Effects of Environmental Conditions on Reproduction in Experimental Populations of *Chironomus tentans* (Postprint)

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Abstract

Using Chironomus tentans as the research subject, this study comprehensively investigated the combined effects of temperature and light intensity on survival rates and developmental rates at various life stages, adult sex ratios, fecundity, and population dynamics. Through F-tests of regression equations and coefficients for the survival rates of chironomid eggs, larvae, and pupae under different temperatures but identical light conditions, it was found that under the same light intensity (800 lx or 2000 lx) and within the temperature range of 15-35 °C, the survival rates of both larval and pupal stages showed significant correlation with temperature, with larval survival being more susceptible to temperature effects than pupal survival; however, temperature changes did not significantly affect egg survival rates, and the two extreme temperatures of 15 °C and 35 °C were unsuitable for chironomid survival and reproduction. Subsequently, through orthogonal experiments, range analysis and two-way ANOVA were conducted on chironomid survival rates and developmental rates under the combined effects of two temperatures (15 °C and 30 °C) and two light intensities (800 lx and 2000 lx), concluding that the optimal conditions for C. tentans population reproduction were 25 °C and 800 lx, and that while neither factor significantly affected the adult sex ratio, temperature had a far greater influence than light intensity on all three parameters (survival rate, developmental rate, and sex ratio). Finally, through one-way ANOVA of results on adult fecundity under different light and temperature conditions, as well as results on adult fecundity under different humidity levels (45%, 65%, 85%, and 95%) at identical light and temperature conditions, it was concluded that C. tentans maintained good vitality and successfully completed reproductive development at 25-30 °C and 800 lx light intensity, and that 85%-95% relative humidity enabled emerged adults to maintain high oviposition levels. Furthermore, based on observed population fecundity data, experimental population life tables were

calculated for combinations of corresponding temperatures (25 °C and 30 °C) and light conditions (800 lx and 2000 lx). Results indicated that under conditions of 800 lx light intensity and 25-30 °C, chironomids could maintain high net reproductive rates (R0) and intrinsic rates of increase (rm). In summary, conditions of 25 °C temperature, 800 lx light intensity, and 85% relative humidity were more suitable for C. tentans population reproduction. These results establish a foundation for developing standardized conditions for indoor breeding of C. tentans, understanding corresponding population development patterns, and constructing toxicity testing methods for native chironomid species.

Full Text

Preamble

Effects of Environmental Conditions on the Reproduction and Development of *Chironomus tentans* (Diptera)

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Abstract

This study investigated the comprehensive effects of temperature and light intensity on the survival and developmental rates of each life stage, adult sex ratio, fecundity, and population dynamics of experimental *Chironomus tentans* populations. F-tests were applied to regression equations and coefficients for survival rates of eggs, larvae, and pupae under different temperatures with constant light intensity. Results revealed that survival rates of both larvae and pupae were significantly correlated with temperature within the same light intensity range, with larval survival being more susceptible to temperature than pupal survival. However, temperature changes did not significantly affect egg survival. The two extreme temperatures (15°C and 35°C) were unsuitable for *C. tentans* survival and reproduction. Orthogonal experiments were subsequently conducted to identify optimal breeding conditions through range analysis and two-way ANOVA of survival rates and developmental rates. The optimal conditions were determined to be 25°C and 800 lx, with both factors showing no significant influence on adult sex ratio, though temperature exhibited a far greater effect than light intensity on survival, developmental rate, and sex ratio. Single-factor ANOVA of adult fecundity under different light and temperature conditions, combined with humidity effects (45%, 65%, 85%, 95%) at constant temperature and light, demonstrated that *C. tentans* maintained robust vitality and completed reproductive development at 25–30°C and 800 lx. A relative humidity of 85%–95% was necessary to maintain high oviposition levels in emerged adults. Life tables of experimental population reproductive characteristics were constructed based on observed fecundity data, revealing that *C. tentans* could

maintain high net reproductive rates and intrinsic rates of increase at 25–30°C and 800 lx. These results establish a foundation for developing standardized indoor breeding conditions, understanding corresponding population development patterns, and constructing toxicity testing methods for native chironomid species.

Keywords: *Chironomus tentans*; temperature; light intensity; reproduction and development; population dynamics

Introduction

Environmental conditions such as temperature, light, and humidity significantly influence the survival and reproduction of aquatic organisms. For chironomid midges, which undergo multiple metamorphoses and transition between aquatic and aerial habitats, temperature and light are particularly critical factors throughout their life cycle. The generation time for chironomids typically ranges from 20 to 65 days, with temperature and light intensity being the most significant factors affecting growth and reproduction. Although different chironomid species have established breeding protocols, species-specific environmental adaptations necessitate systematic studies on native species to develop stable indoor breeding and toxicity testing methods.

Chironomus tentans is widely distributed across China, particularly in Xinjiang, making it a representative native species for ecotoxicological research. However, the lack of systematic studies on its reproductive and developmental requirements has hindered the establishment of standardized toxicity testing methods, especially for life-cycle and multi-generational assays. Given the advantages of using chironomids in aquatic toxicity testing—including shorter test durations and lower costs compared to fish—and the importance of geographic distribution in ecological risk assessment, investigating the effects of environmental factors on *C. tentans* is essential for evaluating chemical hazards to native Chinese species. This study systematically examined the influence of temperature, light intensity, and humidity on the survival, development, sex ratio, and population dynamics of *C. tentans* to propose standardized breeding conditions and inform the development of toxicity testing protocols for native chironomid species.

1. Test Organism

Chironomus tentans was obtained from the Shanghai Chemical Industry Research Institute and has been cultured in our laboratory for dozens of generations. Culture conditions followed those established for *Chironomus riparius*. Larvae were maintained in glass aquaria filled with test substrate and water (volume not exceeding half the aquarium capacity), covered with mesh netting. Powdered fish food was provided daily, and overlying water was replaced weekly using a siphon to avoid disturbing larvae and their tubes. This established experimental population served as the test organism source.

2. Test Substrate

The test system consisted of Elendt M7 medium and artificial sediment. The artificial sediment formulation comprised 75% quartz sand (1 mm), 20% kaolin clay, and 5% peat (50–200 m). Peat was dried, ground into fine powder, and suspended in deionized water using a rotary mixer. The suspension was conditioned at (20±2)°C for pH stabilization and microbial community establishment, then combined with Elendt M7 medium to create a homogeneous water-sediment system with a 1:1 volume ratio. This standardized substrate offers reproducible conditions independent of seasonal sediment variations and eliminates the need for pre-treatment to remove indigenous fauna.

3. Test Methods

Temperature (15, 25, 30, 35°C), light intensity (800 lx, 2000 lx), photoperiod (16:8 L:D), and humidity (45%, 65%, 85%, 95%) were controlled in climate chambers (MMM Climacell). Test containers were randomly positioned within chambers. Newly laid egg masses were placed in petri dishes with Elendt M7 solution and maintained at test conditions. Hatching was monitored, and approximately 100 first-instar larvae from each egg mass were transferred to beakers containing the water-sediment system that had been pre-conditioned to test temperature. Beakers were gently aerated, sealed with mesh, and larvae were fed powdered fish food daily (0.25–0.5 mg/larva initially, increasing to 0.5–1 mg/larva as they grew). Each treatment had four replicates. Developmental stages, mortality, and emergence were recorded daily. Emerged adults were transferred to fresh beakers with Elendt M7 solution for oviposition. Sex ratio, adult lifespan, oviposition duration, and fecundity were recorded. Egg counts and hatching success were monitored to assess reproductive output.

Based on initial results, the two extreme temperatures (15°C and 35°C) were excluded, and orthogonal experiments were designed using an $L4(2^2)$ array with two temperature levels (25°C, 30°C) and two light intensities (800 lx, 2000 lx) . To further examine humidity effects on adult fecundity, four humidity levels (45%, 65%, 85%, 95%) were tested under the optimal temperature and light conditions identified from orthogonal experiments.

4. Data Processing

SPSS 19.0 (IBM, USA) was used for statistical analyses. F-tests were applied to regression equations for survival rates under different temperatures at constant light intensity. Two-way ANOVA analyzed developmental time, total survival rate, and sex ratio from orthogonal experiments. One-way ANOVA examined fecundity under different temperature, light, and humidity combinations. Significance level was set at =0.05 for all analyses. Life table parameters—including net reproductive rate (R), intrinsic rate of increase (r), finite rate of increase ( ), mean generation time (T), and population doubling time (Dt)—were calculated following established methods [10–11] for populations at 25°C and 30°C

under both light intensities.

Results

1. Single-Factor Experiments

At 800 lx, regression analysis revealed that survival rates of larvae and pupae were significantly correlated with temperature across 15–35°C ($R>0.99$, $p<0.05$), while egg survival showed no significant temperature dependence . Larval survival was more temperature-sensitive than pupal survival. Both extreme temperatures (15°C and 35°C) inhibited development; at 35°C, larvae ceased development or progressed only to third instar, while at 15°C, development was arrested. Survival rates peaked at 25–30°C, reaching 92.5% for larvae and 98.87% for pupae. Egg survival remained high (98.9%) across all temperatures, with maximum mortality of only 5.36%.

At 2000 lx, similar patterns emerged . Larval survival was lower than at 800 lx, particularly at 15°C. Optimal temperatures remained 25–35°C, with survival rates significantly correlated with temperature ($R>0.99$, $p<0.05$). These results demonstrate that while temperature strongly affects larval and pupal survival, egg survival is largely independent of temperature within the tested range.

2. Orthogonal Experiments

Since extreme temperatures prevented complete life cycle development, orthogonal experiments focused on 25°C and 30°C combined with 800 lx and 2000 lx. Range analysis identified optimal conditions: 25°C and 800 lx yielded the highest survival rate (85.43%), while 30°C and 2000 lx produced the fastest development rate . Temperature exerted a greater influence than light intensity on both parameters. Two-way ANOVA confirmed that temperature had a highly significant effect on survival and developmental rate ($p<0.05$), whereas light intensity did not ($p>0.05$) . The combined analysis established 25°C and 800 lx as the optimal breeding conditions.

Sex ratio analysis showed no significant effects from either temperature or light intensity, though temperature influence was greater than light . Under optimal conditions (25°C, 800 lx), the sex ratio was approximately 1.05:1 (female:male), ensuring stable population dynamics.

3. Multi-Factor Effects on Adult Fecundity

Fecundity analysis under four temperature-light combinations revealed that higher temperatures and light intensities shortened oviposition duration . At 25°C, fecundity was slightly higher at 800 lx (321±24 eggs/female) than at 2000 lx (325±29 eggs/female), though differences were not significant. At 30°C, fecundity decreased to 281±27 and 285±22 eggs/female under 800 lx and 2000 lx, respectively.

Humidity significantly affected fecundity [Figure 3: see original paper]. At 25°C and 800 lx, oviposition was highest at 85–95% relative humidity, with fecundity significantly greater than at 45% or 65% RH. This indicates that high humidity is essential for maintaining reproductive output in emerged adults.

4. Effects of Temperature and Light Intensity on Experimental Population Parameters

Life table parameters calculated from observed fecundity data showed consistent trends across light intensities . At 25°C and 800 lx, the net reproductive rate (R) was 56.16, mean generation time (T) was 29.58 days, intrinsic rate of increase (r) was 0.136, and finite rate of increase ( ) was 1.145. At 30°C and 800 lx, R decreased to 44.26 while r increased to 0.193 and T shortened to 19.65 days. Population doubling time ranged from 3.59–5.10 days across optimal conditions. These parameters indicate that *C. tentans* populations grow geometrically under favorable conditions, with temperature modulating the trade-off between reproductive output and generation time.

Discussion

The results demonstrate that temperature and light intensity significantly affect survival and developmental rates of larval and pupal stages, but not egg survival. The insensitivity of eggs to temperature likely reflects the protective function of the chorion, which shields embryos from environmental fluctuations. The larval stage exhibited the highest sensitivity to temperature, confirming its suitability as the target stage for acute toxicity testing.

Optimal breeding conditions of 25–30°C and 800 lx ensure complete life cycle development and stable population dynamics. Within this range, increasing temperature accelerates development and shortens generation time, while 800 lx light intensity maintains a balanced sex ratio. The combination of 85–95% relative humidity is critical for maximizing adult fecundity. These findings align with reported environmental requirements for other chironomid species such as *C. riparius*, *C. dorsalis*, and *C. kiieensis*, though specific optimal parameters vary among species.

The establishment of life table parameters under controlled conditions provides a baseline for assessing population-level effects in toxicity studies. The observed ability of *C. tentans* to adjust its intrinsic rate of increase in response to temperature demonstrates its capacity to adapt to environmental changes, a crucial consideration for ecological risk assessment.

Conclusion

This systematic investigation of *Chironomus tentans*, a widely distributed Chinese chironomid species, revealed that:

1. Within the tested range (15–35°C), survival rates of larvae and pupae were significantly correlated with temperature at both 800 lx and 2000 lx, with larvae being more sensitive than pupae. Egg survival was not significantly affected by temperature.
2. Orthogonal experiments identified 25°C and 800 lx as the optimal conditions for population breeding, with temperature being far more influential than light intensity. Neither factor significantly affected adult sex ratio.
3. Conditions of 25–30°C, 800 lx, and 85–95% relative humidity were optimal for maintaining vigorous life activity, complete reproductive development, and high oviposition rates.
4. *C. tentans* larvae represent the most sensitive life stage to environmental stress, making them appropriate test organisms for acute toxicity assessments.

These results establish the foundation for standardized indoor breeding protocols, elucidate population development patterns, and support the development of toxicity testing methods for native chironomid species in China.

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Note: Figure translations are in progress. See original paper for figures.