrugineus*-infested palms (7 dpi RPW and 14 dpi RPW, respectively) (see table 1). C and MI values were compared with a dependent t-test for paired samples (df = 11 in all cases) and each of these values was subsequently compared with 7 and 14 dpi RPW with 1-way, see Table 2).

	C palms compared with MI	RPW-injured palms compared (*F*; *P*) with	
	(*t*; *P*)	C	MI
SA	1.082; 0.302	14.44; <0.001	16.02; <0.001
IAA	-1.278; 0.227	42.56; <0.001	36.3; <0.001
CA	0.671; 0.516	7.76; 0.002	8.28; 0.001
FA	-1.984; 0.073	20.12; <0.001	7.91; 0.002
ChA	0.030; 0.976	0.29; 0.7501	0.25; 0.7803
Ci	-0.400; 0.697	NA	NA
ABA	-3.819; 0.003	17.59; <0.001	12.84; <0.001
ABAGlu	-2.857; 0.016	23.12; <0.001	18.58; <0.001
SAG	2.515; 0.029	31.18; <0.001	33.32; <0.001
JA	2.349; 0.039	3.59; 0.039	6.62; 0.004
JA-Ile	NA	20.28; <0.001*	10.12; <0.001

SA: Salicylic Acid; IAA: Indole-3-acetic Acid; CA: Caffeic Acid; FA; Ferulic Acid; ChA: Chlorogenic Acid; CiA: Cinnamic Aid; ABA: Abscisic Acid; ABAGlu: ABA-glucoside; SAG: Gulcosyl-salicylate;JA: Jasmonic Acid; JA-Ile: JA-isoleucine; UD: undetectable; NA: not applicable.

* Means were compared with a t-test (df = 11).

Additionally, an increase of auxins and, remarkably, phenolic compounds, as precursors of phenylpropanoid phytoalexins, was observed in infested palms and this was not the case of mechanically wounded palms. These results are in agreement with Giovino et al (2015), who documented the upregulation of phenylalanine metabolism and phenylpropanodiol biosynthesis genes in *P. canariensis* from the middle stage of infestation. Phenolic compounds are known to possess insecticidal properties (Lattanzio, Lattanzio, & Cardinali, 2006) as they are involved in the formation of physical barriers as components of lignin and reducing the palatability of the plant (Lattanzio et al, 2006; Burghardt, Proksch, & Fiedler, 2001). Our results, which show that these substances can be detected as early as 7 days after infestation, demonstrate that *P. canariensis* activates defense responses against *R. ferrugineus* soon after oviposition/feeding damage starts, much earlier than reported by Giovino et al (2015). In agreement with these authors, the response of *P. canariensis* against *R. ferrugineus* cannot counterbalance the attack. Nevertheless, this response may offer a unique signature to detect early infestations. Because the observed changes appeared soon after exposure to adult ovipositing females, substances as caffeic acid and JA could be further exploited as early warning signs of infestation and provide an extremely useful tool for the management of this deadly pest.

ACKNOWLEDGEMENTS

The authors thank M. Piquer and C. Llopis (UJI) for their collaboration during the assays. This research was partially funded by the European FP7 (project KBBE.2011.1.2-12) and the Valencian Conselleria d'Agricultura, Pesca i Alimentació (project IVIA-5611).

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Received: February 14, 2017 *Accepted: March 07, 2019*

J. Entomol. Res. Soc., 21(1): 11-16, 2019
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

From Southern Balkans to Western Russia: Do First Polish Records of *Pantala flavescens* (Fabricius, 1798) (Odonata: Libellulidae) Indicate a Migration Route?

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ABSTRACT

Pantala flavescens, probably the most widespread dragonfly on Earth, has been recorded for the first time in Poland. Two single specimens (males) were observed in middle-eastern and northern part of the country in Summer 2016. Both observation sites are the valuable completion of knowledge about the distribution of this migratory species, which had been previously found only once in Central-Eastern Europe. New data indicates possible migration routes of this species in this region.

Key words: Odonata, *Pantala flavescens*, migration route, Europe, Poland.

Buczyński, P., Buczyńska, E., & Michalczuk, W. (2019). From southern Balkans to western Russia: Do first polish records of *Pantala flavescens* (Fabricius, 1798) (Odonata: Libellulidae) indicate a migration route?. *Journal of the Entomological Research Society,* 21(1), 11-16.

INTRODUCTION

The circumtropical species *Pantala flavescens* is found in all continents except Antarctica (Dijkstra & Lewington, 2006). The species is an obligate migrant. Northeastern African immigrants, as a part of multigenerational migratory circuit, start to migrate in Spring over the distance of even 14-18,000 km to Central Asia. In late Summer and Autumn the adults of the second generation migrate back to the tropics (NE Africa) (Kalkman & Monnerat, 2015). The migratory range in the Palearctic is smaller than in the Nearctic: within the Palearctic it is especially small in Europe which is explained by the presence of the Sahara. The desert conditions generate unfavourable dry winds which make dragonfly passage almost impossible (Corbet, 1999). The core distribution area of *P. flavescens* in Europe covers only the vicinities of the Bosphorus; migratory individuals were recorded relatively regularly only in the south of the Balkans, and, the boundary of the migration area has been defined by the site on Krk Island in Croatia until recently (Finkenzeller, 2010). In 2013, *P. flavescens* was found in the Baltic exclave of Russia - the Kaliningrad Oblast, that is 1,100 km north of Krk Island. This is the northernmost site of this species in the Palearctic (Buczyński, Shapoval, & Buczyńska, 2014). Taking the above into consideration, the specific questions are as follows: (i) do such long-distance migrations of *P. flavescens* happen more often; (ii) where is the possible route of these migrations? In this light, the present records of this species in Central-Eastern Europe seem to be particularly interesting and possibly important parts of the migration puzzle of the species in the western Palearctic (Fig. 1).

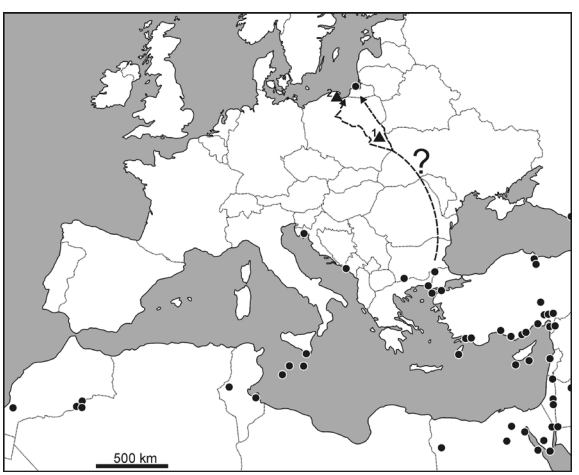

Fig.1. Sites of *Pantala flavescens* (Fabricius, 1798) in Europe and adjacent areas; circles - literature data (De Knijf, 2015; Kalkman & Monnerat, 2015; Galasso, Marletta, & Corso, 2017), triangles - new records (numbers as in the text). Arrows show the possible migration routes.

MATERIAL AND METHODS

This paper is based on data collected during faunistic studies on dragonflies of eastern and northern Poland, conducted in 2016. Adults were observed with the naked

eye and with the use of binoculars. We determined their number per 100 m-long shore or transect, together with potential reproductive and hunting behaviour. Photographic documentation of both sites and pictures of the specimen from northern Poland (Lębork) are available on the website https://wazki.pl/wazki_pantala_flavescens.html (Buczyński & Michalczuk, 2016).

RESULTS

Pantala flavescens was recorded in two sites in Poland.

(1) Nasutów (51°22'32.6" N, 22°30'59.8" E, UTM: FB09), June 8, 2016. A 20-25-metre-wide dry fallow field, bordering to the west on pine forests and to the east on ploughed field. The nearest surface waters are the River Minina (distance of 1.3 km) and fish ponds (distance of 1.5 km), which are separated from the observation site with a wide belt of clumped forest stand. The weather was sunny, the sky clear. The maximum air temperature in the shade was 24°C, light breeze was blowing from the south-west, however, the fallow field was completely covered from the wind with the forest. In this situation, with full insolation and dry sandy ground, it was very hot: air temperature was certainly higher than 30°C.

One male of *Pantala flavescens* was observed directly and through binocular between 10^{30} and 11^{00}. It was hunting for insects flying near the forest, mainly Diptera. The male was flying vigorously, from the edge of the fallow field to the first row of trees, at altitudes of 3-6 meters, from time to time lowering its flight to the altitude of 2 meters. The flight was almost continuous: the male briefly disappeared from our sight in treetops only twice, probably crouching with prey on the branches reaching several meters above the ground.

Other dragonfly species observed were as follow: *Ophiogomphus cecilia* (Fourcroy, 1795) (about 10 individuals), *Orthetrum albistylum* (Selys, 1848) (2 ind.) and *O. cancellatum* (Linnaeus, 1758) (about 10 ind.). There were no interactions observed between those and *Pantala flavescens*: in contrast to the species discussed they were hunting at lower altitudes (up to 1-2 m) and perching very often on the ground (sand or mosses) or pine branches lying on the ground or hanging low.

(2) Lębork (54°33'20.2" N, 17°44'47.5" E, UTM: XA74) (26 km from the Baltic shore, 200 km from the site on the Courish Spit and 478 km from the site in Nasutów), August 13, 2016. Clay excavation with two permanent water bodies about 1.5 m deep, supplied from ground water. In 2016, at slightly higher ground level, a few small and shallow (up to ca. 0.3 m) temporary pools appeared, filled with rainwater.

In the morning it was cloudy and windy. During observation, from about 13^{00}, cloud cover decreased to approx. 50%, but the wind was still strong. The maximum air temperature in the shade was about 24°C. One male of *Pantala flavescens* was spotted on one of the small temporary pools (size of ca. 10x10 m, depth of ca. 0.2 m). It was flying from shore to shore or along the shore, mostly at an altitude of ca. 1 m above the water, from time to time rising or almost crouching on the water in pursuit

of insects. It looked as if it searched through the pool, which is described as a typical patrol flight of a male (Abbott, 2016). Several times the male flew away from the water and after a while came back. Any perching behaviour was observed.

Pantala flavescens was less sensitive to weather conditions than co-occurring species represented by males of *Orthetrum albistylum* and *Libellula depressa* Linnaeus, 1758. Both species perched immediately just after it became cloudy in contrary to *Pantala flavescens* which continued its flight despite the lack of sun and quite strong, chilly wind. About 15^{00} the described specimen flew away from the water and was not observed any more.

The observed male was mature: the abdomen was yellow with red coating on top. The eyes were chestnut brown (upper parts) and yellowish (lower parts). This is a less common form of the eye coloration in this species (Dijkstra & Lewington, 2006).

DISCUSSION

Our data, as a link between the records from the Balkan Peninsula and the Kaliningrad Oblast, may indicate potential routes of the migration of *Pantala flavescens* to Central-Eastern Europe (Fig.1). Taking into consideration the arrangement of mountain ranges (especially the Alps and Carpathians), which are the barrier for the migrations of animals, the most likely seems to be the route from the vicinities of the Bosphorus through Bulgaria and Romania, and next, the western Ukraine, outside the arc of the Carpathians. Further north, up to Central Poland, the migration route may lead either longitudinally along the valley of the River Bug, very favourable for thermophilous dragonfly species, or along the parallel valley of the Vistula. Two regions of Poland: Roztocze and the Sandomierska Basin, can be especially advantageous route for the species. When reaching the Baltic Sea, *P. flavescens* may migrate along the sea coast, as proved by the record from the Coursih Spit (Buczyński, Shapoval, & Buczyńska, 2014).

Our data may indicate that *P. flavescens* migrating from tropical and subtropical areas chooses thermally advantageous, very warm sites during its migration in Europe. Such sites should be taken as starting points in searching for this species. This is confirmed by the fact that at both sites discussed in this paper, we found very thermophilous *Orthetrum albistylum*. The site of the occurrence of this species in Lębork is currently the northernmost in Poland (Bernard, Buczyński, Tończyk, & Wendzonka, 2009) and it is probably the last (towards the north) on which reproductive (territorial) behaviour was observed. Further north, in the Kaliningrad Oblast (Shapoval & Buczyński, 2012) and Lithuania (Gliwa, 2013), only single, probably migrating adults were observed.

The arrangement of mountain barriers for the specimens migrating from Balkans can be the reason why there is still no data on *P. flavescens* from western Europe. However, outside the islands in the Mediterranean Sea, it has not been recorded here, even at the southern edges of the continent (De Knijf, 2015; Kalkman & Monnerat, 2015; Galasso et al, 2017). The key role for a wider range of migration on the east

seems to be the valley of the Nile, which is the migration route of dragonflies through desert areas (Dumont, 1977). Bigger number of sites in north-eastern Africa is a better source of migrant individuals. Recently, dragonflies have been fostered by more and more warmer and drier climate as well as tropical air currents from Africa, beneficial for migrations towards the north (EEA, 2012). This might explain why *P. flavescens* has recently moved outside the Balkans. In general, these are weather phenomena like winds, on which the distribution of *P. flavescens* is related to a large extent (Troast, Suhling, Jinguji, Sahlén, & Ware, 2016). Taking into consideration the growing importance of the current and expected temperature rise conditioned by the inflow of warm air masses to Central-Eastern Europe including Poland (MERP, 2013), the next records of the species in this area are highly probable. Nevertheless, the route to western Europe can be bounded by the Alps. This is symptomatic that Krk Island, situated on the shortest route in this direction (along the Adriatic) and the northernmost site of the occurrence of *P. flavescens* on this sea (Finkenzeller, 2010), is actually located in the foothills of the Alps. The best solution for the resolving the problem of the real migration routes of the species would be genetic studies, however, in the current situation, the main obstacle is the lack of the specimens for such analysis. Assuming that the year temperatures are getting higher and higher, we can expect the occurrence of the new specimens which allow to clarify another unanswered questions in the present: (i) does the species migrate back from Europe to Africa; (ii) does the species reproduce in Europe; (iii) do such long distance migrations of *P. flavescens* happen more frequently?

ACKNOWLEDGEMENTS

We would like to thank to anonymous Reviewer for the help in improving our manuscript.

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Received: July 21, 2017 *Accepted: April 05, 2018*

J. Entomol. Res. Soc., 21(1): 17-35, 2019 *Research Article*
Print ISSN:1302-0250 *Online ISSN:2651-3579*

Body Size Patterns in Stream Communities: A Test of Holling's Textural Discontinuity Hypothesis

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ABSTRACT

Theoretical studies of the resilience of ecological systems to environmental change predict that the size distributions of species in ecosystems should have discontinuities that reflect similar discontinuities in ecosystem processes. Body size distributions should have many peaks and troughs (modes) for natural, undisturbed ecosystems, but that as disturbances increases, so the number of modes declines. If so, this prediction has implications for assessing the quality of real ecological systems and has potential for environmental monitoring.

This paper explores the relationship between water quality and body size patterns in stream communities in order to establish the potential of size based indicators for assessing environmental conditions as well as testing Holling's (1992) proposition that lumpiness occurs in body size distributions across a broad range of spatial and temporal scales. Samples of the stream benthos were collected at different station in River Aire, Yorkshire, UK, which varies in water quality. All sites showed skewed distributions towards smaller size classes and most had two very obvious modes at medium and large size classes except for most polluted habitats. Analysis of the number of gaps using Holling's (1992) BMDI, revealed wide variation in clean and intermediate water quality sites, though the most polluted site had the fewest gaps. However other disturbed sites had more gaps and for some clean site had fewer gaps. It is clear that size distributions in stream communities are lumpy in the sense that most sites showed more than one mode or many gaps but the number of gaps (discontinuities) is not correlated with disturbances, at least for freshwater quality.

Key words: Size patterns, ecosystem resilience, benthic communities, water quality.

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INTRODUCTION

Body size is an important parameter in ecological studies (Blackburn & Gaston, 1994), being a key determinant of a wide range of ecological and evolutionary parameters. Body size determines many aspects of life history such as metabolic efficiency, generation time and metabolism (Morse, Stork, & Lawton, 1988). The relationship between body size and abundance has been shown a useful tool for describing patterns across a wide range of taxa and habitats (Blackburn & Gaston, 1999) with a diversity of biotic and abiotic factors influencing these patterns (Maurer & Brown, 1988; Cyr, Peters, & Downing, 1997). Body size has strong potential to determine environmental impacts on community composition (Ptacnik, Moorthi, & Hillebr, 2010). The composition of small species due to relatively short generation time and high growth rate can quickly track the changes of local environment (Korhonen, Soininen, & Hillebrand, 2010). Departures from expected body mass distributions may provide an indication of disturbance in communities and insights into resilience (Damuth, 1992; Baho et al, 2015).

One approach to exploring body size and resilience has been developed by Holling (Holling, 1992). His Textural Discontinuity Hypothesis proposes that organisms develop specific physical and behavioural characteristics in response to the environmental texture which varies across scales and which is reflected in discontinuities in their body size distributions. In a wide range of marine ecosystems, the body size distribution of benthic organisms are tri-modal (Schwinghamer, 1988) and in planktonic systems biomass size spectra models indicate that size distributions are also multi-modal (Sheldon, Prakash, & Sutcliffe Jr, 1972; Thiebau & Dickie, 1992). The data from stony stream suggests that the body size distributions is dynamic and does not always falls into a single pattern (Stead, Jenny, Peter, & Alan, 2005) while bimodal size distribution was also reported across meio- to macrobenthos size range (Bett, 2013). Such discontinuities in the distributions of body size indicates self-organizing processes within ecosystems and may provide a tool to assess ecosystem resilience (Allen, Gunderson, & Johnson, 2005). These observations lead to the development of resilience theory and related concept, such as adaptive cycles of ecosystem processes operating at specific scales of space and time (Fig. 2) which consist of 4 phase: exploitation, conservation, release and re-organization.

There are multiple competing hypotheses regarding the determinants of body mass distributions of species. Community interactions (Hutchinson, 1959) and related ecological processes (Brown, Marquet, & Taper, 1993), the energetic hypothesis based on the allocation of energy for species growth and reproduction processes which are limited by the energy availability from the environment and by the subsequent transformation of energy into offspring (Marquet, Navarrete, & Castilla, 1995; Lovegrove & Haines, 2004; Allen et al, 2006); the phylogenetic hypothesis, reflecting different evolutionary histories of species (Cassey & Blackburn, 2004; Smith et al, 2004); the biogeographical hypothesis, which suggests that multiple modes in body size distributions are due to restricted set of species present in a given community (Silva, Brimacombe, & Downing, 2001). Many studies have found a relationship between body mass distributions and geographical range (Gaston & Blackburn, 1996; Pyron, 1999).

An alternative explanation is the textural discontinuity hypothesis which argues that species respond to biotic and abiotic processes across different scales (micro, meso, and macro -scale) in time and space producing discontinuous distributions in their body sizes (Holling, 1992). The adaptive cycles operate at all spatial and temporal scale in a forest (Fig. 1). Many authors have argued that different landscapes and biomes with different ecological structures produce different patterns of body size distributions (Allen, Forys, & Holling, 1999; Havlicek & Carpenter, 2001) and studies on freshwater fish found a relationship between gaps in body size distributions and habitat structure (Fu, Wu, Wang, Lei, & Chen, 2004). If the body mass pattern is controlled by landscape architecture, differences in phylogenetics, biogeography, energenitics, and community interactions should not significantly change patterns in body mass configuration (Allen et al, 2006).

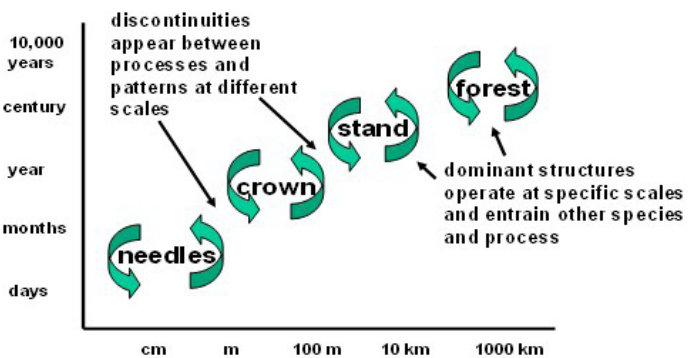

Fig. 1. Patchiness occurs at a range of spatial and temporal scales in nature, as shown in this example of a spruce forest ecosystem. In such systems, dominant structures (from needles to forests) operate over different spatio-temporal scales. The cycles of life and death for each of these structures may follow adaptive cycle dynamics (see Fig. 2), and these may entrain other ecological processes (Raffaelli & Frid, 2010).

Direct tests of these ideas are difficult but can be tested indirectly using surrogates of ecosystem processes, the body sizes of the organisms in the ecosystem; because body sizes are a reflection of processes operating at different scales. Thus, in Holling's plots of the adult body sizes of birds and mammal species from North American grasslands and forests, many modes are apparent which he claimed were associated with ecosystem processes operating at specific scales (although the identities of these processes were not known, only suspected). The regions between modes, the so-called "gaps", were claimed to represent the discontinuities between ecosystem processes. Holling further argues that these gap regions would be the most susceptible to disturbance and where species losses would be most likely. These ideas were further tested by examining how the body sizes of invasive species in the Florida Everglades (Allen et al, 1999) and elsewhere fitted in to the existing body size distributions of the community being invaded, reviewed in (Allen et al, 2006) and references therein. The study found that invasive species tended to have body sizes that were immediately adjacent to the gap regions, and that species which were lost due to disturbance were close to these gaps, consistent with Holling's predictions.

(Raffaelli, Hall, Emes, & Manly, 2000) also tested this idea for a marine intertidal community and found that the body size distributions were multimodal as suggested by Schwinghamer (Schwinghamer, 1981b) for marine sediments and that at least one kind of disturbance, which were applied experimentally, organic enrichment, had the greatest impact on body sizes in and adjacent to one the troughs between modes.

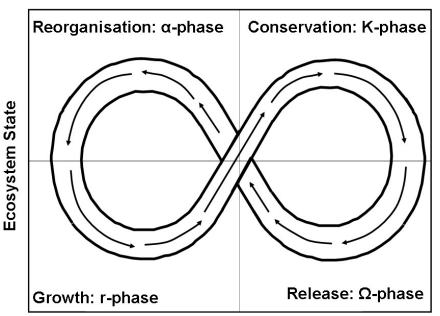

Fig. 2. The adaptive cycle view of ecosystem development and change. In this perspective, collapse of the system is inevitable, whereupon the system components may re-assort and begin development again as a broadly similar system or one which is very different (Raffaelli & Frid, 2010).

From the above, it can be seen that one of the effects of disturbance on body size distributions may be first to deepen the troughs between modes (make them more pronounced), if that disturbance only leads to species loss, as in the case of eutrophication (Fig. 3). However, the response of ecological systems to eutrophication or enrichment is not usually a “simple, or monotonic”. At moderate enrichment, there may be an increase in the abundance of all species (and body sizes), but at higher levels of enrichment, the positive effects may be overtaken by the negative effects as some species intolerant of low oxygen concentrations brought about by a high BOD may be excluded and smaller taxa which are more tolerant of pollution dominate. In such cases, the body size distributions may at first maintain their structure and modality, but as pollution increases, the larger taxa will become excluded and the size structure becomes more skewed towards smaller animals altering modality. Such changes in body size distributions are well-documented in aquatic communities as empirical observations (Warwick, 1984), but their consequences for, and relationships with, changes in ecosystem processes at different scales have not been explored in the context of Holling’s theories.

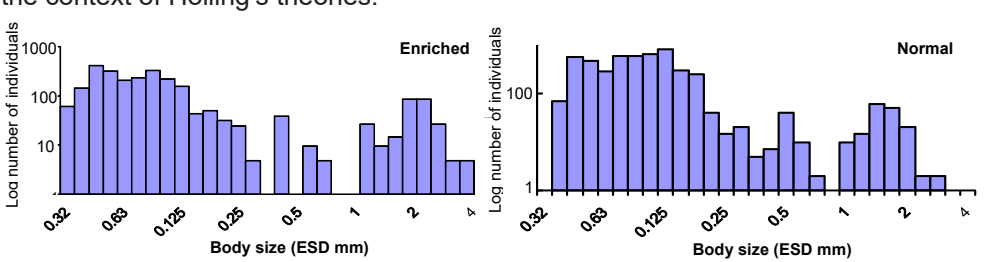

Fig. 3. The effect of pollution on body mass distributions on benthic communities (Raffaelli et al, 2000).

As far there have been no previous experimental or empirical published studies looking at the degree of modality in body size distributions (as a reflection of the heterogeneity in ecosystem processes) and disturbance (in this case pollution). A similar loss of larger body size modes (reduction in modality) can be seen in comparisons of the Pleistocene fauna of North America with the present day, due to over-exploitation of the megafauna by early man (Smith et al, 2004), but that research was not placed in the context of Holling's hypothesis. This paper therefore represents the first empirical test of this aspect of Holling's theory.

In this paper the hypotheses regarding body size distributions in relation to the stability /stress of freshwater stream communities have been explored. Specific objectives of the paper included;

- Establish the modality of benthic body size distributions across a gradient of environmental stress (sites with differing water quality).

- Explore the relationship between the number of gaps in body size distributions and water quality using a gap finding approach proposed by Holling (1992).

- Assess whether the modality and number of gaps has potential for assessing the ecological health (resilience) of streams.

METHODS

Samples of benthic invertebrates were taken from sites along the River Aire, North Yorkshire in September and October of 2008 and 2009. The sites were pre- selected according to their water quality previously determined by the Environment Agency UK (Table 1). The RIVPACS data was provided by environmental Agency for most of the sites (Table 4). At each site, 5 repeat Surber samples (0.25m^2 base area, 200µm net) were taken to allow collection to be dispersed over a wide spatial extent. The fauna collected was preserved in ethanol, identified to the lowest taxonomic level and the body size (mass) of all individuals estimated from morpohometric- based formulae (Table 3) or, for larger individuals, by water displacement. Water quality was also assessed using invertebrate samples as the biotic index Average Score Per Taxon (Mason, 2002a).

Table 1. Sites sampled on the River Aire and their general grade as assessed by environment agency.

	Sites	**General water quality**
1	Winterburn	Very good
2	Airton	Very good
3	Otterburn	Good
4	Hetton	Good
5	Gargrave	Fair
6	Carlton bridge	Fair
7	Esholt village	Fairly good
8	Calverley bridge	Poor
9	Thwaites mill	Bad

Body size distributions were plotted for each site in order to evaluate the degree of modality. In addition, individuals (and taxa) were ranked in increasing body size and the body mass difference index (BMDI) calculated between consecutive rankings using Holling's (1992) formula:

$$BMDI=(M_{n+1}-M_{n-1})/(M_n)^{\gamma}$$

Where Mn is the body mass of n^{th} species in a rank order of increasing size and γ is exponent sufficient which values 1.1 as the invertebrates exploit their resources with dimension 1, i.e, finding a path of a certain width. The mean BMDI was calculated as well as the mean +2SE criterion line in order to estimate the number of significant gaps in the distributions. Two consecutive differences values above the mean +2SE; followed by four value below the line is a considered conservative and robust method to detect gaps (Holling, 1992).

RESULTS

The water quality at the site, as determined from the ASPT estimates, was broadly similar to the classification provided by Environment Agency. Winterburn was cleanest on the ASPT range and Thwaites mill and Calverly bridge had the poorest water quality (Fig. 5). The fauna found at each of these sites is shown in Table 2, Fig. 4. Winterburn was dominated by stoneflies (Leucrtidae and Perlodidae), Haliplidae, Chironomidae and Simulidae. In Airton large number of Haliplidae, Chironomidae, Gyrinidae, Diptera and Oligochaeta was recorded. Otterburn had many Haliplidae, Gyrinidae, Chironomidae, Diptera and Oligochaeta. The dominant taxa in Hetton were Haliplidae Baetidae, Chiromidae, Oligochaeta and Diptera, while in Gargrave high abundances of Chironomidae, Dixidae, Haliplidae, Oligochaeta and Diptera was recorded. In Carlton bridge the dominant taxa were Nematomorpha, Chironomidae, Oligochaeta, Haliplidae and Baetidae. The most abundant species in Esholt village are Oligochaeta, Chironomidae, Hydrosychidae, Asellidae,and Nematomorpha. Calverly bridge was dominated by Oligochaeta, Chironomidae, Hydropsychidae, Nematomorpha and Asellidae. The site with poor water quality Thwaites mill had abundant Oligochaeta, Chironomidae, Asellidae, Hydrodiidae and Viviparidae. Thus our analyses confirm a gradient of water quality in the River Aire at these sites.

Table 2. Community abundance of River Aire, showing the numbers of each fauna present at each sites.

Taxa	Winterburn	Airton	Otterburn	Hetton	Gargrave	Carlton bridge	Esholt village	Calverley bridge	Thwaites mill
Baetidae	7	37	21	53	20	15	50	40	1
Ephemerillidae	61	17	1	5		2	15	10	
Heptagonidae				2	2	1	28	13	
Potamintidae				1	5				
Leuctridae	400	41	18	10		2			
Perlodidae	90			5	1		1		
Haliplidae (L)	131	295	545	75	73	57	4		

Table 2. Continued.

Taxa	Winterburn	Airton	Otterburn	Hetton	Gargrave	Carlton bridge	Esholt village	Calverley bridge	Thwaites mill
Diptera	29	53	123	16	21	10	7		
Oligocheata	12	48	76	43	37	62	700	771	92
Nematomorpha						68	104	104	
Tipulidae	1	8	20	1		1	8	7	
Chironomidae	127	65	132	52	487	63	632	375	30
Haliplidae (A)				1					
Gyrinidae	7	55	151	1	13	5	2		
Dytiscidae	9	6	4			1		2	
Trichoptera		29	11	4	1		9		
Hydropsychidae	34		23	6	10	9	153	110	1
Rhyacophilidae	6					3			
Ceratopogonidae			2	1		6	1		
Simuliidae	44	12		1		2	1	1	
Dixidae	4	10	19		91	5	21	7	
Gammaridae	1	19			3		2		
Glossiphoniidae	1	1	6		1	5	60	19	
Erpobdellidae			9					3	
Hymenoptera				7					
Hydracarina									
Sphaeriidae							3		
Viviparidae		4							2
Valvatidae									1
Hydrobiidae		3	26						2
Asellidae							132	53	7
Arachnida		1	3				1		
Hemiptera		1					2		
Hydrometridae		1						2	
Chalcididae	1	3				4	2	6	
Cladocera		7	9						
Veliidae	1								
Polycentropodidae	32	2							
Planorbiidae	1								
Ecdyonuridae		1	1						
Ancylidae	1	2							

Table 3. Regression equations for body mass determination of Stream communities where DM is the dry mass of the organism (mg), DW is dry weight (mg) of the organism, L is the length of the organisms (mm), HW is head width of the organism (mm), volume (V) of the organisms expressed in (nL), Wt is weight of the organisms (mg).

Family/Class	Regression equation to determine body mass of fresh water invertebrates	References
Baetidae Heptageniidae Caenidae Ephemerellidae Ephemeridae Potamanthidae Hymenoptera Ecdyonuridae	Dw(mg)=aL(mm)^{b} Dw(mg)=3.8x10^{-3}L(mm)$^{2.918}$	(Stead et al, 2003) and reference within
Diptera	DM(mg)=aL(mm)^{b} DM(mg)=1.3x10^{-3} L(mm)$^{2.851}$	
Leuctriodae Perlolidae	Dw(mg)=aL(mm)^{b} DW(mg)=2.5x10^{-3}L(mm)$^{2.744}$	
Gammaridae	ln DM(mg)=lna +b lnL(mm) ln DM(mg)=-4.95 +2.83 lnL(mm)	
Tipulidae	DW(mg)=aL(mm)$^{2.851}$ Dw(mg)=1.3x10^{-3} L(mm)$^{2.851}$	
Chironomidae Caratopogonidae	DM(mg)=a L(mm)^{b} DM(mg)=6.0x 10^{-4} L(mm)$^{2.770}$	
Hemerobiidae	Log DM(µg)=a+b log HW(mm) Log DM(µg)=2.68+2.9 log Hw(mm)	
Trichoptera Rhyacophilidae Hydropsychidae	ln DM(mg)=lna+b lnL(mm) ln DM(mg)=-6.037+2.82 ln L(mm)	
Simulidae	ln DM(mg)=lna+b Hw(mm) ln DM(mg)=-4.5009+2.0742 HW(mm)	
Arachinida Argulidae	DM(µg)=aL(µm)^{b} DM(µg)=1.1x10^{-5}L(µm)$^{1.89}$	
Oligochaeta	DM(nl)=a L(µm)^{b} DM(nl)=3.5 x10^{-3} L(µm)$^{2.1}$	
Dixidae	DM(mg)=aL (µm)^{b} DM(mg)=6.62x10^{-4} L(µm)$^{2.59}$	
Cladocera	lnDM(µg)=lna+b lnL(mm) lnDM(µg)=ln1.7512+2.653L(mm)	
Asellidae	DM(mg)=aL(mm)^{b} DM(mg)=7.2x10^{-3}L(mm)$^{2.785}$	
Nematomorpha	DM(µg)=a L(µm)^{b} DM(µg)=6.0x10^{-5} L(µm)$^{0.8205}$	
Turbullaria	V(nL)=L(mm)xW^{2}mm)x C V(nL)=L(mm)W^{2}(mm)x550 V(nL) ×1.05 = dry weight= µg µg/1000=mg	(Feller & Warwick, 1988)
Piscicolidae Erpobdellidae Glossiphonidae	V(nL)=L(mm)×π(W/2)^{2}×530 V(nL)×1.13=dry wight(µg)	
Valvatidae Uniondae Planorbidae	**Water Displacement** V(nL)=WD(µL)x1000 Wt(µg)=v(nLx1.05) Mass(mg)=µg/1000	(Leaper et al, 2001)
Hydrobiidae Physidae Viviparidae	V(µL)=L(mm) (0.851)$^{1.91}$ Wt(µg)=v(µLx 1.05) Mass(mg) = µg/1000	
Ancylidae Sphaeriidae	**Approximate a geometric shape (cone)** V(µL)=1/3πr^{2} (mm) h(mm) V(nL)=µLx1000 Wt(µg) = nLx1.05 Mass (mg) = µg/1000	

Table 3. Continued.

Family/Class	Regression equation to determine body mass of fresh water invertebrates	References
Corixidae Notonectidae Mesovellidae Veliidae Hydrometridae Hemiptera	ln W(mg)=lna+b lnL ln W(mg)=-4.200+2.60 lnL(mm)	(Smock, 1980)
Gyrinida Dyticidae Haliplidae	Dw(mg)=lna+b lnL(BL(mm) or HW(mm) Dw=-2.0076+3.2271 lnL(BL-Dw) Dw =3.1102 +2.5412 lnL(HW-DW) converted BL to HW by using HW:BL	(Tower et al, 1994)

Habitats at the sites

There are six categories of general quality assessment (GQA) of water: very good, good, fairly good, fair, poor and bad. These classes have been determined by the Environment Agency in England, by combining two parameters, an ecological quality index based on the ASPT measure described above and the taxa present in the water body (Mason, 2002b). The computer model, RIVPACS (River Invertebrates Prediction and Classification System), has been developed to assess environmental stress based on the physical, geographical and chemical characteristics of a site, and what the invertebrate fauna of that site would look like in the absence of pollution. A comparison of the predicted macroinvertebrates communities with those actually observed allows calculation of ecological quality indices (EQI). The most relevant EQIs in describing biological quality are based on the number of macroinvertebrate taxa and ASPT as follows:

EQI taxa = Observed number of taxa present on given habitat (Predicted from RIVPACS)

EQI ASPT = Observed ASPT for the present taxa on given habitat (Predicted from RIVPACS)

RIVPACs habitat data for the different sites on the River Aire were supplied by the Environment Agency and the most revelvent variables are shown in Table 4. These data show that all the sites are stony shallow riffles which do not differ greatly in their substrate composition. Any between-site differences in BMWP and body size distributions are thus unlikely to be due to differences in stream bed characteristics or to bank vegetation as indicated by the shading score.

The RIVPACS data sheets also provide information on conductivity, related to dissolved solids and suspended material and general chemical characteristics of natural water (Hem, 1985). Significant changes in water conductivity could indicate pollution, pure (low conductivity) water being a good conductor of electric current. Thus, a positive relation has been found between pollution levels and conductivity (Ali, Ahmed, Othman, & Othman, 2009). In the present study, conductivity values were available for most sites on the Aire (Table 4) and there is a clear relationship between conductivity and water quality (ASPT) (Fig. 9).

Table 4. RIVPACS data for different sites on River Aire provided environmental agency leeds.

Winterburn	Light	Slight	None	8	20	208	None	Unstable	5	65	15	5	5	0	Yes
Hetton	Moderate	Slight	None	6.2	20	275	None	Stable	10	60	15	10	5	0	Yes
Gargrave	None	Clear	None	25	25	355	None	Stable	5	60	20	10	5	0	Yes
Carlton Bridge	None	Clear	None	14	20	283	None	Unstable	0	40	40	10	10	0	Yes
Esholt Village	Light	Slight	None	15	40	381	None	Unstable	0	70	30	0	0	0	Yes
Calverley Bridge	None	Clear	None	35	30	664	None	Unstable	5	45	40	5	5	0	Yes
Thwaites Mill	None	Clear	None	20	60	348	None	Stable	5	55	30	0	10	0	Yes

The body size distributions of benthic fauna at the sites are shown in Fig. 6. The data here are shown on a linear body mass scale (0.25-5 mg), but the shape is similar across a range of bin sizes and transformations. All sites show a skewed distribution towards smaller size classes and none of the sites can be adequately described by a single uni-modal distribution. In addition to the left- skewed mode, there is often a clear mode around 1-2 mg and possibly another mode in the largest size classes, although neither are apparent for Thwaites mill.

In contrast to left-skewed distributions, analysis by Kernel Density Estimate (KDE) revealed multiple modes for the most clean site Winterburn (Fig. 7, Table 5), and a single mode for the most polluted site (Thwaites Mill). However there was no consistency in the number of modes in body size spectra for intermediate quality water sites. The sites which are considered cleaner often had fewer modes while less clean sites had more modes. Thus, the number of modes for intermediate quality water are more variable, but all sites present at least bimodality, except for the most polluted habitat.

Analysis of the number of gaps, following Holling's methods, shows wide variation in the number of body size gaps detected (Fig. 8). The highest numbers of gaps occurred at the Esholt site which has moderate water quality and the lowest number of gaps was recorded in Carlton bridge having a fair water quality according to the Environment Agency and ASPT analysis. Consequently, there is no clear relationship between the number of gaps and water quality (Fig. 10, $R^2 = 0.0014$, $p>0.5$).

Table 5. Results of the test of significance for the sites spectra from the kernel density estimation and smoothed bootstrap re-sampling where h is smoothing constant used in kernel estimation, m is the smallest number of modes for which the bootstrap test was not significant at the 5% level, P level of significance for each distribution (mode number).

Sites	**h**	**m**	**p**		**Sites**	**h**	**m**	**p**
Winterburn	0.126	3	0.496		Carlton Bridge	0.353	2	0.103
Airton	0.178	2	0.292		Esholt Village	0.128	3	0.467
Otterburn	0.47	2	0.23		Thwaites mill	0.153	1	0.802
Hetton	0.173	2	0.338		Calverly Bridge	0.194	3	0.055
Gargrave	0.137	4	0.095					

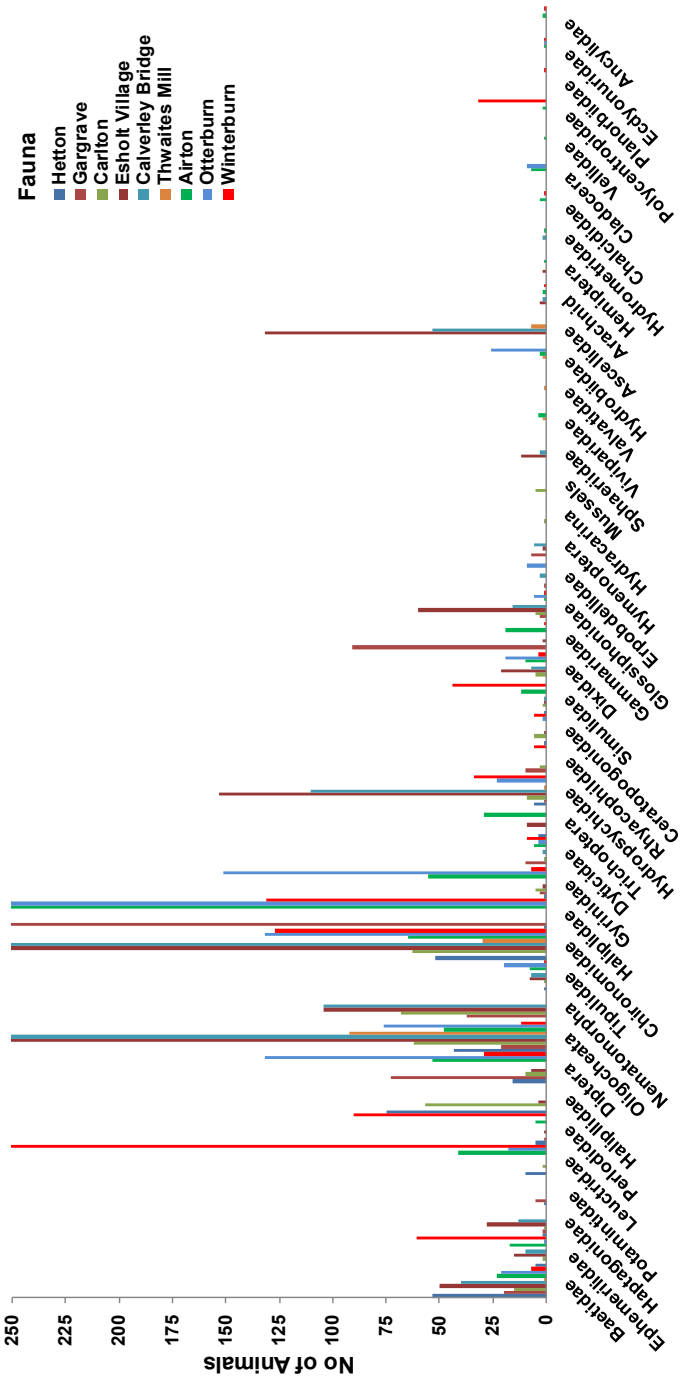

Fig. 1. Community abundance of macro invertebrates for different sites at river Aire, with species types (taxa) at X-axis and number of individuals at Y-axis.

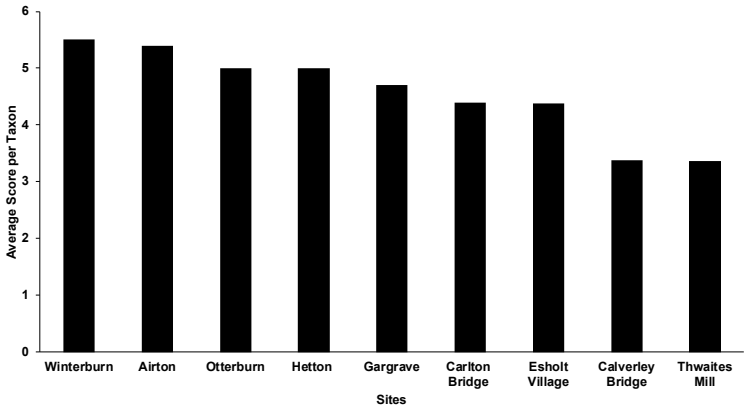

Fig. 2. Average score per taxon for different sites on the river Aire, varying in water quality. The score declines with decreasing water quality.

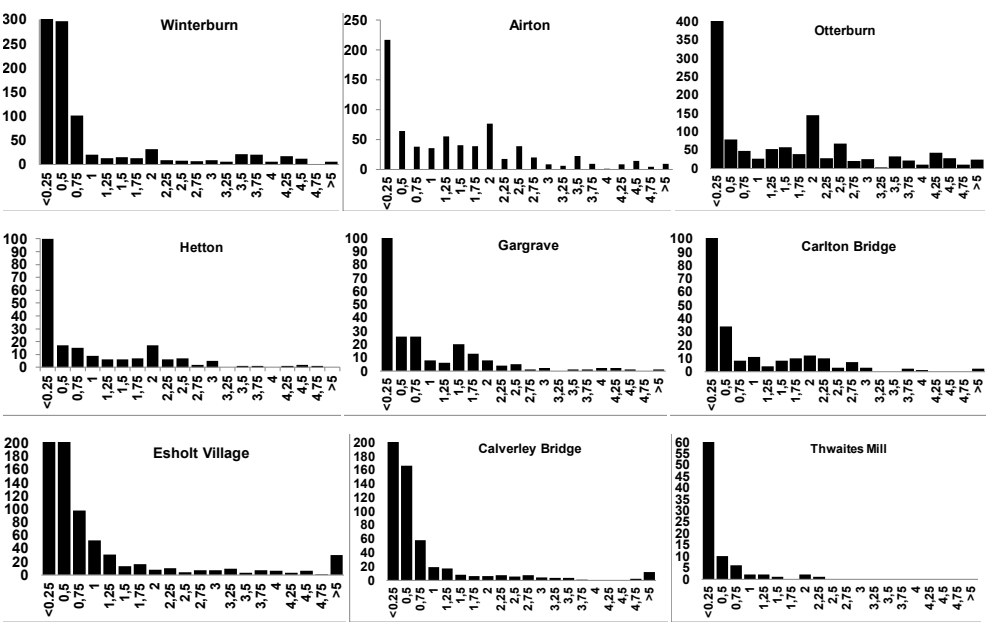

Fig. 3. Body size distribution for invertebrates of river Aire flows from Winterburn to Thwaites mill. Body mass were measured in mg, ranging from <0.02 to >5, plotted at x-axis while number of individuals are shown at y-axis. The distribution of body masses presents multimodality several sit.

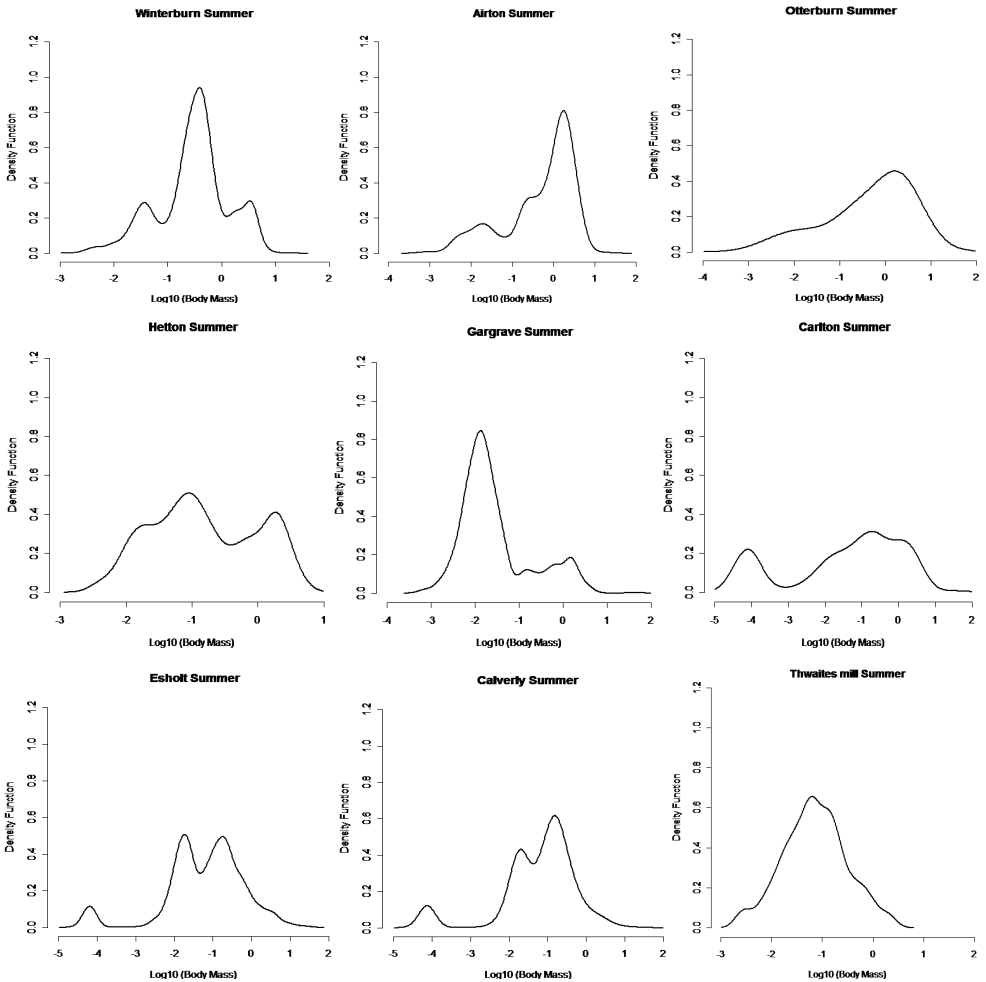

Fig. 4. The fitted distributions for density-body size estimated by Kernel density estimation and bootstrapped re-sampling at river Aire. Both axes are scaled as log to base 10 of the original data. Density function=number of individuals per core.

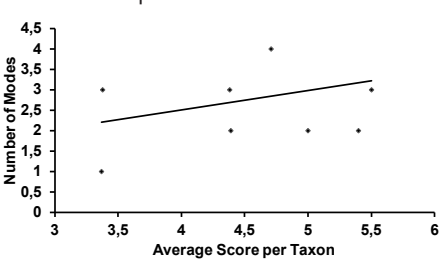

Fig. 5. The relationships between water qualities determined by biotic index (ASPT) and number of body mass modes determines by Kernel density estimates.

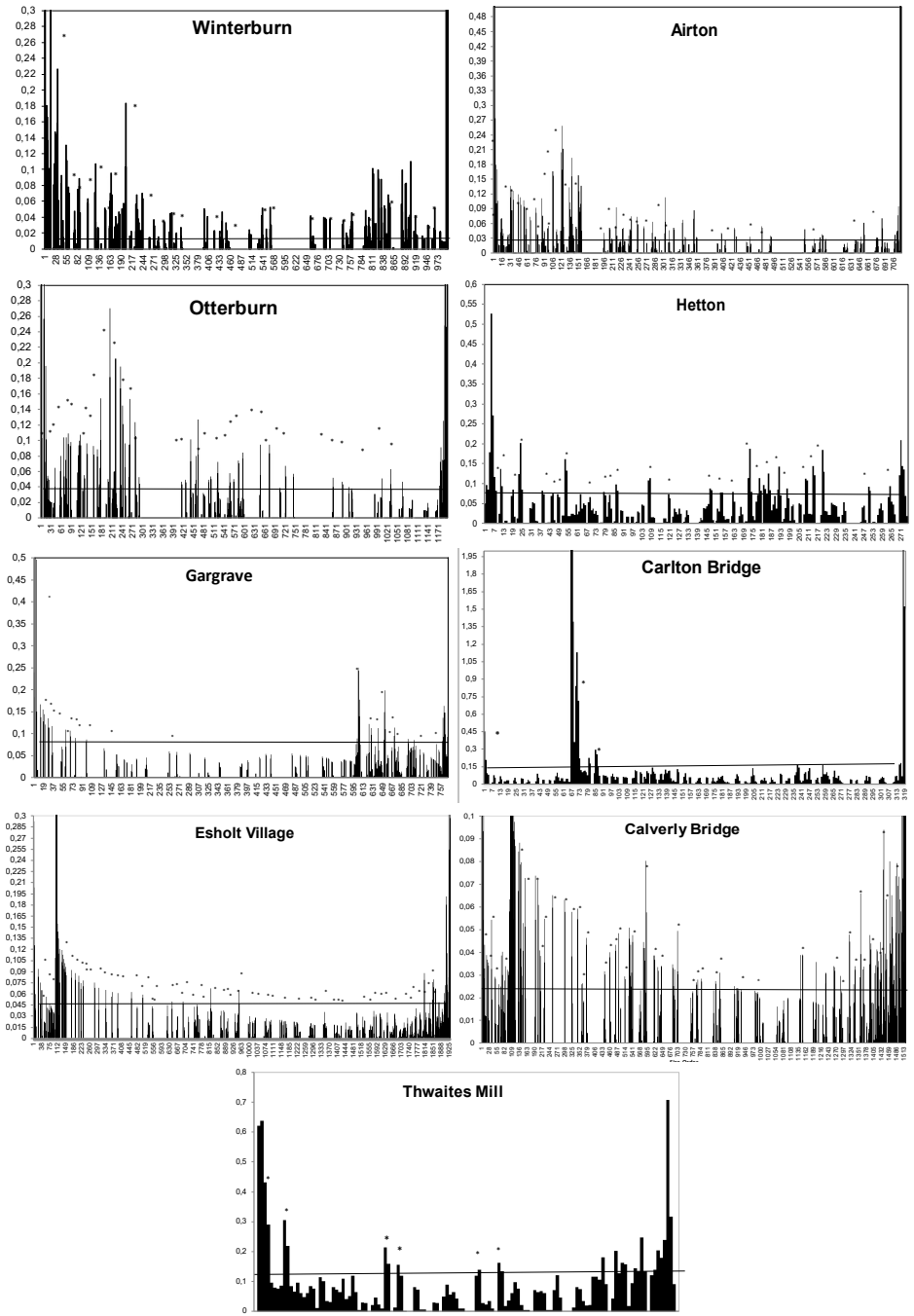

Fig. 6. Distributions of body mass gaps for river Aire stream communities. The horizontal line is the mean +2SE and asterisks (*) show significant body mass gaps, identified as at least two values of BMDI.

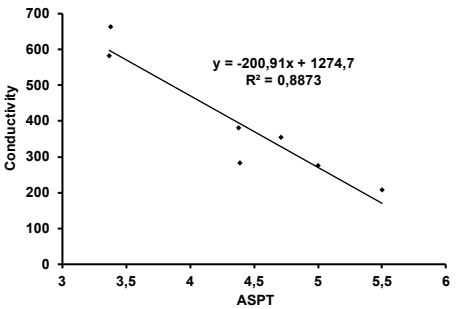

Fig. 7. The relationship between water quality (ASPT) and water conductivity.

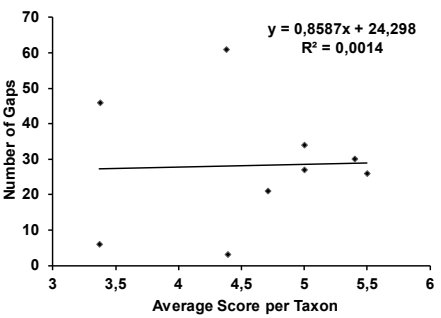

Fig. 8. The relationship between the number of gaps and average score per taxon for different sites on the river Aire. The vertical arrow indicates the number of gaps detected in body mass distributions.

DISCUSSION

The study was carried out to assess the effects of water quality on body mass distributions in stream communities. Benthic fauna were sampled across different site on the River Aire varying in water quality, to establish the potential of size based indicators for assessing environmental condition. Water quality had been classified by the Environment Agency UK and also assessed by us using invertebrate samples as the Average Score per Taxon (Mason, 2002). The ASPT estimates for the sites were similar to the classification provided by Environment Agency. The highest score of 5.5 was for the cleanest site and scores decreases with decreased water quality with the lowest score of 3.37 for the most polluted sites as reported in another studies of reducing score of the index with decreasing the quality of water (Ariella & Atiek, 2017).

The analysis of the size distributions showed that most sites were clearly not unimodal with respect to their body size distributions as claimed by many authors that body mass distributions in communities are multimodal (Schwinghamer, 1981a; Poff et al, 1993; Matthews, Borges, & Whittaker, 2014), although some do show uni-modal size spectra (Solimini, Benvenuti, D'Olimpio, Cicco, & Carchini, 2001). In the River Aire, body size distributions showed at least bimodality for invertebrates, mainly in the cleaner sites which supported a wide range of body sizes. The most polluted sites

(Calverly bridge and Thwaites mill) were better described as unimodal. There were a large number of small size individuals within these sites.

Whilst the above presents some evidence for changes in modality with water quality, this was not reflected in the BMDI analysis. For gap analysis, more discontinuities (body size gaps) imply less disturbed communities (Holling, 1992), but in our study there was no relationship between number of gaps and water quality as measured by ASPT. Many gaps were observed at Esholt village which had a fairly good quality compared to the cleaner site (Winterburn). 61 gaps were recorded in Esholt village followed by Calverly bridge with 46 gaps. Otterburn, Airton and Hetton are good quality habitats but in our gap analysis these sites revealed 34, 30 and 27 gaps, respectively. At the cleanest site (Winterburn), the number of gaps was 26 while in Gargrave 21 gaps were recorded. One of the lowest numbers was recorded in the polluted site (Thwaites mill) with 6 gaps, but in contrast a fair quality habitat (Carlton bridge) had only 3 gaps. Thus, there was no clear relationship between ASPT and the number of gaps. Finding gaps using the BMDI approach seems to be sensitive to the presence of exceptionally large values of BMDI that increase the mean value hugely making it almost impossible to detect the gaps which occur amongst the lower BMDI values.

In this research work the leading competing hypotheses were categorized which explaining body size distributions in ecosystems. The scale varies for each hypothesis and there is no evidence that one scale is superior to other scales of analysis (Vermaat, Eppink, van den Bergh, Barendregt, & van Belle, 2005) because different processes are important at different scales, and so no single theory can explain the patterns across different scales (Gaston, Chown, & Mercer, 2001). To link the body mass patterns to the processes affecting those patterns, multiscale analysis is critical (Krawchuk & Taylor, 2003), but there is evidence of multimodality (Havlicek & Carpenter, 2001) and discontinuity in body mass distribution in a range of ecosystems (Allen et al, 1999). The present study compares adjacent systems with different environmental conditions, so that the taxonomic identities of species and their evolutionary histories will be similar, thus phylogenetics are held constant. The system is spatially connected and allows species to disperse across the habitats which varies from clean to pollute and existence of body mass patterns cannot be due to biogeographical separation. The community interaction hypothesis predicts that changes in the patterns of body size are because of different taxa present in the system, but such taxonomic differences are restricted to the species level, not the higher-level taxa dealt with here. The textural discontinuity hypothesis predicts changes in body size patterns because the habitat available to the animals differs. The presence of multiple modes and gaps in cleaner sites reflects the hierarchical physical structure of the system and shows that multiple processes are responsible to structure a dynamic ecosystem. At the most polluted sites (Thwaites mill), fewer modes might be a sign of a disturbed and less resilient system, although the number of gaps for other polluted sites is higher and for some clean site is lower.

In conclusion, it is clear that body size distributions in stream communities are “lumpy”, in the sense that most site shows more than one mode or many gaps. The

most polluted site in our study had the fewer modes and gaps and the cleanest site had many gaps and were clearly multimodal. In term of Holling's (1992) textural discontinuity hypothesis, these patterns could reflect the dynamic processes operating at particular scales, no other competing hypotheses seeming plausible. However, it is also clear that the number of gaps (discontinuities) is not well correlated with disturbance, at least water quality, but further empirical exploration of such relationships is needed given their compelling theoretical basis (Holling, 1992).

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Received: July 20, 2017 *Accepted: February 25, 2019*

J. Entomol. Res. Soc., 21(1): 37-56, 2019
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

Ethology of *Proctacanthus longus* (Wiedemann, 1821) (Diptera: Asilidae) in Northeastern Florida, U.S.A.

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ABSTRACT

Proctacanthus longus (Wiedemann, 1821) forage from the soil, dead vegetation on the soil (detritus), and vegetation, capturing and immobilizing prey in flight. Identified prey came from seven insect orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Neuroptera, Lepidoptera, and Orthoptera), with Diptera and Orthoptera making up 43.3 and 24.3%, respectively. Mating initially occurs in the male-over-female position and then the pair straightens out into the tail-to-tail position. Females oviposit in the soil, typically in the shade of vegetation. This species exhibits a daily rhythm of activity for feeding, mating, and ovipositing. Grooming behavior resembles that described for other species of Asilidae. Habitats, resting behavior, and predators and parasites also are discussed.

Key words: Behavior, robber flies, prey, Diptera, Asilidae

Dennis, D.S. (2019). Ethology of *Proctacanthus longus* (Wiedemann, 1821) (Diptera: Asilidae) in Northeastern Florida, U.S.A. *Journal of the Entomological Research Society*, 21(1), 37-56.

INTRODUCTION

There are 19 described species of robber flies in the genus *Proctacanthus* in the United States of America (U.S.A.; Geller-Grimm, 2017). Of these species only the ethology of *P. brevipennis* (Wiedemann, 1828) (Dennis, 2012), *P. fulviventris* Macquart, 1850 (Dennis, 2015), *P. micans* Schiner, 1867 (Dennis & Lavigne, 1975; Rogers & Lavigne, 1972), and *P. nearno* Martin, 1962 (Lavigne & Dennis, 1979) have been described in detail. Dennis (2012) listed other publications that reported observations on habitat and/or prey for the other species.

This paper provides detailed information on the ethology of *P. longus* (Wiedemann, 1821) in the Moses Creek Conservation Area (MCCA) in St. Augustine in northeastern Florida, U.S.A. *P. longus* is 32-39 mm in length and its body is grayish to brownish pollinose. The proboscis is slender and the mystax is yellowish-white. The wings are uniform brown, wide and long, extending to at least the middle of the posterior margin of abdominal segment 7 (Figs. 1 and 2).

Fig. 1. Male *P. longus* on detritus on soil (Photograph: D.S. Dennis, 11.06.2012, 9:11 AM).

MATERIALS AND METHODS

P. longus is widely distributed in Florida and, depending on location, generally occurs from May through August. Observations were made over a period of 7 years, from: 21.06.2011-16.08.2011; 04.05.2012-05.07.2012; 01.07.2013-16.08.2013; 26.05.2014-29.07.2014; 27.05.2015-22.07.2015; 13.05.2016-27.07.2016, and 12.06.2017-17.08.2017. The author observed *P. longus* in the MCCA in the mowed edges of roads in upland mixed forest and mesic flatwoods vegetation communities, and in mowed scrub, scrubby flatwoods, and in sandhill vegetation communities, including their roads.

The times when *P. longus* was most abundant in the previously mentioned vegetation communities determined the periods of study. Up to 16 robber flies were observed per day with an average of three, with observations of individuals lasting

as long as 163 minutes. Total number of hours of observation equaled approximately 138, not including the time spent looking for *P*. *longus* to study.

Fig. 2. Female *P. longus* on saw palmetto leaflet (Photograph: D.S. Dennis, 22.05.2012, 8:44 AM).

During the study the author sat on the soil (mostly sand) or stood and observed single flies, mating pairs, and ovipositing females for as long as possible in order to collect information on their various behaviors and diurnal activities. In addition, after gathering sufficient data on their behavior, the author slowly walked through a study area and recorded the activities of as many flies as possible. During these walks data also was collected on prey selected and numbers of times specific behaviors occurred.

Collected prey was placed in glass vials with the following information: sex of predator (if observed); date; time, and location. The author sent prey that he could not identify to the U.S. Department of Agriculture, Agricultural Research Service, Systematic Entomology Laboratory, Beltsville, Maryland, U.S.A. for identification. Prior to shipment, prey was measured to the nearest 0.5 mm using a clear, plastic ruler.

Ovipositing females were observed for as long as they exhibited oviposition behavior or until they moved out of sight. When a female ceased to oviposit or the author lost visual contact, he dug up the oviposition site with a small hand shovel. Then he visually examined the soil in the laboratory and eggs, if found, were removed. Those eggs that were recovered (from four ovipositions) were placed in 95% ethyl alcohol for subsequent examination and measurement to the nearest 0.1 mm. Equipment used was a Wild Heerbrugg M8 stereomicroscope with a transmitted light base, a 1.6x objective, and a 20x-focusing eyepiece for magnifications up to 160x. The eyepiece was equipped with a 5-mm/100-division reticle for measuring the eggs. The reticle was calibrated using a dual axis 1 mm/100 division/0.01 mm and linear 50-mm/500 division/0.1 mm multi-function scale/stage micrometer.

While in the field a hand held Taylor thermometer and a Cooper-Atkins DPP400W Digital Thermometer were used to take air, and surface and subsurface soil temperatures. A Dwyer Hand-Held Wind Meter measured wind speed.

RESULTS AND DISCUSSION

Habitat

The St. Johns River Water Management District (District) owns and operates the MCCA. To restore, maintain, and protect natural communities and diversity, the District uses a combination of prescribed fire and mechanical (roller chopping and mowing) vegetation management in the scrub, scrubby flatwoods, and sandhill communities. The District also mows along roads and the sides or edges of roads in these communities, and the upland mixed forest and mesic flatwoods vegetation communities to facilitate access to the MCAA. Most *P. longus* were found and studied in the mowed scrub community (Fig. 3).

Fig. 3. *P. longus* habitat in mowed scrub vegetation community (Photograph: D.S. Dennis, 31.05.2014, 8:34 AM).

The *P. longus* study areas have the plants associated with the vegetation communities shown in Table 1. The dominant plants in all areas are 30 cm to 1 m tall saw palmetto, scrub oak, staggerbush (*Lyonia* spp), grasses (*Andropogon* spp.), and sedges (*Cyperus* spp.). Over time, the same plant species have invaded the mowed areas in the various habitats.

Bromley (1950) recorded that *P. longus* occurred in Florida in sandy fields and pastures, and on Panama City beach and dunes. Previously, Bromley (1934) had indicated that in Texas this species "Occurs in sandy fields and pastures near the larger water courses." Bromley (1928) made the general statement that *Proctacanthus* "…inhabit dry fields or pastures, several being restricted to dry sandy plains." Hull (1962) observed that *Proctacanthus* are found in "…rank grassland and shrubs on the edges of woodlands in swampy country and some prefer sandy river banks." Dennis (2012) found *P. brevipennis* primarily on sand roads in the MCCA, and *P. fulviventris* (Dennis, 2015) in a mowed scrub community and the mowed edges or roads in scrub, scrubby flatwoods, and upland mixed forest vegetation communities.

Table 1. Vegetation communities in which *P. longus* was studied in the Moses Creek Conservation Area.

Vegetation Type	Mowed Edges of Roads in Vegetation Communities		Mowed Vegetation Communities and Edges of Roads		
Family/Genus or Species/Common Name	Upland Mixed Forest	Mesic Flatwoods	Scrub	Scrubby Flatwoods	Sandhill
Agavaceae					
Yucca filamentosa L./ Adam's needle	—[1]	X	—	—	X
Amaranthaceae					
Froelichia floridana (Nutt.) Moq./cottonweed (plains snakeweed)	X	—	—	X	X
Annonaceae					
Asimina sp./pawpaw	X	X	X	X	X
Aquifoliaceae					
Ilex glabra (L.) A. Gray/ gallberry	—	X	X	—	—
Arecaceae					
Serenoa repens (W. Bartram) Small/saw palmetto	X	X	X	X	X
Asteraceae					
Carphephorus corymbosus (Nutt.) Torr. & A. Gray/ coastalplain chaffhead (Florida paintbrush)	—	X	X	X	—
Carphephorus odoratissimus (J. F. Hamel) H. Hebert/ vanillaleaf (Deer's tongue)	—	X	X	X	—
Erechtites hieraciifolius (L.) Raf. ex DC./ American burnweed (fireweed)	—	—	X	—	—
Eupatorium sp./fennel	X	X	X	X	X
Liatris gracilis Pursh/ slender gayfeather	X	—	—	—	—
Liatris tenuifolia Nutt./ shortleaf gayfeather	X	X	X	X	—
Pityopsis graminifolia (Michx.) Nutt./ narrowleaf silkgrass	X	X	—	X	X
Solidago spp./goldenrod	X	X	X	X	X
Cactaceae					
Opuntia humifusa (Raf.) Raf./ Eastern prickly pear cactus	X	X	X	X	X
Convolvulaceae					
Ipomoea sp./morning glory	—	—	—	—	X

Table 1. Continued.

Vegetation Type	Mowed Edges of Roads in Vegetation Communities		Mowed Vegetation Communities and Edges of Roads		
Family/Genus or Species/Common Name	Upland Mixed Forest	Mesic Flatwoods	Scrub	Scrubby Flatwoods	Sandhill
Cyperaceae					
Cyperus spp./flatsedge	X	X	X	X	—
Dennstaedtiaceae					
Pteridium aquilinum L. (Kuhn) var. *pseudocaudatum* (Clute) Clute ex A. Heller/ tailed bracken	X	X	X	X	X
Ericaceae					
Bejaria racemosa Vent./ tar flower (flyweed)	—	X	X	X	—
Ceratiola ericoides Michx./ Florida rosemary (sand heath)	—	—	X	—	—
Lyonia ferruginea (Walter)Nutt./rusty lyonia	X	X	X	X	X
Lyonia fruticosa (Michx.) G. S. Torr./ coastalplain staggerbush	—	—	X	—	—
Lyonia lucida (Lam.) K. Koch/fetterbush	X	X	X	X	—
Vaccinium corymbosum L./ highbush blueberry	—	X	X	X	—
Vaccinium myrsinitas Lam./ shiny blueberry	—	X	X	X	—
Eriocaulaceae					
Lachnocaulon spp./ bogbutton	—	—	X	X	—
Euphorbiaceae					
Cnidoscolus stimulosus Michx. Engelm & A. Gray/tread-softly	—	X	X	X	X
Fabaceae					
Centrosema virginianum (L.) Benth./spurred butterfly pea	—	X	—	—	X
Galactia elliottii Nutt./Elliott's (white) milkpea	X	X	X	X	X
Galactia spp./milkpea	X	—	X	—	—
Mimosa sp./sensitive plant	—	X	—	—	X

Table 1. Continued.

Vegetation Type	Mowed Edges of Roads in Vegetation Communities		Mowed Vegetation Communities and Edges of Roads		
Family/Genus or Species/Common Name	Upland Mixed Forest	Mesic Flatwoods	Scrub	Scrubby Flatwoods	Sandhill
Fagaceae					
Quercus incana W. Bartram/ bluejack oak	X	—	X	—	—
Quercus laevis Walter/turkey oak	—	—	—	—	X
Quercus virginiana (P. Mill.)/live oak tree	X	X	X	X	X
Quercus sp. /scrub oaks	X	X	X	X	X
Magnoliaceae					
Magnolia grandiflora L./southern magnolia	—	X	—	—	—
Pinaceae					
Pinus clausa(Chapm. ex Engelm.) Vasey ex Sarg./sand pine	X	X	X	X	X
Pinus elliottii Engelm./slash pine	X	X	X	—	—
Pinus palustris Mill./ longleaf pine	—	X	—	X	X
Poaceae					
Andropogon glomeratus (Walter) Britton et al./ bushy bluestem	X	X	X	X	X
Andropogon virginicus L./broomsedge bluestem	X	X	X	X	X
Aristida stricta Michx. Var. beyrichiana (Trin. & Rupr.) D. B. Ward/ wiregrass	X	X	X	X	X
Cenchrus sp./sandbur	—	X	X	—	—
Setaria spp./foxtail	X	X	X	—	—
Sorghastrum secundum (Elliott) Nash/lopsided indiangrass	—	X	—	—	—
Other grasses	X	X	X	X	X
Saururaceae					
Saururus cernuus L./ Lizard's tail	X	X	X	X	X

Table 1. Continued.

Vegetation Type	Mowed Edges of Roads in Vegetation Communities		Mowed Vegetation Communities and Edges of Roads		
Family/Genus or Species/Common Name	Upland Mixed Forest	Mesic Flatwoods	Scrub	Scrubby Flatwoods	Sandhill
Smilaceae					
Smilax auriculata Walter/earleaf greenbrier	—	X	X	—	X
Smilax bona-nox L./saw greenbrier vine	X	X	X	X	X
Smilax glauca Walter/ cat greenbrier	X	X	X	X	X
Vitaceae					
Vita rotundifolia Michx./muscadine	X	X	X	X	X
Zamiaceae					
Zamia integrifolia L./ Florida arrowroot (Coontie)	X	—	X	—	—

Footnote: — = not present; X = present.

Resting behavior

P. longus rests on the soil, on dead vegetation on the soil (detritus), and on the stems and leaves of live vegetation. In early morning, when on the soil or dead vegetation on the soil, individuals would flatten themselves against the substrate with their dorsal surface to the sun; turn so that one of their sides faced and was slightly elevated to the sun; or face the sun and elevate themselves on their fore tarsi so that their bodies are at a 45-degree angle. Flattening against the soil is particularly common when the surface soil temperatures are 28-30˚C or less and/or the wind is gusting 4.8-9.6 km/hr.

When resting on the soil or vegetation, individuals tend to groom themselves. At the same time, they generally ignore other insects flying by, even insects as close to the asilid as 15-30 cm.

P. longus generally started to move from the soil onto vegetation between 9:30 to 11:30 AM when the soil temperature reach 35-37˚C and the air temperature is 31-36˚C. When moving to vegetation to continue grooming or foraging, a *P. longus* would usually land on vegetation in the sun; whereas, if it moved to continue resting, it would land in the shade of vegetation with its body at a 45-degree angle, parallel to the vegetation, or vertical to the soil. After moving to vegetation, one male rested in the shade with its body at a 45-degree angle to a grass blade for 43 minutes. Both *P. brevipennis* (Dennis, 2012) and *P. fulviventris* (Dennis, 2015) show similar movement from the soil to vegetation as the day progresses and soil temperatures increase.

P. longus apparently maintain their body temperature by changing their position and flattening themselves against the substrate that they are on (in particular while on the soil), and moving to vegetation that is in shade. *Proctacanthus brevipennis*

(Dennis, 2012), *P. fulviventris* (Dennis, 2015), *P. nearno* (Lavigne & Dennis, 1979), and *P. micans* (Dennis & Lavigne, 1975) also maintain their body temperature by postural adjustments and microhabitat selection. Similar behavior is shown by many other species of robber flies (Dennis & Lavigne, 1975; Morgan, Shelly, & Kimsey, 1985; Morgan & Shelly, 1988).

While resting, abdominal pumping of the first one to two segments was observed in both male and female *P*. longus. This was observed for one male during feeding. In the laboratory *Promachus giganteus* Hine, 1911 pumped haemolymph into the abdomen to regulate thoracic temperatures (Morgan & Shelly, 1988). *P. longus* may exhibit similar behavior in the field to regulate its body temperature. Abdominal pumping or contractions during feeding generally have been attributed to the injection of proteolytic enzymes into prey and food pumping (Musso, 1968; Lavigne & Holland, 1969).

Foraging and feeding behavior

P. longus foraged from the soil, detritus on the soil, and from vegetation, often with their bodies at a 45-degree angle and facing the sun. Both *P*. *brevipennis* (Dennis, 2012) and *P*. *fulviventris* (Dennis, 2015) exhibited similar foraging behavior, and *P*. *micans* (Dennis & Lavigne, 1975) and *P*. *nearno* (Lavigne & Dennis, 1979) foraged from vegetation with their bodies at a 45-degree angle. These foraging postures presumably allow the robber flies to better see potential prey, and when they face the sun this may highlight potential prey by backlighting. Other authors have made similar observations for a number of species of robber flies (Dennis, 2012, 2015).

P. longus make flights around a forging position that are not directed at potential prey (i.e., orientation flights) and flights directed at potential prey without coming into contact with them (i.e., investigatory flights). Over periods of 9-18 minutes (average 13.5 minutes), individuals made orientation or investigatory flights 3-8 times (average 5 times). As part of an investigatory flight, often times the robber fly would follow or hover near the potential prey. *P. longus* also make foraging flights when they hit or capture and release potential prey.

Orientation flights took place within 2.5 cm-13.7 m (average 1.6 m) of an individuals foraging location. Investigatory flights were for distances of 5.0 cm-2.1 m (average of 74.0 cm) behind, above, to the side of or in front of a foraging position, and 5.0 cm-1.2 m (average 45.5 cm) above the soil.

The majority of *P. longus* foraging flights are made in front of an individual's foraging location, but they also take place to the side or behind a foraging location. Foraging flights occur from 5.0 cm-3.0 m (average 73.7 cm) around an individual's location and are conducted 5.0-75.0 cm (average 40.6 cm) above the soil.

After orientation, investigatory, and foraging flights, individuals often re-land at or near (within 15.0 cm) their starting location. If they move to a new foraging location, it is 22.9 cm -6.1 m (average 3.5 m) from its original location. Robber flies may move to new foraging locations to increase the probability of finding prey (Dennis, 2016).

P. longus captured all of its prey in the air when the prey were within 12.7-91.4 cm (average 54.4 cm) in front of, to the side of or behind its foraging location, and

within 15.0-61.0 cm (average 31.8 cm) above the soil. *Proctacanthus fulviventris* also captured all of its prey in flight; whereas, *P. brevipennis* (Dennis, 2012), *P. micans* (Dennis & Lavigne, 1975), and *P. nearno* (Lavigne & Dennis, 1979) captured most of their prey in flight.

When capturing prey, *P. longus* would either immediately insert its proboscis in the dorsal or dorsolateral part of the prey's thorax or hover and manipulate the prey with all six tarsi before inserting its proboscis. After initially landing on soil or vegetation in the sun, the asilids would generally then fly to the shade of nearby vegetation to feed.

Most *P. longus* move to a new location one to four times while feeding on prey. When they move it is to a location up to 1.8 m from the previous location, and it is often from an area in the sun to the shade of vegetation.

During feeding, *P. longus*, (1) did not manipulate prey, (2) manipulated prey in a hover above the feeding site, or (3) held prey against vegetation and crawled on them before reinserting their proboscises. In general, smaller prey [e.g., 11.5 mm long *Blauta falli* Brown, 1936 (Coleoptera: Elateridae)] were not manipulated or were manipulated in a hover; whereas, larger prey [20 mm long *Mydas maculiventris* Westwood, 1835 (Diptera: Mydidae)] were crawled on. *Proctacanthus fulviventris* manipulated prey in a hover above the feeding site (Dennis, 2015). *Proctacanthus brevipennis* (Dennis, 2012), *P. micans* (Dennis & Lavigne, 1975; Rogers & Lavigne, 1972), and *P. nearno* (Lavigne & Dennis, 1979) manipulate prey during a hover and held larger prey against the soil or vegetation and crawl on them before reinserting their proboscises.

When *P. longus* were feeding, prey less than 13 mm long generally hung free from the asilid's proboscis without support by the tarsi or being held against vegetation. For longer prey greater than approximately 18 mm, an asilid used its body to hold prey against vegetation while grasping the vegetation with all six tarsi.

Only four *P. longus* complete feedings were observed and these ranged from 17-119 minutes with an average of approximately 60 minutes. Based on this limited data, the time spent feeding depended on prey length. *Blauta falli* with a length of 11.5 mm was fed on for 52 minutes and *Mydas maculiventris* with a length of 20 mm was fed on for 119 minutes. Other researchers also have observed that for a number of robber fly species the time spent feeding usually depends on prey length (Dennis, 2016).

Male *P. longus* captured prey that were slightly longer than those captured by females. Mean prey length for males was 19.8 mm (n=12) with a range from 6.6-32.0 mm; whereas, for females it was 18.5 mm (n=14) with a range from 5.5-31.0 mm. The overall mean prey length was 19.1 mm with a predator to prey ratio of 1.7:1.0 which indicates that *P. longus* was between 1.5 to 2 times as large as its prey. Mean predator to prey ratios for *P. brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), and *P. micans* (Dennis & Lavigne, 1975), were 3.0:1.0, 1.9:1.0, and 2.0:1.0, respectively. Mean predator to prey ratios for other species of robber flies range from 0.9:1.0 to 8.4:1.0 (Dennis, 2016) with a mean of 2.9:1.0.

At completion of feeding, each individual *P. longus* discarded prey in one of four ways: (1) it dropped prey in flight as it moved to a new location; (2) it pushed prey

off its proboscis with one or both fore tarsi while it was still at the feeding site; (3) it allowed prey to drop-off its proboscis at the feeding site; or (4) it dropped prey during a hover at the feeding site Other species of *Proctacanthus* use similar methods to discard prey (Dennis, 2015). In addition, *P. micans* pushed prey off its proboscis with the fore tarsi during a flight to a new location (Dennis & Lavigne, 1975).

Interfeeding times (time between feedings) for *P. longus* were extremely difficult to obtain because of the speed and distance flown by individuals after feeding. Additionally, the asilids tended to be lost to sight as they weaved in and out of vegetation. Consequently only one partial interfeeding time of 44 minutes was recorded.

One can calculate the theoretical number of prey an individual *P. longus* could feed on in one day if we assume that: (1) it continually forages and feeds between 9:00 AM and 3:00 PM (the period when individuals were found with prey), and (2) it captures and feeds on prey every 104 minutes (based on the average feeding time and the one partial interfeeding time). Thus, over a 6-hour period an individual could feed on approximately 3 to 4 prey. Dennis (2012, 2015) estimated 7 to 8 and 3 to 4 prey per day for *P. brevipennis* and *P*. *fulviventris*, respectively. Dennis & Lavigne (1975) calculated that *P. micans* could feed on approximately 6 to 7 prey per day. Other investigators have estimated that robber flies feed on from 1 to 35 prey per day (Dennis, 2016).

P. longus feeding on fewer prey per day than many other species of robber flies may be because they have longer feeding and interfeeding times and they feed on larger prey as shown by the lower predator to prey ratio. *Proctacanthus micans* (Dennis & Lavigne, 1975) also had a low predator to prey ratio (2.0:1.0), but it had average shorter feeding (46 minutes) and interfeeding times (21 minutes) than *P*. *longus*.

Prey

P. longus fed on Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Orthoptera. However, the majority of prey was Diptera (43.3%) and Orthoptera (24.3%) (Table 2). Other researchers record *P. longus* feeding on Diptera, Hemiptera (Homoptera), Hymenoptera, Lepidoptera, Odonata, and Orthoptera (Clauson, 1940; Bromley, 1934, 1950; Lavigne, Nelson, & Schreiber, 1994).

Both *P. brevipennis* and *P. fulviventris* occur in some of the same habitats with *P. longus*. Dennis (2012) reported *P. brevipennis* feeding on six insect orders (Coleoptera, Diptera, Hymenoptera, Isoptera, Lepidoptera, and Orthoptera); whereas, he (2015) indicated that *P. fulviventris* fed on only Diptera and Hymenoptera.

Male and female *P. longus* generally fed on the same insect orders and approximately the same number of prey was collected for both sexes. However, many investigators have reported collecting more females than males with prey (Dennis, 2016). For *P. brevipennis* and *P. rufus* Williston, 1885, Bromley (1923) attributed this to the females being "…larger and more powerful than the males…"

Table 2. Number and percent composition of orders of prey captured by *P. longus*.

	Male		Female		Total	
Order	Number	Percent	Number	Percent	Number	Percent
Coleoptera	2	11.8	2	10.0	4	10.8
Diptera	9	52.8	7	35.0	16	43.3
Hemiptera	1	5.9	0	0	1	2.7
Hymenoptera	2	11.8	3	15.0	5	13.5
Lepidoptera	0	0	1	5.0	1	2.7
Neuroptera	1	5.9	0	0	1	2.7
Orthoptera	2	11.8	7	35.0	9	24.3
Totals	17	100.0	20	100.0	37	100.0

The following is a list of prey taken by *P. longus*. Number and sex of the predator is indicated following the prey record.

COLEOPTERA, Elateridae: *Blauta falli* Brown, 1936, 13.06.2012 (1 ♂), 10.06.2014 (1 ♂, 1 ♀), 30.06.2014 (1 ♂). Scarabaeidae: *Melanocanthon* sp. prob. *granulifer* (Schmidt, 1920), 29.06.2012 (1 ♀). DIPTERA, Asilidae: *Efferia tabescens* (Banks, 1872), 17.06.2014 (1 ♀), 17.08.2017 (1 ♀); *Polacantha gracilis* (Wiedemann, 1828), 06.06.2014 (1 ♀); *P. longus,* 07.05.2012 (1 ♀), 11.07.2017 (1 ♂); *Proctacanthus rufus* Williston, 1885, 09.07.2015 (1 ♀); *Promachus bastardii* (Macquart, 1838), 20.07.2017 (1 ♂); unidentified, 30.06.2014, (1 ♂). Mydidae: *Mydas maculiventris* Westwood, 1835, 11.06.2012 (1 ♀), 04.06.2014 (1 ♂), 10.06.2014 (1 ♂, 1 ♀), 12.06.2014 (1 ♂), 13.06.2014 (2 ♂♂), 16.06.2014 (1 ♂). HEMIPTERA, Heteroptera: unidentified, 14.06.2012 (1 ♂). HYMENOPTERA, Apidae: *Bombus* sp., 03.07.2014 (1 ♀), 18.07.2014 (1♀). Formicidae: *Tetramorium* sp., 12.06.2012 (1♂). Scoliidae: unidentified, 20.06.2012 (1 ♂). Unidentified: 07.05.2012 (1 ♀). LEPIDOPTERA: Blastobasidae, *Holcocera immaculella* (McDunnough, 1930), 02.07.2015 (1 ♀). NEUROPTERA, Myrmeleontidae: *Myrmeleon* sp., 29.06.2012 (1 ♂). ORTHOPTERA: Acrididae, *Chortaphaga australior* Rehn and Hebard, 1911, 27.05.2015 (1 ♀); *Orphulella pelidna* (Burmeister, 1838), 3-VI-14 (1 ♂); *Psinidia fenestralis* (Serville, 1839), 25.07.2017 (1 ♀); *Spharagemon crepitans* (Saussure, 1884), 11.06.2014 (1 ♀); unidentified, 04.06.2014 (1 ♀), 07.07.2014 (1 ♀), 15.07.2014 (1 ♀), 11.07.2017 (1 ♂), 01.08.2017 (1 ♀).

Mating behavior

Male *P. longus* performed searching flights for receptive females with which to mate. Flights consisted of males making one to five vertical undulations as they flew a straight or zigzag pattern above vegetation or they weaved in and out of vegetation. They flew for distances of 1-12 m and 7.5 cm-2.4 m above the soil. Male searching flights in vertical undulations have been reported for *P. brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), and *P. micans* (Dennis & Lavigne, 1976). One male *P. longus* buzzed his wings during his searching flights and a few males had their abdomens slightly curved up without buzzing their wings.

As part of searching for females with which to mate, males frequently flew up to investigate other male *P. longus* without coming into contact. A few males also would either briefly hover in front of or circle each other and sometimes come into contact before landing on the soil or vegetation.

P. longus usually initiated matings in-flight when the male would land on the dorsum of the female's thorax and then clasp the female's genitalia. One male grasped and released a female in flight when she played dead (i.e., thanatosis) and then fell to the soil where she remained for 30 seconds before flying off. Another male landed on the dorsal surface of a female resting on a dead saw palmetto leaflet, approximately10 cm above the soil. The female immediately fell to the soil on her back with her legs extended and bent inward at the joints between the femora and tibiae. She remained in this position for 149 seconds, then stood up and flew off. A female also played dead when captured in a small jar. Dennis & Lavigne (1976) commented that when female *Efferia varipes* [(Williston, 1885); as *Erax*)] played dead, "…the males did not receive the necessary stimuli to continue mating attempts."

After mating started, the pair would fly in the male-over-female position to nearby vegetation and land up to 2.5 m above the soil (e.g., on the trunk of a live oak tree), in the shade of surrounding vegetation or a plant stem. In the male-over-female position the male's abdomen curved to the right or left of the female's abdomen and clasped her genitalia from below. The wings of at least the female were usually spread at a 30 to 45-degree angle to her body. The female's wings in this position usually passed between the male's mid and hind legs so that the male's mid tibiae were over the female's wing bases. One female's wings passed over the male's mid and hind legs. The male's wings were either closed or intermittently, briefly opened to a 30 to 45 degree angle to his body. The males fore tarsi rested on the female's eyes/head, on the anterior part of her thorax or on vegetation. The male's mid tarsi held on to the female's thorax or on to the anterior part of her abdomen. The male's hind tarsi held on to the females abdomen or his hind legs passed around the female's abdomen with the hind tarsi holding onto vegetation.

Fifteen partial matings and two complete matings were observed. The mating pairs generally remained in the male-over-female position for 10-52 minutes and then assumed the tail-to-tail position while in flight to another location on vegetation (Fig. 4). The female of one mating pair appeared agitated and after being in the male-over-female position for 5 minutes, she initiated flight and the pair straightened out in to the tail-to-tail position. When in the tail-to-tail position, both the male and female periodically had their wings spread at 30 to 45-degree angles, although the previously mentioned agitated female held her wings straight down at a 90-degree angle to her body.

Fig. 4. Mating pair of *P. longus* in the tail-to-tail position (Photograph: D.S. Dennis, 16.06.2014, 2:27 PM).

The two complete matings lasted 116 minutes and 106 minutes. Matings occurred when the air temperature at the height where the mating pair rested on vegetation ranged from 28.5-32.5ºC (average 30.1ºC) in the shade and 29.5-33.5ºC (average 31.4ºC) in the sun. *Proctacanthus brevipennis* mated for 78 to 111 minutes with an average of 90 minutes (Dennis, 2012); *P. fulviventris* mated for 30 to 63.5 minutes with an average of 40.6 minutes (Dennis, 2015); and *P. micans* mated for 23 to 66 minutes with an average of 42 minutes (Dennis & Lavigne, 1975).

Proctacanthus fulviventris (Dennis, 2015) and *P. micans* (Dennis & Lavigne, 1975) mated in the male-over-female position. *Proctacanthus nearno* started mating in the male-over-female position and shortly after the initiation of mating assumed the tail-to-tail position (Lavigne & Dennis, 1979). *Proctacanthus brevipennis* mated in the tail-to-tail position (Dennis, 2012).

At the completion of mating, like *P. fulviventris* (Dennis, 2015), male *P. longus* released the female and both flew off or the pair flew into the air in the tail-to-tail position and then separated. Towards the end of mating, females did not flex or stroke their abdomen such as was observed for two matings of *P. fulviventris* (Dennis, 2015).

Oviposition behavior

The females of all described species of *Proctacanthus* (Bromley, 1946; Hine, 1911) have spines (acanthophorites) on their ovipositors and oviposit in the soil. Female *P. longus* ovipositions occurred in the soil, often in sugar sand (fine silt made up of ultrafine mineral sand mixed with a large percentage of organic granules), in the shade of vegetation (Fig. 5). *Proctacanthus brevipennis* (Dennis, 2012), *P. fulviventris* (Dennis, 2015), and *P. micans* (Dennis & Lavigne, 1975), also oviposit in the soil in the shade of vegetation.

Fig. 5. Female *P. longus* withdrawing her abdomen from oviposition hole in sugarsand (Photograph: D.S. Dennis, 22.07.2013, 10:01 AM).

Seventeen ovipositions were observed. Air temperatures 30 cm above the oviposition sites in the shade ranged from 29.0-37.0°C with an average of 33.6°C. In comparison, air temperatures in the sun ranged from 32.0-33.0°C with an average of 32.7°C. Soil surface temperatures at the oviposition sites ranged from 29.0-36.0°C with an average of 33.8°C; temperatures beneath the surface of the soil where ovipositions occurred also ranged from 29.0-36.0°C with an average of 33.7°C.

P. longus females either landed on the soil and immediately inserted their ovipositors into the soil or walked along the soil and probed with their ovipositors in order to find a suitable place to deposit their eggs. They inserted their ovipositors into the soil with a lateral action for up to 38 seconds with an average of 20 seconds. One female exhibited a tamping action while inserting her ovipositor. The actual deposition of eggs took 69 to 135 seconds with an average of 107 seconds. Following deposition of eggs, females withdrew their ovipositors from the soil with a sweeping action that continued on the soil surface around the oviposition hole for 25 to 183 seconds, with an average of 114 seconds. Average time for complete ovipositions was 240 seconds with a range from 211 to 276 seconds.

Like *P. fulviventris* females (Dennis, 2015), the depth that a *P. longus* female inserts her abdomen in the soil depends on the dryness of the soil and/or the amount of organic matter/roots in the soil. In dry soil a female would typically insert her abdomen in to the soil to about 1/2 its length.. In this position the female's abdomen was gently curved outward and her wings were folded over her abdomen, often with the wing tips buried in or touching the soil.

In damp soil, following a rain when the soil was presumably more compacted, or soil-containing lots of organic matter/roots, a female would barely insert her abdomen into the soil. The female then kept her wings folded over her abdomen and the tips were not buried in the soil.

The length of time for depositing eggs, withdrawing ovipositors and sweeping the soil around the oviposition hole was about the same in dry and damp soil. Dennis (2015) also observed this for *P. fulviventris*.

One female *P. longus* oviposited four times over a 26-minute period before being lost to sight. Female *P. fulviventris* oviposited up to five times over a 15 to 20 minute period (Dennis, 2015). Rogers & Lavigne (1972) observed a female *P. micans* oviposit six times over 31 minutes.

Two to six eggs in a "packet" were recovered from each of four *P. longus* ovipositions. For these ovipositons more eggs (four and six eggs) were deposited in damp than dry soil (two eggs).

Eggs are shiny, glistening white to creamy-white, and are similar to those of many other species of robber flies (Dennis, Barnes, & Knutson, 2013) including, *P. brevipennis* (Dennis, 2012), *P. micans* (Dennis & Lavigne, 1975), and *P. fulviventris* (Dennis, 2015). The eggs of *P. longus* range in length from 2.0-2.6 mm, with an average of 2.3 mm; range in width is from 0.7-1.2 mm, with an average of 0.9 mm. These are the same dimensions as the eggs of *P. fulviventris* (Dennis, 2015).

Grooming

P. longus groomed themselves in much the same way as reported for other species of *Proctacanthus* and robber flies in general (Dennis, 2012, 2013, 2015; Dennis & Lavigne, 1975). They used the fore legs to groom their faces, and the hind legs for grooming their wings, abdomen and genitalia. Sometimes *P. longus* would groom their forelegs prior to rubbing the dorsolateral part of the face and eyes with the inside of and distal part of either one or both front femora and proximal 1/2 of the tibiae.

P. longus often rub their hind tarsi together prior to grooming the abdomen, genitalia, and wings. They then curve their abdomen down up to a 90-degree angle, and groom the abdomen, genitalia, and tops and bottoms of the wings with their hind tibiae and proximal part of the tarsi. Generally they groom the posterior 1/2 of the abdomen and often their wings when they are slightly spread. Grooming of the wings and abdomen was always from anterior to posterior as observed for *P. brevipennis* and *P. fulviventris* by Dennis (2012, 2015).

Grooming was common while resting and between foraging flights. Grooming of the face was also particularly common after feeding, as was grooming of the abdomen and genitalia after mating and ovipositing.

P. longus never groomed its thorax as was observed for *P. fulviventris* (Dennis, 2015).

Daily rhythm of activity

P. longus exhibited a diurnal or daily rhythm of activity between 9:00 AM and 3:00 PM for mating, ovipositing, and feeding (Fig. 6). Most of these behaviors occurred between 9:00 AM to 2:00 PM with 93.3%, 94.2%, and 97.3% for mating, ovipositing, and feeding, respectively.

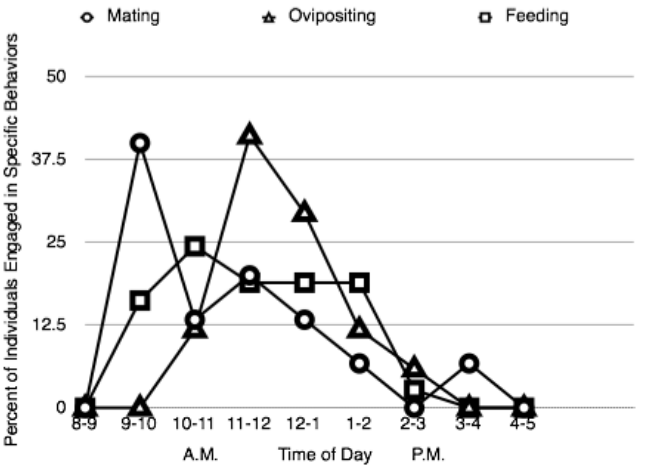

Fig. 6. Daily rhythm of activity of *P. longus* based on 15, 17, and 37 observations for mating, ovipositing, and feeding, respectively.

The number of mating pairs peaked early in the day between 9:00 to 10:00 AM, with a smaller peak from 11:00 AM to 12:00 noon. Also, ovipositing peaked during the latter time period. Both mating and ovipositing declined from 10:00 to 11:00 AM when feeding peaked. Then, from 12:00 noon to 2:00 PM more *P. longus* engaged in feeding than mating and ovipositing. After 3:00 PM all three behaviors generally steadily declined.

Dennis & Lavigne (1975) observed that most of the species of robber flies they studied engaged in feeding before their peak periods of mating and ovipositing. This also was the case for *P. brevipennis* (Dennis, 2012), but not for *P. fulviventris* (Dennis, 2015) or *P. longus*.

Robber flies are most active when the sun is shining. However, when the sky was overcast and the author could still see a very light shadow, both *P. longus* and *P. fulviventris* (Dennis, 2015) continued to forage, mate, and oviposit. This may be because as long as air and soil temperatures are high enough, these species continue with their normal behaviors.

Predators and parasites

Both male and female *P. longus* preyed on males. One male grabbed another male in flight and the pair fell to the soil where they separated facing each other. They then quickly grabbed each other and one of the males inserted his proboscis in the left side of the other male's thorax.

Two female *P. bastardii* preyed on male *P. longus*. Also, a female *Diogmites crudelis* Bromley, 1936 preyed upon a female *P. longus.*

After a mating pair of *P. longus* straightened out in the tail-to-tail position on the trunk of a live oak tree, a regal jumping spider (Salticidae: *Phidippus regius* C.L. Koch, 1846) captured the female. When the author captured the spider, the male *P. longus*

took flight with the dead female hanging behind him. The male did not release the female until the author captured the pair and took the dead female.

Mites are often found on robber flies (Lavigne, Dennis, & Gowen, 2000), in particular on their thorax, but no mites were found on *P. longus.*

There are a number of ants (Formicidae, *Formica* spp. and *Solenopsis invicta* Buren, 1972) in the same habitats as *P. longus*. When the ants crawl on the asilids' tarsi, the asilids would shake their tarsi and then usually either walk or fly to a new location. One male stood up on its tarsi so that the ants could walk underneath and another male buzzed his wings when disturbed by ants.

Lizards may attack robber flies (Lavigne et al, 2000). In the MCCA the six-lined racerunner [*Cnemidophorus sexlineatus* (Linnaeus, 1766)] is very common and one caused a female *P. longus* to move to a new location. Racerunners are known to be insectivorous but they did not attack *P. longus*.

CONCLUSIONS

There exists detailed information on the ethology of only four of 19 species of robber flies in the genus *Proctacanthus* (*P. brevipennis*, *P. fulviventris*, *P. micans*, and *P. nearno*) in the United States. This paper provides information on a fifth species, *P. longus*. This species rested on the soil, on dead vegetation on the soil, and on the stems and leaves of live vegetation. *P. longus* maintains its body temperature by positioning itself on the soil or in the shady side of vegetation, depending on the air and soil temperature, and location of the sun. Foraging is from the soil, detritus on the soil, and from vegetation in an attitude or posture that presumably allows the asilids to better see prey. All prey are captured in flight and consist of Coleoptera (10.8%), Diptera (43.3%, including cannibalism), Hemiptera (2.7%), Hymenoptera (13.5%), Lepidoptera (2.7%), Neuroptera (2.7%), and (Orthoptera 24.3%). During feeding, *P. longus* sometimes did not manipulate prey, manipulated prey while hovering above its feeding site or held prey against vegetation and crawled on them before reinserting their proboscises. There was no courtship prior to mating, which occurrs in the male-over-female position and then the tail-to-tail position. Female's oviposite in the soil, and 2 to 6 eggs were recovered from each of four ovipositions. Peak period for mating is from 9:00 to 10:00 AM, feeding is from 10:00 to 11:00 AM, and ovipositing was from 11:00 AM to 12:00 noon. Grooming was in much the same manner as other asilids. Two other species of robber flies (*Promachus bastardii* and *Diogmites crudelis*), and a regal jumping spider (Salticidae: *Phidippus regius*) preyed on *P. longus*.

ACKNOWLEDGEMENTS

The author thanks the staff of the St. Johns River Water Management District for the issuance of the Special Use Authorization that allows the study of robber flies in the Moses Creek Conservation Area (MCCA).

With gratitude, the author acknowledges the following with the Systematic Entomology Laboratory (SEL), Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland, U.S.A. for their identification of prey of other species of robber flies that the author compared for identification of *P. longus* prey: Alexey Tishechkin (Elateridae), Matt Buffington (Formicidae), and Norman E. Woodley (Mydidae). The author also would like to thank the SEL's Michele Touchet for handling of the prey and the prompt Identification Reports.

I also thank the anonymous reviewers for their constructive comments on the manuscript.

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Received: October 10, 2017 *Accepted: January 22, 2019*

J. Entomol. Res. Soc., 21(1): 57-63, 2019 *Research Article*
Print ISSN:1302-0250 *Online ISSN:2651-3579*

Comparison of Different Sampling Procedures for Population Monitoring of Important Citrus Aphids on Two Orange Species

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ABSTRACT

Sampling is a key aspect of integrated pest management program. In this study, different sampling procedures for population monitoring of three important citrus aphids, *Aphis spiraecola*, *A. gossypii* and *Toxoptera aurantii* were compared on two citrus trees, Satsuma mandarin and Thomson navel orange, in order to determine the most appropriate one. The samplings were performed from different heights, main directions and inner or outer foliage layer of the trees. Also, efficacy of two traps, yellow sticky and yellow basin traps, for monitoring of the aphids were evaluated. Results showed that the best sampling procedures were different according to aphid and host plant species. Except *T. aurantii* on Satsuma mandarin, both traps were not efficient for estimating population of the aphids under field condition. The findings can be used in an IPM program of the aphids in citrus orchards.

Key words: Aphididae, host plants, traps, IPM.

Alizadeh Kafeshani, F., Rajabpour, A., Aghajanzadeh S., Gholamian E., & Farkhari, M. (2019). Comparison of Different Sampling Procedures for Population Monitoring of Important Citrus Aphids on Two Orange Species. *Journal of the Entomological Research Society,* 21(1), 57-63.

INTRODUCTION

Citrus (Family: Rutaceae) is one of the world's major fruit crops with global availability and popularity in human diets (Liu, Heying, Tanumihardjo, 2012). Citrus pests are important problem confronting the citrus grower in Iran (Farahbakhsh, 1996). Aphids (Hom., Aphididae) are important citrus pests in Iran (Rajabi, 1986). Large amount of broad spectrum insecticides (BSIs) are applied to control the pests in north of Iran (Farahbakhsh, 1996). BSIs cause many problems including reduced profits from high insecticide costs, destruction of non-target organism, development of resistance in populations, pest replacement, pest resurgence, environment pollution and etc. (Pedigo, 2002). Establishment of suitable integrated pest management (IPM) program is critical for sustainable pest control and reduction of BSIs in citrus orchards (Farahbakhsh, 1996). Sampling for decision making is a key aspect of IPM. Due to cost and time consuming of sampling, growers must know how to gather enough information about pest abundance to able make precise decisions without incurring excessive costs. Selection of the best sampling procedure has a crucial role in sampling program (Binns & Nyrop, 1992; Pedigo, 2002). Estimation of population densities in highly aggregated insect species in complex and variable habitats can be difficult to estimate efficiently, accurately, and with minimal variance (Whitaker, Mahr, & Clayton, 2006). Appropriate sampling procedure is especially important in situations where heterogeneity in pest density is suspected (Binns & Nyrop, 1992). Also, traps as sampling tools were extensively used to estimate population density (Pedigo, 2002). Among traps, yellow sticky trap (Heathcote, 1957) and yellow water trap (Heathcote, 1957; O'Loughlin, 1963) were previously applied to population monitoring of some aphid species. There have not been made any effort to determine the best sampling procedure for population monitoring of citrus aphids. Therefore, the objectives of the present experiment were selecting the best sampling procedures of various citrus aphids and efficacies of two traps, yellow sticky and water traps, for population monitoring of the aphids.

MATERIAL AND METHODS

The experiments were performed in an experimental citrus orchard of citrus and subtropical fruits research center, 20 hectares, in Ramsar, Mazandaran province, north of Iran, 36°54'24.2"N 50°39'26.7"E from January 2016 to August 2017. No pesticides were applied during the study period.

Sampling

Samplings for estimating aphid density were weekly performed. At each sampling date, ten Thompson navel orange, *Citrus sinsensis* L. (20 years old), and ten Satsuma mandarin, *Citrus unshiu* Markovich, trees (20 years old) were randomly chosen. Different samples were collected at three heights (1, 1.5 and 2 meters), four directions (north, south, east and west) and on two foliage layers (inner and outer layers). From each sampling procedure, one shoot and totally 24 shoots from each tree, were randomly taken. The samples were separately transferred to the

entomological laboratory and number of each species was separately recorded under stereomicroscope. For monitoring of alate aphid population, two types of traps were used. 1- Yellow sticky trap (30×10 cm) (Russel IPM, UK) and 2- yellow basin trap (25 cm in diameter) which was half filled with water + detergent. Eight of each traps was used per hectare and number of trapped aphids was weekly recorded.

Data analysis

Regression analysis between aphid numbers of each sampling procedure and total aphid number on each tree were used to select the best sampling procedure. The regression between mean aphids of each trap and mean aphids per tree were performed to investigate efficacy of the traps for population monitoring,. All analyses were done by SPSS (version 16.0) software.

RESULTS

Results of regression analysis for selecting the best foliage layer (inner or outer) of Satsuma mandarin and Thomson navel orange for population monitoring of Aphis spiraecola Patch, *A. gossypi*i Glover and Toxoptera aurantii Boyer de Fonscolombe are showed in table 1. There was a significant correlation between the population of both layers and total aphid population on Satsuma mandarin. Therefore, both foliage layers can be used for monitoring the aphids of Satsuma mandarin. But, there were not significant regression between density of *A. gossypii* collected from inner layer and total density of Thompson navel orange. Hence, inner foliage layer of the orange is not suitable sampling procedure. Also, results of selection of the best height for sampling *A. spiraecola, A. gossypii* and *T. aurantii* on Satsuma mandarin and Thomson navel orange are presented in table 2. The results of the present experiment imply that an estimate of population density of the aphids in Satsuma mandarin can be obtained regardless of the height at which the traps are positioned. Similarly, significant correlations were found between aphid density of each heights and total population on Thompson navel orange. The only exception was *T. aurantii* on 2m height of Thomson navel foliage which did not provide a good estimate of the population density.

There were significant correlations between the aphids population from each main direction (north, south, west and east) and total population (Table 3). The data showed that each main direction could be used for monitoring of *A. spiraecolae*, *A. gossypii* and *T. aurantii*. But there was not significant correlation between *T. aurantii* in south direction of Thomson navel orange and total density of the aphid. Therefore, the sampling procedure is not appropriate for the aphid sampling.

Regression analysis for evaluating efficacy of the yellow sticky trap and the yellow basin trap are showed in table 4 and 5, respectively.

The results showed that both traps, yellow sticky and yellow basin traps, could be used to monitor *T. aurantii* in Satsuma mandarin. There were no significant correlations between the trapped individuals of other aphid species on Satsuma mandarin. Also, both traps are not suitable for monitoring of all species in Thomson navel orange.

Table 1. Regression analysis for selecting the best sampling procedure in inner and outer layer of the Satsuma mandarin and Thomson navel orange foliage.

	Aphid species	Foliage layer	N	Intercept±SE	Regression slope LINE±SE	R^2	$F_{(df)}$	$P_{regression}$
Satsuma mandarin	A. *spiraecolae*	Outer	35	0.13±0.158	1.415±0.036	0.979	$1521_{(1,33)}$	<0.0001
		Inner	35	-0.13±0.158	0.585±0.036	0.888	$260.4_{(1,33)}$	<0.0001
	A. gossypii	Outer	35	-0.022±0.025	0.0395±0.016	0.948	$619.4_{(1,33)}$	<0.0001
		Inner	35	0.022±0.025	1.605±0.016	0.997	$10235.2_{(1,33)}$	<0.0001
	T. aurantii	Outer	35	-0.007±0.015	1.903±0.042	0.984	$2082.5_{(1,33)}$	<0.0001
		Inner	35	0.007±0.015	0.097±0.042	0.142	$5.4_{(1,33)}$	<0.026
Thompson navel orange	*A. spiraecolae*	Outer	35	0.03±0.026	1.734±0.026	0.992	$4327.8_{(1,33)}$	<0.0001
		Inner	35	-0.017±0.022	0.278±0.024	0.809	$139.9_{(1,33)}$	<0.0001
	A. gossypii	Outer	35	0.0±0.001	1.996±0.005	1	185325	<0.0001
		Inner	35	0.001±0.001	0.004±0.005	0.158	0.85	0.363
	T. aurantii	Outer	35	3.2×10^{-11}±0	2±0	1	-	-
		Inner	35	-	-	-	-	-

Table 2. Regression analysis for selection of the best sampling procedure in different heights of Satsuma mandarin and Thomson navel orange foliage.

	Aphid species	Foliage height	N	Intercept±SE	Regression slope LINE±SE	R^2	$F_{(df)}$	$P_{regression}$
Satsuma mandarin	A. *spiraecolae*	1 m	34	0.043±0.053	0.4±0.012	0.971	$1076.3_{(1,32)}$	>0.0001
		1.5 m	34	-0.0108±0.14	1.08±0.032	0.973	$1132.2_{(1,32)}$	>0.0001
		2 m	34	0.065±0.147	1.511±0.034	0.984	$1991.3_{(1,32)}$	>0.0001
	A. gossypii	1 m	34	0.018±0.012	0.295±0.008	0.978	$1437.1_{(1,32)}$	>0.0001
		1.5 m	34	-0.029±0.021	1.052±0.013	0.995	$6611_{(1,32)}$	>0.0001
		2 m	34	0.011±0.02	1.653±0.012	0.998	$17702_{(1,32)}$	>0.0001
	T. aurantii	1 m	35	0.001±0.047	1.478±0.129	0.8	$131.6_{(1,33)}$	>0.0001
		1.5 m	35	-0.001±0.013	0.514±0.037	0.855	$194.8_{(1,33)}$	>0.0001
		2 m	35	0.0±0.034	1.008±0.092	0.784	$120_{(1,33)}$	>0.0001
Thompson navel orange	*A. spiraecolae*	1 m	35	-0.086±0.07	0.995±0.074	0.847	$183.1_{(1,33)}$	>0.0001
		1.5 m	35	0.067±0.062	0.29±0.065	0.375	$19.9_{(1,33)}$	>0.0001
		2 m	35	0.018±0.066	1.715±0.069	0.949	$619.6_{(1,33)}$	>0.0001
	A. gossypii	1 m	35	0.005±0.006	0.443±0.034	0.837	$169.6_{(1,33)}$	>0.0001
		1.5 m	35	-0.003±0.016	1.131±0.098	0.802	$133.57_{(1,33)}$	>0.0001
		2 m	35	-0.002±0.016	1.42±0.96	0.869	$218.4_{(1,33)}$	>0.0001
	T. aurantii	1 m	35	-	-	-	-	>0.0001
		1.5 m		-8.9×10^{-5}±0	3±0.021	0.998	$2079.1_{(1,33)}$	>0.0001
		2 m	35	8.9×10^{-5}±0	0±0.021	0	$0_{(1,33)}$	0.989

Table 3. Regression analysis for selection of the best sampling procedure in different direction (North, south, west and east) of Satsuma mandarin and Thomson navel orange foliage.

	Aphid species	Direction	N	Intercept±SE	Regression slope LINE±SE	R^2	$F_{(df)}$	$P_{regression}$
Satsuma mandarin	A. *spiraecolae*	North	35	-0.027±0.069	0.568±0.016	0.976	$1312.2_{(1,33)}$	>0.0001
		South	35	0.118±0.152	0.789±0.034	0.943	$527.8_{(1,33)}$	>0.0001
		East	35	0.165±0.265	1.311±0.06	0.937	$478.6_{(1,33)}$	>0.0001
		West	35	0.074±0.155	1.331±0.35	0.978	$1452_{(1,33)}$	>0.0001

Table 3. Continued.

	Aphid species	Direction	N	Intercept±SE	Regression slope LINE±SE	R^2	$F_{(df)}$	$P_{regression}$
Satsuma mandarin	*A. gossypii*	North	35	-0.055±0.5	0.909±0.039	0.944	$557.1_{(1,33)}$	>0.0001
		South	35	0±0	1±0	1	-	-
		East	35	0.029±0.075	1.128±0.058	0.919	$376.8_{(1,33)}$	>0.0001
		West	35	-0.082±0.071	1.746±0.055	0.968	$1009.1_{(1,33)}$	>0.0001
	T. aurantii	North	35	0.001±0.107	1.471±0.301	0.419	$23.8_{(1,33)}$	>0.0001
		South	35	0.001±0.066	1.25±0.186	0.579	$45.33_{(1,33)}$	>0.0001
		East	35	0.001±0.009	0.388±0.025	0.88	$241_{(1,33)}$	>0.0001
		West	35	-0.003±0.035	0.388±0.025	0.712	$81.6_{(1,33)}$	>0.0001
Thompson navel orange	B. *spiraecolae*	North	35	-0.001±0.49	0.725±0.051	0.858	$199.2_{(1,33)}$	>0.0001
		South	35	0.062±0.075	1.072±0.079	0.848	$184.57_{(1,33)}$	>0.0001
		East	35	-0.012±0.077	1.216±0.081	0.83	$227.1_{(1,33)}$	>0.0001
		West	35	0.076±0.86	0.987±0.091	0.781	$117.7_{(1,33)}$	>0.0001
	A. gossypii	North	35	0.0±0.017	1.267±0.108	0.806	$137.1_{(1,33)}$	>0.0001
		South	35	0.001±0.013	0.83±0.8	0.766	$108.3_{(1,33)}$	>0.0001
		East	35	-0.001±0.007	0.99±0.046	0.934	$467.8_{(1,33)}$	>0.0001
		West	35	0.001±0.014	0.913±0.085	0.778	$115.5_{(1,33)}$	>0.0001
	T. aurantii	North	35	7.27×10^{-5}±0.002	1.77±0.357	0.428	$24.65_{(1,33)}$	>0.0001
		South	35	0.0±0.0	0.0±0.28	0	$0_{(1,33)}$	0.989
		East	35	-	-	-	-	-
		West	35	0.0±0.002	2.22±0.357	0.542	$38.9_{(1,33)}$	>0.0001

Table 4. Regression between mean densities of citrus aphids on Satsuma mandarin and Thomson navel orange trees and trapped aphids by yellow sticky traps.

	Aphid species	N	Intercept±SE	Regression slopeLINE±SE	R^2	$F_{(df=1,5)}$	$P_{regression}$
Satsuma mandarin	*A. spiraecolae*	7	2.502±2.26	0.494±0.26	0.419	3.599	0.116
	A. gossypii	7	2.52±1.32	0.486±0.376	0.251	1.675	0.252
	T. aurantii	7	0.332±0.464	2.039±0.601	0.697	11.513	0.019
Thompson navel orange	*A. spiraecolae*	7	10.96±9.79	10.01±36.3	0.015	0.076	0.794
	A. gossypii	7	10.5±6.42	1.36±1.82	0.101	0.56	0.488
	T. aurantii	7	-	-	-	-	-

Table 5. Regression between mean densities of citrus aphids on Satsuma mandarin and Thomson navel orange trees and trapped aphids by yellow basin traps.

	Aphid species	N	Intercept±SE	Regression slope LINE±SE	R^2	$F_{(df=1,5)}$	$P_{regression}$
Satsuma mandarin	*A. spiraecolae*	7	11.15±7.03	1.266±0.839	0.313	2.278	0.192
	A. gossypii	7	10.5±6.42	1.362±1.828	0.101	0.56	0.488
	T. aurantii	7	0.055±0.055	1.277±0.071	0.985	320.8	<0.0001
Thompson navel orange	A. *spiraecolae*	7	10/96±9.79	10.01±36.3	0.015	0.076	0.794
	A. gossypii	7	4.46±5.4	21.8±15.45	0.286	2.002	0.216
	T. aurantii	7	-	-	-	-	-

DISCUSSION

Our finding indicated the best sampling procedure (from different heights, main directions and inner or outer layer foliage) for population estimating of three important citrus aphids on two citrus trees, Satsuma mandarin and Thomson navel orange. The best sampling procedures were different according to aphid or host plant species. The differences may be due to different behavior of various aphid species on same or different host plants. Janzen (1973) demonstrated that many factors including host plant and pest species, seasons, time of day and etc influences sampling program. Our finding agrees with Hajek & Dahlsten (1986) who showed that three coexisted aphids select different ecological niche for feeding on *Betula pendula* Roth. Similarly, it is demonstrated *Aphis pomi* De Geer selects different leaf position along the apple shoots (Whitaker et al, 2006). Trumble (1982) showed that the best sampling procedure of aphids are different in broccoli. Yarahmadi, Soleyman Nejadian, Mohisseni (2008) reported that the wheat aphids (*Sitobion avenae* Rodani, *Schizaphis graminum* Fabricus and *Diuraphis noxia* Mordviko) choose different parts of wheat during their feeding activity and the behaviors affected their suitable sample universes.

The yellow sticky and yellow basin traps are only suitable for population monitoring of *T. aurantii* on Satsuma mandarin. For other aphid and host plants, the traps are not appropriate for estimating population. In contrast, Marroquin et al (2004) used yellow sticky and yellow basin traps for monitoring citrus aphids in various citrus orchards in Spain. Our result is in line with Han, Han, Zhang, & Byers (2012) who showed that *T. aurantii* attracted to yellow sticky traps. Straw et al. (2011) demonstrated that the trap color significantly affected the capture of aphid alate. Therefore, other color of sticky or basin trap may be efficient for monitoring of the aphids population on Satsuma mandarin or Thomson navel orange.

In conclusion, the best sampling procedures of *A. spiraecola*, *A. gossypii* and *T. aurantii* were significantly influenced by aphid or host plant species. Also, yellow sticky and yellow basin traps are nearby not suitable for population monitoring of the aphids on Satsuma mandarin and Thomson navel orange.

ACKNOWLEDGEMENTS

The research was financially supported by Agricultural Sciences and Natural Resources University of Khuzestan and Citrus and Subtropical Fruits Research Center of Iran.

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Received: November 08, 2017 *Accepted: February 27, 2019*

J. Entomol. Res. Soc., 21(1): 65-93, 2019
Print ISSN:1302-0250

Research Article
Online ISSN:2651-3579

An Overview of the Calliphoridae (Diptera) of Saudi Arabia with New Records and Updated List of Species

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ABSTRACT

Despite the species richness of the blow fly (Calliphoridae: Diptera) fauna (1600 species), the relevant environmental, medical, agricultural, and forensic knowledge of these species found in Saudi Arabia is limited. As part of a study on the biodiversity of Diptera of south-western Saudi Arabia a survey of the Diptera fauna of Jazan, Asir and Najran was performed between 2010-2016 at 17 sites, mainly using Malaise traps, sweep nets and baited traps. Eighteen species of Calliphoridae were identified and recorded in this study, seven of which were recorded for the first time. This makes the total number of Calliphoridae species in Saudi Arabia (including 26 species previously recorded and excluding two species which were synonymized namely: *Rhyncomya zumptii* Peris 1952; *Chrysoma regalis* Robineau-Desvoidy 1830) to be 44. A list of all species of Calliphoridae recorded from Saudi Arabia is provided. Images of five species are presented. Biological information on each species (where known) and geographical distribution are included. In addition to the results of the identifications all available literature about Calliphoridae of Saudi Arabia is summarized and analysed. The species recorded in this study are more Afrotropical in origin than they are to other regions. The need for further field and laboratory work and surveillance is highlighted.

Key words: Calliphoridae, Saudi Arabia, afrotropical, new records.

Ali Dawah, H., Abdullah, M. A., & Kamran Ahmad, S. (2019). An overview of the Calliphoridae (Diptera) of Saudi Arabia with new records and updated list of species. *Journal of the Entomological Research Society,* 21(1), 65-93.

INTRODUCTION

Calliphoridae is the most common family of higher flies of diverse biology with considerable impact on environment, agriculture and health of human beings and cattles (Deeming, 1995; Dawah & Abdullah, 2009). It is a family of large sized, dull or coppery coloured, shiny and metallic bodied flies, often gleaming green, blue, black, purple or coppery colored thoraces and abdomens (Kettle, 1992). There are about 1600 described species in 150 genera distributed worldwide (Grella et al, 2015; Thompson, 2013). They occur almost all over the world and may be found in tropical rain forests, deserts, oceanic islands, temperate lands and Arctic wastes. Calliphoridae is absent from Antarctica only (Erzinçlioğlu, 1996). There is a dispute over the tribes and the subfamilies of this family. However, at least 12 subfamilies are recognizable, i.e., Calliphorinae, Luciliinae, Chrysomyinae, Toxotarsinae, Melanomyinae, Polleniinae, Helicoboscinae, Ameniinae, Plumosiinae, Aphyssurinae, Bengaliinae and Prosthetosomatinae (Marshall, 2012). The calliphorinae commonly occur around houses in search of breeding material and food and are attracted to meat, carrion, feacal matter and dead animals for egg laying and to cheese for food (Jadav & Sathe, 2014). Adult calliphorids are reported to cluster in dark parts of buildings where they overwinter (e.g. *Pollenia* Robineau-Desvoidy) (Szpila & Draber-Mońko, 2008; Rognes, 1987a; b).

The biology of Calliphoridae has been extensively studied due to its forensic importance but this has been limited to a few genera only. The females are dependent on a protein rich substrate for larvae development, as provided by animal-associated tissues or by-products (Stevens, 2003). The females of the majority of species lay large numbers of whitish rice-like eggs on any decomposing organic matter or flesh, these hatch into larvae rasping the food substrate along with bacteria. However, macrolarviparity occurs in a variety of diverse species of Calliphoridae (Ferrar, 1987). Fully mature larvae pupate in soil (Draber-Mońko, 2004; Marshall, 2012). The larvae of some calliphorid species (e.g. *Chrysomya* Robineau-Desvoidy and *Lucilia* Robineau-Desvoidy) have considerable importance to public health and may be mechanical vectors of pathogens of humans, causing facultative myiasis in animals and humans, as well as being used in studies of legal medicine as forensic science (Zumpt, 1965; Greenberg, 1971, 1991; Furlanetto, Campos, & Harsi, 1984; Guimarães & Papavero, 1999; Thyssen, Moretti, Ueta, & Ribeiro, 2004; Maldonado & Centen, 2013). Calliphoridae are known as the initial colonizers in the faunal succession on human cadavers (Smith, 1986; Vélez & Wolff, 2008; Liu et al, 2011). Therefore, their larvae are very useful in forensic investigations in solving the problems related to postmortem interval/estimations of time of death, murder, suicide, sexual molestation, child neglect and abuse etc., (Greenberg, 1991; Erzinçlioğlu, 1996; Jadav & Sathe, 2015; Roe & Higley, 2015). Many calliphorid species are parasites of earthworms, snails, oothecae of orthopteran insects and Noctuid moths (Deeming, 1996; Rognes, 2010; Coupland & Barker, 2004; Bowser, 2015). Moreover, the species *Chrysomya megacephala* Fabricius is responsible for economic losses in fishery businesses in Southeast Asian countries, where the fish being exposed to the sun to dry (Esser, 1990; 1991). In case of sever infestation, the Calliphoridae can cause an 80%

reduction in mammal population (Parchami-Araghi, 2013). Furthermore, some calliphorid larvae (e.g. *Protocalliphora* Hough) live in birds' nests and feed on the blood of nestlings (Bennet & Whitworth, 1991). Many calliphorid species contaminate food with pathogens (e.g. bacteria, viruses, protozoans and helminthes) which they carry, causing various enteric and other diseases in man. Therefore, such species are of the greatest hygienic importance and pose a permanent threat to human health (Rognes, 1998).

Despite its drawbacks, Calliphoridae has also been utilized for human betterment. The maggots due to their ability to consume flesh appear to possess exceptional healing properties against chronic osteomyelitis, caused by *Streptococci* and *Staphylococci* bacteria (Baer, 1931). The use of maggots for therapeutic purposes has been replaced by antibiotics but, when in vogue, the practice was extensive and, in North America alone, over 5000 cases of osteomyelitis and related disorders were treated with maggots in the early 1930s (Leclercq, 1969). Adult calliphorids were said to have been used therapeutically as a cure for eye ailments and baldness although no record appears to exist as to the efficacy of the treatments (Heath, 1982).

Some calliphorids are regular visitors of certain plants for sugars resulting in cross pollination, but it is not known how their abilities compare with those of bees. Some flowering plants (e.g. *Hedera helix* L.) may be completely dependent on flies for pollination (Faegri & van der Pijl, 1971). There are records of the use of calliphoridae in cages and sleeves as crop pollinators on onions and then in self and cross- fertilization experiments with carrots (Jones & Emsweller, 1934; Clement, Hellier, Elberson, Staska, & Evans, 2007; Vasconcelos & Salgado, 2014). On the other hand, some African and Chines natives used to bury meat to breed maggots (Bodenheimer, 1951). They were said to be very fond of this, to Western stomachs, repulsive delicacy. Calliphoridae maggots have been used as fishing bait in past (Heath, 1982) and still are by freshwater fishermen.

In the Middle East, a single case of myiasis in a 14 year old female was reported (Ansari & Oertley, 1982) followed by 12 cases of myiasis in sheep, both caused by *Chrysomyia bezziana* Villeneuve (AlAhmed, 2002). Calliphoridae are also known from Bahrain, Kuwait, Qatar, Oman, United Arab Emirates, Iraq and Iran (AlAhmed, 2004). Abdul-Rassoul (1976) recorded six species of Calliphoridae from Iraq. Al-Houty (1989) reported five species of Calliphoridae from Kuwait. Deeming (1996; 2008) reported 21 species of Calliphoridae from Oman and 16 species from United Arab Emirates, respectively. Al-Ahmadi & Salem (1999) and Abu-Zoherah, Al-Taher, & Tilkian (1993) listed 22 species of Calliphoridae in combination from Saudi Arabia. Deeming (2008) listed 17 species of Calliphoridae from United Arab Emirates, Oman, Saudi Arabia and Yemen. Dawah & Abdullah (2009) recorded 20 species of Calliphoridae of which 13 were new to Saudi Arabia. Setyaningrum & Aldhafer (2014) recorded 34 species of Calliphoridae from Saudi Arabia. Rognes (2002) listed 43 species of Calliphoridae in Palestine and adjacent areas. The objective of this paper is to present our investigation of Calliphoridae in south-western Saudi Arabia, report new records and provide a checklist of Calliphoridae species recorded from Saudi Arabia, with some biological information, taxonomic remarks and world-wide distribution of species.

MATERIALS AND METHODS

Collection methods and sites

With the aim of exploring the biodiversity of Diptera in Southwest Saudi Arabia, insect specimens for the present study were collected with Malaise traps, set up by the authors at 17 sites in Jazan, Asir and Najran (Saudi Arabia) (Table 1), during 2010-2016. Malaise trap is a commonly used sampling technique for low flying insects (e.g. Diptera). It does not need to be observered throughout the day, hence it is economical and time saving (Malaise, 1937). Some specimens were also collected sporadically from wild environments, at dams, wadis and road margins using sweeping nets and pooters. The Malaise traps were situated in a variety of habitats, and at various altitudes up to 2685 meters above sea level. The types of habitat were a rather barren coastal plain which was subject to seasonal rains, wadis, mountains with rain at any month of the year resulting in pasture and furthermore supporting cattle, thus have added fertility (Fig. 1). Wherever possible these traps were located in farms where they could be protected. The farms were visited at three week intervals to collect the insects and add more alcohol to the collecting containers fitted in Malaise traps. In addition specimens collected using Malaise traps and sweeping from 2002-2004 are referred to with full information in the text and listed in the table of the sites collection. Calliphorid flies had to be sorted from mixed diptera samples, dried (by passing the specimens through alcohols to absolute to remove water, then into ethyl acetate for few days, then air dry to protect the tomentum on the surface). They were pinned and labelled before being studied. Where species are not recorded for the first time, reference is made to the first published records and others.

Identification and deposition of the of flies

Specimens of Calliphoridae were identified to species level using Zumpt (1965); Crosskey & Lane (1993); Deeming (1996); Rognes (2002) and various papers covering the Palaearctic, Oriental and Afrotropical fauna. Where necessary genitalia dissection were made to establish correct identity. The technique employed for preparation of male and female genitalia which is a prerequisite to identification of some species, was as described by Dawah & Abdullah (2006).Voucher specimens of some species collected have been deposited at the National Museum of Wales, Cardiff (NMWC) and in the Centre for Environmental Research and Studies (CERS), Jazan University (Saudi Arabia).

Distribution and nomenclature

The distribution sections and nomenclature of the species are based on the *Catalogue of the Diptera of the Afrotropical Region* (Pont, 1980) and the *Catalogue of Palaearctic Diptera* (Schumann, 1986) or other references which are listed in the text. The Calliphoridae classification to subfamilies is according to Rognes (2002). Information on biology, distribution and taxonomic remarks on other species of Calliphoridae (Table 2) of Saudi Arabia can be found in Dawah & Abdullah (2009) and Setyaningrum & Aldhafer (2014).

Photography and illustrations

Photographs of five species were taken in the National Museum of Wales, Cardiff, UK (NMWC) using images from a video camera and "Synaptics Automontage" software to produce a montage image of the species.

Table 1. List of sampling localities with coordinates, altitude and methods (Serial number S.N.).

S.N.	Locality	Coordinates	Altitude	Method
1	Asir, Abha, Maraba, Al-Hudaithy fruit farm, mainly mango, banana and wild plants, 60 km south of Abha.	17º51'N42º23'E	0226m	Malaise trap, Sweeping, Sand fly trap
2	Asir, Keratha, Al-Ethrebany fruit farm, mainly mango, banana and wild plants, 38 km south of Abha	18º04'N 42º31'E	0994m	Malaise trap, Sweeping
3	Asir, Abha, Hay Al-Nusub (Abha Farm Centre), various vegetables and wild plants	18º13'N 42º30'E	2199m	Malaise trap, Sweeping
4	Asir, Abha, Hay Al-Menhel, various vegetables and wild plants	18 º13'N42º30'E	2150m	Malaise trap, Sweeping
5	Asir, Al-Souda, BaniMazen, various grasses	18º11'N 42º30'E	2199m	Malaise trap, Sweeping
7	Asir, Abha, Madinate Al-Ameer Sultan, Hay Al-Sad, fruit plants, vegetables, wild plants and grasses	18º17'N 42º37'E	2042m	Malaise trap, Sweeping
8	Khaybar Al-Janob, Hay Al-Salam,	18°47'N42°52'E	1150m	Sweeping
9	Jazan, Abu Aresh, Al-Mahdage Village	17º00'N42º50'E	80m	Malaise trap, Sweeping and Baiting trap
10	Jazan, Fifa, Al-Tatweer Centre	17º17'N43º08'E	800m	Malaise trap, Sweeping
11	Jazan, Harob, Wadi Lajab	17º27'N42º52'E	529m	Sweeping, Malaise trap
12	Jazan, Farasan Island, Al-Maraq	16º41'N 42º05'E	12m	Rearing, Sweeping and Malaise trap
13	Jazan, Sabya, Basahy Farm, Mango farm	17º07'N 42º37'E	29m	Malaise trap
14	Jazan, Sabya, Al Husseini farm	17º15'N 42º68'E	58m	Baiting trap
15	Jazan, Sabya, Al-Sunef Mango farm	17º07'N 42º30'E	60m	Malaise trap
16	Jazan, Beish	17º37'N 42º54'E	81m	Baiting trap
17	Najran, Al-Shurfa, SalehMaqbol Farm	17º31'N 44º15'E	1261m	Malaise Trap and Sweeping

RESULTS

Eigteen species of Calliphoridae were identified and recorded in this study, seven of which were recorded for the first time from south-western Saudi Arabia. This makes the total number of Calliphoridae species in Saudi Arabia (including 26 species previously recorded and excluding two species which were synonymised *Rhyncomya zumptii* Peris 1952; *Chrysoma regalis* Robineau-Desvoidy 1830) to be 44. A list of all species of Calliphoridae recorded from Saudi Arabia is provided. Images of five species are presented. Biological information on each species (where known) and geographical distribution are included. In addition to the results of the identifications, all available literature about Calliphoridae of Saudi Arabia has been summarized (Table 2). The species recorded in this study are more Afrotropical in origin than they are to other regions (Fig. 7).

Table 2. List of Calliphoridae species recorded from Saudi Arabia (Serial number S.N.).

S.N.	Species	References	Origin
Subfamily Rhiniinae			
1	*Cosmina aenea* (Fabricius, 1805)	Abu-Thuraya, 1982	A
2	*Cosmina arabica* Robineau-Desvoidy, 1830	Dawah & Abdullah, 2009; El-Hawagry, et al, 2017	P
3	*Cosmina fishelsohni* Rognes, 2002	This study	A,P
4	*Cosmina fuscipennis* Robineau-Desvoidy, 1830	Dawah & Abdullah, 2009	A
5	*Cosmina prasina* (Brauer and Bergenstamm, 1889)	Dawah & Abdullah, 2009; El-Hawagry, et al., 2017	A, P
6	*Cosmina viridis* (Townsend, 1917)	Abu-Thuraya, (1982); El-Hawagry, et al, 2013; 2017;This study	A, P
7	*Isomyia terminata* (Wiedemann,1830)	Dawah & Abdullah, 2009; El-Hawagry, et al, 2017	A, P
8	*Pararhynchomyia cribriformis* Becker,1910	Abu-Zoherah et al, 1993	A
9	*Rhinia apicalis* (Wiedemann, 1930)	Dawah & Abdullah, 2009; El-Hawagry, et al, 2017	A,O, P
10	*Rhinia nigricornis* (Macquart, 1843)	This study	A.
11	*Rhyncomya aravaensis* Rognes, 2002	This study	P
12	*Rhyncomya bullata* Deeming, 1996	Dawah & Abdullah, 2009	P
13	*Rhyncomya callopis* (Loew, 1856)	This study	P
14	*Rhyncomya cassotis*Walker, 1849	This study	A
15	*Rhyncomya desirtica* Peris, 1951	Abu-Zoherah et al, 1993	A, P
16	*Rhyncomya jordanensis* Peris, 1951	Dawah & Abdullah, 2009	P
17	*Rhyncomya nigripes* (Séguy,1933)	Dawah & Abdullah, 2009	P
18	*Rhyncomya seguyi* (Grunin,1957)	Abu-Zoherah et al, 1993	A
19	*Rhyncomya sinaiensis* Rognes, 2002	Setyaningrum & Aldhafer, 2014	P
20	*Rhyncomya tristis* Séguy,1933	Abu-Zoherah et al, 1993	A
21	*Rhyncomya varifrons* Becker,1910	Abu-Zoherah et al, 1993	A
22	**Rhyncomya zumptii* Peris, 1952	Setyaningrum & Aldhafer, 2014	A
23	*Stomorhina chapini* Curran, 1931	This study	A
24	*Stomorhina cribrata* (Bigot, 1874)	Dawah & Abdullah, 2009	A, P
25	*Stomorhina lunata* (Fabricius, 1805)	Dawah & Abdullah, 2009	A,O, P
26	*Stomorhina rugosa* (Bigot, 1888)	Dawah & Abdullah, 2009; El-Hawagry, et al, 2017	A
27	*Metalliopsis arabica* Deeming, 2008	Deeming, 2008	P
Subfamily Chrysomyinae			
28	*Chrysomya albiceps* (Wiedemann, 1819)	Shalaby, 1962; El-Hawagry, et al, 2013; 2016; 2017; This study	A,O, P
29	*Chrysomya bezziana* Villeneuve,1914	Ansari & Oertley, 1982; Al-Ahmad, 2002	A,P, O
30	*Chrysomya chloropyga* (Wiedemann, 1881)	Büttiker et al, 1979	A, P

*Co: Cosmopolitan; A: Afrotropical; P: Palaearctic; O: Oriental. Notes for*Chrysoma regalis; **R. zumpti*; see text under *C. marginalis* and *R. tristis*.

Table 2. Continued.

S.N.	Species	References	Origin
31	*Chrysomya marginalis* (Wiedemann, 1830)	Büttiker et al, 1979; This study	A,O, P
32	*Chrysomya megacephala* (Fabricius, 1794)	Ramadan & Al-Bihari, 1980; This study	CO
33	*Chrysomya putoria* (Wiedemann, 1830)	Abu-Zoherah et al., 1993 as: *C. chloropyga*; El-Hawagry, et al, 2016; 2017	A
34	**Chrysoma regalis* Robineau-Desvoidy, 1830	Dabbour, 1979; El-Hawagry, et al, 2013; 2016; 2017	A, P
Subfamily Calliphorinae			
35	*Bengalia minor* Malloch, 1927	Abu-Thuraya, 1982; This study	
36	*Calliphora croceipalpis* Jaennicke, 1867	Abu-Zoherah, et al, 1993; El-Hawagry, et al., 2016; 2017; This study	A
37	*Calliphora vicina* Robineau-Desvoidy, 1830	Abu-Zoherah, et al, 1993; El-Hawagry, et al, 2016; 2017; This study	CO
38	*Hemipyrellia pulchra* (Wiedemann, 1830)	This study	A
Subfamily Luciliinae			
39	*Lucilia cuprina* (Wiedemann, 1830)	Büttiker et al, 1979; El-Hawagry, et al, 2017; This study	CO
40	*Lucilia sericata* (Meigen, 1826)	Walker & Pittaway, 1987; El-Hawagry, et al, 2013; 2016; 2017; 2018; This study	CO
41	*Pericallimyia greatheadi* Zumpt, 1971	Setyaningrum & Aldhafer, 2014; This study	A
Subfamily Auchmeromyiinae			
42	*Cordylobia anthropophagi* (Blanchard &Berenger –Féraud, 1872)	Büttiker et al., 1979; This study	A, P
Subfamily Polleniinae			
43	*Pollenia dasypoda* Portschinsky, 1881	Dabbour 1979	P
44	*Pollenia hungarica* Rognes, 1987	Setyaningrum & Aldhafer, 2014; El-Hawagry, et al, 2013; 2016	P
45	*Pollenia pediculate* Macquart, 1834	Dawah & Abdullah, 2009	CO
46	*Pollenia rudis* (Fabricius, 1794)	Dawah & Abdullah, 2009; El-Hawagry, et al, 2016; 2017	CO

*Co: Cosmopolitan; A: Afrotropical; P: Palaearctic; O: Oriental. Notes for*Chrysoma regalis; **R. zumpti*; see text under *C. marginalis* and *R. tristis*.

Species Account

Subfamily Rhiniinae

Cosmina aenea (Fabricius, 1805)

Dictya aenea Fabricius, 1805: *Systema antliatorum secundum ordines*, *genera*, *species adiectis synonymis*, *locis*, *observationibus*, *descriptionibus*: 328.

Distribution: This species was first recorded from Saudi Arabia by Abu-Thuraya (1982). It was described from Guinea. A widespread in the Afrotropical Region (Pont, 1980).

Remarks: Biology unknown.

Cosmina arabica Robineau-Desvoidy, 1830

Cosmina arabica Robineau-Desvoidy, 1830: *Essai sur les Myodaires*, 2: 424.

Material examined: Saudi Arabia: 2♂♂, 2♀♀, Asir, Karatha, Al-Ethrebany Fruit Farm, 01-25.05.2013, Malaise trap, H.A. Dawah (CERS); 14♂♂, 8♀♀, Asir, Maraba, Al-Hudaithy Fruit Farm, 01-17.06.2003, Malaise trap, H.A. Dawah (NMWC; CERS).

Distribution: This species was first recorded from Saudi Arabia by Dawah & Abdullah (2009); El-Hawagry, Abdel-Dayem, El-Sonbati, & Al-Dhafer (2017). A species described from the Arabian Peninsula and is recorded from Oman, United Arab Emirates and Yemen (Pont, 1980; Schumann, 1986; Deeming, 1996; 2008).

Remarks: *C. arabica* is not known from Israel (Rognes, 2002). Other published records from Israel such as the one in Pont (1980: 780) stem from Peris (1951) and are based on misidentification which is explained in Rognes (2002: 27).

Fig. 1. The most sampled sites: (a) Jazan, Abu Aresh, Al-Mahdag village; (b) Jazan, Fifa, Al-Tatweer Centre, (photo by Habib Khemira); (c) Asir, Maraba, Al-Hudaithy Fruit Farm (d) Asir, Abha, Hay Al-Nusub; (e) Asir, Abha, Madenate Al-Ameer Sultan (f) Asir, Al-Souda, Bani Mazen (photo by Othman Abdullah).

***Cosmina fishelsohni* Rognes, 2002**

Cosmina fishelsohni Rognes, 2002: *Entomologica Scandinavica* (=*Insect Systematics & Evolution*) supplement, 59: 21-24.

Material examined: Saudi Arabia: 1♂, Jazan, Abu Aresh, Al-Mahdag Village, 01.02-03.04.2011, Malaise trap, H.A. Dawah (CERS).

Distribution: This is first record from Saudi Arabia. The species was described from Israel and Palestine and is further recorded from United Arab Emirates (Deeming, 2008).

***Cosmina viridis* (Townsend, 1917)**

Synamphoneuropsis viridis Townsend, 1917: *Record of the Indian Museum* (=*Records of the Zoological Survey of India*),13: 199.

Material examined: Saudi Arabia: 1♂, Jazan, Abu Aresh, Al-Mahdag Village, 21.02.2013, Malaise trap, H.A. Dawah (CERS); 1♂, same data but, 01.07-30.08.2010 (CERS); 2♂♂, 3♀♀, Jazan, Sabya, Al-Sunef Mango Farm, 08.05.-17.06.2003, H.A. Dawah (NMWC; CERS).

Distribution: This species was first recorded from Saudi Arabia by Abu-Thuraya (1982); El-Hawagry, Khalil, Sharsf, Fadl, & Aldawood, 2013; El-Hawagry et al, 2017). It was described from India. Known from the Palaearctic Region: Iran; Afrotropical Region: Ethiopia, Oman and Yemen; Oriental Region: India (Pont, 1980; Schumann, 1986; Deeming, 1996; 2008). In addition there are some specimens in NMWC collected from Gambia, Mali and Yemen.

Remarks: Biology; unknown.

***Isomyia terminata* (Wiedemann, 1830)**

Musca terminata Wiedemann, 1830: *Aussereuropäischen Zweiflügeligen Insekten*, 2: 414.

Material examined: Saudi Arabia: 1♂, Jazan, Abu Aresh, Al-Mahdag Village, 01.02-03.04.2011, Malaise trap, H.A. Dawah (CERS); 1♂, 1♀, Asir, Abha, Madenate Al-Ameer Sultan, 25.02-25.05.2002, Malaise trap, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia by Dawah & Abdullah (2009); El-Hawagry et al (2017). It was described from Sierra Leone. Known from West Africa to Congo basin (the sedimentary basin of the Congo River) and Uganda (Pont, 1980; Deeming, 1996). There are specimens in NMWC collected from Oman and Yemen.

Remarks: Biology; unknown.

***Pararhynchomyia cribriformis* Becker, 1910**

Pararhynchomyia cribriformis Becker, 1910: *Denkschriften der Wissenschaften Wien*, 71: 143.

Distribution: This species was first recorded from Saudi Arabia by Abu-Zoherah et al (1993). This species was described from Socotra and further recorded from Kenya and Tanzania.

***Rhinia nigricornis* (Macquart, 1843)**

Idia nigricornis Macquart, 1843: *Mémoires de la Société* (*Royale*) *de Sciences de l' Agriculture et des Arts à Lill*e 1842, 2(2): 281.

Material examined: Saudi Arabia: 1♀, Asir, Maraba, Al-Hudaithy Fruit Farm, 16.09.2014, sweeping trap, H.A. Dawah (CERS).

Distribution: This is the first record from Saudi Arabia. It was described from Senegal. It is widespread in the Afrotropical Region (including Madagascar), though first recorded in region from Arabia and United Arab Emirates by Deeming (2008).

***Rhyncomya aravaensis* Rognes, 2002**

Rhyncomya aravaensis Rognes, 2002: *Entomologica Scandinavica* Supplement, 59: 35.

Material examined: Saudi Arabia: 1♂, Asir, Abha, Madenate Al-Ameer Sultan, 01-20.11.2013, Malaise trap, H.A. Dawah (CERS).

Distribution: This is the first record from Saudi Arabia. The species was described from a desert region of southern Palestine (Rognes, 2002).

Remarks: Though *R. aravaensis* and *R. cassotis* (Walker) appear to be closely related, they can easily be separated by the structure of the male fifth (pregenital) sternite. Rognes (2002: 94; Fig 105) shows that the lateral lobes of *R. aravaensis* to be short, with a pair of short blunt tooth-like projections on the truncate apices medially situated. In *R. cassotis*, however, the apices as figured by Zumpt (1958: 182; Fig. 60) are very deeply concave with a single long projection at their medial end, where as it is not so in *R. aravaensis.*

***Rhyncomya bullata* Deeming, 1996**

Rhyncomya bullata Deeming, 1996: *Fauna of Saudi Arabia*, 15: 270.

Material examined: Saudi Arabia: 1♀, Asir, Maraba, Al-Hudaithy Fruit Farm, 01-30.05.2004, Malaise trap, H.A. Dawah (NMWC); 2♂♂, Jazan, Farasan Island, Al-Maraq, 1.06.2016, Malaise trap, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia by Dawah & Abdullah (2009). It was described from Oman and further recorded from United Arab Emirates (Deeming, 1996; 2008).

***Rhyncomya callopis* (Loew, 1856)**

Idia callopis Loew, 1856: *Programm Koniglichen Realschule zu Meseritz*, 1856: 49.

Material examined: Saudi Arabia: 1♂, Khybar Al-Janob, Hay Al-Salam, 01-15.01.2011, sweeping trap, M. Al-Shahrany (CERS).

Distribution: This is the first record from Saudi Arabia. This species was described from Egypt and further recorded from Algeria, Egypt, Europe (widespread), Iran, Israel, Morocco, Tunisia and Western Sahara (Spanish Sahara) (Zumpt & Tsacas, 1976; Schumann, 1986; Deeming, 1996).

Remarks: Rognes (2002) split off from *R. callopsis* of authors three good species, being *negevi*, *sinaiensis* and *yahavensis*. The species description and very good figures 117-131 in Rognes (2002: 96-99) of the male genitalia of *R. callopis* clearly separate it from the related species in the same paper.

Rhyncomya cassotis (Walker, 1849) (Fig. 2)

Tachina cassotis Walker, 1849: *List of the specimens of dipterous insects in the collection of the British Museum*, 4: 761.

Material examined: Saudi Arabia: 1♂, Jazan, Harob, Wadi Lajab, 08.06.2015, sweeping, H.A. Dawah (CERS).

Distribution: This is the first record from Saudi Arabia. This species was described from Sierra Leone and is further recorded as being widespread on the Afrotropical mainland (Pont, 1980).

Rhyncomya desertica Peris, 1951

Rhyncomya desertica Peris, 1951: *Eos*, *Revista Española de Entomologia*, 27: 243.

Distribution: This species was first recorded from Saudi Arabia by Abu-Zoherah et al (1993). It was described from Algeria and is further recorded from the Palaearctic Region: Egypt, Libya, Palestine and Tunisia: Afrotropical Region; Chad, Mauritania, Niger, Saudi Arabia, and United Arab Emirates (Peris, 1952; Zumpt & Tsacas, 1976; Pont, 1980; Schumann, 1986; Abu-Zoherah et al, 1993; Deeming, 1996; 2008).

Fig. 2. *Rhyncomya cassotis* Walker 1849. Male.

Rhyncomya jordanensis Peris, 1951

Rhyncomya jordanensis Peris, 1951: *Eos*, *Revista Española de Entomologia*, 27: 242.

Material examined: Saudi Arabia: 2♂♂, Jazan, Fifa, Al-Tatweer Centre, Malaise trap, 07-28.01.2016, H.A. Dawah (CERS); 1♀, Asir, Maraba, Al-Hudaithy Fruit Farm, 01-30.05.2004, Malaise trap, H.A. Dawah (NMWC).

Distribution: This species was first recorded from Saudi Arabia by Dawah & Abdullah (2009). This species was described from Jordan and is further recorded from Egypt, Israel, Oman, United Arab Emirates and Yemen (Schumann, 1986; Deeming, 1996; 2008; Rognes, 2002).

Remarks: Biology: Unknown.

***Rhyncomya sinaiensis* Rognes, 2002**

Rhyncomya sinaiensis Rognes, 2002: *Entomologica Scandinavica* (=*Insect Systematics & Evolution*) supplement, 59: 49-51.

Distribution: This species was first recorded from Saudi Arabia by Setyaningrum & Aldhafer (2014). It was described from Egypt (Sinai) and is further recorded from Palestine (Rognes, 2002).

Remarks: Rognes (2002: 50) found in two females of *R. sinaiensis* that he dissected a large uterine first instar larva filling the whole length of the abdomen. Such macrolarviparous reproduction is found in tsetse flies (Glossinidae), in which the larva is protected within the uterus of its parent, being fed on an oil-bearing solution until it is as large as its mother and when finally deposited immediately pupates (Pollock, 1992). A similar strategy is found in the chloropid genus *Pachylophus* Loew (Deeming, 2018) and Hippoboscidae.

***Rhyncomya tristis* Séguy, 1933**

Rhyncomya tristis Séguy, 1933: *Memórias e Estudosdo Museu Zoológico da Universidade de Coimbra*, 67: 67.

Material examined: Saudi Arabia: 3♂♂, Jazan, Abu Aresh, Al-Mahdag Village, 05-20.06.2011, Malaise trap, H.A. Dawah (CERS); 3♂♂, same data but, 01.02-03.04.2011, Malaise trap, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia Abu-Zoherah et al (1993). This species was described from Mozambique. An afrotropical species known from Botswana, Chad, Rhodesia and South Yemen (Pont, 1980).

Remarks: Setyaningrum & Aldhafer (2014) recorded recently *Rhyncomya zumpti* Peris, 1952 from Saudi Arabia. *R. zumpti* has been placed under synonymy of *R. tristis* (see Zumpt & Stimie, 1965: 9-11; Pont 1980: 786 also K. Rognes; pers. Comm; 2016). Abu-Zoherah et al (1993: 227) recorded *R. tristis* from Saudi Arabia.

***Rhyncomya varifrons* Becker, 1910**

Rhyncomya varifrons Becker, 1910: *Denkschriften der Wissenschaften Wien*, 71: 141.

Distribution: This species was first recorded from Saudi Arabia Abu-Zoherah et al (1993). It was described from Socotra (Pont, 1980).

***Villeneuviella seguyi* Grunin, 1957**

Villeneuviella seguyi Grunin, 1957: *Entomologicheskoe Obozrenie*, 36: 543.

Distribution: This species was first recorded from Saudi Arabia by Abu-Zoherah et al (1993). This species was described from Iran and is further recorded from Iran,

Israel, Oman, Saudi Arabia, United Arab Emirates and Yemen (Pont, 1980; Schumann, 1986; Deeming, 1996; 2008).

Remarks: The synonomy of the genus *Villeneuviella* Austen as being a junior synonym of *Rhyncomya* Robineau-Desvoidy as proposed by Pont (1980) is not accepted by Schumann (1986), nor Rognes (2002), nor Deeming (1996) and are followed here. The justification given by Deeming (1996: 274) are based upon highly unusual larval characters.

Stomorhina chapini Curran, 1931

Stomorhina chapini Curran, 1931: *American Museum Novit*, 506:16.

Material examined: Saudi Arabia: 1♀, Jazan, Fifa, Al-Tatweer Centre, 16.12.2015-06.01.2016, Malaise trap, H.A. Dawah (CERS).

Distribution: This is the first record from Saudi Arabia. This species was described from Zaire. This species is widespread in West and East Africa.

Stomorhina rugosa (Bigot, 1888)

Rhinia rugosa Bigot, 1888: *Bulletin de la Société Zoologique de France*, 12(5-6) (1887): 591.

Material examined: Saudi Arabia: 1♀, Asir, Maraba, Al-Hudaithy Fruit Farm, 13.01.2013, Malaise trap, H.A. Dawah (CERS); 1♀, Asir, Abha, Madenate Al-Ameer Sultan, 25.02.-25.05.2002, Malaise trap, H.A. Dawah (NMWC).

Distribution: This species was first recorded from Saudi Arabia by Dawah & Abdullah (2009); El-Hawagry et al (2017). It was described from Sierra Leone. It is known from South Africa and Guinea (Pont, 1980).

Remarks: Females were reported to oviposit in freshly excavated termite mounds and the eggs hatched immediately (Cuthbertson 1934, as *S. mitis* Curran "sic").

Metalliopsis arabica Deeming, 2008

Metalliopsis arabica Deeming, 2008: *Arthropod Fauna of the UAE*, 1: 726.

Material examined: Saudi Arabia: 2♂♂, Asir, Maraba, Al-Hudaithy Fruit Farm, 15.04.2014, Malaise trap, H.A. Dawah (CERS; NMWC); 1♀, same data but, 17.06.2003, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia by Dawah & Abdullah (2009). It was described from the United Arab Emirates and Saudi Arabia (Deeming, 2008).

Subfamily Chrysomyinae

Chrysomya albiceps (Wiedemann, 1819) (Fig. 3)

Musca albiceps Wiedemann, 1819: *Zoologisches Magazin*. *Kiel*, 1(3): 38

Material examined: Saudi Arabia: 1♂, Asir, Abha, Hay Al-Nusub (Abha Farm Centre), 13.03-02.04.2015, Malaise trap, H.A. Dawah (CERS); 1♀, same data but, 03-06.2001 (CERS); long series of ♂ and ♀, Jazan, Abu Aresh, Al-Husseini Farm, baited traps, 15.04.2013; same but 13.02.2014, N.M. Gamal and M.F. Sallam (KSU, CERS).

Distribution: This species was first recorded from Saudi Arabia by Shalaby (1962); Dawah & Abdullah (2009); El-Hawagry et al, 2013; El-Hawagry, Abdel-Dayem, Elgharbawy, & Al-Dhafer, 2016; El-Hawagry et al, 2017). This species was described from South Africa and further recorded from the Afrotropical Region: Angola, Benin, Botswana, Burkino Faso, Burundi, Cameroun, Cape Verde Islands, Congo, Djibouti, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Guinea, Ivory Coast, Kenya, Lesotho, Liberia, Malawi, Mali, Niger, Nigeria, Madagascar, Mauritius, Mauritania, Mozambique, Oman, Ruanda, Réunion, Rodriguez, St Helena, Senegal, Seychelles, Sierra Leone, Socotra, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, United Arab Emirates, Yemen, Zambia and Zimbabwe; Palaearctic Region: Afghanistan, Albania, Algeria, Armenia, Austria, Azerbaijan, Azores, Bahrain, Bosnia, Herzegovina, Bulgaria, Canary Islands, Croatia, Cyprus, Czech Republic, Egypt, France, Germany, Greece, Hungary, Iran, Iraq, Israel, Italy, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Libya, Macedonia, Madeira, Malta, Moldova, Montenegro, Morocco, Palestine, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzeland, Syria, Tajikistan, Tunisia, Turkey, Turkmenistan, Ukraine, Uzbekistan and Western Sahara; Oriental Region: India and Pakistan; Neotropical Region: Argentina, Bolivia, Brazil, Ecuador, Paraguay, Peru and Puerto Rico (Shalaby, 1962; Pont, 1980; Verves, 2003; 2004; Harten, 2005; Deeming, 2008).

Remarks: Larvae necro- and coprophilous, predators of larvae of other Diptera (e.g., *Musca stabulans* Fallén, 1817) (Omar, 1995). It is very closely related to *C. rufifacies* (Macquart) and may be confused with it by inexperienced taxonomists (Verves, 2004). *C. albiceps* prefers hot and moist conditions (Büttiker, Attiah, & Pont, 1979). It is reported to breed in carrion (e.g., leopard, dog and porcupine) and dung (e.g., sheep and goats) and produces facultative cutaneous myiasis in livestock, goats, donkey, sheep, camels and men (Greenberg, 1971; 1973), following an initial strike by *Lucilia* species. Adults could be a nuisance in houses, markets, food shops, hospitals and slaughter-houses (Büttiker et al, 1979). Summarized information on biology, distribution and synanthropic significance of *C. albiceps* can be found in Zumpt (1965); Madeira (2001); Rognes (2002); Verves (2004); Hall & Smith (1993: 449) and Dawah & Abdullah (2009).

Chrysomya bezziana Villeneuve, 1914 (Fig. 4)

Chrysomya bezziana Villeneuve, 1914: *Revue Zoologicalique Africaine*, 3: 430.

Material examined: Saudi Arabia: 2♂♂, 2♀♀, Jazan, Beish, baited traps, 15.06.2013, N.M. Gamal and M.F. Sallam (CERS).

Distribution: This species was first recorded from Saudi Arabia by Ansari & Oertley (1982) and AlAhmed (2002). It was described from Africa. It is widespread in Asia, tropical Africa, the Indian sub-continent and Southeast Asia from Taiwan in the north to Papua New Guinea in the south (Pont, 1980).

Remarks: This species is more common in India than in Africa (Zumpt, 1965). The female lays 150-500 eggs at a time at wound sites or in body orifices (nose, mouth, ear and orbit) of live mammals as obligate parasitic flies requiring a host to complete

their development (Hall & Smith, 1993). The larvae feed on host tissue, attracted to blood and after completing their developments they drop to the ground to pupate. The adults are rarely found in the field (Zumpt, 1965). The adults feed on decomposing corpses, decaying matter, excreta and take nectar from flowers. Therefore the adult flies can be a mechanical vector for pathogens because of their diet. This species has not been used in maggot therapy because the larvae aggressively burrow through living tissue and can cause permanent tissue-damage. It is not suitable for use in forensics because it can cause myiasis on a live mammal and this means the time of colonization is not always concurrent with the time of death (Sukontason et al, 2005).

Fig. 3. *Chrysoma albiceps* (Wiedemann, 1819). Male.

Fig. 4. *Chrysomya bezziana* Villeneuve, 1914. Female.

***Chrysomya chloropyga* (Wiedemann, 1881)**

Musca chloropyga Wiedemann, 1881: *Zoologisches Magazin*, 1(2): 44.

Material examined: Saudi Arabia: 1♂, 1♀, Asir, Abha, Hay Al-Nusub, (Abha Farm Centre), 03.06.2001, Malaise trap, H.A. Dawah (NMWC; CERS); 1♂, 1♀, same data but, 03-30.05.2014 (MNWC); 3♀♀, same data but, 03-24.07.2014 (NMWC); 1♀, same data but, 19.06.-09.07.2013 (CERS); 1♂, 1♀, Asir, Abha, Hay

Al-Menhel, 12.05.-03.06.2015, Malaise trap, H.A. Dawah (NMWC); 1♂, same data but, 23.05.-12.06.2013 (CERS).

Distribution: This species was first recorded from Saudi Arabia by Büttiker et al (1979); Abu-Zoherah et al (1993). It was described from Cape of Good Hope in South Africa (Vorgebirge der guten Hoffnung) andfurther recorded from the Afrotropical Region; Cameroon, Democratic Republic of Congo (Zaire), Ethiopia, Kenya, Lesotho, Saudi Arabia, South Africa, Tanzania, Yemen and Zimbabwe: Palaearctic Region; Canary Islands, Egypt: and Neotropical Region (Schumann, 1986; Rognes & Paterson, 2005).

Remarks: All the south-western Saudi Arabian specimens of *C. chloropyga* were collected from Asir, which is above 2600m. These differ from other specimens of *C. chloropyga* in NMWC from Nigeria, Kenya and Ethiopia in having a broad line extending from lower eye margin to mouth margin which is almost completely shiny in contrast to the surrounding grey-dusted gena. In characters of development of aedeagus and cerci they exactly fit *chloropyga*, rather than *putoria*. In terms of mesonotal markings they are rather indistinct, having a dark blue mesonotal ground colour.

Chrysomya marginalis (Wiedemann, 1830)

Musca marginalis Wiedemann, 1830: *Aussereuropäischen Zweiflügeligen Insekten*, 2: 395.

Material examined: Saudi Arabia: 1♂, Asir, Maraba, Al-Hudaithy Fruit Farm, 13.01.2013, Malaise trap, H.A. Dawah (CERS); 1♂, same data but, 01-30.05. 2004, Malaise trap, H.A. Dawah (CERS).

Distribution: It was described from South Africa and first recorded from Saudi Arabia by Büttiker et al (1979); Dawah & Abdullah (2009); El-Hawagry et al (2013; 2016; 2017 as *C. regalis*). It is widespread within the Afrotropical Region (Pont, 1980; Rognes, 2002). In the Middle East, it was recorded from Bahrain, Egypt, Oman, Pakistan, Palestine, Syria, United Arab Emirates and Yemen (Deeming, 1996; 2008; Harten, 2005).

Remarks: *C. marginalis* breeds carrion breeder. It is well established in Israel (Rognes, 2002) and Egypt (Schumann, 1986). Zumpt (1965: 96) reported that the breeding record of larvae found in the malformed horns of a dying ox in Kenya as being the only reliable one for *C. marginalis*. *C. regalis* Robineau-Desvoidy was recorded from Saudi Arabia by Dabbour (1979), but this species has been placed in synonymy of *C. marginalis* (Rognes, 2002: 13).

Chrysomya megacephala (Fabricius, 1794)

Musca megacephala Fabricius, 1794: *Entomologia Systematica*, 4: 317.

Material examined: Saudi Arabia: 2♂♂, Asir, Maraba, Al-Hudaithy Fruit Farm, 13.01.2013, Malaise trap, H.A. Dawah (CERS); 1♂, same data but, 25.07.2013, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia by Ramadan & Al-Bihari (1980). It was described from South Africa and is further recorded from the Afrotropical Region: Angola, Benin, Botswana, Burkino Faso, Burundi, Cameroun,

Cape Verde Islands, Congo, Djibuti, Equatorial Ghana, Eritrea, Ethiopia, Gabon, Gambia, Guinea, Ivory Coast, Kenya, Lesotho, Liberia, Malawi, Mali, Madagascar, Mauritius, Mauritania, Mozambique, Namibia, Niger, Nigeria, Oman, Ruanda, Réunion, Rodriguez, Senegal, Sierra Leone, Somalia, South Africa, Saudi Arabia, Sudan, Tanzania, Togo, Uganda, United Arab Emirates, Yemen, Zambia and Zimbabwe; Palaearctic Region: Afghanistan, Canary Islands, China, Egypt, Iran, Japan, Libya, Korea, Russia; Oriental Region: Bangladesh, Borneo, Brunei, Cambodia, China, India, Indonesia, Japan (Ryukyu Island), Laos, Malaysia, Malaya, Myanmar, Nepal, Pakistan, Phillippines, Busuanga, Cebu, Leyte, Lozon, Mindanao, Negros, Palawan, Pany, Samar, Sulu Arch, Singapore, Thailand, Taiwan, Vietnam; Australsian/Oceanian Region: Admiralty Islands, Australia, Belaug, Bonin Island, Christmas Island, Cook Island, Easter 1, Eastern Somoa, Fiji, French Polynesia, Hawaiian Island, Henderson and Rapa Island, Marquesas Island, Society Island, Tuamotu Arch, Tubai Island, Marianas, Marshall Island, Micronesia, Kiribati, New Caledonia, New Zealand, Niue, Norfolk 1, Palau, Papau New Guinea, Pitcairn Island, Solomon Island, Tongo, Vanuatu, Vokano Island, Western Samoa; Nearctic Region: USA; Neotropical Region: Brazil, Equador, Honduras and Puerto Rico (Deeming, 1996; 2008; Hall & Smith, 1993; Pont, 1980; Verves, 2003). One of us (HAD) has examined specimens of *C. megacephala* in NMWC collected from Oman and the United Arab Emirates.

Remarks: This species is a widespread and common scavenger breeding in dung, decaying meat, carrion, corpses of pigs, dogs, toads, rats, frogs, essentially saprophagous, breeding in decomposing animal matter (Verves, 2003). It is occasionally a causative agent of cutaneous myiasis of different living mammals and man (Zumpt, 1965; Hall & Smith, 1993). It is known as the Oriental latrine-fly. It is of little use in forensics because it can cause myiasis in the absence of necrotic tissue and therefore, it can be difficult to determine the time of colonization (Sukontason et al, 2005). It is a nuisance when it is present in large number in fish markets, slaughterhouses and open-air meat markets (Hall & Smith, 1993). Adults swarms on meat and sweets, with notable attraction to fish. Under insanitary conditions, it is likely to transmit enteric pathogens and parasites (Zumpt, 1965; Greenberg, 1971; 1973; Kurahashi, 1982; Kurahashi & Chowanadisai, 2001).

***Chrysomya putoria* (Wiedemann, 1830)**

Musca putoria Wiedemann, 1830: *Aussereuropaischen Zweiflugeligen Insekten*: 403.

Distribution: This species was first recorded from Saudi Arabia by Abu-Zoherah et al (1993: 227) as *C. chloropyga* form *putoria* (Wiedemann, 1881); (El-Hawagry et al 2016, 2017). It was described from Sierra Leone and further recorded from Botswana, Cameroon, Democratic Republic of Congo (Zaire) Gambia, Ghana, Kenya, Madagascar, Mauritius, Réunion, Seychelles, South Africa, Swaziland, Tanzania and Zambia (Rognes & Paterson, 2005).

Remarks: *C. putoria* is recorded from Saudi Arabia by Büttiker et al (1979) but this species has been placed as a junior synonym of *C. chloropyga* (see Pont, 1980: 788). Rognes & Paterson (2005) revised the taxonomic status of *C. chloropyga*, *C. putoria*

and formally re-established them as being two different species on the charactersof adult external morphology and the genitalia.

Subfamily Calliphorinae

***Bengalia minor* Malloch, 1927**

Bengalia minor Malloch, 1927: *Annals and Magazine of Natural Histor*y, (9) 20: 408.

Material examined: Saudi Arabia: 3♂♂, 4♀♀, Jazan, Fifa, 03-24.11.2015, Malaise trap, H.A. Dawah (CERS; NMWC); 1♂, Asir, Maraba, Al-Hudaithy Fruit Farm, 06-27.08.2013, Malaise trap, H.A. Dawah (NMWC).

Distribution: This species was first recorded from Saudi Arabia by Abu-Thuraya (1982). It was described from Democratic Republic of the Congo and is further recorded from Mali (Pont, 1980).

***Calliphora croceipalpis* Jaennicke, 1867**

Calliphora croceipalpis Jaennicke, 1867: *Abhandlungen herausgeben von der Senckenbergischen Naturforschenden Gesellschaft*, 6: 376.

Material examined: Saudi Arabia: 3♀♀, Asir, Abha, Hay Al-Menhel, 07-31.12.2014, Malaise trap, H.A. Dawah (CERS); 1♂, Asir, Abha, Hay Al-Nusub (Abha Farm Centre), 03-24.07.2013, Malaise trap, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia by Abu-Zoherah et al (1993); El-Hawagry et al (2016, 2017). It was described from Ethiopia and is further recorded as being widespread from East Africa to southern Africa as well as Gough I., St Helena, Yemen, and Subantarctic islands (Pont, 1980).

***Calliphora vicina* Robineau-Desvoidy, 1830**

Calliphora vicina Robineau-Desvoidy, 1830: *Mémoires Présentés par divers* Savants *a l' Académie Royale des Sciences de l' Institut de France*, 2: 435.

Material examined: Saudi Arabia: 1♂, Asir, Abha, Hay Al-Nusub (Abha Farm Centre), 20.04.2013, Light trap, H.A. Dawah (CERS).

Distribution: This species was first recorded from Saudi Arabia by Abu-Zoherah et al (1993); El-Hawagry et al (2013, 2016, 2017). This species was described from USA (Philadelphia) and further recorded from the Palaearctic Region: Canary Islands, China, Mongolia and Japan; Afrotropical Region: Mauritius and South Africa; Nearctic Regions: Oriental Region: Northern India; Australiasian Region: Australia and New Zealand. It is a holarctic species and found in association with humans elsewhere (Pont, 1980; Schumann, 1986; Deeming, 1996).

***Hemipyrellia pulchra* (Wiedemann, 1830)**

Musca pulchra Wiedemann, 1830: *Aussereuropaischen Zweiflügeligen Insekten*, 2: 406.

Material examined: Saudi Arabia: 1♀, Jazan, Sabya, Basahy Farm, 24.07.2013, Malaise trap, H.A. Dawah (CERS); 1♀, Asir, Maraba, Al-Hudaithy Fruit Farm, 01-16.03.2013, Malaise trap, H.A. Dawah (CERS);1♂, Jazan, Abu Aresh, Al-Mahdag Village, 09-30.12.2013, Malaise trap, H.A. Dawah (CERS); 4♂♂, Jazan, Fifa, Al-Tatweer Centre, 14.04-06.05.2014, Malaise trap, H.A. Dawah (CERS); 1♀, same data but, 01-18.07.2013 (CERS).

Distribution: This is first record for Saudi Arabia. This species was described from Egypt?. It is widespread in West Africa to East Africa, Sudan: Oriental Region; India (Pont, 1980; Schumann, 1986).

Remarks: It may be an insect of forensic importance. In Thailand, a low percentage of this species was found among carrion calliphorids (Moophayak et al, 2014).

Subfamily Luciliinae

Lucilia cuprina (Wiedemann, 1830) (Fig. 5)

Musca cuprina Wiedemann, 1830: *Aussereuropaischen Zweiflügeligen Insekten*, 2: 654.

Material examined: Saudi Arabia: 1♂, Asir, Karatha, Al-Ethrebany Fruit Farm, 19.02.2014 Malaise trap, H.A. Dawah (CERS); 1♂, Asir, Abha, Hay Al-Nusub (Abha Farm Centre), 03.06.2015, sweeping trap, H.A. Dawah (CERS); 7♂♂, same data but, 19.02.2014 (CERS).

Distribution: The species was described from China and first recorded from Saudi Arabia by Büttiker et al (1979); El-Hawagry et al (2017). It has been recorded from Afrotropical Region: Madagascar, Mauritius, Réunion; Mediterranean Region to Oriental Region: Australasian Region: Nearctic Region and Neotropical Region (Büttiker et al, 1979; Pont, 1980; Rognes, 1994). In the Middle East it is known from Egypt.

Remarks: Most species in the genus *Lucilia* Robineau-Desvoidy are saprophagous in vertebrate carrion, with some tending primarily to attack live sheep and one (possibly more) that attacks live Amphibia (Ferrar, 1987). Ferrar (1987) reported that *L. cuprina* is the sheep blowfly, particularly of Australia and South Africa, where it is a major veterinary pest in sheep-raising areas. It causes primary myiasis of previously uninjured sheep and this damage may then be secondarily invaded and enlarged upon by other Calliphoridae. The species breeds in carrion to a much lesser extent, but is principally an agent of myiasis. The flies are attracted to sheep which have areas of soiled fleece or are suffering from bacterial decomposition of the fleece in fleece rot. The female lays her eggs in sores or cuts in the sheep's skin and the larvae develop there, eating the sheep's flesh away with alarming rapidity. Heavy infestations may kill sheep. The adults feed on fallen fruit, nectar, the honeydew of aphids but also on faeces of sheep and other animals to obtain a protein meal, which is important for maturing the eggs (Webber, 1958). The larvae rarely develop in faeces and the adults only occasionally land on man to feed on sores or secretions (Hall & Smith, 1993). Kettle (1992: 249) summarised existing knowledge of the biology, behaviour, bionomics and the effect of *L. cuprina* on sheep.

Fig 5. *Lucilia cuprina* (Wiedemann, 1830). Male.

Lucilia sericata (Meigen, 1826)

Musca sericata Meigen, 1826: *Systematische Beschreibung der Bekannten Europaischen Zweiflügeligen Insekten*, 5: 53.

Material examined: Saudi Arabia: long series of ♂♂ and ♀♀, Jazan, Abu Aresh, Al-Husseini Farm, baited traps, every month from April 2013-March 2014, N.M. Gamal and M.F. Sallam (KSU).

Distribution: This species was described from Austria. It is Holarctic species (Northern Hemisphere) found throughout the world (Büttiker et al, 1979). It is widespread in Afrotropical, Palaearctic, Oriental, Australasian, Nearctic and Neotropical Regions (Pont, 1980). It is recently recorded in Australia and in several South and Central American countries (Rueda, Ortega, Segura, Acero, & Bello, (2010). In the Middle East, it has been first recorded from Saudi Arabia by Shalaby (1962); El-Hawagry et al (2013; 2016; 2017); El-Hawagry, Abdel-Dayem, & Al Dhafer, 2018): Kuwait (Hira et al, 2004) and Iran (Youssefi, Rahim, & Marhab, (2012).

Remarks: There are several factors which play a role in development of *L. sericata* including the temperature, food source and humidity (Tarone & Foran, 2006). The adults are diurnal and are attracted by the solid or wet fleece of sheep, open wounds, carrion and to a lesser degree by faeces, in which the larvae can also complete their development. Adults are very fond of sweet or fermenting materials and are found on flowering plants. The females need a protein meal for the development of the eggs (Büttiker et al, 1979). After mating, females lay up to 200 eggs at a time, on the host or carcass. *L. sericata* plays an important role in: (a) veterinary medicine as feeding by larval *L. sericata* can cause a form of myiasis known as a sheep strike or blowfly strike, mainly in Northern and Central Europe with substantial losses in animals and production (Strikewise, 2007), (b) medical treatment using the larvae of *L. sericata* to heal injuries by not only eating the decomposing tissue but also secreting and producing antimicrobial enzymes while in the wound (Horobin, Pritchard, & Shakessheff, 2002; Rueda et al, 2010), (c) forensic science as the larvae help to determine the period of insect colonization, as it relates to the time of death, aiding

law enforcement in their investigations (Rueda et al, 2010).

***Pericallimyia greatheadi* Zumpt, 1971**

Pericallimyia greatheadi Zumpt, 1971: *Novos Taxa Entomologicos*, 99: 3.

Material examined: Saudi Arabia: 1♀, Asir, Abha, HayAl-Nusub (Abha Farm Centre), 19.06-09.07.2014, Malaise trap, H.A. Dawah (CERS); 1♂, same data but, 20.04.2013 (CERS); 4♂♂, Asir, Abha, Hay Al-Menhel, 19.02.2014, Malaise trap, H.A. Dawah (CERS); 2♂♂, Al-Baha, Hay Al-Dhofair, 03.11.2015, Malaise trap, H.A. Dawah (CERS)

Distribution: This species was first recorded from Saudi Arabia by Setyaningrum & Aldhafer (2014). This species was described from Eritrea and is further recorded from Saudi Arabia (Pont, 1980; Setyaningrum & Aldhafer, 2014).

Remarks: Deeming (1996) recorded it breeding in the tree-snail *Euryptyxis latireflexa* (Reeve) in Dhofar, Oman, and described the puparium.

Subfamily Auchmeromyiinae

***Cordylobia anthropophaga* (Blanchard and Berenger-Féraud in Larrey, 1872) (Fig. 6)**

Ochromyia anthropophaga Blanchard and Berenger-Féraud in Larrey, 1872: *Comptes rendes Hebdomadaires des seances de l"Academie des Sciences*: 1133-1134.

Material examined: Saudi Arabia: 4♂♂, 6♀♀, Jazan, Abu Aresh, Al-Mahdag Village, 21.01.2015, Malaise trap, H.A. Dawah (CERS); 1♂, 2♀♀, same data but, 03.03.2015 (CERS); 1♂, 2♀♀, same data but, 10.11.2014 (CERS); 1♂, same data but, 26.11.2014 (CERS); 1♂, 2♀♀, same data but, 30.06.2010 (CERS); 1♂, same data but, 01.06.2013 (CERS); 1♀, Asir, Al-Souda, Bani Mazen, 27.05.2014, Malaise trap, H.A. Dawah (CERS).

Distribution: It was first recorded from Saudi Arabia (Büttiker, Habayeb, & Zumpt, 1980; Dawah & Abdullah, 2009; Setyaningrum & Aldhafer, 2014). It was described from Senegal and it is widespread in mainland Afrotropical Region (Pont, 1980).

Remarks: Known as the Tumbu fly, the larva of this species causes subcutaneous myiasis in humans, dogs and other domestic and wild animals (the rats form the main reservoirs of the fly in the field) in many parts of the Afrotropical Region. Roberts, Boyce, & Lyerly, (1982) reported a technique for rearing larvae of *C. anthropophaga*. Blacklock & Thompson (1923); Zumpt (1965) and Dawah & Abdullah (2009) gave a good account of the life history, biology and pathogenesis of the Tumbu fly.

Fig. 6. *Cordylobia anthropophaga* (Blanchard and Berenger-Féraud 1872).

***Pollenia hungarica* Rognes, 1987**

Pollenia hungarica Rognes, 1987: Syste*matic Entomology*, 12: 483.

Distribution: This species was first recorded from Saudi Arabia by Setyaningrum & Aldhafer (2014); El-Hawagry et al (2013, 2016). This species was described from Hungary (Albertirsa). It is known from the Palearctic Region: Austria, Norway, Sweden and Switzerland (Rognes, 1987a; b). Based on the known distribution of this species it would seem prudent to confirm the identification of this species from Saudi Arabia using photographs of the hind tibial vestiture and genitalia.

Remarks: This species has been reared from the earthworm *Eisenia rosea* Savingy (Lumbricidae) by Professor Zicsi (HNHM) (Rognes, 1987b: 484). Adults are on the wing in the field most of the year (February to November). In Central Europe this species has been captured at altitude up to 1600m (Rognes, 1987b: 485).

DISCUSSION

Eighteen known species of Calliphoridae were identified and recorded in this study, seven of which were recorded for the first time. This makes the total number of Calliphoridae species in Saudi Arabia (including 26 species previously recorded and excluding two species which were synonymized namely: ***Rhyncomya zumptii*** Peris, 1952; ***Chrysoma regalis*** Robineau-Desvoidy, 1830) to be 44. A list of all species of Calliphoridae recorded from Saudi Arabia is provided. A checklist is essential for (1) biodiversity studies, by providing them with: (a) useful information about regional biodiversity (comparison of species numbers in a group with other regions) and general distribution of species, (b) to give indications of which species have expanded their range and how quickly they did so by comparing recent and old checklists of the same country (Siepel, Bink, Broekhuizen, Stumpel, & van Wingerden, 1993), (2) faunistics studies by defining species by their recent names including synonyms or misidentifications,

information about where the describer published the description of species, page number and the location of the type specimens and distribution, (3) nature conservation studies as they provide information to determine species under threat or a target species, by the comparison of checklists (species composition) of one country with other countries.

The species recorded in this study are more Afrotropical in origin than ther are to other regions. Dawah & Abdullah (2009) found the situation of Calliphoridae in the southwest of Saudi Arabia is almost the reverse as the Palaearctic element prevails, with some species from the Afrotropical and Oriental regions. Although it is tempting to draw assumptions from these percentages of species distribution (Fig. 7), it must be acknowledged that all parts of Saudi Arabia have not been surveyed to the same extend, with some areas having had very little sampling. Therefore, further collecting, surveying and trapping of insects from the North (Palaearctic) (at the border with Jordan; most of Saudi Arabia, including northern and eastern Jeddah), Oriental Region (the southeastern area and a part of the eastern provinces of Al-Hassa and Al-Dammam are considered as representing the Oriental Region, to develop a better picture of the zoogeographical affinity of the Calliphoridae of Saudi Arabia.

Some species of Calliphoridae (e.g., *C. anthropophaga*; *Chrysomya albiceps*; *I. terminata*) were found living at unexpected levels of both high and low altitude in Jazan and Asir. Such insects demonstrate that they have a successful adaptation. Decreased oxygen availability and lower temperatures make life at such altitudes challenging though many species have adapted successfully. Such adaptations (which depend on morphology and phylogeny) include oxygen uptake and better oxygen delivery to tissues (Mani, 1968; Mani & Giddings, 1980; Hodkinson, 2005). It is not easy to determine whether a given insect is a true high altitude species. The difficulty is in part also due to the vagueness of the expression “high altitude” (Mani & Giddings, 1980; Hodkinson, 2005). It is evident from examination of a large number of Malaise trap samples from different altitudes in Jazan, that introductions of species from elsewhere succeed in establishing populations more readily at higher altitudes. It is well known that Saudi Arabia is situated in the transitional zone of three zoogeographic regions (Palaearctic, Afrotropical and Oriental). The highlands of Asir and Najran are of great entomological interest and as it is here that insects blown in from other regions are brought by convection currents and deposited.

A notable feature of the Calliphoridae fauna of Saudi Arabia is the frequency of so called tramp species (six species: or 14% of the species listed in table 2) that have been dispersed around the world through the agency of man. Arabia has always been involved in trade and interchanges with distant nations. These movements have increased out of all proportion in the last hundred years. Not only has the ancient trade in frankincense been eclipsed by the modern oil trade but the pilgrimage to Makkah by Muslims has brought ever increasing numbers by aeroplanes from every corner of the globe. All this increases the opportunities for the transport of small insects to and from Arabia. In our present state of knowledge, it is frequently not possible to assess the likely original distribution of the tramp species. The future use of molecular phylogenetics may be able to provide the answers. This study has added new

records to the Saudi Arabia checklist of Calliphoridae which will provide the basis for systematic studies and fauna analyses of future works on Calliphoridae. The Saudi Arabian Diptera list continues to increase in line with recording effort and is clearly far from complete, even for long-established native species.

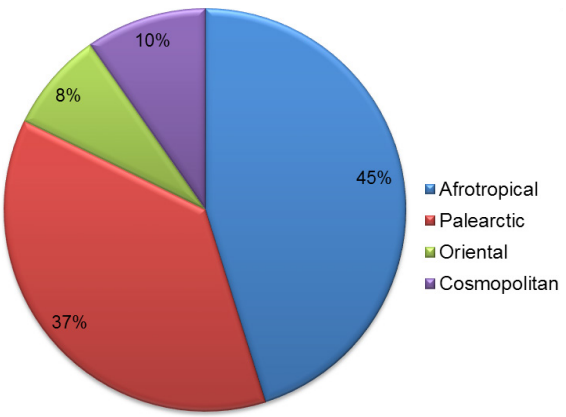

Fig. 7. Distributional elements in the *Calliphoridae* found in Saudi Arabia.

ACKNOWLEDGEMENTS

This project was supported by the King Abdul Aziz for Science and Technology, grant number (AT-32-0023), in the Kingdom of Saudi Arabia for which we are very grateful. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.Our sincere thanks are due to the following, without whose kind help this paper would not have been written: to Jazan University for providing us with research facilities and to Professor Abdullah Basahy, the former supervisor of the Centre for Environmental Research and Studies for all the support and encouragement; to Dr J.C. Deeming, for his help in identification, commenting on the manuscript and taxonomic advice; Prof. K. Rognes for reading the manuscript and useful comments andtaxonomic advice; to Mrs. H.J. Dawah for English correction; to Dr Alrajab, Mr. G. Jervis and Mr. M. Bouka for their help with literature search; to the National Museum of Wales for the facilities and permission to use the insect collection; to Jazan Agriculture Research Centre for facilities and permission to erect Malaise traps in the field of the Centre; to Mr. A. R. Hobany and O. S. Sanlocan for technical and field help; to Mr. S. Makbol, Mr. S. Al-Rahela Al-Kahtani and his family for allowing us to erect the Malaise traps on their farms andtaking care of them during their operation.

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Received: September 26, 2017 *Accepted: January 15, 2019*

J. Entomol. Res. Soc., 21(1): 95-108, 2019
Print ISSN:1302-0250
Research Article
Online ISSN:2651-3579

The Effects of Oxyclozanide on Survival, Development and Total Protein of *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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ABSTRACT

We investigated that the effects of oxyclozanide on survival rate, development time, adult longevity and the amount of total protein (TP) in different developmental stages (7th-instars larvae, pupae and adult) of greater wax moth *Galleria mellonella* L. First-instar larvae of insects were reared on 0.003, 0.03 and 0.3 g oxyclozanide in 100 g artificial diets. When compared to all tested concentrations of oxyclozanide and control diet without oxyclozanide, it was confirmed that this anthelmintic drug significantly reduced survival rate of 7th-instar larval stage, pupal and adult stage of *G. mellonella*. While 7th-instars larval rate is 91.25 ± 6.21 % in the control diet without oxyclozanide, this rate has been determined 28.75 ± 3.24 % in the 0.3 % concentration of oxyclozanide. TP of the insect increased in response to all concentration of oxyclozanide in comparison to control diet. This considerable increase was expressed almost two-fold especially at the 0.003 and 0.03 % of oxyclozanide concentrations in all developmental stages. The results obtained from this research support that the oxyclozanide has effects on the survival, development, adult longevity and TP of the insect.

Key words: Galleria mellonella, oxyclozanide, survivorship, development, total protein.

Çelik, C., Büyükgüzel, K., & Büyükgüzel, E. (2019). The effects of oxyclozanide on survival, development and total protein of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *Journal of the Entomological Research Society,* 21(1), 95-108.

INTRODUCTION

Lepidoptera species, especially the larval stage of these insects causes significant economic losses by damaging to agricultural and industrial plants. These species include *Achroia grisella* (lesser wax moth) and *Galleria mellonella* (greater wax moth). Since the larvae of these insects feed on products such as honeycomb, honey, beeswax and pollen residues, they cause serious economic losses in apiculture industry (Uygur & Girişgin, 2008; Ellis, Graham, & Mortensen, 2013). Several studies were conducted on the life cycle, biology, behavior, ecology, molecular biology, physiology of the insect to control this pest with economic significance (Akyol, Yeninar, Şahinler, & Ceylan, 2009; Ellis et al, 2013; Erdem, Küçük, Büyükgüzel, & Büyükgüzel, 2016; Büyükgüzel & Büyükgüzel, 2016).

A variety of physical, chemical, biological and certain other methods are used in the control of agricultural pests to reduce their populations to a certain level and not to eliminate their existence (Kılınçer et al, 2010; Sezer & Ozalp, 2011). Various chemical (sulfur, acetic acid, formic acid, aluminum phosphate, methyl bromide, ethylene dibromide, naphthalene, sulfur and carbon dioxide), physical (hot or cold) applications and biological insecticides (*Bacillus thuringiensis*) are used for the protection of raised honeycombs against the damages caused by greater wax moth (Akyol & Korkmaz, 2008; Akyol et al, 2009; Babarinde et al, 2013). In recent years, studies on the insecticide properties of antibacterial, antiprotozoal, antifungal and anthelmintic drugs with different chemical structures and modes of action by culturing insects with artificial diets in laboratory environment have been increased (Büyükgüzel & Kayaoğlu, 2014; Kılıç, Büyükgüzel, & Büyükgüzel, 2015; Çalık, Büyükgüzel, & Büyükgüzel, 2016; Sugeçti, Büyükgüzel, & Büyükgüzel, 2016; Hız, Erdem, Büyükgüzel, & Büyükgüzel, 2016; Sefer & Büyükgüzel, 2018).

Studies on the effects of various factors on the protein, lipid and carbohydrate content in insects were demonstrated in different insect species (Büyükgüzel & Kalender, 2008; Sönmez & Gülel, 2008; Sharma, Mohan, Dua, & Srivastava, 2011; Clark, Zera, & Behmer, 2015). In a study by Boz & Gülel (2012) who examined the effect of post-parasitizing time and temperature on the total protein, lipid and carbohydrate content of mediterranean flour moth *Ephestia kuehniella* larvae, it was reported that the protein concentration in the hemolymph of mediterranean flour moth parasitized by parasitoid *Venturia canescens* increased with time after parasitizing, while total lipid and carbohydrate content decreased. When the effect of temperature was examined, it was determined that protein and carbohydrate concentrations increased, however the lipid content did not change. Aksoy, Bahadıroğlu, & Kayabaşı (2015a) conducted to determine the effects of different doses of X-ray (50-200 Gy) on the protein, carbohydrate and lipid levels in *Sesamia nonagrioides* larvae were investigated and the best results were obtained as 150 Gy between the applied doses. It was reported that increases in the amount of these molecules (protein, carbohydrate and lipid) caused deterioration in the physiological and biochemical activities in *S. nonagrioides*, and interrupted the biologic stages and prevented the female larvae to enter the pupa stage.

Oxyclozanide and *G. mellonella*

Galleria mellonella is commonly used as a model organism in several fields such as clinical drug trials and investigation of the activity of disease factor microorganisms due to its high ecological adaptation, short life cycle, high egg-laying capacity and it is a species that could breed high number of healthy individuals (Junqueira, 2012; Harding, Schroeder, Collins, & Frankel, 2013; Jacobsen, 2014; Champion, Wagley, & Titball, 2016). Furthermore, similar to certain other species, the larvae and pupae of these insects are also used as pseudo-hosts in propagation of parasitoid insects under laboratory conditions (Bernardi, Haddad, & Parra, 2000; Büyükgüzel, 2006).

The oxyclozanide that was used in the study is a salicylanilide derivative anthelmintic drug (Kang, Wakabayashi, & Kim, 2016). Oxyclozanide is effective against adults of *Fasciola hepatica* and *Fasciola gigantica* (liver flukes), which belong to trematodes, an important group of parasites (Coles & Stafford, 2001; Power et al, 2013). Its effect on parasites is demonstrated by breaking down the oxidative phosphorylation chain and degrading the energy metabolism (Hrckova & Velebny, 2013). There is no information on the use of oxyclozanide, a salicylanilide derivative anthelmintic drug, in the control of agricultural pest species. Thus, the greater wax moth *G. mellonella*, a Lepidoptera species, was used as a model organism in the present study to investigate the effect of oxyclozanide on insects, which constitute a significant group of invertebrates. The final aim of the present study was to contribute to the control methods against this species and to investigate the availability of anthelminthic drugs for chemical control of the species in the Lepidoptera order. Furthermore, information on the availability of oxyclozanide as an insecticide in pest control is significant since it would contribute to the preservation and the yield of agricultural crops, improvement their quality and development of new methods. In the present study, the effects of oxyclozanide on the survival rate, growth period, adult longevity and total protein content of the larvae were investigated by supplementing the diets that were provided for *G. mellonella* with oxyclozanide in the laboratory environment.

MATERIAL AND METHODS

Culturing *G. mellonella* in laboratory environment

G. mellonella (Lepidoptera: Pyralidae), the greater wax moth, was cultured in the university insect culture laboratory to maintain the stock insect culture. For the propagation of the insect culture, newly hatched larvae were grown in artificial medium (Bronskill, 1961). The culture was conducted in an incubator (Nüve, FN 400) that was set to 28 ± 2°C temperature and 65 ± 5 % relative humidity and continuously in darkness.

Obtaining the *G. mellonella* larvae

The *G. mellonella* larvae to be used in diet experiments were obtained by the hatching of the eggs laid by female individuals grown in the stock culture. The first stage larvae that were freed with the hatching of the eggs were transferred to medium size wire-mesh glass jars with a metal lid (60x120 mm) using a soft-tip moistened brush (No: 0, Goya Toray). Thus, the effects of different concentrations of oxyclozanide,

ingested directly by the larvae via diets, on the survival rate until adult stage, growth period, adult longevity and total protein (TP) content of the larvae were investigated.

Determination of oxyclozanide concentrations

Concentration of the quantities tested in diet tests conducted by adding oxyclozanide [2,3,5-trichloro-N-(3,5-dichloro-2-hydroxyphenyl)-6-hydroxybenzamide] (Jo et al, 2011), a salicylanilide derivative, was determined as gram quantity (%) supplemented to 100 grams of diet. Three different concentrations, namely 0.003, 0.03 and 0.3 g were used to investigate the effect of oxyclozanide on the insect except for the control diet (without oxyclozanide). The concentrations were determined based on the preliminary experiments that were conducted based on the concentrations utilized in previous studies that investigated the effect of certain antibiotics on *G. mellonella* (Büyükgüzel & Kalender, 2007, 2008, 2009). Twenty 1st stage larvae were transferred into each jar prepared for the experiment using a fine-tipped brush. The jars were covered with lids that were cut out and replaced with thin metal mesh and placed in incubators where insects were cultured. Experiments were repeated four times.

Experiments on *G. melonella* survival rate, developmental time and adult longevity

All experiments (jars media where the first-stage larvae were kept and containers prepared for the 7th stage larvae to become pupae) were kept in the dark at all times except for a brief daily inspection period. The number of mature (7th stage) *G. mellonella* larvae that completed the development was counted and noted and they were placed in 30 ml plastic sample containers (Orlab, L190030, 35x55 mm) that were layered with tissue paper to provide a dry environment for pupae. After the number of individuals that reached the pupal stage and and the number of individuals that matured from these pupae were noted and the survival rates of the larvae, pupae and adults were calculated. The longevity of individuals who reached the maturity stage was also calculated.

Determination of TP in *G. mellonella* 7th stage larvae, pupae and adults

G. mellonella 7th stage larvae, pupae and adult individuals cultured with diets that contained different concentrations of oxyclozanide were taken to 1.5 ml Eppendorf tubes and stored in a deep freeze (-80°C) until the analyses were conducted. Experiments were repeated four times using five 7th larvae, pupae and adults for each trial. All development stage of *G. mellonella* collected for each repeat were placed in 1.5 ml phosphate buffer (K_2HPO_4) (pH: 8) and initially grinded in 24000 rpm tissue grinder homogenizer (Ultra Turrax, Ika T18 basic NC, USA), and then in an ultrasonic homogenizer (10 sec, 30 w) (Bandelin Sonoplus HD2070, Berlin, Germany). Homogenized 7th stage larvae and others were transferred to 2 ml Eppendorf tubes and centrifuged at +4°C for 10 minutes at 1000x g. The supernatant was used to determine the TP after the sediment was discarded. Bovine serum albumin solutions were prepared for use as standard protein solution and a standard graph was plotted. TP content was calculated based on this standard graph. The absorbances of the

samples were measured with the Folin-Lowry (Lowry, Rosebroug, Farr, & Randall, 1951) method at 600 nm with a spectrophotometer (Shimadzu 1700 UV / Vis, Kyoto, Japan).

Statistical analyses

Percentages of *G. mellonella* individuals that reached different stages (7^{th} stage, pupa and adult) were defined as the survival rate, development as the time required to reach these stages in days, the time they survived as adults after they reached the adult stage in days and TP content in mg in tissue extract.

One-way analysis of variance (ANOVA) (SPSS 1997) was used to evaluate the data on the duration of development, adult longevity and TP content of the insect, and the "LSD Test" was used to determine the significance of the difference between the mean values. In the evaluation of the survival rate data, "$\chi 2$ (Chi square) test" (Snedecor and Cochran, 1989) was used. Correlation analysis was also performed to test the correlation between tested concentration and survival rate of insect (SPSS 1997). The significance of the means was assessed at the 0.05 level of significance.

RESULTS

The effect of oxyclozanide on the survival rate and developmental time of *G. mellonella* larvae, pupae and adults

Comparison of the concentrations of oxyclozanide, a anthelmintic substance, used in the study group with the control group demonstrated that the increase in oxyclozanide concentration decreased the larval survival rate (Figs. 1-3). While 91.25 ± 6.21 % of the larvae in the control diet that did not contain oxyclozanide reached 7^{th} stage, in 0.003, 0.03 and 0.3 g oxyclozanide concentrations, only 77.50 ± 8.19 %, 61.25 ± 7.97 % and 28.75 ± 3.24 % of the larvae reached the 7^{th} stage, respectively (Fig. 1). We obtained significantly relationship between the tested concentration of oxyclozanide and survival rate. Tested concentrations were negatively correlated with survival rate of 7^{th}-instar larva ($R^2 = 0.97$, $P > 0.05$), pupa ($R^2 = 0.95$, $P > 0.05$) and adult ($R^2 = 0.96$, $P > 0.05$) stages of *G. mellonella.*

A decrease was observed in all oxyclozanide concentrations when the effect of oxyclozanide on the rate of transformation of greater wax moth *G. mellonella* into pupa was compared between the study and control groups. The highest decrease was observed with 0.3 g oxyclozanide concentration. While 83.75 ± 8.54 % of the larvae grown with the control diet reached the pupal stage, only 77.50 ± 8.19 %, 58.75 ± 7.15 % and 26.25 ± 5.11 % of the larvae cultured with 0.003 %, 0.03 % and 0.3 % oxyclozanide concentrations added to the artificial diet reached the pupal stage, respectively, and it was found that the decrease was statistically significant when compared to the control (Fig. 2).

Oxyclozanide, the anticholinergic substance added to the artificial diets, significantly affected the percentage of pupae that reached adulthood, similar to the rate of larvae that reached the 7^{th} stage and pupa stage (Fig. 3). In the control group, 80.00 ± 8.66 %

of the first stage larvae reached adulthood, while in 0.3 g oxyclozanide concentration, only 25.00 ± 4.67 % of the larvae reached the adulthood.

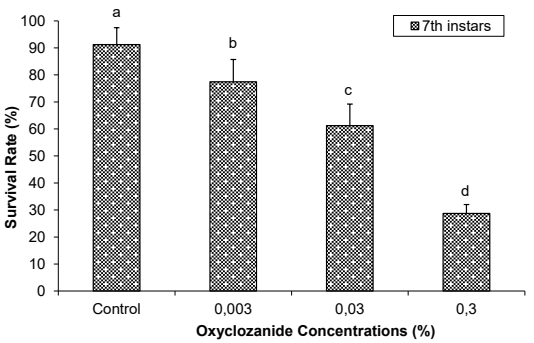

Fig. 1. Effects of oxyclozanide on *G. mellonella* 7th stage survival rate. Four replicates with 20 larvae per replicate were used. Values labelled with the same letter are not significantly different from each other within each survival rate, P > 0.05 (χ2 test). Control diet (without oxyclozanide).

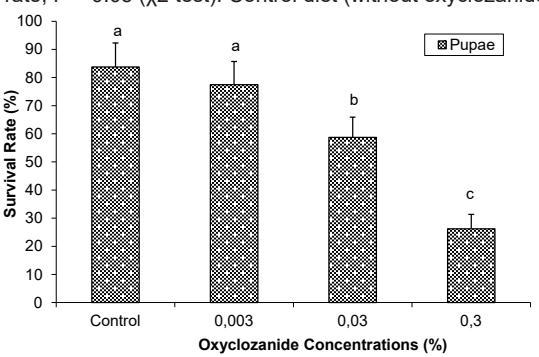

Fig. 2. Effects of oxyclozanide on *G. mellonella* pupal survival rate. Four replicates with 20 larvae per replicate were used. Values labelled with the same letter are not significantly different from each other within each survival rate, P > 0.05 (χ2 test). Control diet (without oxyclozanide).

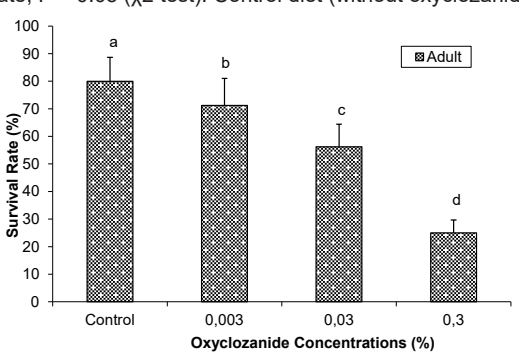

Fig. 3. Effects of oxyclozanide on *G. mellonella* adult survival rate. Four replicates with 20 larvae per replicate were used. Values labelled with the same letter are not significantly different from each other within each survival rate, P > 0.05 (χ2 test). Control diet (without oxyclozanide).

Oxyclozanide and *G. mellonella*

There were no significant differences between the oxyclozanide concentration applications based on the rate of *G. mellonella* larvae that reached to 7th, pupae and adult stages. Although this ratio of oxyclozanide in the diet delayed the time that was required for the 1st stage larvae to reach the 7th stage for 4 days, the difference was not statistically significant (Fig. 4).

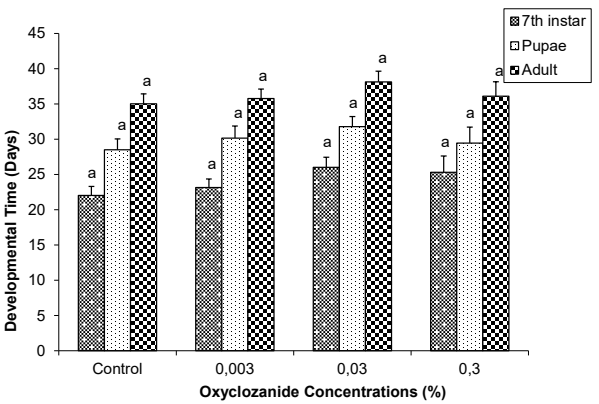

Fig. 4. Effects of oxyclozanide on *G. mellonella* larval, pupal and adult developmental time. Four replicates with 20 larvae per replicate were used. Values labelled with the same letter are not significantly different from each other within each developmental time, P > 0.05 (LSD test). Control diet (without oxyclozanide).

The effect of oxyclozanide on adult longevity of *G. mellonella*

When the effect of oxyclozanide on adult longevity of the insect was examined, it was determined that there was no statistically significant difference based on the results (Table 1).

Table 1. Mean (±SE) adult longevity of *G. mellonella* treated with Oxyclozanide

Oxyclozanide(%)	Adult Longevity (Day)
	(Mean ±SE)*
0.000§	10.03 ± 0.92a
0.003	10.55 ± 0.50a
0.03	10.56 ± 0.29a
0.3	9.38 ± 0.43a

Four replicates with 20 larvae per replicate. *Means within a column followed by the same lowercase letter are not significantly different, P > 0,05 (LSD Test).§ Control diet (without Oxyclozanide)

The effect of oxyclozanide on TP content in *G. mellonella* 7th stage larvae, pupae and adults

All oxyclozanide concentrations in the diet increased the TP content in larval, pupal and adult stages of the insects, and the differences were statistically significant. In

the control group, the 7th stage larva TP content was recorded as 39.00 ± 1.87 mg / ml, the pupal TP content was 20.75 ± 1.08 mg / ml and the adult TP content was 12.25 ± 1.86 mg / ml.

Comparison of the results obtained with 0.03 % oxyclozanide group with that of the control group demonstrated that the TP content in the larvae was almost double in the oxyclozanide group. It was determined that TP content was 84.00 ± 2.57 mg / ml in the larvae cultured with the diet that contained 0.03 g oxyclozanide (Fig. 5). Similar to the larval TP content, the TP content increased to 57.00 ± 1.54 mg / ml in the pupae cultured with the diet that contained 0.03 % oxyclozanide concentration (Fig. 6). A similar finding was also observed in the adult stage. It was determined that the TP content was 12.25 ± 1.86 mg / ml in adult phase in the control group and this value increased to 41.25 ± 1.43 mg / ml with the diet that contained 0.03 % oxyclozanide (Fig. 7).

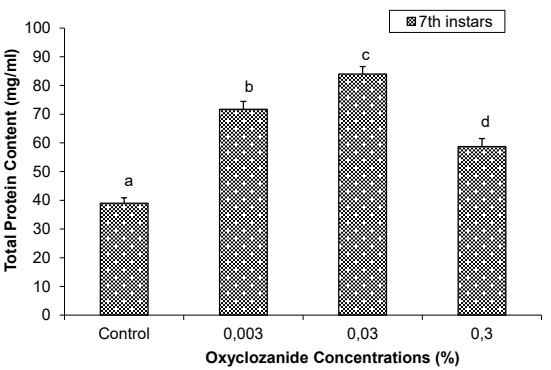

Fig. 5. Effects of oxyclozanide on *G. mellonella* larval total protein content. The data are given as the mean of four replicates. Five larvae were used for each replicate test. There were no differences between the values indicated with the same letter, P > 0.05 (LSD Test). Control diet (without oxyclozanide).

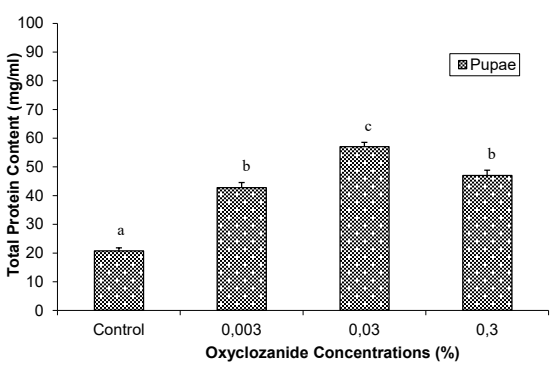

Fig. 6. Effects of oxyclozanide on *G. mellonella* pupal total protein content. The data are given as the mean of four replicates. Five pupae were used for each replicate test. There were no differences between the values indicated with the same letter, P > 0.05 (LSD Test). Control diet (without oxyclozanide).

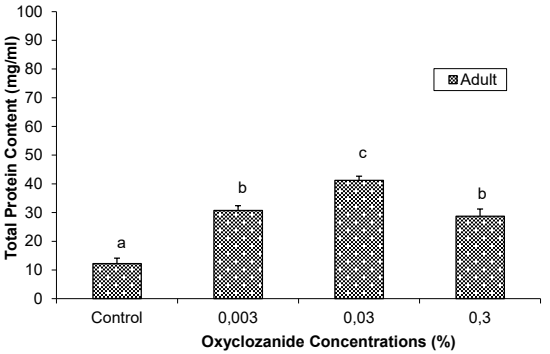

Fig. 7. Effects of oxyclozanide on *G. mellonella* adult total protein content. The data are given as the mean of four replicates. Five adult were used for each replicate test. There were no differences between the values indicated with the same letter, P > 0.05 (LSD Test). Control diet (without oxyclozanide).

DISCUSSION

The insects (Insecta), located in the Hexapoda subphylum of the Arthropoda phylum, include approximately 70 % of all species living in the world. Furthermore, new species that are scientifically identified every passing year are added to this count. Several insect species harm one third of the global crops, thus they have both ecological and economical significance (Aydın, 2011).

In the present study, the effects of oxyclozanide that was added to an artificial diet on survival, growth and TP content of *G. mellonella* were investigated. It was determined that all oxyclozanide concentrations demonstrated significant effects on all parameters investigated for the insect. Similar results were obtained with previously studied anthelminthic substances (niclosamide from the salicylanilide group, triclabendazole, mebendazole and oxfendazole from the benzimidazole group) (Büyükgüzel & Kayaoğlu, 2014; Kılıç et al, 2015; Çalık et al, 2016; Sugeçti et al, 2016).

In a study conducted with niclosamide, an anthelmintic substance from the salicylanilide group, it was determined that niclosamide reduced the survival rate of the insect in 7th stage larva, pupa and adult stages, 1.0 g concentration increased the adult growth period and increased the longevity of male individuals (Büyükgüzel & Kayaoğlu, 2014). In the present study, it was also determined that the survival rate decreased in the 7th stage larvae, pupae and adult insects, however the differences in the growth period and adult longevity of the insect were statistically insignificant. In previous studies that supported the results of the present study, benzimidazole group anthelmintic drugs, namely triclabendazole, mebendazole and oxfendazole, were used and it was concluded that administration of anthelmintic drugs decreased the percentage of the insect larvae that reached the 7th, pupal and adult stages (Çalık et al, 2016; Sugeçti et al, 2016). Comparison of the results obtained in 0.3 g oxyclozanide administered and control groups demonstrated that oxyclozanide significantly reduced the rate of the insects that reached larval, pupal and adult stages. Similarly, it was determined that 0.1 % g triclabendazole

concentration significantly decreased the rate of the insects that reached pupal and adult stages (Kılıç et al, 2015).

The physical structure of holometabolous insects have the ability to store diets ingested during the whole larval period. The diets ingested and stored during the larval period are utilized during the metamorphosis and the adult stages (Aksoy, Bahadıroğlu & Toroğlu, 2015b). Furthermore, previous studies demonstrated that the chemical substances added to insect diets had an adverse effect on the biologic properties of the insect by damaging the quality of the diets (Nielsen & Toft, 2000; Kılıç et al, 2015). The holometabolous *G. mellonella* utilizes the diet intake in the larval stage until the adult stage. Based on the results of the present study, the fact that the chemical substance ingested with the diet had negative effects on the survival and development of the insect could be due to the negative effects of this chemical substance on the consumption of the diets by reducing the quality of the diet.

Lipids, carbohydrates and proteins are the major biomolecules that required for the physiological functions of insects (growth, development and reproduction) (Van der Horst, Vroemen, & Van Marrewijk, 1997). It was demonstrated that oxyclozanide had positive effects on the TP content in 7th stage larvae, pupae and adult *G. mellonella*. This increase could be due to the active utilization of the diets ingested with oxyclozanide by the insect. The determination of changes in the TP content in the whole body or a specific tissue of an insect is important in determining whether the substances in the diet were effectively used by the insect and whether it was effective on the growth of the insect (Büyükgüzel, 2002; Büyükgüzel & İçen, 2004; Sak, Uçkan, & Ergin, 2006; Sak, Ergin, Uçkan, Rivers, & Er, 2011). Some studies have shown that synthetic chemicals, herbal pesticides and biopharmaceuticals are effective on the body weight of insect by altering the amount of lipid, carbohydrate and protein (Vijayaraghavan & Chitra, 2002; Wang et al, 2005; Sezer & Ozalp, 2011).

It is known that chemical substances have important effects on proteases that provide free amino acids in insects and also these substances have important effects on the formation of enzymes which help to eliminate the toxic effect (Ramaswamy, 1987). Many studies have shown that there is a relationship between the tolerance of toxic properties of insecticidal chemical substances and total body protein (Nath, Suresh, Varma, & Kumar, 1997; Ahmed, Wilkins, & Mantle, 2002; Guedes, Oliveira, Guedes, Ribeiro, & Serrao, 2006). All tested oxyclozanide concentrations demonstrated a positive effect on the TP levels in larvae, pupae and adult stages of the insect, which could be due to the development of a tolerance for the chemical substance added to the diet.

The total amount of protein is known to be an important biomolecule in all stages of development of the insect. Our total protein content results have clearly showed that the effects of oxyclozanide on insect. It was determined that increasing concentrations of the tested chemical substance had adverse effects on the biological parameters of the insect. Since the tested 0.3 % oxyclozanide concentration had significant negative effects on the survival and developmental stages of the insect, the said substance could be evaluated for use as an alternative insecticide.

Oxyclozanide and *G. mellonella*

Increasing number of studies are conducted on the effects of anthelmintic substances on insects (Büyükgüzel & Kayaoğlu, 2014; Kılıç et al, 2015; Çalık et al, 2016; Sugeçti et al, 2016). In the present study, where the salicylanilide group anthelmintic oxyclozanide was used, it was determined that increasing oxyclozanide concentrations had negative effects on important parameters of greater wax moth *G. mellonella*, an agricultural pest, such as survival rate and development and also the changes in TP content in all developmental stages were identified in the study. The fact that the results were obtained with very low oxyclozanide concentrations (0.003, 0.03 and 0.3 g / 100 g) could make it possible to control the target pest without harming humans, the environment and non-target organisms. These results are also significant in terms of identifying the capacity oxyclozanide as an insecticide with further studies. However, in order to fully identify and understand the effects of this substance on *G. mellonella* and its uses in the field in practice as an insecticide, further detailed experiments should be conducted.

ACKNOWLEDGEMENTS

This study was supported by Zonguldak Bülent Ecevit University, Research Fund (Project No: 2015-50737594-03).

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Received: December 18, 2017 *Accepted: January 22, 2019*

J. Entomol. Res. Soc., 21(1): 109-114, 2019
Print ISSN:1302-0250
Research Article
Online ISSN:2651-3579

A New Record of the Non-Biting Midge Larvae *Heterotrissocladius marcidus* (Walker, 1856) (Diptera: Chironomidae) for Turkey with Notes on Their Ecology

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ABSTRACT

Larvae of the chironomid genus *Heterotrissocladius* and its species *H. marcidus* are recorded in Turkey for the first time. The chironomid samples were collected from natural lake Nazlıgöl in Yedigöller National Park located in the northern part of Bolu Province. Within the scope of this study nine specimens of *H. marcidus* were identified.

Key words: Diptera, Orthocladiinae, taxonomy, limnofauna.

Akyıldız, G.K., Bakır, R., & Duran, M. (2019). A new record of the non-biting midge larvae Heterotrissocladius marcidus (Walker, 1856) (Diptera: Chironomidae) for Turkey with notes on their ecology. *Journal of the Entomological Research Society,* 21(1), 109-114.

INTRODUCTION

The chironomids are the most common and abundant insect group in both aquatic and terrestrial environments. Despite the growing effort of revealing the chironomid richness in Turkey (Özkan & Ahıska, 2017; Akyıldız, Taşdemir, & Ustaoğlu 2015; Bakır, Akyıldız & Duran, 2012; Taşdemir, 2010; Arslan, Ayık, & Şahin, 2010, Arslan, & Şahin, 2006), these efforts have not yet been completed. The Turkish chironomid fauna has been studied with almost 200 species in several running and still waters so far (Akyildiz, 2013; Bakır, 2012; Çağlar & İpekdal, 2004). *Heterotrissocladius* (Spärck, 1923), which has not been yet reported in Turkey, is a genus of non-biting midges in the subfamily Othocladiinae of the bloodworm family Chironomidae. So far the most comprehensive work has been done by Saether (Saether, 1975). It is known that larval *Heterotrissocladius* (Spärck, 1923) occurs in lakes (littoral to profundal), ponds, puddles, and in all sizes of flowing waters (Cranston et al, 1983). The genus is Holarctic, with up to 15 species. With this study, both a new record of the genus *Heterotrissocladius* (Spärck, 1923) has been reported for Turkey and a new contribution has been made to the ecological preferences of the new record species *H. marcidus* (Walker, 1856) from Turkey.

MATERIAL AND METHODS

Nine specimens were collected from lake Nazlıgöl (40.937748° N, 31.741877° E) which is one of the natural ponds of Yedigöller National Park located in the northern part of Bolu Province. The lake is located within the national park protection area. Chironomidae larvae were collected by using a hand net (500µm mesh size) in August 2014. The samples were preserved in 70% ethanol and sorted to morphotypes under the stereomicroscope. Species identification was performed by using a binocular microscope at high magnification (40x and 100x objectives). Larval stages of chironomids were decided to follow Schmid (1993). Euparal was used in the current study to obtain permanent slides. The required equipment and the mounting media are described in Epler (2001). The preparations are stored in the laboratory archives. Photograph images showing morphological characters were taken with using a camera and measurements and comparisons could be made. The software ImageJ (Schneider, Rasband & Eliceiri, 2012) was used to measure body parts. The identification up to the species level was done with the aid of the taxonomic keys of Saether (1975), Cranston et al, (1983), Klink & Moller-Pillot (2003) and some other taxonomic descriptions. In general the terminology follows Saether (1971, 1974).

RESULTS

In the following description the measurements are given as ranges followed by a mean value when four or more measurements have been taken, followed by a number in parentheses giving the number measured (n). The fourth instar larvae were taken into consideration for the measurements and proportions in the diagnosis. Antenna, labrum, mandible, mentum, maxilla and body are used in the description of the genus and the species (Fig. 1).

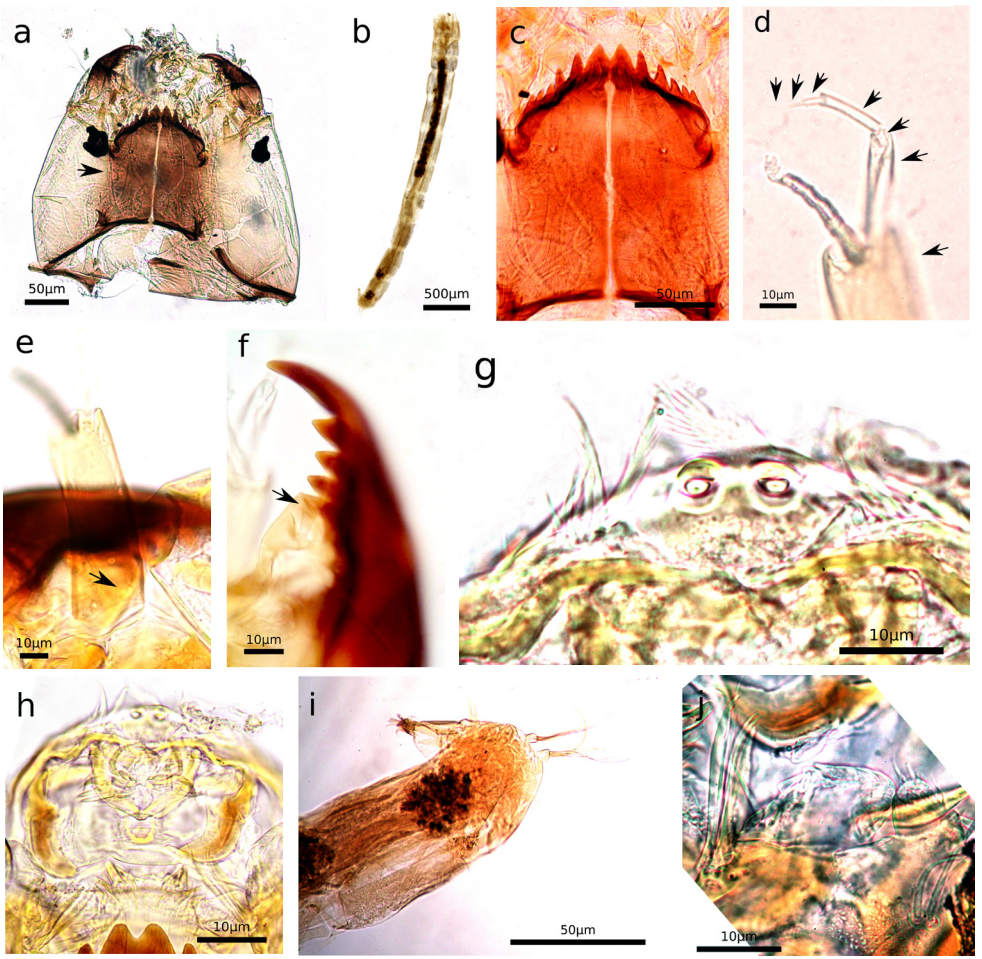

Fig. 1. *Heterotrissocladius marcidus* a. Head capsule, b. Thorax, abdomen c. Mentum, d. Antennal segments, e. Ring organ, f. Mandible, seta subdentalis, g. Labral lamella and SI, h. Labral lamella and premandibles, i. posterior parapods, anal setae, j. Maxillary palp.

Larva: The coloration of the body is yellowish brown and with brownish black submentum which is conspicuously darker than the surrounding areas of the head capsule. The head capsule width is about 430 µm (9). The mean body length of the larvae is measured as 6.20 mm (8). Procercus is well developed. Anal tubules are shorter than posterior parapods. Body setae are inconspicuous.

Antenna: Antenna with 7 segments. Segment lengths are not distributed in an hierarchical order. The third and the seventh segments should be considered in diagnosis. The third segment is the shortest segment, and the seventh segment is hair-like and vestigial. The ratio of the length of the basal antennal segment divided by the length of the combined apical segments (AR) is 1.18 (6). Basal antennal

segment 3.1 (6) times as long as wide. Ring organ placed in basal ¼ of the first segment. Blade is shorter than segment four and the length is 40µm (3) at the apex. Lauterborn organs are absent.

Labrum: Labral lamella is present and apically rounded. SI is in a plumose form. Horse shaped basal sclerite of ungula is well developed. Pecten epiharyngis is weakly sclerotised and consisting of 3 serrated scale-like spines. Chaetulae laterales are simple. Premandible is bifid, without a brush and 72µm (6) long and 16.5µm (6) wide.

Mandible: The coloration of the mandible is dark brown. Mandible is with 3 inner teeth. Apical tooth is relatively shorter than the combined width of inner teeth. The inner teeth are in the same order and size. The ratio of the length of the apical tooth divided by the length of the combined inner teeth is 0.95 (6). Seta interna is present with 6-7 plumose branches. Seta subdentalis is 18µm (6) relatively long, extending beyond the first inner tooth and several seta-like toothlets bearing at its basis.

Mentum: The colouration of the mentum is dark as it is in mandibles. Mentum is with two median teeth and it is extending beyond the apex of the first lateral tooth. The first and second lateral teeth distinctly longer than the outer three laterals. Median tooth width is about 20µm (8) and it is about twice as broad as first lateral tooth. Ventromental plate bulbous at the apex and protruding outside the line of teeth and its width is about 25µm (8). Beard is absent. Distance between two seta submenti is about 80µm (8).

Maxilla: Maxilla with broad anterior lacinial chaeta. Chaetulae of palpiger and 2 seta maxillaris are visible. Pecten galearis is present with distinct teeth. Maxillary palp is relatively short, the ratio of the length to the width is about 1.06 (6).

DISCUSSION AND CONCLUSION

H. marcidus (Walker, 1856) is a common species that can be considered as spreading in the Nearctic and Palearctic regions and its larvae are among the most common chironomids in alpine and subalpine lakes (Goffova, Bitusik, Ciamporova-Zatovicova, Bukvova, & Hamerlik, 2015; Mousavi & Amundsen, 2012; Lods-Crozet, Oertli, & Robinson, 2012; Saether, 1975). However, it had not been reported from Turkey before. Through this study, the larvae of chironomid genus *Heterotrissocladius* (Spärck, 1923) and its species *H. marcidus* (Walker, 1856) were identified and presented in Turkey for the first time. The measurements and the ratios are also given in the study. In addition, the region, where the larvae were identified, is very important for understanding the ecological preferences of the species. Lake Nazlıgöl is located between Bolu-Zonguldak provinces and between the forests of Western Black Sea. The highest elevation in the region is 1488 m a.s.l. and the lowest level is 465 m a.s.l., while Nazlıgöl is located at 878 m a.s.l. altitude in that region where the Black Sea climate dominates. The lake is fed only by the Black Creek, and various organic materials are carried by the falling rain and snow waters. Kazanci & Türkmen (2008) have reported that the water quality of this region is in I-II quality class and the lake is in the mesotrophic condition. Although not in terms of altitude,

considering the ecological characteristics of the habitat where *H. marcidus* (Walker, 1856) was found, it overlaps with the water quality and ecological characteristics of several studies (Moubayed-Breil, Ashe, & Langton, 2012; Rieradevall, Chave & Prat, 2007; Goffova et al, 2015) and it is known that food quality and quantity can be more important than the direct effect of temperature on larval growth (Anderson & Cummins, 1979). Therefore, we are able to say that the ecological preferences of this species such as nutrient and environmental conditions are more effective than the effects of altitude.

This study contributes to the Turkish limnofauna by introducing the new record genus *Heterotrissocladius* (Spärck, 1923) and the species *H. marcidus* (Walker, 1856). It is obvious that *H. marcidus* (Walker, 1856) can be widely collected in Turkey with more detailed field studies. It is also possible that the other *Heterotrissocladius* (Spärck, 1923) species are distributed in Turkey.

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Received: January 23, 2018 *Accepted: January 23, 2019*

J. Entomol. Res. Soc., 21(1): 115-127 2019 *Research Article*
Print ISSN:1302-0250 *Online ISSN:2651-3579*

Host-associated Genetic Differentiation of the Green Citrus Aphid, *Aphis spiraecola* (Hemiptera: Aphididae) in Algeria

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ABSTRACT

The green citrus aphid, *Aphis spiraecola* Patch, is an important pest attacking many plant species, including citrus. We have analyzed the genetic variability among green citrus aphid adults sampled from six citrus cultivars grown in Algeria (an orange, a grapefruit, a lemon and three mandarin cultivars), using the random-amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. Analysis of molecular variance (AMOVA), based on RAPD markers, indicated a significant difference between the studied samples, correlated to the host plant species, while host cultivar and geographical origin had no significant impact on the genetic diversity. Two-dimensional PCO analysis confirmed AMOVA results, showing the grouping of the different insects into four major clusters according to their host plant species. The Neighbor-Joining dendrogram constructed based on the Euclidian distance grouped the accessions into four main clusters according to their host plant species genotypes, giving insight into the coevolution of insect strains with their corresponding citrus species. In order to investigate any possible relationship between the genetic aggregation of insect genotypes and the leaf morphology in citrus species, we carried out leaf morphological characterization and surveyed the degree of infestation of the studied citrus cultivars. Leaves of grapefruit and orange were the most similar morphologically and the most attacked by aphids, suggesting that genetically close biotypes would be compatible with these two species. Results of this study are a step toward the development of an integrated controlling strategy against *A. spiraecola* in North Africa, taking into account the host specialization that seems to play a key role in shaping the genetic diversity of *A. spiraecola*.

Key words: Citrus, *Aphis spiraecola*, RAPD-PCR, genetic structure, host strains.

Lebbal, S., Mezghani-Khemakhem, M., Bouallegue, M., Ben Amara W., Khalfallah Y., Makni, M., & Bouktila, D. (2019). Host-associated genetic differentiation of the green citrus aphid, *Aphis spiraecola* (Hemiptera: Aphididae) in Algeria. *Journal of the Entomological Research Society*, 21(1): 115-127.

INTRODUCTION

Aphis spiraecola Patch 1914 is a cosmopolitan pest (Barbagallo, Cocuzza, Cravedi, & Komazaki, 2007). It can colonize a very wide range of secondary hosts belonging to more than 20 plant families, including Caprifoliaceae, Asteraceae, Rosaceae, Rubiaceae and Rutaceae (Blackman & Eastop, 2006). Citrus species are the most important hosts for this insect; therefore, another scientific name, *A. citricola* auctt. nec Van der Goot 1912 has been used (Andreev, Rasheva, & Kutinkova, 2009). The primary economic impact of this insect not only arises from foliage distortion, but also from promoting growth of sooty mold and attracting ants which fend off natural enemies of aphids; especially, spring-time attacks are the most deleterious in young citrus orchards (Satar & Uygun, 2008). In addition, it can transmit phytoviruses such as cucumber mosaic virus (CMV), plum pox virus (PPV) and zucchini yellow mosaic virus (ZYMV) (Blackman & Eastop 2007) and may also involve in the transmission of citrus Tristeza virus (CTV) (Yahiaoui, Djelouah, D'Onghia & Catara, 2012).

Hermoso de Mendoza, Arouni, Belliure, Carbonell, & Pérez-Panadés (2006) considered *A. spiraecola* among the most harmful citrus aphids in the Mediterranean region. In Algeria, it is very harmful to citrus fruits (Saharaoui, Benzara, & Doumandji-Mitiche, 2001). Its occurrence in different citriculture regions has been reported by several authors (Benoufella-Kitous, Doumandji, & Hance, 2014; Lebbal & Laamari 2015; Lebbal & Laamari 2016; Ali Arous, Guenaoui, & Djelouah, 2017). It is attacking different citrus species and varieties such as orange (Labdaoui & Guenaoui, 2015), Clementine (Mostefaoui, Allal-Benfekih, Djazouli, Petit, & Saladin, 2014), mandarin (Lebbal, 2017) and lemon (Saharaoui & Hemptinne, 2009). The spreading of *A. spiraecola* has been facilitated by the existence of few effective parasitoids of this aphid (Labdaoui & Guenaoui, 2017). Even though it has been frequently reported, there is a deep lack of studies focusing on the population genetic structure of *A. spiraecola* in Algeria. Therefore, a clear understanding of the potential paths of gene flow could provide useful insights for the management of this pest.

There is a growing awareness of the importance of natural selection in driving population genetic divergence in agricultural pests, and several cases of this differentiation are provided by the existence of genetically distinct host forms in phytophagous insects. The genetic differentiation of insect communities usually occurs through feeding and/or oviposition site choices. These choices are usually driven by the genetic variation in host plants, leading to host-associated differentiation potentially driving insect evolution and speciation (Zytynska et al, 2014). Herbivorous insects are known to feed on a restricted range of plants, and herbivore preference and performance can vary among host plants within a species due to genetically based traits of the plant (e.g. defensive compounds) or among a range of host species. In a natural system, genetic variation within both plant and herbivore communities is a major factor that influences species interactions. Sap-sucking insects, such as aphids, experience an intimate relationship with their host plant and many aphid species exhibit genetic variation in host preference and performance on different host plants (Nikolakakis, Margaritopoulos, & Tsitsipis, 2003). Different aphid genotypes have

also been found to preferentially colonize different host-plant genotypes (Zytynska & Preziosi, 2011). The term 'biotype' can be defined as an intraspecific classification, segregating individuals by a divergent phenotypic response to an ecological variable (Wenger & Michel, 2013). Biotypic differentiation is a common phenomenon in many insect pests, which is also of major concern in identification and deployment of host resistance genes in crop plants (Weng, Perumal, Burd, & Rudd, 2010).

Molecular markers have greatly enhanced our understanding of the genetic diversity and population structure of many insects (Charaabi et al, 2008; Mezghani-Khemakhem, Bouktila, Kharrat, Makni, & Makni, 2012; Kharrat, Mezghani-Khemakhem, Bouktila, Makni, & Makni, 2012; Abdallah, Mezghani-Khemakhem, Bouktila, Makni, & Makni, 2012, 2013; Béji et al, 2013; 2015). The Random-Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique (Williams, Kubelik, Livac, Rafalski & Tingey, 1990) has been frequently used in several studies, to evaluate the genetic diversity of some major invasive species, such as the Mediterranean fruit fly, *Ceratitis capitata* Wiedmann (Haymer, He, & McInnis, 1997), the potato whitefly, *Bemisia tabaci* Gennadius (Hasan, 2006) and the date palm root borer, *Oryctes agamemnon* Burmeister (Abdallah et al, 2012). This technique is rapid, simple, and has the advantage that no prior knowledge of the genome is necessary (Babu, Rajesh, Samsudeen, Minoo, Suraby, Anupama, & Ritto, 2014). In addition, it requires only small amounts of DNA (Kumar & Gurusubramanian, 2011).

The study of the specialization of aphids to a botanical species can contribute to a better understanding of the host plant-aphid interaction, and thus to improve the methods of controlling these bio-aggressors. In this study, an attempt has been made to evaluate the genetic variability within individuals of the green citrus aphid from different citrus species and varieties in Algeria, and characterize the eventual relationship between insect infestation and host plant leaf morpho-metric variables.

MATERIALS AND METHODS

Sampling procedure

During the study period, from January to December 2013, *A. spiraecola* insects as well as citrus leaves were sampled from citrus trees planted in the experimental orchard of the "institut technique de l'arboriculture fruitière (ITAF)" (Emjez Edchich, Skikda). This orchard (36° 42' N and 6° 47' E) is located on an altitude of 200 m, and includes an area of 73.12 ha, reserved to fruit trees.

For citrus leaves, 16 young infested leaves were taken from four trees representing each citrus species, at the rate of four leaves / tree, following the four cardinal points. These young leaves were collected at the tips of young shoots and *A. spiraecola* aphids were counted on each leaf. The same sampling technique has been already applied by Fadamiro et al (2008), Yoldas et al (2011), Kamel (2010) and Mostefaoui et al (2014), to study aphid infestation on vegetative organs of crop trees, in the United States, Turkey, Egypt and Algeria, respectively.

For *A. spiraecola* aphids, only one aphid per tree was analyzed and samples were taken from trees that were at least 10 m apart from each other, in order to avoid biases caused by collecting individuals resulting from the same clone. In total, seventeen adults of *A. spiraecola* were kept for the molecular analysis. These specimens were collected from trees of sweet orange (cv. Thomson Navel), grapefruit (cv. Shambar), lemon (cv. Eureka) and mandarin (cv. Commune, cv. Ortanique and cv. Carvalhal). Two additional individuals were used for comparison purposes, which were collected on lemon cultivar (cv. Eureka) grown in the region of Menzel Bouzelfa, in Tunisia (36° 69' N; 10° 67' E). Aphids were stored in micro tubes in 75% ethanol at 20°C prior to DNA extraction. The botanical relationships between citrus species used for sampling as well as the numbers of *A. spiraecola* samples collected, by host species, are shown in figure 1.

DNA extraction and RAPD-PCR analysis

The DNA was extracted from 19 *A. spiraecola* adults using cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987). The DNA extracted from the different samples was suspended in 30 µl of autoclaved distilled water. DNA concentration was quantified by the Qubit apparatus (Qubit® 3.0 Fluorometer, Invitrogen). DNA amplification from each individual insect, was carried out in a total volume of 25 µl, containing the DNA extract, 5 µl of buffer (5x Green Gotaq®) of the polymerization enzyme, 25 mM of $MgCl_2$, 10 mM of each dNTP (dATP, dGTP, dTTP, and dCTP), 10 µM of each primer, 5 U of *Taq* DNA polymerase. The PCR process was carried out in a 2720 thermocycler (Applied Biosystems, Carlsbad, California, USA), programmed as follows: an initial denaturation (3 min at 94 °C.), followed by 35 cycles of amplification (1 min of denaturation at 94 °C, 1 min of annealing at 36 °C, 1 min of extension at 72 °C) and a final extension (7 min at 72 °C).

Initially, a total of 10 oligonucleotides (Operon Technologies, Almeda, CA, USA) were tested as RAPD-PCR primers. These oligonucleotides were OPA09 (5'- GGG TAA CGC C- 3'), OPA12 (5'- TCG GCG ATA G-3'), OPA14 (5'- TCT GTG CTG G-3'), OPA19 (5'- CAA ACG TCG G-3'), OPH11 (5'-CTT CCG CAG T-3'), OPH18 (5'- GAA TCG GCC A- 3 '), OPH20 (5'- GGG AGA CAT C-3'), OPD9 (5'-CTC TGG AGA C-3'), OPD14 (5'-CTT CCC CAA G-3 '), OPD17 (5'-TTT CCC ACG G-3'). On the basis of the number of markers generated by each one, four primers were retained, namely OPA9, OPA12, OPH18 and OPH20. All amplifications were conducted twice to check for their reproducibility. RAPD-PCR products were subjected to electrophoresis on 1.5% agarose gels, in Tris-Borate-EDTA buffer, then visualized under UV light, after staining in 0.5 µg mL ethidium bromide, and photographed using a photo documentation system (Vilber, Marne-la-Vallée, France). A molecular weight marker (1 Kb Ladder, Invitrogen, Carlsbad, California, USA) was used as a standard in electrophoresis.

RAPD data analysis

RAPD-PCR profiles for each aphid sample were identified visually by scoring the presence or absence of all reproducible bands. The finalized fragment data from all four

primers were pooled to define a single binomial phenotype for each of the 19 samples. Analysis of molecular variance (AMOVA) was performed using GenAlEx v. 6.502 (Peakall & Smouse, 2012), with a test of significance by 999 permutations, in order to compare differences among the aphid samples according to the host-plant, host cultivar and geographical origin. Pairwise Euclidian distances between pairs of samples were calculated using PAST 2.17c (Hammer et al, 2001) and subjected to principal coordinates analysis (PCO) and dendrogram construction by the neighbor-joining (NJ) method, to study the variation among *A. spiraecola* populations.

Leaf morphological characterization and infestation assessment

In order to analyze the relationship between the morphology of citrus leaf and infestation by *A. spiraecola*, young leaves sampled as described above, were used to assess 11 leaf descriptors: (a) the length and (b) width of the leaf; (c) the length of the petiole; (d) the presence of wings and (e) their width; (f) green color intensity; (g) emargination; (h) leaf shape in cross section; (i) leaf twisting, (j) blistering and (k) margin undulation. Parallel to these descriptors, we have evaluated the degree of infestation of each citrus species. Infestation rates as well as morphological characters were submitted to analysis of variance (one way ANOVA) with Student-Newman-Keuls test, using SPSS software (version 10).

RESULTS

RAPD polymorphism assessment

All amplification products used in the statistical analysis were reproducible. Each of the four primers used in the RAPD-PCR technique generated a different number of DNA markers according to the genotypes of the analyzed aphids. A wide range of markers, varying in size between 100 bp and 1,500 bp, was obtained, indicating that the amplification involved diverse loci, among which 32 were polymorphic from the 19 aphids analyzed with the four primers. The number of polymorphic markers obtained by each primer, separately, ranged from 2 (OPH-18) to 15 (OPH-20) with an average of 10 markers per primer (Table 1).

Table 1. Total number of randomly amplified polymorphic DNA (RAPD) fragments (N), number (P), percentage (%P) and size range of polymorphic fragments generated by four RAPD primers.

Primer	Nucleotide sequence (5'-3')	N	P	%P	Size range
OPA-09	5'-GGG TAA CGC C-3'	10	8	80.00	100 pb - 1500 pb
OPA-12	5'- TCG GCG ATA G-3'	8	7	88.00	100 pb – 1020 pb
OPH-18	5'- GAA TCG GCC A- 3'	4	2	50.00	150 pb - 1020 pb
OPH-20	5'- GGG AGA CAT C-3'	16	15	94.00	250 pb - 1160 pb
All primers		**38**	**32**	**84.00**	**100 pb – 1500 pb**

Analysis of molecular variance (AMOVA)

AMOVA analysis obtained from the distance matrix enables the partitioning of the overall RAPD variation between defined groups. Three sources of variation were defined according to the geographic origin (Skikda, Algeria vs. Menzel Bouzelfa, Tunisia), the host plant species (Mandarin vs. orange vs. grapefruit vs. lemon), and the host plant cultivars (cv. Eureka vs. cv. Thomson Navel vs. cv. Shambar vs. cv. Commune vs. cv. Ortanique vs. cv. Carvalhal). Results of AMOVA did not indicate any genetic differentiation associated with the geographical areas or host plant cultivars ($p = 0.460$ and 0.2, respectively). However, the host-plant species had a significant effect on the partitioning of the total genetic diversity ($p < 0.05$) (Table 2).

Table 2. AMOVA analysis of the different sources of polymorphism variation.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p-value
(A) Among Host plant species within individuals	3	29.7	1.23	22%	**0.01 (p < 0.05)**
(B) Among Host plant cultivars within individuals	5	41.98	0.00	25%	0.2 (NS)
(C) Among host plant countries within individuals	1	5.24	1.36	0%	0.460 (NS)

DF: Degrees of freedom.

Factorial analysis

The representation of genetic relatedness was depicted by two-dimensional principal coordinates analysis (PCO) based on Euclidian similarity which revealed diversity among different *A. spiraecola* samples. The first and second coordinates revealed 24.62 % and 17.60 % of the variations in the standardized data set of the 19 samples. The PCO showed significant grouping of the insects that were plotted into four sub-plots, representing each group of *A. spiraecola* with distinctive features (Fig. 2). The groupings were largely in accordance with their host species, irrespective of the origin of samples or the host cultivar. It is noteworthy that insects colonizing mandarin were the most heterogeneous genetically according to PCO axes 1 and 2, and this heterogeneity was not dependent on the mandarin variety. Such result would be, most likely, due to the fact that the host species (mandarin) is a direct genitor of sweet orange and indirect genitor of both grapefruit and lemon (Fig. 1) within the Rutaceae family.

Cluster analysis

The Neighbor-Joining dendrogram showed four clusters corresponding to the four studied citrus species (Fig. 3). Genetic clusters did not show any sub-grouping of the analyzed insects with respect to the host plant cultivars or the geographical location as *A. spiraecola* samples collected on lemon from Tunisia did not cluster distinctly from those collected on the same citrus species from Algeria. This pattern of genetic grouping supports results of PCO, further suggesting that insect genotypes within the same cluster would have co-evolved with the gene pool of the corresponding host citrus species.

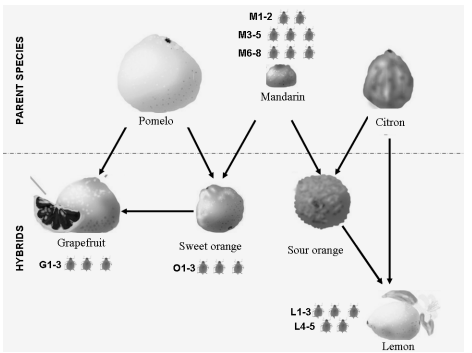

Fig. 1. Botanical relationships, within the family of Rutaceae, between citrus species used for sampling, and numbers of samples of *Aphis spiraecola*, collected by host species or cultivar. M1-2: aphids sampled on mandarin cv. Commune, M3-5: aphids sampled on mandarin cv. Ortanique; M6-8: aphids sampled on mandarin cv. Carvalhal; G1-3: aphids sampled on grapefruit (cv. Shambar); O1-3: aphids sampled on sweet orange (cv. Thomson Navel); L1-3: aphids sampled on lemon (cv. Eureka); L4-5: aphids sampled on lemon (cv. Eureka) from Tunisia.

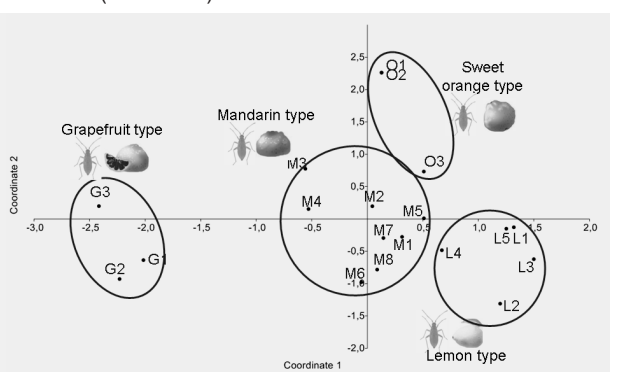

Fig. 2. Bidimensional principal coordinates analysis (PCO) scatter plot showing the pattern of diversity among 19 *Aphis spiraecola* samples collected on four citrus species and six cultivars, based on 32 polymorphic RAPD markers (populations are designated according to their labels in fig. 1).

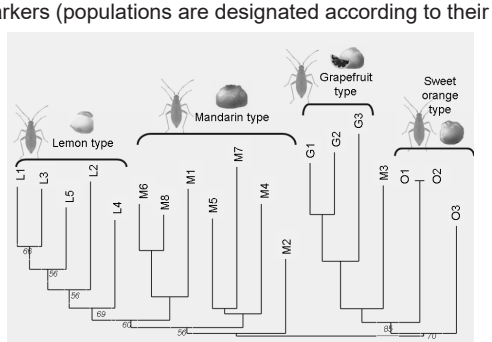

Fig. 3. Neighbor-Joining dendrogram illustrating pattern of genetic diversity of 19 *Aphis spiraecola* samples collected on four citrus species, based on 32 RAPD markers (populations are designated according to their labels in fig.1).

Morphological characterization of citrus species

Morphological markers correspond to the visually scoring of qualitative traits as well as measurements of quantitative traits that are influenced by plant biology. The selected accessions were characterized phenotypically in this study by comparing the leaf characteristics. The ANOVA analysis revealed a significant difference concerning the quantitative traits between the four species examined (p=0, p=0, p=0.009 and p=0.023, for the length of petioles, width of petiole wings, width of leaves and length of leaves, respectively) and for the leaf infestation degree (p=0.026). Sweet orange and grapefruit appeared to be relatively close morphologically to each other (similar dimensions of the leaf, similar length of the petiole, dark color of the leaf, presence of wings on petiole, a weakly concave leaf, and a weakly undulated leaf margin), and were the most heavily infested species (Table 3).

Table 3. Leaf morphological characteristics and degree of leaf infestation by *Aphis spiraecola*, in four citrus species.

Descriptors		Sweet orange	Grapefruit	Lemon	Mandarin
Leaf morphological characteristics	a. Mean length of leaves (cm)	8.7 [a]	9.27 [a]	7.52 [a]	5.61 [a]
	b. Mean width of leaves (cm)	4.47 [ab]	5.77 [a]	3.89 [ab]	2.60 [b]
	c. Mean length of petioles (cm)	1.07 [b]	1.82 [a]	0.60 [c]	0.81 [bc]
	d. Presence of petiole wings	+	+	-	-
	e. Mean width of petiole wings (cm)	0.30 [b]	1.05 [a]	0 [c]	0 [c]
	f. Leaf color	Dark green	Dark green	Light green	Dark green
	g. Emargination	+	+	+	+
	h. Leaf shape in cross section	Weakly concave	Weakly concave	Straight	Straight
	i. Twisting of leaf	Weak	Weak	Intermediate	Strong
	j. Blistering of leaf	+	+	-	-
	k. Undulation of leaf margin	Weak	Weak	Intermediate	Weak
Leaf infestation	Mean number of aphids / leaf in April 2015	95.37 [a]	50.75 [ab]	29.50 [b]	26.98 [b]

DISCUSSION

Analysis of the genetic diversity of *A. spireacola*, collected from different citrus species and varieties from Skikda (Algeria) and Menzel Bouzelfa (Tunisia), was performed based on 32 polymorphic DNA markers. Analysis of molecular variance (AMOVA), factorial analysis (PCO) and cluster analysis (NJ) did not show any significant difference among adult samples concerning their geographical origin (Algeria vs. Tunisia). This result may be related to the ability of winged aphids to move. In fact, the migration phenomenon has been well documented in several aphid species such as *Sitobion avenae* (Liewellyn, Loxdale, Harrington, Clark & Sunnucks, 2004) and *Elatobium abietinum* (Halldórsson et al, 2004). Several authors (Zitoudi, Margaritopoulos, Mamuris, & Tsitsipis, 2001; Halldórsson et al, 2004; Figueroa et al,

2005; Béji et al, 2013; Jun, Michel, Wenger, Kang, & Rouf Mian, 2013) mentioned that the region has no significant effect on genetic variability in many aphid species. Further, some populations of Italian peach-potato aphid, *Myzus persicae* were reported to have similar insecticide resistance profiles, independent of geographical origin (Monti et al, 2016). In addition, Callejas, Beitia, Gobbi, Velasco, & Ochando (2005) have shown that populations of whitefly *Bemisia tabaci* collected from several Canary Islands are of the same biotype.

Our results suggest that genetic distance between *A. spiraecola* individuals is rather shaped by the citrus species from which they were collected. This fact is probably due to the selection pressure exerted by the nutritional requirements of each genotype of this aphid, which can only be assured by well-defined plant species. For example, Auclair & Boisvert (1980) reported significant differences in vitamin requirements in two biotypes of *Acyrthosiphon pisum* Harris. Similarly, the results of Caillaud & Via (2000) suggested that the major determinant of host specialization for pea aphids is the behavioral acceptance of a plant rather than the toxicity of the food source. In the case of *Aphis gossypii,* as previously reported by Carletto et al (2009), specialization occurred on three very different plant families, Cucurbitaceae, Solanaceae and Malvaceae, which exhibit a different phenology and probably a great variability in their sap compounds that might act as selective factors for host acceptance by aphids. This specialization process may be seasonal for some aphids such as *Aphis fabae* Scopoli (Powell & Hardie, 2000), while the pea aphid complex illustrates how insect biotypes blend into species by gradual reduction of gene flow (Peccoud et al, 2009).

Mezghani-Khemakhem et al, (2012) reported that the genetic variation of *A. spiraecola* populations, in Tunisia, was governed exclusively by the host plant species. Such a genetic variation strongly depending on the host plant has also been reported in *Sitobion avenae* F. aphids (Lushai, Markovitch, & Loxdale 2002), *Rhopalosiphum maidis* (Blackman, Halbert, & Carroll, 1990), *Aphis gossypii* (Charaabi et al, 2008), *Acyrthosiphon pisum* (Peccoud et al, 2009) and *Myzus persicae* (Rubiano-Rodríguez et al, 2014).

The genotypes of *A. spiraecola* analyzed in this study, showed no differences depending on the cultivars of mandarin. Apparently, the three cultivars used are genetically similar, leading to an offer of similar suitability and thus hosting the same aphid biotypes. Several authors have demonstrated genetic similarities between mandarin varieties (Uzun, Yesiloglu, Aka-Kacar, Tuzcu, & Gulsen, 2009; Youseif et al, 2014). Besides, some mandarin species, such as willow leaf mandarin, *Citrus deliciosa* Ten., were shown to have a low genotypic variability (Herrero et al, 1996).

The genetic analysis allowed classifying the individuals of *A. spiraecola* colonizing orange and grapefruit in close genetic groups. These two species of citrus are very similar. Grapefruit is the result of hybridization between orange and pummelo (*Citrus maxima*) (Luro, Gatto, Costantino, & Pailly, 2011), while the lemon tree is relatively distant (Youseif, El-Halwagi, Sayed, & El-Itriby, 2014). We have found that these two species were similar to each other with respect to the leaf morphological traits and were the most infested, suggesting that they are attacked by well adapted biotypes.

Recognition of a plant as a suitable host and subsequent feeding initiation by an aphid depends on a complex interaction between aphid and plant traits (Züst & Agrawal, 2016). Plants possess a great repertoire of traits (e.g. color, shape, odour, etc.) that insects could utilize to make oviposition decisions (Carrasco, Larsson, & Anderson, 2015). For instance, leaf color intensity (related to the photosynthesis ability) is important for attracting several fly and aphid species (Bernays & Chapman, 1994). In our case, it seems that large dimensions of grapefruit and orange leaves, compared with mandarin and lemon, were responsible for the attraction of winged *A. spiraecola*, providing a sufficient nutrition for proliferation.

In conclusion, our study revealed evidence for patterns of genetic structuring among *A. spiraecola* populations related to their host plants, suggesting a possible host specialization that should be taken into account in the development of integrated controlling strategies that should be varied, following the disrupted gene flow between populations colonizing different citrus species. Our results provide baseline information for monitoring the genetic structure of *A. spiraecola* in Algeria. The conclusions herein presented will need to be analyzed in depth, and extended to a larger geographical zone, based on larger sample sizes and combining several molecular markers.

ACKNOWLEDGEMENTS

Authors would like to thank the staff of the Technical Institute of Fruit Trees (ITAF, Skikda, Algeria), for their valuable help.

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Received: February 05, 2018 *Accepted: September 11, 2018*

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Material examined: Ankara, Altındağ, Çubuk Dam Lake, 900 m, 29.06.1998, 1 ♂; Kalecik, 600 m, 24. 07. 2001, 2 ♀♀, Kalecik, 800 m, 25. 07. 2001, 3 ♀♀
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