

## **BIOLOGY AND EFFECT OF TEMPERATURE ON LARVAL DEVELOPMENT TIME OF *CHIRONOMUS RIPARIUS* MEIGEN (DIPTERA: CHIRONOMIDAE) UNDER LABORATORY CONDITIONS**

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**ABSTRACT:** Biology of *Chironomus riparius* was studied at 26±1 °C. Larval development time of *C. riparius* was also studied at six temperatures (18 ± 1, 22 ± 1, 24 ± 1, 26 ± 1, 28 ± 1 and 30 ± 1 °C, respectively). The sex ratio of this chironomid was 0.58 ± 0.033. Mean number of eggs in each gelatinous egg mass and percentage of egg hatch were 509.38 ± 42.27 and 97.47 ± 0.64, respectively. Females preferred sites with red colors for oviposition. Incubation period before hatching of eggs, larval and pupal development period and adult longevity were 3.76 ± 0.39, 22.5 ± 1.38, 1 ± 0 and 2.30 ± 0.17 days at 26 ± 1 °C, respectively. The shortest and the longest larval development period were at 22, 24±1 and 18 ± 1 °C, respectively. Developmental rate increased with increasing temperature to 26 °C.

**KEY WORDS:** Developmental rate, *Chironomus riparius*, oviposition sites, percentage of egg hatch, sex ratio.

The ability of chironomid species to exist under certain conditions, such as low levels of dissolved oxygen, wide range of temperature, pH, salinity and depth of water has been achieved largely by behavioral and physiological adaptation with relatively slight morphological change. The short life cycles and high densities of many species have provided basic information on productivity and population dynamics (Cranston, 1995). The aquatic larvae of non-biting midges (Diptera: Chironomidae) are widely used in fresh water environmental monitoring. They are sensitive to many pollutants, easy to culture and have a short life cycle, which make them suitable for biomonitoring (Ingersoll and Nelson, 1990). In a study, it was revealed that *Chironomus riparius* was fairly tolerant to low pH and high iron, although sensitivity among stages varied. For example, midge eggs were more tolerant than larvae. Conversely, midge larvae were the least tolerant life stages to iron. It was concluded that the greater tolerance of midge eggs to iron may be due their encasement in a gelatinous egg mass (Roush et al., 1997). It has also been found that the persistence of this fast growing opportunistic chironomid species (i.e. *Chironomus riparius*) in organically enriched aquatic ecosystems is independent of the contamination level of the sediment (Ristola, 2000; De Haas et al., 2006).

Tube-building is initiated by first and second instar larva with sediment of nutrients at the bottom of aquarium. Larvae of *C. riparius* construct tubes using the mouthparts and salivary secretions to gather sediments and attach them together (Edgar & Meadows, 1969). The ability of chironomids to construct tubes decreases the risk of predation by vertebrates and invertebrates (Walde & Davies, 1984; Hershey, 1985, 1987; Macchiusi & Baker, 1991). Halpern et al. (2002) showed that larvae without the tube were significantly more sensitive to copper and chloramines than larvae with salty and silty tube. They concluded that in

addition to its role in feeding, respiration and anti-predator shelter, the tube protects its habitant from toxic substances.

There are some factors that make chironomid larvae as a rich food for many organisms such as both juvenile and adult fish (Armitage, 1995). These parameters are: high value of protein (56% for chironomids larvae) (Sugden, 1973), high digestibility 73.6% (De La Noue and Choubert, 1985), high capability of reproduction, and the apparent function in small quantities as a growth promoter in fish diet (Yashouv, 1956; Yashouv & Ben-Shachar, 1967).

Quality and quantity of food and habitat quality play an important role in determining the growth, development rate and survival of chironomid larvae (Johannsson, 1980; Sankarperumal & Pandian, 1991; Parren et al., 1993; Stanko-Mishic et al., 1999; De Haas et al., 2006; Vogt et al., 2007). However, temperature constitutes a major controlling factor in larval growth (Tokeshi, 1995). A number of studies have demonstrated that growth rate of larvae increased at higher temperatures. For example, Menzie (1981) reported that in a laboratory rearing experiment, *Cricotopus sylvestris* (Fabricius) completed larval development in 28 days at 15 °C and in 10 days at 22 °C, while Kostantinov (1985) documented for the same species 21 days at 18 °C and 14 days at 22 °C. Larvae of *Chironomus crassicaudatus* Malloch were reared at nine constant temperatures. The slowest development was at 15 °C, with developmental rate peaking between 25 and 27.5 °C. However, developmental rate increased rapidly as temperature increased up to 20 °C, slowed between 20 and 27.5 °C and there was a decrease at temperature higher than 27.5 °C (Frouz et al., 2002).

*Chironomus riparius* Meigen, is one of the most important species used in fish cultivation and it has been reported that larvae of *C. riparius* are most often associated with high nutrition/low oxygen condition (Ferrington & Crisp, 1989). This chironomid is widely distributed in Guilan province (North of Iran, Southern coast of Caspian Sea). The aim of this study was to investigate biological parameters and the influence of temperatures on larval development of *C. riparius*.

## MATERIAL AND METHODS

Glass aquariums (50×25×30 cm) containing dechlorinated tap water 25 cm in depth were used to rear the larvae of *C. riparius*. The tap water chemistry values were: pH 7.1, Ca 112 ppm, K 3.8 ppm, Na 112.6 ppm, Mg 17.76 ppm, HCO<sub>3</sub><sup>-</sup> 244 ppm, Cl 177.5 and conductivity 1.18 mS/cm. Water temperature was adjusted with a heater tube at 26±1 °C. Each aquarium was aerated with an air pump connected to an air stone. Water temperature was also checked by a thermometer attached to the front side of aquarium.

In order to study biological parameters of *C. riparius*, aquariums (25×16×35 cm) filled up to about 30 cm with water were used. The aquariums were covered with a cloth net and the water temperature and oxygen was adjusted as above. All experiments were conducted in a glasshouse (5.60×4.30×2.45 m) at day light regime so that adult insects could swarm and mate readily (Armitage, 1995). Several plastic water pans (50×15 cm) filled with water placed in the floor of glasshouse for adult females to oviposit. Sex ratio was determined by recording the number of adults emerging from each aquarium daily.

In order to establish rearing of *C. riparius* in the glasshouse, collection was made in two following ways:

- 1) Water pans (50×15 cm) containing water with 20 cm in depth were placed outside the glasshouse to collect the insect gelatinous egg masses. They were then

observed daily and egg masses were detached from water pan sides by forceps and transferred to rearing aquariums. After egg hatching, chicken manure was added to the aquariums for larvae to feed (Sahragard & Rafati Fard, 2006).

2) Larvae of *C. riparius* were directly collected from the bottom of standing water (ponds and ditches) in the area using an aquarium net (9×11.5 cm). To do this, the bottom of the pond was first agitated and floated larvae were then collected with an aquarium net. These larvae were treated as above.

Keys (Cranston, 2002) were used to identify the chironomid to the genus level. Dr. John Martin of Melbourne University in Australia made species identification. This was done through cytotoxicology by obtaining both polytene chromosome squashes and DNA typing.

### **Effect of color on female oviposition**

In order to determine the effect of color on the rate of oviposition, plastic water pans (31 cm D and 10cm H) containing water with 5 cm in depth were used. Water pans were white, red, blue and yellow in color. The experiment was carried out in 4 treatments (colors) and 3 replicates in a completely randomized design. The water pans were randomly placed on the glasshouse floor. Females flying in the glasshouse were allowed to oviposit in these water pans. The water pans were observed daily and number of egg masses was recorded in each pan. This observation was made for five consecutive days.

### **Number of eggs in each egg mass and percentage of egg hatch**

Thirteen egg masses were randomly taken from water pans and the number of eggs per each egg mass was determined under a stereomicroscope. The egg masses were observed twice a day (8:00 am and 14:00 pm) in order to determine number and percentage of egg hatch.

### **Effect of temperature on larval development**

In order to determine the effect of different temperatures on larval development period, each egg mass was separately introduced to rearing units (aquariums) with different level of temperatures ( $18 \pm 1$ ,  $22 \pm 1$ ,  $24 \pm 1$ ,  $26 \pm 1$ ,  $28 \pm 1$ , and  $30 \pm 1$  °C) regimes. After egg hatch, the larvae were fed on chicken manure (Sahragard and Rafati Fard, 2006) and were observed daily till pupation. Appearance of pupae was considered as a criterion for the larval development period. This was continued until the last pupae appeared. Egg incubation, pupal period and adult longevity were measured at  $26 \pm 1$  °C.

Data analysis was performed with SAS 6.12 and SPSS 9. Means were compared with Duncan's multiple range test.

## **RESULTS**

### **Effect of color on female oviposition**

Female *C. riparius* preferred significantly sites with red colors ( $4.67 \pm 1.89$ ) for oviposition, while the preference for blue, yellow and white colored water pans were  $0.2 \pm 0.2$ ,  $0.2 \pm 0.2$  and  $0 \pm 0.0$  (Mean  $\pm$  S.E.) egg masses, respectively.

Mean development time of different stages of *Chironomus riparius* at  $26 \pm 1$ °C are shown in (Table 1). Mean pupal period of *C. riparius* was very short compared to larval development time. Sex ratio (female proportion) of this chironomid was  $0.58 \pm 0.033$  (n=30).

### Number of eggs in each egg mass and percentage of egg hatch

Adult males and females of *C. riparius* differed in color and size, females were darker and brown ( $5.2 \pm 1.46$  mm long), but males were greenish ( $6.4 \pm 0.54$  mm long). Antennae in males were plumose and more obvious than those of females. Results showed that *C. riparius* female adult produced a single egg mass. Eggs were  $336 \pm 2.43$   $\mu$ m long and  $122 \pm 1.4$   $\mu$ m wide (n=20). Mean number of eggs per each egg mass was  $509.38 \pm 42.27$  (n=13). Percentage of eggs hatch in *C. riparius* was  $97.78 \pm 0.68$ .

### Effect of temperature on larval development

Mean larval developmental time of *C. riparius* significantly varied at different constant temperatures ( $p < 0.01$ , Table 2), the shortest was at  $24 \pm 1$  or  $22 \pm 1$  °C, and the longest at  $18 \pm 1$  °C. Larval developmental rate increased with increasing temperature up to 26 °C (Fig.1), but it seemed to be constant from 22 to 26 °C.

## DISCUSSION

Eggs of *C. riparius* were deposited in a gelatinous matrix arranged helically and attached to water pan at the water edge by a slender stalk at one end. Only in the Telmatogetoninae, eggs are laid singly, without benefit of a gelatinous matrix (Nolte, 1993). *C. riparius* produced a single egg mass as many other chironomids, but Wensler and Rempel (1962) deduced from a study of ovarian structure of *Chironomus plumosus* L. that this chironomid was capable of producing up to three egg masses. Eggs of *C. riparius* are elliptical in shape and newly laid eggs are green brownish. According to Nolte (1993) chironomid eggs are most commonly elliptical or reniform although they are deltoid in *Eukiefferiella claripennis* (Lundbeck), *Telmatogeton japonicus* (Tokunaga) and some *Orthocladius*. Nolte (1993) reported that egg size varied considerably depending on the species. The smallest eggs, those of *Corynoneura* and *Thienemanniella*, were around 170  $\mu$ m long and 70  $\mu$ m wide, while *Tanypus punctipennis* (Meigen), a large Tanypodine, produced eggs that may be as long as 612  $\mu$ m and 135  $\mu$ m wide. The egg size of *C. riparius* ( $336 \pm 2.43$   $\mu$ m long and  $122 \pm 1.4$   $\mu$ m wide) is in the range of other Chironomid species.

Typical egg masses of freshwater chironomids contain from 20 or 30 eggs in the case of some smaller species, to 2000 or more for larger species. The highest recorded number of eggs in a single mass was 3300, for *Chironomus (Camptochironomus) tentans* (Fabricius) (Nolte, 1993). Based on the number of eggs laid in each egg mass (i.e.  $509.38 \pm 42.27$  or less than 2000), this species is categorized as larger species.

High percentage of eggs hatch (i.e.  $97.78 \pm 0.68$ ) was obtained for *C. riparius* at  $26 \pm 1$  °C which is in accordance with other studies. As Williams (1981) reported that mortality among *Thienemanniella vittata* (Edwards) eggs was strongly influenced by temperature; the survival rate with 90% hatching successfully was obtained at 15 °C, but only 28% at 20 °C. Eggs of *Chironomus pulcher* hatched after only 48 hours with a mere 1% mortality at 25 - 30 °C (DeJoux, 1971).

Research has shown that the temperature influences embryonic development of insects, as eggs of *T. vittata* hatched in a minimum of 4 days at 20°C, 6 days at 15 °C, 13 days at 10 °C and 31 days at 5 °C. *C. tentans* eggs developed fully in 17.5 days at 8.8 °C and 3 days at 22.1 °C (Pinder, 1995). Frouz et al. (2002) found that the male eggs of *Chironomus crassicaudatus* (Malloch) hatched in 48h at 25 °C and in 39h at 27.5 °C. They also found that the female eggs hatched in 48 and 36h

at 25 and 27.5 °C, respectively. Eggs of *C. riparius* hatched in a range of 2-6 days at 26±1 °C which is somehow different with other species.

Larval development time of *C. riparius* was significantly influenced by temperature, as it was shortest at 22 ±1 or 24 ±1 °C, and the longest at 18 ± 1°C. Similar results have been found in other chironomids. Comparison of growth in *Paratendipes albimanus* (Meigen) at three different temperatures (10, 15 and 20 °C) also proved that larval growth was enhanced at higher temperatures (Ward and Cummins, 1979). Larval developmental period of *Glyptotendipes paripes* (Edwards) at 20, 22.5, 25, 27.5 and 30 °C was 30, 30, 33.7, 22 and 21 days, respectively (Lobinske et al., 2002). Frouz et al. (2002) reported that larval developmental time in males of *C. crassicaudatus* at mentioned temperatures was 38, 36, 35, 31 and 31 days, respectively whereas in females was 42, 38, 41, 37 and 41 days, respectively. Stevens (2003) studied development and survival of *Chironomus tepperi* (Skuse) from 12.5 to 37.5 °C (2.5 °C interval) and showed that developmental rate increased with increasing temperature up to 32.5 °C, but fell at 35 °C. There was an increase in larval developmental rate of *C. riparius* as temperature increased but there was a curvilinear relationship in the whole range of temperatures tested. Variable and similar relationships have also been found in other chironomid species. Pery and Garric (2006) at five temperatures from 15 to 27 °C found that the growth rate of *C. riparius* increased linearly with temperature as a consequence of quicker food ingestion. Frouz et al. (2002) showed that relationship between temperature and developmental rates of *C. crassicaudatus* varied among different developmental stages. In fourth instar larvae, the developmental rate increased at low temperatures, peaked at 20 °C, and then decreased. They also showed that relationship between temperature and developmental rate follows a bell-shaped curve. In the other words, a wide optimum at higher temperatures was observed, whereas developmental rate was reduced at lower temperatures as described by Maier et al. (1990) for *Chironomus decorus* (Johannsen). A similar wide plateau of developmental rate at higher temperatures has also been observed in other aquatic insects (Rueda et al., 1990). The developmental rates may be affected by many factors, e.g. manipulation of chironomids during the rearing process (Nolte, 1995) and rearing isolated larvae or colony rearing (Beiver, 1971; McLachlan, 1983).

Pupal duration has been recorded from few hours to several days as in long-lived Podominae (Cranston, 1995). Mean pupal developmental time in male of *C. crassicaudatus* at 25 and 27.5 °C was 24 and 22 hours, respectively. This period for female pupa was 23 and 27 hours, respectively (Frouz et al., 2002). Lobinske et al. (2002) reported that mean pupal duration of *G. paripes* at 25 and 27.5 °C was 355 ± 73 and 363 ±109 hours, respectively. The pupal period obtained for *C. riparius* in this study was different with that of latter species and very close to former ones.

Mean adult longevity of *G. paripes* at 25 and 27.5 °C was 42 and 39 hours, respectively (Lobinske et al., 2002). Longevity of *C. riparius* adult females was very similar to *G. paripes*.

**Conclusion:** According to the results, temperature affected highly larval development time and developmental rate of *C. riparius*. Larval developmental time was significantly varied at different constant temperatures, as the most favorable condition for larval development was at 22-26 °C.

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Table 1. Development time (Mean  $\pm$  S.E.) of different stages of *Chironomus riparius* at 26  $\pm$ 1°C (in days).

Developmental stage	Min.	Max.	Mean $\pm$ S.E.
Egg	2	6	3.76 $\pm$ 0.39
Lava	12	33	22.5 $\pm$ 1.38
Pupa	1	1	1 $\pm$ 0
Female adult	1	3	2.30 $\pm$ 0.17

Table 2. Larval development time (Mean  $\pm$  S.E.) of *Chironomus riparius* at different constant temperatures (in days).

Days	Temperature (°C)					
	18 $\pm$ 1	22 $\pm$ 1	24 $\pm$ 1	26 $\pm$ 1	28 $\pm$ 1	30 $\pm$ 1
Min.	30	13	12	12	14	14
Max.	41	30	31	33	56	38
Mean $\pm$ S.E.	35.5 $\pm$ 1.04b	21.5 $\pm$ 1.25a	21.5 $\pm$ 1.32a	22.5 $\pm$ 1.38a	35.0 $\pm$ 1.9b	26.0 $\pm$ 1.47a

Data in column followed by different letters are significantly different ( $p < 0.01$ ) (Duncan test)

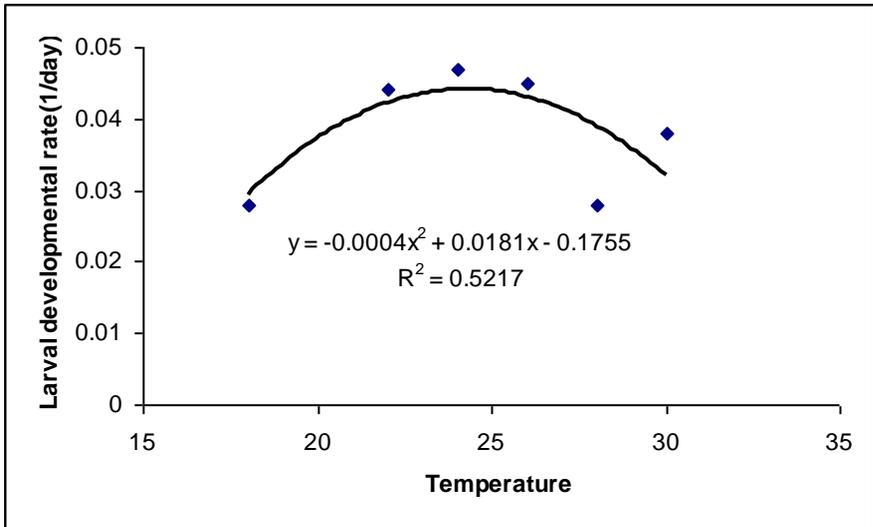


Figure 1. Larval developmental rate of *Chironomus riparius* at different constant temperatures (°C).