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Raising *Hydropsyche instabilis* Curtis, 1834 and *Hydropsyche bulbifera* McLachlan, 1878 (Hydropsychidae, Trichoptera) Larvae in the Laboratory: Larval Morphology and Male Genital Characterization

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ABSTRACT
Observing of life cycle of aquatic insects can be easier and more reliable information can be collected by raising them in a controlled medium in the laboratory. There is insufficient information about larvae and other life stages in many species of aquatic insects. In order to identify and determine them properly, stages of larvae and pupa should be linked with their adult forms. In this study, the larvae of *Hydropsyche instabilis* Curtis, 1834 and *Hydropsyche bulbifera* McLachlan, 1878 (Hydropsychidae, Trichoptera) which were collected from the two rivers were reared in the laboratory. Additionally, it was collected detailed information about the morphological characters of larvae and adult forms. Moreover, it has been observed the process of metamorphosis in larvae and the emergence of adults. The morphological characters of the larvae and the male genital structures of the adults were photographed and explained. During the study, raising success of *H. instabilis* and *H. bulbifera* species was determined as 17.85% and 15.65%, respectively.

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Hydropsyche instabilis Curtis, 1834 ve *Hydropsyche bulbifera* McLachlan, 1878 (Hydropsychidae, Trichoptera) Larvalarının Laboratuvarda Yetiştirilmesi: Larva Morfolojisi ve Erkek Genital Karakterizasyonu

ÖZET
Sucul böceklerin laboratuvarda kontrollü bir yetiştirme ortamında yetiştirilmesi ile yaşam döngülerinin gözlenmesi çok daha kolay bir hale gelebilir ve çok daha güvenilir bilgiler toplanabilir. Birçok türün larva ve diğer yaşam evreleri hakkındaki bilgiler yetersizdir. Türlerin sağlıklı olarak teşhis edilmesi ve belirlenmesi için larva ve pupa evrelerinin, yetişkin formları ile ilişkilendirilmesi gerekmektedir. Yapılan bu çalışmada belirlenen iki akarsudan toplanan *Hydropsyche instabilis* Curtis, 1834 ve *Hydropsyche bulbifera* McLachlan, 1878 (Hydropsychidae, Trichoptera) larvaları laboratuvarda yetiştirilmiştir. Ayrıca larva ve yetişkin formlarına ait morfolojik karakterleriyle ilgili ayrıntılı bilgilerin toplanmıştır. Larvaların metamorfozu ve erginlerin pupadan çıkma süreci gözlenmiştir. Larvaların morfolojik karakterleri ve erginlerin erkek genital yapıları fotoğraflanarak kısaca açıklanmıştır. Çalışma süresince *H. instabilis* ve *H. bulbifera* türlerinin yetiştirme başarısı sırasıyla %17.85 ve %15.65 olarak belirlenmiştir.

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INTRODUCTION

Aquatic insects play important ecological roles in almost all freshwater ecosystems. Stream-dwelling insects are vital to aquatic ecosystems as they support food webs in the in-stream, riparian, and floodplain, process organic matter, and transfer energy along the stream bed, vertically to the stream bed, and laterally to the floodplain (Hynes, 1970; Malmqvist, 2002; Boulton & Lake, 2008). Overuse of water has negative consequences for all freshwater biodiversity, especially for vertebrates and fish (Dudgeon et al., 2006). In particular, aquatic insects are involved in all water regime changes, from the most stable upstream source to the most dynamic streams in deserts, which sometimes dry up and sometimes flood. These habitat changes mean that aquatic insects are subject to certain pressures (Lytle, 2008). For this reason, it has great importance to determine the ecological conditions of fresh water resources, especially rivers, to protect the high quality ones, to make improvement studies for the resources that are not in good condition and to take the necessary precautions (Verdonschot & Nijboer, 2004).

In order to learn the factors affecting the distribution of aquatic insects, it is necessary to know their ecological knowledge very well (Greve et al., 1998). The life cycle of aquatic insects can be observed much better and more reliable information can be obtained by raising them in a controlled medium in the laboratory (Keiper & Foote, 1996). For this purpose, various techniques have been used to raise benthic macroinvertebrates in a laboratory environment (Bjarnov & Thorup, 1970; Wiley & Kohler, 1980; Mackay, 1981).

Trichoptera (Caddisflies), are the largest insect order, most of which larvae are aquatic and adults are terrestrial (Wiggins, 2004; Malicky, 2004). Recently, the study of various features of caddisflies and other freshwater invertebrates has provided a wealth of information about possible causes of ecological degradation in surface waters and the effects of climate change (Durance & Ormerod, 2007; Graf et al., 2008; Durance & Ormerod, 2010; Watts et al., 2015; Morse et al., 2019). It has been revealed that Ephemeroptera, Plecoptera and Trichoptera species are less tolerant to organic and other pollutions in waters than other taxa found in fresh waters. Therefore, changes in the abundance and diversity of these three taxa, which are frequently used in freshwater biomonitoring studies, inform us about the pollution status of the water (Morse et al., 2019).

Aquatic larvae are widely distributed in freshwater habitats worldwide, thanks to their silk secretions and uses (Wiggins & Mackay, 1978; Mackay & Wiggins, 1979). Except for members of the Rhyacophilidae family, almost all Trichoptera live in a case or a silk tube into which they use to retreat (Bouchard, 2009). Larvae use a wide variety of food resources and exhibit almost all the feeding behaviors that exist in aquatic ecosystems (Wiggins, 2004).

When the larvae complete their growth stage and mature, they usually build fixed shelters in which they spin various kinds of cocoons or transform their portable cases into fixed shelters and then turn into pupa form and prepare to pupate. The pupal stage is completed after a few weeks. The mature pupa, which has almost turned into an adult in the pupal skin, uses to come out cutting the silken cocoon and the anterior end of the pupal shelter. Pupae emerging from the shelter can swim rapidly to the surface of the water, or they reach the surface by crawling towards the bottom materials that exceed the water surface. Here, the pupa quickly sheds its skin, unfolds its folded wings, and flies towards coastal plants or rocks that will protect it (Morse et al., 2019).

New caddisfly species are being described a great number in various parts of the world. Identification of new species is carried out through adults, mostly males. The inability to identify the larvae and other life stages of many species in the light of available information requires that they be associated with their adult forms (Morse et al., 2019). The lack of taxonomic studies on the larval stages of caddisflies and other benthic groups in Turkey makes it difficult to use them in biomonitoring studies (Ekingen & Kazancı, 2021). New molecular research contributes to revealing unknown species and establishing relationships between their life stages. These relationships make it possible to discover diagnostic characters and determine species for identification of benthic forms. As the larvae and other life stages of more species become identifiable, the possibilities will increase significantly to study their functional characteristics, ecological services, life cycles and habitat requirements and use them in biomonitoring programs (Morse et al., 2019). Therefore, we still need information about caddisflies and studies; to obtain this information will always be important. In this study, the larvae of *Hydropsyche instabilis* Curtis, 1834 and *Hydropsyche bulbifera* McLachlan, 1878 (Hydropsychidae, Trichoptera) collected from the two rivers were reared in the laboratory and also aimed to collect detailed information on the morphological characters and the different stages in their life cycles.

MATERIAL and METHOD

Experimental Design

A standard fish aquarium of 80x40x20 cm was used to raise the larvae. SOBO WP-100F brand internal filter, ELİTE 801 brand air motor and fine-grained aquarium

sand were used in the raising medium. First of all, the aquarium where the samples will be placed was cleaned. Before the larvae were placed in the raising medium, 19 lt of water was put into the aquarium. The internal filter, air motor and ground gravel were carefully washed, cleaned and placed in the aquarium. Fine white aquarium sand was used on the aquarium floor to allow better examination of Trichoptera larvae. Before the larvae were placed in the aquarium, the mains water was dechlorinated by running the aquarium empty for 48 hours. In order to complete the amount of water lost during the study, 19 liter water containers were filled with water and left open to remove chlorine. A cage equivalent to the aquarium size (80x40x20 cm) was used to prevent the Trichoptera samples that matured in the aquarium from escaping over the aquarium. This study was carried out in two periods between 29.06.2017-23.07.2017 and 04.08.2017-28.08.2017, and the raising setups prepared were named as Aquarium-1 and Aquarium-2, respectively.

Study Area and Collection of Samples

H. instabilis and *H. bulbifera* larvae to be reared in the laboratory were collected from Karacehennemboğazı Creek on 29.06.2017 and from Daday Creek on 04.08.2017, respectively. D-frame bottom net with 500 µm mesh-opening was used for sampling. Benthic macroinvertebrates at the bottom were allowed to enter the net by holding the net against the flow and mixing the ground with the foot (kick-net method). Then, the net was removed from the stream and all samples were placed in a white plastic container (60x30 cm), and Trichoptera larvae were separated from other benthic macroinvertebrates. Live larvae samples were transported to the laboratory in plastic containers, in a small amount of stream water, together with some bottom debris and plants in the environment. Details of the locations where caddisfly larvae were collected are given in Table 1.

Table 1. Areas where Trichoptera larvae were collected.
Çizelge 1. Trichoptera larvalarının toplandığı alanlar.

Province	Town	Brook/Creek	Latitude	Longitude	Altitude (m)
Kastamonu	Küre	Karacehennemboğazı Creek	41°50'22.98"K	33°43'46.24"D	607
Kastamonu	Daday	Daday Creek	41°28'28.72"K	33°31'42.16"D	845

Placing and Examining the Samples in the Experimental Aquarium

Trichoptera larvae collected from the field and brought to the laboratory were placed in the aquarium with 10 liters of water brought from the field. Larvae were fed using Tetra brand granulated fish food every two days and 19 lt of aquarium water was replaced with previously prepared rested water every five days.

Larvae, pupa and adult stages of the samples were observed by monitoring both aquarium setups daily. Dead larvae seen in the aquarium were removed daily from the aquarium.

RESULTS and DISCUSSION

This study was carried out on raising 140 *Hydropsyche instabilis* larvae collected from Karacehennemboğazı creek in Kastamonu, Küre between 29.06.2017-23.07.2017 and 115 *Hydropsyche bulbifera* larvae collected from Daday creek in Kastamonu, Daday, between 04.08.2017-28.08.2017. The morphological characters of the larvae and the male genital structures of adults were photographed and briefly explained.

25 adult individuals (19 females and 6 males) were reared from 140 *H. instabilis* larvae placed in Aquarium-1 for 25-days. The emergence time of adults from pupa is given in Figure 1.

The first individual seen in Aquarium-1 was 1 ♂ on 08.07.2017. The last individuals, 1 ♂ and 1 ♀, were observed on 23.07.2017. The emergence times of *H. instabilis* larvae brought to the laboratory were on the 10th, 12th, 14th, 19th-21st, 23rd and 25th days, respectively. While the most adult emergence was seen on 17.07.2017 with 6 ♀♀, the least adult emergence was observed on 08.07.2017 with 1 ♂ individual.

18 adult individuals, (7 females and 11 males) were reared from 115 *H. bulbifera* larvae placed in Aquarium-2 during 25-days. The emergence time of adults from pupa is given in Figure 2.

The first individual seen in Aquarium-2 was 1 ♂ on 11.08.2017. The last individuals, 2 ♂♂ and 2 ♀♀, were observed on 21.08.2017. The emergence times of *H. bulbifera* larvae brought to the laboratory were on the 8th, 11th, 14th, 16th, 18th and 20th days, respectively. While the most adult emergence was observed on 13.08.2017 with 2 ♀♀ and 4 ♂♂, the least adult emergence was on 11.08.2017 with 1 ♂ individual.

Morphological Characterization of Larvae

Hydropsyche instabilis Curtis, 1834

The head, pronotum, mesonotum, and metanotum are brown (Fig. 3a). There are branched gills ventral to the abdominal segments (Fig. 3b). In the dorsal view of the head capsule, there are areas of lighter color in the frontclipeal apotome compared to its surroundings. In the middle of these light-colored areas, there is an arrow-shaped darkness extending from front to back. The anterior part of its apotome has two lateral light-

colored areas, while the posterior part has a U-shaped light-colored area. The anterior part of the frontoclypeus is flat (Fig. 3c). In the ventral view of the head capsule, light colored areas are seen towards the anterior part (Fig. 3d). The coloration pattern of the posterior prosternites is shown in Figure 3e. The submentum is the same color as the head. The submentum is equilateral triangle-shaped, short and broad (Fig. 3f).

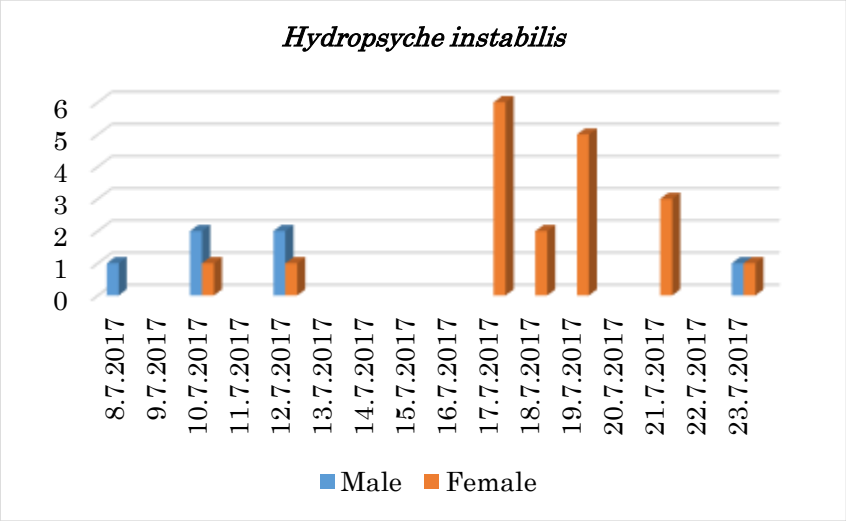

Figure 1. The emergence time of the larvae placed in Aquarium-1 from pupa
Şekil 1. Akvaryum-1'de yetiştirilen larvaların pupadan çıkış zamanları

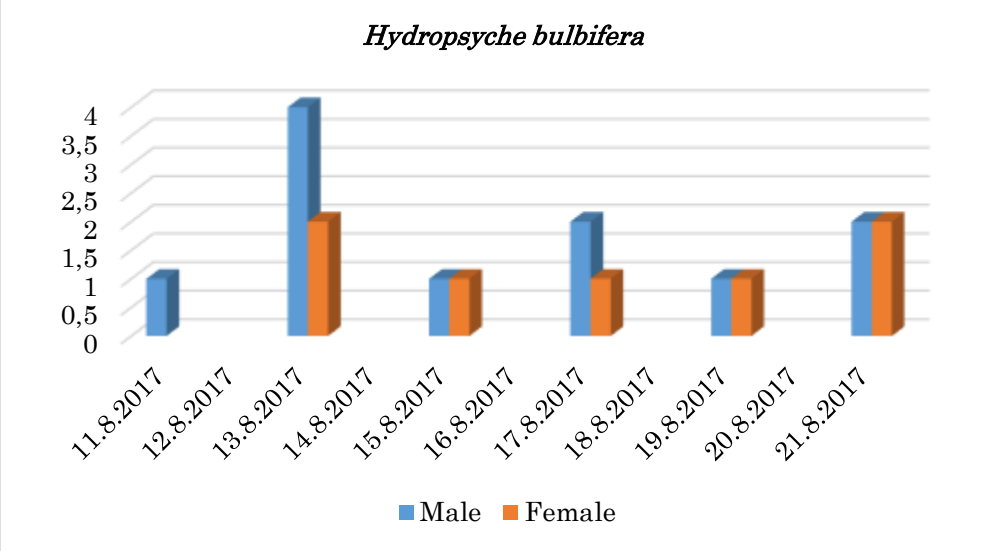

Figure 2. The emergence time of the larvae placed in Aquarium-2 from pupa
Şekil 2. Akvaryum-2'de yetiştirilen larvaların pupadan çıkış zamanları

Hydropsyche bulbifera McLachlan, 1878

The head, pronotum, mesonotum, and metanotum are light brown (Fig. 4a). There are branched gills ventral to the abdominal segments (Fig. 4b). In the dorsal view of the head capsule, there are two lateral light-colored areas anteriorly and a circular light-colored area posteriorly in the frontclipeal apotome. The anterior part of the frontoclypeus is flat. The anterior part of the apotome is glossy (Fig. 4c). In the ventral view of the head capsule, light colored areas are seen towards the anterior part (Fig. 4d). The coloration pattern of the posterior prosternites is shown in Figure 4e. The submentum is black, short and broad to form an equilateral triangle (Fig. 4f).

Characterization of Reared Adults

Hydropsyche instabilis Curtis, 1834

The X segment has finger-shaped extensions, which are long and rod-shaped (Fig. 5a). The tip of the phallus is not membranous and has no differentiated complex lateral extensions (Fig. 5b, d). The subapicolateral phallus teeth (dental phallus) are large enough to be seen from above. The apical part of the phallus (apex phalli) is slightly narrowed apically

(Fig. 5c), and the tip hook (harpago) of the genital foot is markedly shortened (Fig. 5d).

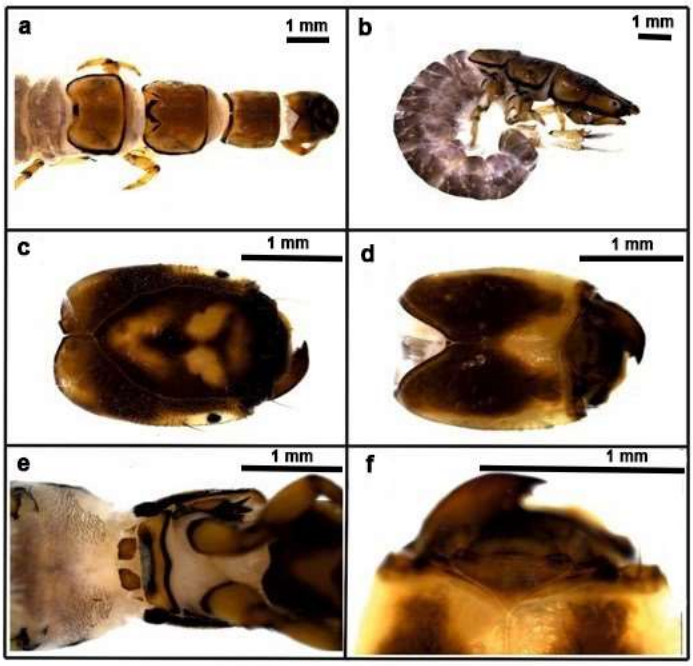

Figure 3. *Hydropsyche instabilis Curtis*, 1834 larva a) Dorsal view of head and thorax; b) lateral view of the larva; c) dorsal view of the head; d) ventral view of the head; e) posterior prosternites; f) submentum

Şekil 3. Hydropsyche instabilis Curtis, 1834 *larvası a) Baş ve toraks dorsal görünümü; b) larvanın lateral görünümü; c) baş dorsal görünümü; d) baş ventral görünümü; e) posterior prosternitler; f) submentum*

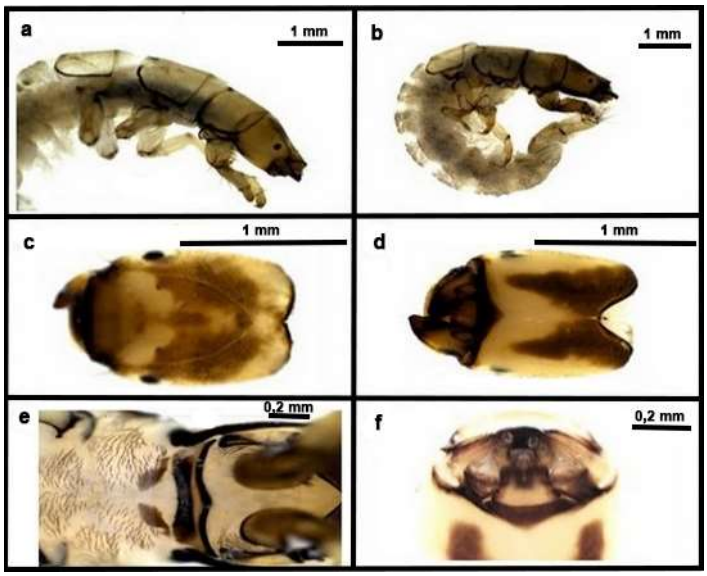

Figure 4. *Hydropsyche bulbifera* McLachlan, 1878 larva a) Dorsal view of head and thorax; b) lateral view of the larva; c) dorsal view of the head; d) ventral view of the head; e) posterior prosternites; f) submentum

Şekil 4. Hydropsyche bulbifera McLachlan, 1878 *larvası a) Baş ve toraks dorsal görünümü; b) larvanın lateral görünümü; c) baş dorsal görünümü; d) baş ventral görünümü; e) posterior prosternitler; f) submentum*

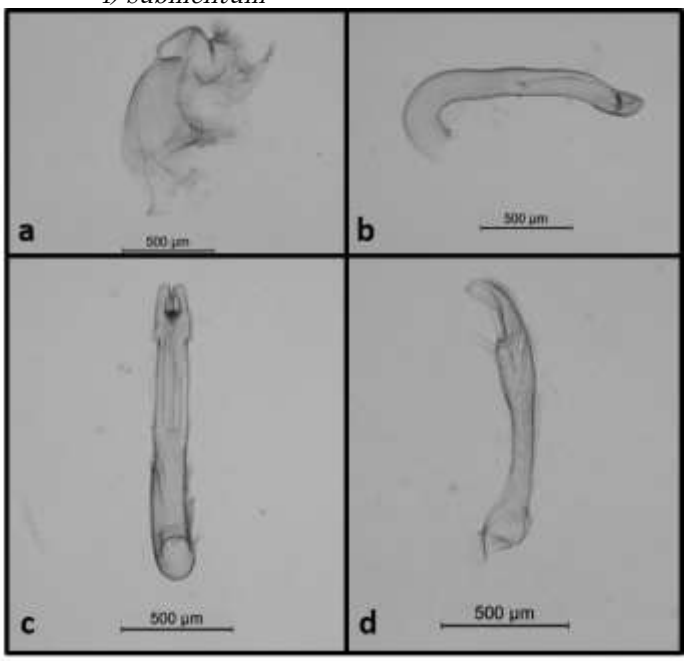

Figure 5. Photograph of *Hydropsyche instabilis* Curtis, 1834 male genital a) Lateral; b) lateral view of the phallus; c) ventral view of the phallus; d) dorsal view of the harpago

Şekil 5. Hydropsyche instabilis Curtis, 1834 erkek genital fotoğrafı a) Lateral; b) fallus lateral görünümü; c) fallus ventral görünümü; d) harpago dorsal görünümü

Hydropsyche bulbifera McLachlan, 1878

There are neither long nor short rod or finger-shaped extensions in the X segment (Fig. 6a). The tip of the phallus is not membranous and lacks differentiated complex lateral extensions. The phallus is flat, with no dorsal or median thickening. The apical portion of the phallus (apex phalli) is characteristically enlarged onion-shaped, narrowing evenly from the base to the phallal shaft (Fig. 6b, c); the tip hook (harpago) of the genital foot is slightly curved and it is like a stick with a slightly tapered corner (Fig. 6d).

The Hydropsychidae family is a family represented by a wide variety of species in streams with different water quality. For this reason, it is accepted as a very tolerant family all over the world (Gordon & Wallace, 1975; Ross & Wallace, 1982; Gallardo-Mayenco et al., 1998). Raising success of *Hydropsyche* larvae was tried to be observed in the aquarium we created in the laboratory, and the larva and adult characterization were made. During this study, raising success of *H. instabilis* and *H. bulbifera* species was determined as 17.85% and 15.65%, respectively.

Studies have clearly shown that exposure to low oxygen concentrations changes the behavioral patterns of *H. angustipennis* larvae (van der Geest, 2007). It has

also been observed that even among the species belonging to the Hydropschidae family, the larvae change their behavioral patterns at low oxygen sensitivities (Philipson & Moorhouse, 1974). In this study, the amount of dissolved oxygen (8.5 mgl^{-1} and 9.1 mg l^{-1}) provided by using an air motor in Aquarium-1 and Aquarium-2 was lower than the natural environments (10.5 mg l^{-1} and 10.8 mg l^{-1}) where we took larvae, which adversely affected the success of raising.

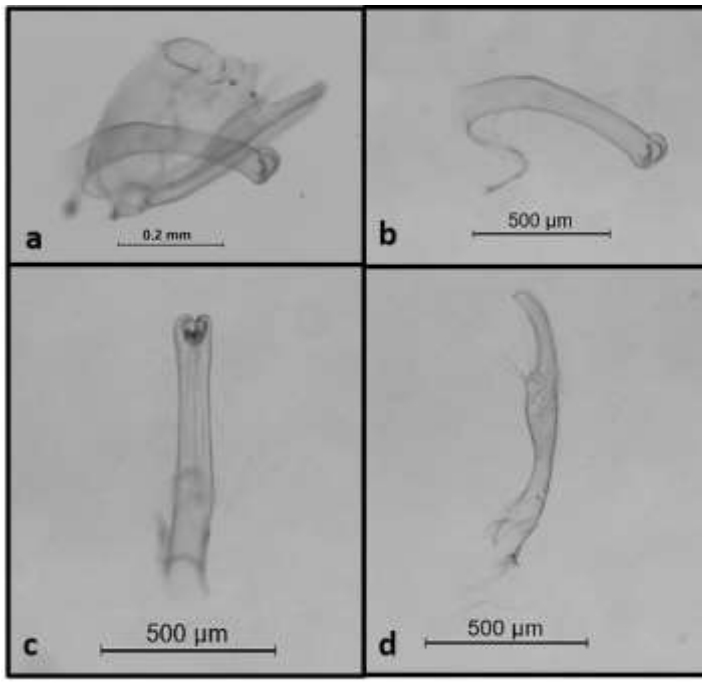

Figure 6. Photograph of Hydropsyche bulbifera McLachlan, 1878 male genital a) Lateral; b) lateral view of the phallus; c) ventral view of the phallus; d) dorsal view of the harpago

Şekil 6. Hydropsyche bulbifera McLachlan, 1878 *erkek genital fotoğrafi a) Lateral; b) fallus lateral görünümü; c) fallus ventral görünümü; d) harpago dorsal görünümü*

Water temperature and change in water temperature are known to be two of the most important environmental factors affecting life cycle patterns, particularly growth rate and seasonal timing of aquatic insects (Sweeney, 1984). In addition, biotic interactions (Westman, 1991), life cycle stage (Verdonschot & Higler, 1992) and the effect of environmental variable also affect the life cycle of larvae (Wiens, 1989). It is also known that the feeding culture of the larvae also affects the development and maturation of the larvae (Anderson et al., 1976). Average water temperatures in the laboratory environment were determined as 30˚C for Aquarium-1 and 28˚C for Aquarium-2. The fact that these values were high was the main factor reducing the success of raising. It is also thought that biotic interactions in a restricted area such as an aquarium and feeding culture of larvae affect the raising success of the species reared in the raising environment.

Laboratory and field studies have shown that some species may have different preferences in different flow rate ranges (Fremling, 1960; Kaiser, 1965; Schwartz, 1972; Malas & Wallace, 1977; Wallace et al., 1977; Boon, 1978). It is also known that Hydropsychidae larvae may have different preferences for different microhabitats (Wu, 1931; Schwartz, 1972; Malas & Wallace, 1977; Oswood, 1979). Although the current was created with the aquarium internal motor, the inability to model the flow rate in the locations where the larvae were collected was one of the other factors that reduced the raising success.

Internal parasites or damage to larvae during collection are possible explanations for larval mortality. Placing too many larvae in the raising environment creates the need for more maintenance, such as removing waste and adding excess food (Keiper & Foote, 1996). Reviewing all the above-mentioned deficiencies in the new raising environments to be created and preparing the raising setup accordingly will increase the success of raising.

Ecological profiles are dynamic assessments that can vary and therefore may be incomplete in small areas or short-term studies (Moretti & Mearelli, 1981). Moreover, environmental variables can also change in terms of time and space (Resh & Unzicker, 1975). Therefore, when ecological profiles are obtained from living space data together with experimental studies, large datasets integrated in time and space will be more appropriate to precisely determine autoecology (Bonada et al., 2004).

CONCLUSION

Here, it was tried to show that Trichoptera larvae can be reared even with a simple setup in the laboratory. In addition, with this study, some useful taxonomic features were obtained for the identification of larvae and adults by characterizing the larvae and adults. Associating the reared adults with the larvae will make it possible to define the larval stage by raising species that have been identified from the adult but whose larval stage is unknown in this way.

This study, which was carried out with a very simple setup, revealed the importance of ecological conditions in raising larvae. Controlling factors such as the amount of dissolved oxygen, water temperature, flow rate, substrate structure and making them suitable for the natural habitat will increase the success of raising larvae. In addition, thanks to the controlled experiments to be carried out on these factors, it will be possible to determine the ecological tolerance values of the aquatic organisms. The use of these raising media will enable Trichoptera and other aquatic

organisms to be used more accurately and widely in biotic indices.

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Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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