http://dergipark.gov.tr/trkjnat
Trakya University Journal of Natural Sciences, 19(1): 101-105, 2018
ISSN 2147-0294, e-ISSN 2528-9691
DOI: 10.23902/trkjnat.356750

Research Article

THE GROWTH OF *Tanypus punctipennis* Meigen (DIPTERA, CHIRONOMIDAE) LARVAE IN LABORATORY CONDITIONS AND THE EFFECTS OF WATER TEMPERATURE AND pH

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Cite this article as:

Gülbunar F., Aydın G.B. & Çamur-Elipek B. 2018. The Growth of *Tanypus punctipennis* Meigen (Diptera, Chironomidae) Larvae in Laboratory Conditions and The Effects of Water Temperature and pH. *Trakya Univ J Nat Sci*, 19(1): 101-105, DOI: 10.23902/trkjnat.356750

Received: 21 November 2018, Accepted: 13 April 2018, Online First: 14 April 2018, Published: 15 April 2018

Abstract: Recent taxonomic studies suggest that findings of larval chironomids should be supported also by adult findings in order to obtain more robust and reliable results on the studied group. Moreover identifications of larvae of some species can be made to genus level only due to similarities of some larval characteristics in different species. In such cases, species level identifications can be achieved by the growth of larvae in laboratory conditions. Also, larval culturing under optimum growth conditions will not only make it easy to provide materials for experimental studies and but also achievement of larvae with a higher biomass value to be used as food in the sector. In this study, *Tanypus punctipennis* Meigen (Diptera, Chironomidae), a very common species in Turkish Thrace, was used as the model organism for culture studies. Individual larvae were cultured from third instar stage to adult form under laboratory conditions. A simple and cheap method is offered for experimental studies on larval growths of chironomids and the effects of water temperature and pH, both with very important roles in larval culturing, were determined. The overall results of laboratory tests showed that the temperature value of 25°C and 7-8 pH interval were the optimal laboratory conditions for culture of *T. punctipennis* larvae.

Key words: Freshwater, midge, environmental conditions, rearing, identification.

Özet: Son zamanlarda yapılan taksonomik çalışmalarda larval chironomidlerin erginleri ile birlikte değerlendirilmesinin daha güvenilir sonuçlar verdiği belirtilmektedir. Ayrıca, bazı türlerin teşhisleri, larval safhadaki bazı karakterlerin türler arasındaki benzerlikleri nedeniyle ancak cins düzeyine kadar yapılabilmektedir. Bu durumda larvaların laboratuvar koşullarında yetiştirilerek erginleştirilmesi sayesinde tür düzeyinde teşhisleri mümkün olabilmektedir. Ayrıca, uygun yetiştirme koşulları belirlenerek yapılan larva yetiştiriciliği, bir taraftan deneysel çalışmalarda gerekli materyalin eldesi için kolaylık sağlarken, diğer taraftan balık yemi sektöründe daha ileri instar evrelerine ve dolayısıyla yüksek biyomass değerine hızla erişecek larva temini için de yararlı olacaktır. Bu araştırmada, yetiştirme çalışmaları için model tür olarak Trakya'da oldukça yaygın olan *Tanypus punctipennis* Meigen (Diptera, Chironomidae) larvaları kullanıldı. Bireyler, üçüncü instar safhasından ergin forma erişinceye dek laboratuvar koşullarında yetiştirildi. Böylelikle, laboratuvar koşullarında larval chironomid yetiştirme amaçlı çalışmalar için basit ve ucuz bir yöntem deneyimlenerek sunulurken, larvaların yetişmesinde oldukça önemli rolü olan su sıcaklığı ve pH gibi çevresel faktörlerin etkileri de araştırıldı. Çalışmanın sonucunda *T. punctipennis* larvalarının gelişiminde 25°C ve 7-8 pH aralığının en uygun koşullar olduğu belirlendi.

Introduction

Chironomidae commonly called as midges or non-biting midges, is a dipteran family in which adult forms live in terrestrial environments while the larval forms adapted to an aquatic lifestyle. The larval period represented with four stages is the longest period of the whole life cycles of these insects (Armitage *et al.* 1995, Specziar 2008). Individual chironomids in metamorphosis leave their exuviae on water surface after a long fourth stage and stay in a relatively short pupal stage to emerge as adults.

Larval chironomids are known as one of the most abundant macroinvertebrate groups in freshwater environments and they often account for the majority of aquatic insects (Epler 2001). Although they have high adaptation abilities to aquatic ecological conditions, species can show different reactions to changing microhabitat conditions (Armitage *et al.* 1995, Maasri *et al.* 2008). Their growth and development can also be influenced by a number of conditions some of which are temperature, food, and photoperiodicity (Maier *et al.* 1990, Vos *et al.* 2000). Furthermore, morphological, physiological or behavioural adaptation abilities of larval chironomids differ from one to another species (Ferrington 2008). Therefore, some environmental variables affect larval dynamics in an aquatic environment in different rates depending on the species

that the larvae belong to, which in turn makes larval chironomids as potential organisms to be considered as indicator organisms for aquatic environments (Kenney *et al.* 2009).

Larval chironomids are one of the most important groups in benthic fauna in terms of their roles in food web since they are live food sources for higher aquatic organism, mainly for fish and macroinvertebrates (Habibi *et al.* 1992, Nath *et al.* 2017). Chironomid larvae are also of paramount importance in aquaculture and they are widely used as live food for fish larvae (Sahandi 2011). They can also be used as food material for commercial fish because of their high protein contents. Therefore, larval chironomids can and are readily reproduced in *in vitro* conditions to be used for this particular aim.

Larval stages of chironomids have been widely used for species identifications in taxonomical studies performed in aquatic ecosystems. However, identifications based on immature specimens sometimes can allow researchers for identification of a specimen up to genus level because of the high taxonomic complexity of the family (for example *Chironomus (Holotanypus)* sp. larvae). One common technique used by researchers from various countries to overcome this problem is larval rearing to obtain adults, but this technique has not been applied in Turkey so far (Namayandeh & Beresford 2012). Recent studies suggest that larval identifications should be supported by adult findings to provide the most reliable results.

Tanypus punctipennis Meigen, is a chironomids species with a wide Palaearctic distribution and is found in freshwater ecosystems. The immature stages of the species are abundant in a wide range of lentic and lotic habitats in Turkey, making the species a suitable material to be used as commercial fish food. *T. punctipennis* chosen as the experimental model organism for this study, is very common in aquatic environments in Turkish Thrace (Özkan & Çamur-Elipek 2006, 2007, Özkan 2006, 2007, Özkan et al. 2010, Çamur-Elipek et al. 2006, 2010, 2012, Aydın & Güher 2017). Although some studies have been performed on growth of *T. punctipennis* larvae in laboratory conditions, effects of environmental conditions on larval growth were not addressed in these studies (Vallenduuk & Lipinski 2009). Moreover, no similar study on growth of larval chironomids has been performed in Turkey so far. We aimed in the present study to provide an inexpensive and rapid method for growth of larval chironomids in laboratory conditions with an experimental study on *T. punctipennis*. We also tested the most suitable water temperature and pH levels for larval growth of the species in laboratory conditions. The effectiveness of the culture apparatus used was tested for possible use of it for chironomids with identification problems. The apparatus allowed us to define optimal culture conditions for experimental studies and fishing worms.

Materials and Methods

Larval chironomids were obtained from surface-sediment samples of an artificial shallow pond (depth less than 2m) in Balkan Campus of Trakya University in Edirne. Samples were collected in the field in May 2017 and were immediately transported to laboratory in their natural sediment and water. A total of 15 *T. punctipennis* larvae in 3rd instar stage were selected under a stereobinoculer microscope to establish the experimental groups. Saether (1980), Fittkau & Roback (1983), Epler (2001) were used in identifications of the larvae. A total of five experimental groups [1 control group and 4 test groups (2 groups for temperature and 2 groups for pH effect tests)] with three larvae in each were obtained and each group was transferred to different aquariums providing conditions similar to their natural conditions. The control group was same for both experimental paradigms. The aquariums were placed in laboratory with an ambient air temperature of +18°C during day and they were covered with a mesh net to retain emerging adults (Fig. 1). An oxygeniser was used to provide two oxygen bubbles per second to each aquarium. All larvae were fed everyday by putting a drop mixture of milk powder and water (1:1) into the aquariums (Namayandeh & Beresford 2012). The experiments were ended when all specimens in the groups reached to adult stage or when all larvae/pupae died. Wiederholm (1989), Armitage *et al.* (1995) and Langton & Pinder (2007) were used in identifications of adults and pupae.

Testing Effects of Temperature: In order to test the effects of ambient temperature on larval growth, three experimental groups were placed in three different temperature conditions. The control group was placed under controlled room temperature (18±2°C) while the two test groups were placed at two climate room temperatures, 25±2°C and 10±2°C, respectively. Temperature levels were measured by an ordinary thermometer at 6 hours intervals during the experiment. The pH levels of water in all groups were measured as 7-8 and were kept stable during the experiments. By doing so, we could determine the effects of temperature on larval growth for each group, considering that all other parameters were adjusted to be same for all groups.

Testing Effects of pH: The pH levels of water in all experimental groups were measured as 8 at the beginning of the experiment. All three groups were placed under controlled room temperature (18±2°C) to provide an environment similar to the natural environments of the larvae. To determine the effects of pH variations on larval growth, the temperature value of 18±2°C was kept the same for each experimental group. The control group of the first experimental condition for which pH was measured as 7-8 was also the control group of this experiment. The pH level of one of the two test groups was decreased to 5-6 with the help of addition of HCl solution and pH level of the second group was increased to 9-10 adding NaOH solution into the environment. The pH levels were measured by a pHmeter at 6 hours intervals during the experiment. Other conditions that have the potential to effect larval growth, i.e. light, pH, food, oxygen, sediment type and water level, were standardized for each group in both testing conditions.

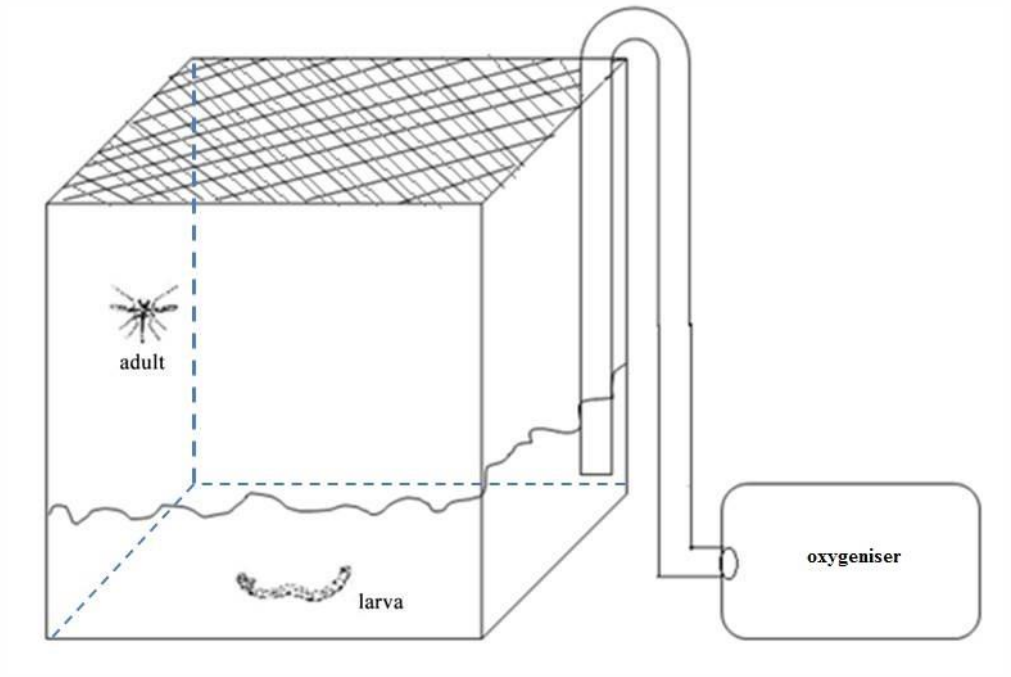

Fig.1. A schematic representation of the experimental setup in the laboratory. The aquariums were covered with a mesh to prevent escape of emerging adults.

Results and Discussion

In this study, a simple and cheap apparatus was tested for the culture of chironomid larvae using *Tanypus punctipennis* as the experimental organism. This apparatus have the potential to be used in culturing of chironomid larvae having identification problems and supplying fishing worm for other studies. The effects of water temperature and pH on larval growth of *T. punctipennis* which is very common chironomid species in Turkish Thrace were also investigated. The results of the experiments testing effects of temperature showed that adult emergence in the control group (+18°C) occurred within 4-5 days. The increasing and decreasing temperature conditions in the test groups revealed different adult emergence periods. All larvae in high temperature (+25°C) group emerged as adults within 2 days, while it took 8-10 days for larvae in low temperature (+10°C) group to emerge as adults (Table 1). In the beginning of the experiments testing effects of pH on larval growth, the natural pH values of the water body containing experimental larvae were measured as 7-8. All larvae of the pH=9-10 group died after changing the pH level in the setup, the larvae placed in the pH=5-6 group survived for only 3 days but no adult emergence occurred after the pupal stage (Table 2).

It is reported that Chironomidae members have relatively a short time period from egg to adult ranging from a few days to one month (Tokeshi 1995). *T. punctipennis* larvae in our study was found to emerge as adults in relatively shorter time period at 25°C compared to control and low temperature conditions. The results in this study showed that this species has high growth ratio at +25°C level.

It is reported that pH levels are also very important in aquatic ecosystems (Makela & Oikari 1992, Courtney & Clements 1998, Weisse & Stadler 2006). The adaptation abilities of benthic macroinvertebrates to the environment can change by pH fluctuations. Increasing alkalinity and pH were reported to have significant effects on the enhancement of protein amounts and body lengths of larval chironomids (Nath *et al*. 2017). Rapid pH changes in an aquatic ecosystem can lead to changes in dynamics of larval chironomid populations, which in turn affect the food chain in a negative manner.

The present results also revealed evidence for that *T. punctipennis* is a species with fast larval growth and high mortality rate in different conditions, and easily maintained in laboratory for experimental studies. More studies on larval chironomids including their growth, feeding, and behaviour are needed for a better understanding of their biology.

This study identified an inexpensive and rapid method that could be used for growth of *T. punctipennis* larvae in laboratory conditions and showed the optimal cultivation conditions of the species. In addition, to provide the natural environment to the larvae, uncontrolled discharges to an aquatic ecosystem must be prevented and monitoring of the water qualities must be made periodically.

Acknowledgement

The preliminary results of the experiments in this study were presented in XIII. Congress of Ecology and Environment with International Participation, held from 12-15 September 2017 in Edirne, Turkey.

Table 1. The results of the experiments on effects of temperature on larval growth.

Embodiments → Days↓	(Control group) (+18°C)	(+25°C)	(+10°C)
1st day	3 alive larvae	3 alive larvae	3 alive larvae
2nd day	3 alive larvae	3 pupae	3 alive larvae
3rd day	1 alive larvae, 2 pupae	3 alive adults	3 alive larvae
4th day	2 pupae, 1 alive adult		3 alive larvae
5th day	3 alive adults		3 alive larvae
6th day			2 alive larvae, 1 pupae
7th day			2 alive larvae, 1 pupae
8th day			2 pupae, 1 alive adult
9th day			2 pupae, 1 alive adult
10th day			3 alive adults

Table 2. The results of the experiments on effects of pH on larval growth.

Embodiments → Days↓	(Control group) (pH=7-8)	(pH=5-6)	(pH=9-10)
1st day	3 alive larvae	3 alive larvae	3 alive larvae
2nd day	3 alive larvae	2 alive larvae, 1 pupae	3 dead larvae
3rd day	1 alive larvae, 2 pupae	3 pupae	
4th day	2 pupae, 1 alive adult	3 dead pupae	
5th day	3 alive adults		

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