Acta Aquatica Turcica
E-ISSN: 2651-5474 15(1), 35-42 (2019) DOI: https://dx.doi.org/10.22392/egirdir.431101

The Influence of Leaf Litter on the Distribution of Aquatic Chironomidae Pupal (Diptera) Fauna in Tunca River (Edirne/Turkey)*

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Research Article

Received 05 June 2018; Accepted 17 July 2018; Release date 01 March 2019.

How to Cite: Özkan, N. (2019). The influence of leaf litter on the distribution of aquatic Chironomidae pupal (Diptera) fauna in Tunca River (Edirne/Turkey). *Acta Aquatica Turcica, 15*(1), 35-42.

Abstract

This study was designed to compare the potential differences in the colonization of Chironomidae pupal fauna in various leaf packs. The study was carried out in Tunca River (Edirne). Three stations were selected in the river and 5 different leaf packs (*Platanus orientalis* L., *Ulmus leavis* Pall., *Morus alba* L., *Juglans regia* L., and artificial *Buxus* sp.) were used to take samples. 20 kg potato bags were used during leaves packaging, prepared 5 packages of each leaves and a total of 25 packets were placed in each of the stations. Chironomidae pupal samples were collected from June 2012 to October 2012. The collected samples were placed in 70% ethyl alcohol, brought to the laboratory and diagnosed under stereo microscope. Then ANOVA test was used to analyse Chironomidae pupae in dates, stations, and leaf packs and 0.05 α statistical significance was used in all tests. When there was a meaningful difference, the reason was revealed by the Tukey test. Chironomidae pupae were found at most 3, 1 and 2. stations respectively and in leaf species of artificial *Buxus* sp. and *Ulmus leavis*. Eight species were identified in leaf packs. The *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804) was found to be the most common species.

Keywords: Chironomid pupae, community structures, leaf pack, Tunca River

Tunca Nehri'nde (Edirne / Türkiye) sucul Chironomidae pupal (Diptera) faunasının dağılımı üzerine yaprak çöpünün etkisi

Özet

Bu çalışma, çeşitli yaprak paketlerinde Chironomidae pupal faunasının kolonizasyonundaki potansiyel farklılıklarını karşılaştırmak için tasarlandı. Çalışma, Tunca Nehrinde (Edirne) gerçekleştirildi. Nehirde üç istasyon seçildi ve örnek almak için 5 farklı yaprak paketi (*Platanus orientalis* L., *Ulmus leavis* Pall., *Morus alba* L., *Juglans regia* L. ve yapay *Buxus* sp.) kullanıldı. Yaprakları paketlemede 20 kg'lık patates torbası kullanıldı, her yaprak çeşidinden 5 paket hazırlandı ve her istasyona toplam 25 paket yerleştirildi. Chironomidae pupal örnekleri Haziran 2012'den Ekim 2012'ye kadar toplandı. Toplanan örnekler %70'lik etil alkole konularak laboratuvara getirildi ve stereo mikroskop altında teşhis edildi. Daha sonra tarihler, istasyonlar ve yaprak paketlerinde Chironomidae pupalarının analizinde ANOVA testi ve tüm testlerde 0,05 α istatistiksel anlamlılık kullanıldı. Anlamlı bir fark olduğunda, Tukey testi tarafından neden kaynaklandığı ortaya çıkarıldı. Chironomidae pupaları sırasıyla 3, 1 ve 2. istasyonda ve yapay *Buxus* sp. ve *Ulmus leavis*'de en çok bulundu. Yaprak paketlerinde sekiz tür tespit edildi. *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804) en yaygın tür olarak bulundu.

Anahtar kelimeler: Chironomid pupaları, komünite yapısı, yaprak paketi, Tunca Nehri

***This research was supported by BAP of Trakya University (project number 2011-130). This work was presented as an oral presentation in the II. International Congress on Fisheries and Aquatic Research held in Nevşehir, Turkey on July 12-15, 2018 and its summary were published.**

INTRODUCTION

The Chironomidae (Insecta: Diptera), commonly known as non-biting midges, are holometabolous flies that typically occur in aquatic environments before emerging as adults on the water's surface. The chironomidae family is species-rich, with approximately 5,000 species described worldwide; however, as many as 20,000 species are estimated to exist. Furthermore, they are often the most abundant and widespread benthic macroinvertebrates in aquatic systems, typically accounting for 50% or more of the species in the community (Ferrington et al., 2008; Armitage et al., 1995).

The family is more than 120 million years old (Armitage et al., 1995) and has undergone extensive adaptive radiation to occupy a wider range of microhabitats at present than any other aquatic insect group. Although many species live in coastal, marine, and terrestrial environments, the Chironomidae are diversed in freshwater habitats (Jacobsen, 2008).

Chironomid midges are excellent indicators of enrichment in lentic environments (Rosenberg, 1993; Ruse, 2002). They are increasingly being used to assess other pollutants and environmental factors (Wright et al., 1996). Because of their high species richness, the great diversity of microhabitats that they occupy, and their wide collective tolerances for physical and chemical conditions, studying midges alone can be as effective as using a larger group of invertebrate taxa in bioassessment (Wright et al., 1996; King and Richardson, 2002).

Pupal exuviae sampling is widely used by benthologists in Europe for studying Chironomid distributions in relation to environmental variables and assessing water quality. Biomonitoring programs using midge pupal exuviae sampling are also being implemented in Madiera and other islands in Macaronesia.

The pupae are the transition phase between larvae and adults, which contains the reorganization of the tissues. When this rearrangement is complete, adult flies develop in the pupa and adult. They float on the water surface for emergence. Subsequently, adult flies are formed by dividing the thorax along the dorsal line. Pupa stage is relatively short (MacDonald and Taylor, 2006). Pupae can remain in the stage for several hours to several days and is relatively shorter than other stages (Oliver, 1971). Most Chironomini pupae live in larval tubers. After passing on the adult form, the remaining pupal sheath is filled with air and remains on the water surface (Ferrington et al., 1991).

According to relative analyses, benthic invertebrates are found both in natural and artificial colonies in rocks and leaf packs (Haapala et al., 2003; Sylvestre and Bailey 2005). One of the major carbon sources of rivers is the leaves that are poured out of the trees and reach to the water. Dried leaves are indispensable carbon sources, especially for the mountain sides (Hunter et al., 2003). It has been proved that the activities of certain benthic invertebrates increase with the amount of rotting leaves (Costa and Melo, 2008). The main source of dissolved organic and inorganic nutrients and particulate matter is litter from high places and from riverside trees. This litter is the basis of the food cycle (Petersen and Cummins, 1974).

Colonization depends on many factors such as movement of invertebrates, substrate composition, competition, nutrient supply, habitat and season. Many studies have been carried out on the mechanism of colony formation in North America and Europe (Townsend, and Hildrew, 1976; Williams, 1980; Nelson, 2000; Royer and Minshall, 2003). In Turkey, similar surveys were done by Duran (2006) and Özkan (2018) in lotic environment.

Although there are many faunistic and limnological studies in the Bulgarian part of the Tunca River (Dimitrov, 1972; Uzunov, 1980; Russev et al. 1984; Javena and Russev, 1985), in the Turkish part of this river there are only two graduate theses (Kavaz, 1997; Öterler, 2003), two phytoplanktonic studies (Öterler et al. 2003; 2004), two faunistic studies (Kırgız et al., 2005; Camur-Elipek et al., 2006), one studies colonization of leaf packs of Chironomidae larvae (Özkan, 2018) and one studies colonization of fresh water leech *Erpobdella octoculata* Linnaeus, 1758 (Annelida: Hirudinida) (Özkan, 2018a). In this study, we aimed to determine whether Chironomidae pupae prefer leaf packaging for nutritional or protective purposes and preferences of and to determine the advantages of leaf packaging in faunistic study.

MATERIALS and METHODS

This study was carried out between May and October 2012 at three stations located in the Tunca River (Figure 1). The first station was Suakacağı Village, the second station was Değirmenyeni Village, and the third station was Tunca Barracks (Edirne) (Figure 1).

Figure 1. Tunca River sampling stations: 1. Suakacağı Village (465409.00 E, 4632519.00 N, 47m); 2. Değirmeniyeni Village (461067.00 E, 4623425.00 N, 40 m); 3. Trakya University Tunca Barracks (Edirne) (462965.63 E, 4619414.88 N, 37m)

Then *Juglans regia* L. (walnut), *Morus alba* L. (mulberry), *Ulmus leavis* Pall. (elm) leaves were collected because of the widespread presence around the river. In addition, *Buxus* sp. (artificial boxwood) and *Platanus orientalis* L. (dried plane leaf) poured from plane trees in the autumn of the previous year were collected. The tree leaves were identified by the author (Dalgıç and Güler, 2015; Mamıkoğlu, 2017). The leaves were then filled into 20 kg potato bags. 5 leaf packages which consisted of 5 different kinds of leaves were prepared for each station. Totally 25 packages were prepared. Packages were regularly placed on the benthic region of the river. Within the following 5 months, a series of leaf packs were collected from sampling stations once a month. They were washed in sieves of different aperture sizes (Nominal Aperture -0,600µ, 300µ and 1,18mm) and the pupae were picked up with some pointed forceps. They were fixed and stored in tubes containing 70% ethyl alcohol. Samples collected from leaf packs were moved to the laboratory and placed in petri dishes under a stereo microscope (Olympus SZ51) and cleaned from the mud.

Samples taken from stations were then diagnosed at least at the genus or species level using the identification keys under the stereo microscope. Jacobsen (2008), Langton (1991), Ruse (2002), Strenzke (1959), Vallenduuk and Langton (2010), Ward and Cummins (1978), Wilson and Bright (1973), Wilson and Ruse (2005), Wright et al. (1996) were used for identification of Chironomidae pupae.

The Analysis of Variance (ANOVA) test was used for total organism analysis in leaf packages according to dates, stations, and leaf types. Tukey post hoc test was conducted if there was a statistical significance of 0.05 α.

RESULTS

The analysis of Chironomidae pupal fauna from aquatic macroorganisms according to different leaf kinds, stations and dates (Table 1, 2, 3, 4) were as follows:

Chironomidae pupae were different from one another in terms of date (Table 1). There was no difference between August, June, and July. There was a difference between August, September, and October. The TUKEY test results showed that August organisms were accumulated 2.2 times more than the October (Table 2).

Table 1. Variability analysis of Chironomidae pupae according to dates

	Sum of squares	df	Mean square	F	Sig.
Between Groups	47.280	4	11.820	3.145	.019
Within Groups	263.067	70	3.758		
Total	310.347	74			

*****The mean difference was significant at the 0.05 level.

Table 2. Multiple comparison analysis of Chironomidae pupae differences according to dates

(I) Date	(J) Date	Mean difference (I-J)	Std. error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
June	**July**	.600	.708	0.915	-1.38	2.58
	August	-1.000	.708	0.622	-2.98	.98
	September	1.000	0.708	0.622	-.98	2.98
	October	1.200	0.708	0.444	-.78	3.18
July	**June**	-0.600	0.708	0.915	-2.58	1.38
	August	-1.600	0.708	0.170	-3.58	.38
	September	0.400	0.708	0.980	-1.58	2.38
	October	0.600	0.708	0.915	-1.38	2.58
August	**June**	1.000	0.708	0.622	-.98	2.98
	July	1.600	0.708	0.170	-.38	3.58
	September	2.000*	0.708	0.047	0.02	3.98
	October	2.200*	0.708	0.022	0.22	4.18
September	**June**	-1.000	0.708	0.622	-2.98	0.98
	July	-0.400	0.708	0.980	-2.38	1.58
	August	-2.000*	0.708	0.047	-3.98	-0.02
	October	0.200	0.708	0.999	-1.78	2.18
October	**June**	-1.200	0.708	0.444	-3.18	0.78
	July	-0.600	0.708	0.915	-2.58	1.38
	August	-2.200*	0.708	0.022	-4.18	-0.22
	September	-0.200	0.708	0.999	-2.18	1.78

*****The mean difference was significant at the 0.05 level.

There was no difference between the Chironomidae pupae according to stations (Table 3).

Table 3. Variability analysis of Chironomidae pupae according to stations

	Sum of squares	df	Mean square	F	Sig.
Between Groups	8.027	2	4.013	0.956	0.389
Within Groups	302.320	72	4.199		
Total	310.347	74			

There was no difference between the Chironomidae pupae according to leaf kinds (Table 4).

Table 4. Variability analysis of Chironomidae pupae according to leaf kinds

	Sum of squares	df	Mean square	F	Sig.
Between Groups	24.880	4	6.220	1.525	.204
Within Groups	285.467	70	4.078		
Total	310.347	74			

DISCUSSION and CONCLUSION

As a result of the field work done in Tunca River, 8 species belonging to 6 genus from 3 subfamilies of Chironomidae family were determined (Table 5).

Table 5. Numerical distribution of Tunca River according to Chironomidae pupae subfamily, genus and species.

Subfamily	Tribus	Genus	Species
Chironominae	Chironomini	2	4
	Tanytarsini	1	1
Orthocladiinae		2	2
Tanypodinae		1	1
Total		**6**	**8**

These species were: *Chironomus (Chironomus) riparius* Meigen, 1804, *Chironomus (Chironomus) anthracinus* Zetterstedt, 1860, *Polypedilum (Polypedilum) nubeculosum* (Meigen, 1804), *Polypedilum (Polypedilum) pedestre* (Meigen, 1830), *Tanytarsus* sp., *Nanocladius bicolor* (Zetterstedt, 1838), *Cricotopus* sp., *Tanypus punctipennis* Meigen, 1818 (Table 6).

The distributions of Chironomidae pupal species according to leaf package kinds were shown ,n Table 6.

Table 6. Distribution of Chironomidae pupal species by leaf package kinds (artificial *Buxus* sp. = A.B, *Juglans regia* = J.R., *Platanus orientalis* = P. 0., *Morus alba* = MA., *Ulmus leavis* = U.L.)

Chironomidae pupae (8)	A.B.	J.R.	P.O.	M.A.	U.L.
Chironomus (*Chironomus*) *riparius*	5	4	3	0	0
Chironomus (*Chironomus*) *anthracinus*	6	3	1	0	1
Polypedilum (*Polypedilum*) *nubeculosum*	4	3	4	1	15
Polypedilum (*Polypedilum*) *pedestre*	1	1	0	1	6
Tanytarsus sp.	2	2	0	0	0
Nanocladius bicolor	1	0	0	0	0
Cricotopus sp.	1	1	0	0	0
Tanypus punctipennis	5	0	0	0	0
Total	**25**	**14**	**8**	**2**	**22**

Nanocladius bicolor (1 individual) and *Tanypus punctipennis* (5 individuals) were found only in artificial *Buxus* sp. and *Polypedilum (Polypedilum) nubeculosum* (27 individuals) in all leaf types (Table 6). Artificial *Buxus* sp. and *Ulmus leavis* leaves were found at high in number of organisms. *Morus alba* leaf is found at least. This shows us that leaves were used for both protection and nutrition. According to the results of Richardson (1992) and Hofer and Richardson (2007), leaves were mainly used as food source and organisms were found in high numbers. According to Duran et al. (2007), Fritz and Feminelle (2011) and Özkan (2018), leaves were mostly used for protection. Table 7 shows the distributions of Chironomidae pupal species by date.

Table 7. Distribution of Chironomidae pupal species by date

Chironomidae pupae (8)	June	July	August	September	October
Chironomus(*Chironomus*) *riparius*	0	9	3	0	0
Chironomus (*Chironomus*) *anthracinus*	0	10	0	1	0
Polypedilum (*Polypedilum*) *nubeculosum*	16	11	0	0	0
Polypedilum (*Polypedilum*) *pedestre*	3	5	1	0	0
Tanytarsus sp.	0	4	0	0	0
Nanocladius bicolor	1	0	0	0	0
Cricotopus sp.	0	4	0	0	0
Tanypus punctipennis	0	3	0	0	0
Total	**20**	**46**	**4**	**1**	**0**

When the distribution of Chironomidae pupae according to date was examined, it was found at most July and then in June (Table 7). These periods are the periods when the transformation of the larvae into pupae was increased in number because of the warm air and nutrient.

Organisms were not observed due to the coolness of the weather in October. Only one organism was found in September. These results are consistent with the process. Pupae mostly have been matured. *Tanytarsus* sp. (July), *Nanocladius bicolor* (June) and *Tanypus punctipennis* (June) were found only in one month. *Polypedilum* (*Polypedilum*) *pedestre* (June, July and August) was the most common species within 3 months. Table 8 shows the distributions of Chironomidae pupal species by stations.

Table 8. Distribution of Chironomidae pupal species by stations

Chironomidae pupae (8)	**1**	**2**	**3**
Chironomus (*Chironomus*) *riparius*	1	2	8
Chironomus (*Chironomus*) *anthracinus*	4	1	7
Polypedilum (*Polypedilum*) *nubeculosum*	13	5	8
Polypedilum (*Polypedilum*) *pedestre*	4	0	5
Tanytarsus sp.	0	2	2
Nanocladius bicolor	0	1	0
Cricotopus sp.	0	0	2
Tanypus punctipennis	1	2	0
Total	**23**	**13**	**32**

When the distributions according to the stations were examined, it was seen that the third station has the maximum number of individuals (Table 8). There was not much organic material in the benthos at that station. The benthos was generally muddy and had a slightly sandy structure. *Chironomus* (*Chironomus*) *riparius*, *Chironomus* (*Chironomus*) *anthracinus* and, *Polypedilum* (*Polypedilum*) *nubeculosum* were found in all stations. *Nanocladius bicolor* and *Cricotopus* sp. were only found at a station.

Özkan (2018) was also found 50 species of Chironomidae larvae in the study with leaf packs in Tunca River. Compared with this study, Chironomidae pupae were quite rare as 8 species. This could be attributed to the short life span of the pupae. In the larval study, *Kiefferulus tendipediformis* (Goethgebuer, 1921) and *Polypedilum* (*Polypedilum*) *convictum* (Walker, 1856) were showed the abundant colonization. In this study, *Polypedilum* (*Polypedilum*) *nubeculosum* was found to be abundant. In addition, *Nanocladius bicolor*, which was not found in the larval study, was found in this study.

Such studies are thought to be important for the explanation of aquatic life and the life forms of organisms.

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