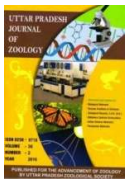
UTTAR PRADESH JOURNAL OF ZOOLOGY

38(1): 20-25, 2018
ISSN: 0256-971X (P)



A STUDY ON THE GROWTH AND BIOMASS OF CHIRONOMUS LARVAE IN DIFFERENT FOOD MEDIA

RAHUL PODDER[1], SUSANTA NATH[2*], CATERINA FAGGIO[3] AND BIPLOB KUMAR MODAK[1]

[1]Department of Zoology, Sidho-Kanho-Birsha University, Purulia, 723 104, West Bengal, India.
[2]Department of Zoology, Government General Degree College Singur, 712 409, West Bengal, India.
[3]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author RP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SN and BKM managed the analyses of the study. Author CF managed the literature searches. All authors read and approved the final manuscript.

ARTICLE INFORMATION

Editor(s):
(1) Marcelo Abidu Figueiredo, Department of Animal and Human Anatomy, Institute of Biological and Health Sciences, Federal Rural University of Rio Janeiro, Brazil.
(2) Idika Kalu Idika, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nigeria.
Reviewers:
(1) E. Mennan Yildirim, Aydın Adnan Menderes University, Turkey.
(2) Khalid Shawky Hamadah, Al-Azhar University, Egypt.

Received: 4th April 2018
Accepted: 8th June 2018
Published: 14th June 2018

Original Research Article

ABSTRACT

Chironomid larvae, popularly known as bloodworm, are considered as preferred food for cultured fish due to their high nutritional content. However, due to less availability and high cost of production, such food is not popular so far. Here, an attempt was made to increase the biomass as well as growth of this insect at low cost. Four culture media were prepared for *Chironomus striatapennis*, using the following, Potato Peel Powder, Bakery Yeast, mixture of Potato peel powder and Bakery yeast, and Commercial Fish Food, as well as along with a Control medium containing natural pond soil and water. The study revealed that production of biomass and growth of bloodworms were higher in the mixed as well as in the medium containing fish food. But, mixed culture medium was considered better than the other three media as well as the control. As potato peel powder, a vegetable waste and bakery yeast are almost of no cost material, giving high yield when biomass of chironomid larvae was concerned.

Keywords: *Chironomus*; body growth; biomass; culture medium; cost effective.

1. INTRODUCTION

The genus *Chironomus* (Order: Diptera; Family: Chironomidae) is the most abundant and widespread members of the bottom mud communities in ponds and lakes, and play a major role in the aquatic food webs, representing a major link among producers and secondary consumers [1]. *Chironomus* larvae are

**Corresponding author: Email: susanta_nath@yahoo.com;*

considered as natural food for different freshwater fishes [2,3] and they are advantageous than artificial food due to presence of digestible protein and high water content. Natural live food is mobile and fishes are attracted to these food. Moreso, water gets dirty by rejected particle of the artificial fish food, whereas, rejection rate of natural live food is less [4]. Larvae are used as live food for many aquarium fish but their artificial culture is really a challenge in the field of aquaculture [5,6]. As a fish food, blood worm is very nutritious but its production is very expensive so far and their use mainly depends upon harvesting from nature [7]. It was reported that fishes supplied with chironomus larvae were found to grow faster and had early spawning. Aquaculture is among the fastest-growing sectors of the world food production [8]. Further development of the aquaculture industry and increasing demand of fish production provokes intensive fish culture [9,10]. This insect has the ability to multiply and compete with other benthic organisms for shelter and food with high tolerance ability of environmental changes [11,12]. Chironomid larvae are opportunistic omnivorous, ingesting a wide variety of food items [13]. Naser and Roy [14] has suggested that these group of organism showed a low degree of selectivity, having a more general food selection habit. Studies on food consumption and growth of chironomid have been mostly carried out in the field, where the effect of quality of food cannot be easily monitored [15]. So far, researchers are trying to invent various techniques to culture this insect in the laboratory as well as for commercial purposes [16]. Very few studies to observe the growth pattern of chironomid in relation to their culture were conducted. The objective of this study was to explore the culture practices of this insect in relation to its growth and biomass, in the laboratory conditions.

2. MATERIALS AND METHODS

2.1 Collection and Stock of Chironomid larvae

Collection of benthic mud, containing tubed larvae of *Chironomous striatapennis* was done from fresh water pond near Kanchrapara (22_56018.664800N, 88_28010.034400E), North Twenty Four Parganas, West Bengal, India. The collected samples transported to the laboratory in aerated plastic bags. The larvae were reared in aquarium (60 cm x 45 cm x 45 cm) containing pond water at the base (20 cm), covered with mosquito net and supplied with fish flakes as food [17]. Adult male and females that emerged in the rearing aquarium were transferred to breeding aquarium (60 cm x 45 cm x 45 cm) covered with mosquito net, containing 3cm autoclaved soil and 10 cm de-chlorinated tap water at the base for laying eggs. Breeding aquarium contains small Water Hyacinth (*Eichhornia crassipes*) to maintain the natural habitat and also to enable the insect lay eggs with ease. First instar was hatched out after 2-3 days and considered for further experiments.

2.2 Preparation of food for Blood worm Culture

Crushed and sieved (106 µm mesh) sun dried potato peel powder mixed with de-chlorinated tap water (5 gl^{-1}), kept in water overnight was considered as culture medium F1. Dried and sieved commercial bakery yeast mixed at the rate of 0.5 gl^{-1} with de-chlorinated tap water was considered as second culture medium (F2). A mixed culture medium (F3) was prepared by mixing F1 and F2 (1:1). Crushed and sieved commercial fish food powder dissolved in de-chlorinated tap water (5 gl^{-1}) and kept overnight was considered as culture medium F4. Freshly prepared media were used for each experiment. A control was maintained by rearing the larvae in a similar tray containing natural pond soil and water.

2.3 Chemical Analysis of Food Items

Carbon and organic matter of the culture media were measured by Walkley-Black Titration Method (Table 1). Protein and carbohydrate contents of food sample were determined according Lowry et al. [18] and Du Bois et al. [19] respectively.

2.4 Measurement of Growth and Biomass

Polythene tray (TARSONS)measuring 45 cm x 30 cm x 20 cm containing autoclaved pond soil at 100 g/ft^2 and de-chlorinated tap water of 10 cm depth was maintained and approximately 1000 1^{st} instar larvae were collected from the stock culture and released in each experimental tray. Each trey was provided with three layers of submerged nylon net (1mm mesh size) for easy collection of the 4^{th} instar larva. Water level was increased up to 15 cm when larvae attained late 3^{rd} instar. Rearing was continued till early 4^{th} instar. Each trey was covered with wooden cages (60 cm x 45 cm x 45 cm) surrounded by nylon net. One side of the cage was facilitated with moving wooden door to maintain easy access of culture tray. Approximate 12L: 12D hour illumination arrangement and 26±1 ˚C water temperature were maintained throughout the experiment [6,20]. Mini air pump was in use for continuous and controlled aeration. Samples of 50 early 4^{th} instar larvae were taken from each culture media to measure the growth of the larva. Body length (BL) was measured from the anterior end to the anus under compound microscope fitted with a calibrated eye piece micrometre. To measure wet body weight (BW), Larvae were placed on filter paper with brush.

After few seconds the weights of these larvae were measured with digital balance (WENSAR). Larvae were collected from each culture medium, dried at 60˚C overnight and biomass was measured by digital balance (WENSAR). The experiments were done in three replicates for each culture medium. A control was maintained by rearing the larva in a similar tray containing natural pond soil and water. Acquired data were subjected to statistical analyses by using computer based software Addinsoft XLSTAT-Premium.

3. RESULTS AND DISCUSSION

Bloodworms or chironomid larvae, were cultivated in four different medium along with a control containing normal pond benthic soil. The biomass (Table 1) along with body length and body weight of the fourth instar larvae were measured and compared (Fig.1). The amount of carbon, organic matter and other inorganic components were highest in F4 (Table 1). The study revealed the highest biomass in larvae exposed to F4 (2984.997 ± 8.50 mg) followed by F3 (2879.41 ± 12.25 mg), F2 (1629.24 ± 11.4 mg) and F1 (1615.61±6.09 mg) in that order. Whereas, only 645.97 ± 3.15 mg of biomass was observed in *Chironomus* present in the control (Table 1). There was significant effect of culture medium on the biomass of *Chironomus* ($F=5774.86$ $p<0.05$ df 3, 17) with a 0.99 strength of association (ω^2). Analyses were done with the mean of dependent variables which showed significant effect of culture medium on BL vs. BW ($t=3.621$, $p<0.05$, df=8), BL vs. Biomass ($t=4.444$, $p<0.05$, df=8) and BW vs. Biomass ($t=4.443$, $p<0.05$, df=8). Also, F4= 90.66%, F3=87%, F2=85.33% and F1=86.33%, when survival rate of the blood worm was concerned (Table 1). Biplot designed from PCA have indicated a close relation between fish food and mixed food medium when compared to F1, F2 and control (Fig. 2). Moreover F3 and F4 have positive influence on biomass of the blood worm, compared to the other food medium. Chemical analyses revealed that percentage of organic matter and carbon were almost equal in F2 and F3 which were nearly similar to the control. Whereas, their percentage were highest in F4 (Table 1).

Commercial fish food like tetramine is generally considered for laboratory culture of Chironomids [21]. In our study, we use some non-conventional material such as dried potato peel powder, dried commercial bakery yeast besides fish food. The present study revealed a significant relationship between body length and body weight of this insect cultured in different media, which in turn also affected the biomass of this insect. When potato peel (F1) and yeast (F2) were used separately, less biomass and growth was observed. Whereas, performance was almost double in the mixed medium (F3). PCA analysis also exhibited the same (Fig. 2). Body weight of 4th instar larvae were found almost similar in both F3 and F4. Similar trend was observed when larval biomass was considered, but F3 was more cost effective than fish food and richer in both protein (34.7%) and carbohydrate (48.5%) as estimated in the laboratory, when compared to used fish food (viz. OPTIMUM) which contains protein (28%). Chemical analyses of the culture media showed that percentage of both carbon and organic matter and other inorganic components were highest in F4. Whereas, biomass was almost similar in F3, though amount of these variables were less. Potato peel powder (F1) was used in our experiment, which contains huge amount of cellulose, but performance of F1 alone was very poor and may be due to the absence of protein which was compensated in the mixed medium. Survival rate of *C. striatapennis*, cultured in F3 was higher than F4 and almost similar to control. This indicated the

Table 1. The biomass and survival rates of *Chironomus* larvae grown on different media, and the chemical constituents of the media

	Control	F1	F2	F3	F4
BIOMASS (mg)(Mean±SE)	645.97±3.15	1615.61±6.69	1629.243±11.40	2879.41±12.25	2984.99±8.50
Survival rate (%)	95.33	89.66	87.66	92.33	88.00
Carbon (%) of the medium	0.505	0.699	0.427	0.445	1.696
Organic matter (%) of the medium	0.870	1.205	0.736	0.767	2.923
Phosphate (ppm)	0.25	2	3	3.5	5
Iron (ppm)	0.15	0.3	0.2	0.2	1
Nitrate (ppm)	0	30	5	5	10
Nitrite (ppm)	0.25	3	0.5	0.5	1
Ammonium (ppm)	0.25	0.25	0.5	0.5	1

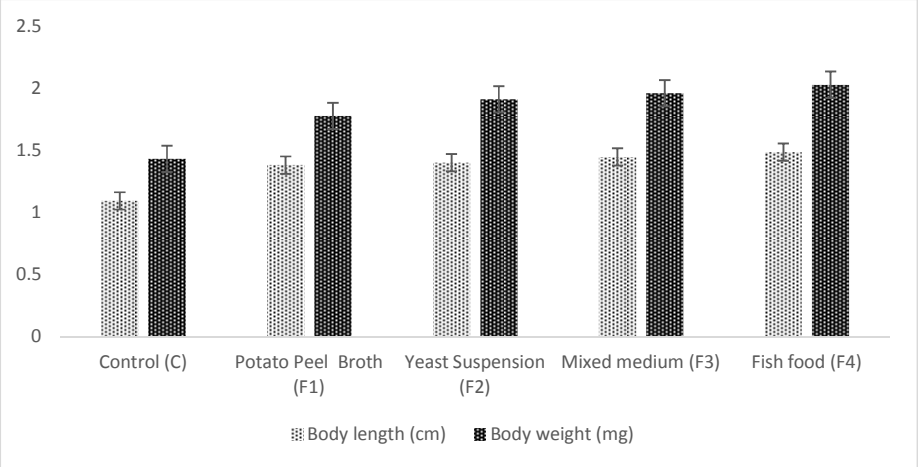


Fig. 1. Comparing body length and weight of *C. striatapennis* larvae (Early 4th instar) in different culture medium

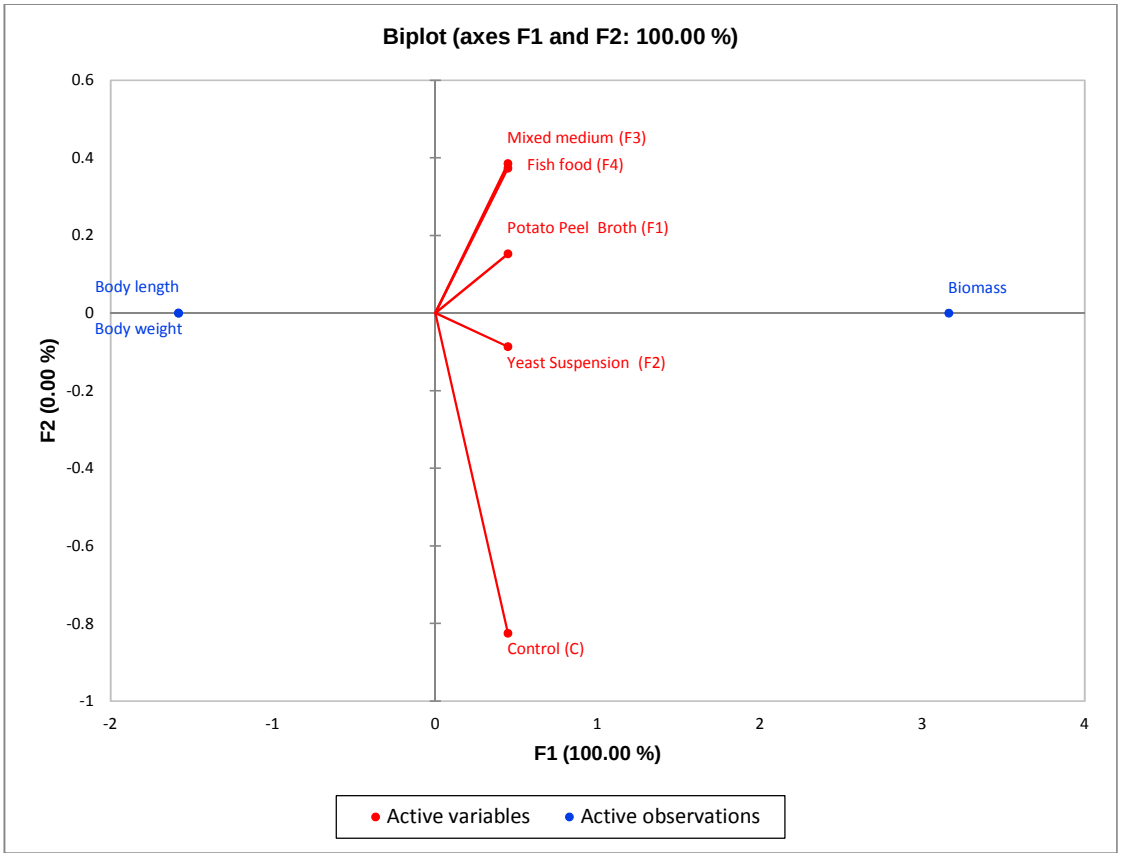


Fig. 2. Biplot showing the ordination of variables

preference of this species to F3. Live food is highly preferred by fish due to its mobility and rapid edibility, permitting the fish to respond to the 'food' movement and their wastage is also less than commercial fish food. Unutilised commercial fish food might contaminate the pisciculture pond being high in dry matter [4]. As a natural food, blood worm is mostly preferred by many freshwater fish species [2,22]. This insect is rich in protein and essential amino acids along with fatty acids, which are suitable

for the fish growth [23]. This insect is available naturally in lentic and lotic habitat of different region of the world, though their availability depends upon different physicochemical parameters [24]. Its production in nature depends upon the pattern of the substrate and their rate of production is influenced by seasonal variations [25,26,27]. But for continuous production of chironomid as fish food, it is necessary to maintain cost effective mass culture in artificial condition. So, it may be commented that for the *Chironomus* culture, F3 was a better option than the other three media as well as the control in the present study. The content of F3 i.e. potato peel powder, a vegetable waste and bakery yeast are almost no cost material but they gave high yield when biomass of chironomid larvae was concerned.

4. CONCLUSION

The present study was conducted to observe the growth and biomass of *Chironomus striatapennis* larvae exposed to different food media namely, Potato Peel Powder, Bakery Yeast, mixed Potato and Bakery yeast, and Commercial Fish Food respectively along with a Control. The mixed culture medium was found most cost effective and better than the other three media as well as the control, when growth and biomass of *Chironomid* larvae was concerned.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Science and Technology, Government of West Bengal for providing financial assistance as Major Project to Dr. Susanta Nath for this work. Authors are also thankful to the Principal, Government General Degree College Singur, for providing laboratory facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tokeshi M. Production ecology. In: Armitage, P. D., Cranston, P. S. and Pinder, L. C. V. (ed) The chironomid biology and ecology of non-biting midges, Chapman and Hall, London. 1995;269-296.
2. Medeiros ESF, Arthington AH. The importance of zooplankton in the diets of three native fish species in floodplain waterholes of a dryland river, the Macintyre River, Australia. Hydrobiologia. 2008; 614(1):19-31.
3. Broyer J, Curtet L. The influence of fish farming intensification on taxonomic richness and biomass density of macrophyte-dwelling invertebrates in French fishponds. Knowl. Managt. Aquatic Ecosyst. 2011;400(10):10p1-10p12.
Available:https://doi.org/10.1051/kmae/2011017
4. Bogut I, Has-Schön E, Adámek Z, Rajković V, Galović D. *Chironomus plumosus* larvae – a suitable nutrient for fresh waterfarmed fish. Poljoprivreda. 2007;13(1):159-162.
5. Kumar D. *Chironomus* larvae culture –A boon to Aquaculture sector. IJCSR. 2016;2(1):239-251.
6. Failla AJ, Vasquez AA, Fujimoto M, Ram JL. The ecological, economic and public health impacts of nuisance Chironomids and their potential as aquatic invaders. Aquat Invasions. 2015;10(1):1–15.
7. Sulistiyarto B, Christiana I, Yulintine. Developing production technique of blood warm (*Chironomid* larvae) in flood plain waters for fish feed. Int. J. Fish. Aquac. 20146(4):39-45.
8. Carbone D, Faggio C. Importance of prebiotics in aquaculture as immune-stimulants. Effects on immune system of *Sparus aurata* and *Dicentrarchus labrax*. Fish Shellfish Immunol. 2016;54:172-178.
9. Faggio C, Piccione G, Marafioti S, Arfuso F, Trischitta F, Fortino G, Fazio F. Monthly variations of haematological parameters of *Sparus aurata* and *Dicentrarchus labrax* reared in Mediterranean land off-shore tanks. Cahiers de Biologie Marine. 2014;55:437-443.
10. Nath S, Matozzo V, Bhandari D, Faggio C. Growth and liver histology of Channa punctatus exposed to a common biofertilizer. Nat. Prod. Res; 2018.
Available:https://doi.org/10.1080/14786419.2018.1428586 in press
11. Kuvangkadilok C. Laboratory studies on the life cycle and breeding of the midges *Chironomous plumatisetigerus* (Diptera: Chironomidae). J. Sci. Soc. Thailand. 1994;20: 125-133.
12. Howarth FG, Oishi DE. The nuisance marine midge, *Kiefferulus longilobus*, is established in Hawai‘i (Diptera: Chironomidae). Occasional paper. 2013;114:59–60.
13. Henriques-Oliveira AL, Nessimian JL, Dorvillé LFM. Feeding habitats of *Chironomid* larvae (Insecta: Diptera) from a stream in the Floresta da Tijuca, Rio de Janeiro, Brazil. Rev. Bras. Biol. 2003;63(2):269-281.

14. Naser MN, Roy D. Feeding Ecology Of Chironomus Larvae (Insecta: Diptera) Collected From Different Habitat Of Dhaka, Bangladesh. Bangladesh J. Zool. 2012;40(1): 129-133.
15. Johannsson OE. Energy dynamics of the eutrophic chironomid *Chironomus plumosusf. semireductus* from the Bay of Quinte, Lake Ontario. Can. J. Fish. Aquat. Sci. 1980;37(8): 1254–1265.
16. Habib MAB, Yusoff SM, Phang SM, Ang KJ, Mohamed S. Nutritional values of Chironomid larvae grown in palm oil mill effluent and algal culture. Aquaculture. 1997;158(1-2): 95-105.
17. Maier KJ, Kosalwat P, Knight AW. Culture of *Chironomus decorus* (Diptera: Chironomidae) and the effect of temperature on its life history. Environ. Entom. 1990;19(6):1681- 1688.
18. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J. Biol. Chem. 1951;193:265–275.
19. Du Bois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956;28(3):350–356.
20. Bhaduri S, Sarkar P, Ghosh C, Midya T. Laboratory rearing of *Chironomus striatipennis* Kieffer (Diptera: Chironmidae). Proc. Zoological Soc. 2012;65(2):121-125.
21. Habashy M. Culture of *Chironomid* larvae (Insecta - Diptera *Chironomidae*) under different feeding systems. Egypt J Aquat Res. 2005;31:403-418.
22. Thipkonglars N, Taparhudee W, Kaewnern M, Iawonyawut K. Cold preservation of *Chironomid* larvae (*Chironomous fuscipes* Yamamoto 1990); Nutritional value and potential for climbing perch (*Anabas testudineaus* Bloch. 1992) larval nursing. Kasetsart University Fisheries Research Bulletin. 2010;34(2):1-13.
23. Kara T. Seasonal variation of vitamin and sterol content of *Chironomidae* larvae. Pak. J. Biol. Sci. 2013;16(22):1579-1583.
24. Podder R, Nath S. Impact of aquatic physicochemical parameters on the diversity and body protein of Chironomids in Proceedings of International Conference on Health Environment & Industrial Biotechnology ICHEIB (Biosangam 2013), McGraw Hill Education (India) Private Limited, New Delhi. 2013;214-219.
25. Saliu JK, Ovuorie UR. The artificial substrate preference of invertebrates in Ogbe Creek, Lagos, Nigeria. Life Sci. J. 2007;4(3):77-81.
26. Nath S, Podder R, Modak BK. Seasonal trend of body protein and growth of Chironomid larvae. Proc. Zool. Soc. 2015;70(1):88-91.
27. Ozkan N, Moubayed-Breil J, Camur-Elipek B. Ecological analysis of Chironomid larvae (Diptera, *Chironomidae*) in Ergene river basin (Turkish Thrace). Turk. J. Fish. Aquat. Sci. 2010;10: 93-99.