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Culture of Chironomid larvae using two different feeds

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Abstract
Rearing of chironomid larvae was conducted under laboratory conditions, to study the effect of two different feed (Yeast and *Scenedesmus*) on the growth rate (weight & length) and hemoglobin content of chironomid larvae. The lengths of Chironomid larvae were measured weekly with the help of a calibrated microscope and the weight was determined using a sensitive weighing balance (SE2 Ultra-microbalance). The hemoglobin concentration was determined using cyano-methemoglobin method according to Van Kampen and Zijlstra (1961) and Tentori and Salvati (1981). Larvae had mean initial length 2.3mm and mean weight 2.0µg. Larvae fed with yeast showed final mean length of 3.75±0.17mm and weight of 4.36±o.21µg, while those fed with scenedesmus showed final mean length of 3.16±0.17mm and weight of 4.09±0.11µg. The hemoglobin count of chironomids fed with yeast was 0.684µgHbmg^{-1} while that of the larvae fed scenedesmus was 0.649µgHbmg^{-1}. Mortality of 5 chironomids was observed in the yeast setup against 8 observed in the setup with scenedesmus, while 41 burrowed chironomids was observed in the scenedesmus setup against 25 observed in the yeast setup. ANOVA reveals significant difference in the effect of both feeds on the growth rate of the chironomid larvae ($P<0.05$). The results among others at the end of the exposure demonstrated that yeast is more suitable for chironomid culture than scenedesmus. This work therefore revealed the suitability of yeast in chironomid culture over scenedesmus.

Keywords: culture, Chironomid, larvae, yeast, *Scenedesmus*, hemoglobin

Introduction

The chironomids are one of the most abundant macroinvetebrate group and they often account for majority of aquatic insects in fresh water environments (Freimuth and Bass, 1994; Epler, 2001).They live in tubes (casts) constructed by themselves with mud or silk secreted from the salivary gland of their body (Swapna *et al.,* 2012).Valuable feeding composition of bloodworms, low expenses and facility of their production tended to be an appropriate live food for cultured aquatics (Farhard *et al.,* 2011). Fish fries supplied with bloodworms has higher body size, growth rate, survival and stocking success (volkman *et al.,* 2004). They are known as bloodworms or red worms due to the presence of hemoglobin in their bodies (Habashy, 2005).

In natural conditions, chironomid larvae feed on decomposed organic sediments and aquatic algae and in laboratory conditions, they may feed on yeast, tetramin etc (Habashy, 2005).

Laboratory rearing of chironomid larvae appears to be problematic because of its dual mode of life in water and in terrestrial environment. Gravid females lay eggs into jelly mass and the eggs hatch into small larvae which spends several days in water and when fully grown is transferred into pupa which leads the aquatic life. Within 2days, the pupa emerges into adult flies which lead the terrestrial life (Swapna *et al.,* 2012).

Vos *et al*. (2000) reported that the nutritional requirement of sediment -feeding invertebrates are poorly understood, thus chironomids are found mostly in the wild and not in culture systems unlike fishes. Also, natural habitat of chironomids and their reproduction areas have gradually been limited due to human activities. Therefore artificial production of bloodworms with economic destination is important (Farhad *et al.,* 2011).

Chironomid larvae are an important food source for both tropical and temperate fishes and birds such as waterfowl (Ciborowski and Corkum, 2003). Many cultured fishes lack natural taste not because they are not well cultured but because they are not fed natural feeds found in their natural habitat. There is therefore an urgent need to study the effects of feeds on their growth and hemoglobin concentration as this will not only provide natural feeds for fishes under culture but will also provide important information on chironomid culture which will enable interested individuals carryout various desired researches on chironomid larvae.

This study is carried out with the following objectives in mind:

- To compare the effects of the two feeds (Yeast and *Scenedesmus*) on the growth (weight and length) of Chironomid larvae.
- To compare the effects of the two feeds (Yeast and *Scenedesmus*) on hemoglobin concentration of Chironomid larvae.
- To identify the healthier of the two feeds for chironomid culture.

The following hypothesis was put forward to guide the research:

- There is no significant difference in the effect of each feed on growth and hemoglobin concentration of Chironomid larvae.

Materials and Methods

Study Area

The study was carried out at the Department of biological sciences, faculty of science, Ahmadu Bello University Zaria. Zaria is located between Latitude 11^o 11'N and Longitude 07^o 38'E, at an altitude of 686 metres above sea level. It lies within the Guinea Savanna zone, and has 3 distinct seasons including harmattan (Nov- Feb), hot (Mar -May) and rainy (Jun- Oct). Its annual rainfall, average temperature and relative humidity are 1055 millimetres, 24.55^o C and 43.6% respectively (Meteorological Unit IAR, 1999).

Larvae Collection

Chironomid larvae were collected from samaru stream along ABU press and acclimatized for 24 hours in collection containers after which hundred (100) larvae were counted into two (2) containers (plastic takeaway plates) each, both containing 2cm thick sediment layer made of prewashed and combusted sand autoclaved at 550^{o}C for 6 hours and stale 500ml of tap water (PH=7.2). The setup was constantly aerated and the work was replicated three (3) times for both treatments.

Growth Experiment

Length and weight determination

Hundred (100) larvae (second instars) were placed in each container using a small eye piece dropper (3 container for each treatment). The rate of feeding was 0.15mg per container twice a week. A total of ten (10) larvae were selected randomly and measured using calibrated microscope and the mean body length was taken on weekly basis. Group of ten (10) individuals of approximately equal size were selected randomly and placed in a small culture dish of water and most of the water was removed. The larvae that accumulated at the bottom were transferred to a piece of filter paper with rubber spoon to avoid killing the larvae. After about eight seconds, the larvae were transferred again to another piece of filter paper and weighed to the nearest 0.001µg using sensitive weighing balance (SE2 Ultra-microbalance) and the larvae mean weight was then estimated.
This, alongside hemoglobin content determination was carried out once in a week for 3 weeks after which the total number of larvae that developed into flies were recorded for each feed.

Hemoglobin Content Determination

The cyano-methemoglobin method described by Van Kampen and Zijlstra (1961) and by Tentori and Salvati (1981) was employed. At present, this method is considered to be the most reliable procedure for the measurement of hemoglobin concentrations (IntPanis *et al.,* 1996). Wet weights were measured on a sensitive weighing balance after blotting the larvae on paper. This method was found to be reproducible and accurate. Lyophilization (freeze-drying) of the larvae prior to the analysis is not necessary and may produce anomalous results (IntPanis *et al.,* 1994b). Hemoglobin determinations were carried out on individual defrosted specimens with a wet weight of more than 2 mg (IntPanis *et al.,* 1996). All hemoglobin determinations were, therefore, carried out on individual defrosted specimens with a wet weight of approximately 3.8mg.The larvae were homogenized with a pestle in an Eppendorf vial containing 200µl of a reagent containing potassium ferricyanide [$K_3Fe(CN)_6$], potassium cyanide (KCN), potassium dihydrogen phosphate (KH_2PO_4), and a nonionic detergent (nonidet P40) . The hemoglobin that is oxidized by the action of $K_3Fe(CN)_6$, binds CN - to give cyano-methemoglobin (Hb+-CN -). KH_2PO_4 keeps the pH at a value at which the reactions are completed within 5 min. The detergent prevents turbidity by proteins. Subsequently, these vials were centrifuged for 30 minutes in an Eppendorf 5414 centrifuge at 4000 rpm. The supernatant was removed and centrifuged again. Spectrophotometric analysis was done in a spectrophotometer after calibrating the ligtith using the reagent containing potassium ferricyanide [$K_3Fe(CN)_6$], potassium cyanide (KCN), potassium dihydrogen phosphate (KH_2PO_4), and a nonionic detergent (nonidet P40). Extinctions of the supernatant were measured at wavelengths of 540 and 650nm. Hemoglobin concentrations were calculated according to equation:

$$[Hb] = \frac{(A(540nm) - A(650N\ nm)) * Mr * 2.0)}{E540nm * 1 * WW}$$

Where [Hb] is the hemoglobin concentration in µg HB mg-1 WW; A(x) is the absorption of the supernatant at x

nm; Mr is the molecular weight of hemoglobin monomer; 2.0 is a dilution factor; E' 40" is the mM extinction coefficient; 1 is the light path in cm and WW is the wet weight in mg.

Determination of Biological Parameters

The number of casts (silky or sandy tubes made by larvae prior to emergence as adult flies) produced by the larvae was counted and recorded.

The number of chironomid larvae that burrowed (penetration into sediment mostly when conditions are not very favourable) was counted and recorded during the three (3) observations.

The number of deaths (mortality of the larvae) was observed and recorded for both treatments. The number of flies that emerged from both treatments was counted and recorded accordingly.

Determination of Water Quality

The quantity of the stale tap water used for the experiment was determined by calculating the dissolved oxygen, temperature and PH.

Dissolved Oxygen Determination

300ml of the water sample was collected into a conical flask. 2ml of magnesium tetraoxosulphate (iv) was added and stirred, after which 2ml of alkaline iodide azide was added. The mixture was shaken thoroughly and allowed to settle for a while.

Concentrated Hydrogen tetraoxosulphate (VI) acid was then added. 100ml of the solution was measured into a conical flask and 0.5ml of starch solution was added and stirred gently, after which titration was carried out using titrant, and readings were taken.

Temperature and pH Determination

The temperature and pH of the water sample were taken using the pH/temperature scale.

Statistical Analysis

Analysis of Variance (ANOVA) was employed to test for significant difference between the effects of the feeds at 0.05 significance level.

Results

Mean weight

Mean weight of chironomids that were fed with *Scenedesmus* was 2.13±0.09µg during the first week of observation and was lower than those of Chironomids that were fed with yeast which had average weight of 2.42±0.11µg on the first day of observation. It was observed during the second week that mean weight for larvae fed with *Scenedesmus* increased to3.37±0.16 µg while the mean weight of the larvae fed with yeast increased to 3.71±0.18µg During the period of three (3) weeks of observation, the final mean weight of larvae fed with *Scenedesmus* was 4.09±0.16µg while those fed yeast had 4.36±0.21µg. Hence increase in weight for larvae fed with yeast between the first and second week of observation was higher in comparism to the increase observed between the second and third week of observation which was higher compared to those fed with *Scenedesmus* as shown in Table 1 below.

Mean length

Table 2 shows that the mean length of chironomid larvae fed with *Scenedesmus* during the first week of observation was 2.36±0.06 mm while for those that were fed with yeast, mean length was 2.77±0.12mm at first week f observation. During the second week of observation, mean length of larvae fed with *Scenedesmus* increased to 2.73±0.14mm and those fed with yeast increased to 3.33±0.16mm. During the third week of observation, mean length for larvae fed with *Scenedesmus* became 3.16±0.20mm. On the other hand, larvae fed with yeast had mean length of 3.75±0.17mm. There was significant difference between the yeast and Scenedesmus feeds for the three weeks of observation ($P<0.05$) but the increase in length observed between the first and second week of observation was considerably higher compared to the increase recorded between the second and third week of observation.

Table 1: Mean Weekly Values of Weight (µg) of Chironomid Larvae fed separately with Yeast and *Scenesdesmus* for three weeks

Diet Type	Week 1	Week 2	Week 3
Yeast	2.42 ± 0.11^{a}	3.71 ± 0.18^{a}	4.36 ± 0.21^{a}
Scenesdesmus	2.13 ± 0.09^{b}	3.37 ± 0.16^{b}	4.09 ± 0.16^{b}

NOTE: Values are represented as mean ± SD. Mean with different superscripted alphabets across the column are different. $P<0.05$

Table 2: Mean Weekly Values of Length (mm) of Chironomid Larvae fed separately with Yeast and *Scenesdesmus* for three weeks.

Diet Type	Week 1	Week 2	Week 3
Yeast	2.77 ± 0.12^{a}	3.33 ± 0.16^{a}	3.75 ± 0.17^{a}
Scenesdesmus	2.36 ± 0.06^{b}	2.73 ± 0.14^{b}	3.16 ± 0.20^{b}

NOTE: Values are represented as mean ± SD. Mean with different superscripted alphabets across the column are different. $P<0.05$

Mean hemoglobin

During the first week of observation, chironomids that were fed with *Scenedesmus* had mean hemoglobin concentration of $0.587\mu gHbmg^{-1}$ while those fed with yeast had $0.561\mu gHbmg^{-1}$. Hemoglobin concentration increased with $0.018\mu gHbmg^{-1}$ and $0.064\mu gHbmg^{-1}$ for *Scenedesmus* and yeast respectively making the hemoglobin concentration during the second week of observation for *Scenedesmus* and yeast to be $0.605\mu gHbmg^{-1}$ and $0.625\mu gHbmg^{-1}$ respectively. During the third week of observation, hemoglobin concentration of chironomids that were fed with *Scenedesmus* increased to $0.650\ \mu gHbmg^{-1}$ and those fed with yeast had $0.686\mu gHbmg^{-1}$ as illustrated in Figure 1.

Mean number of burrows

For the chironomids that were fed with *Scenedesmus*, 2 of the larvae burrowed between the first and sixth day of observation while only 1 larva burrowed from those that were fed with yeast. However, while about 7 chironomids burrowed from the *Scenedesmus* treatment between seventh and fifteenth day of observation, 4 larvae burrowed from yeast fed treatment. 14 and 8 chironomids burrowed respectively from *Scenesdesmus* and yeast fed treatments between sixteenth and the twentieth day of observation. On the twenty first day of observation 18 and 12 chironomids burrowed from the *Scenedesmus* and yeast treatment respectively. The cumulative number of burrows by chironomids fed *Scenedesmus* and yeast therefore was 41 and 25 respectively at the end of the experiment. Figure 2 depicts this observation.

Number of Casts Produced

The number of cast produced by chironomids fed with yeast and *scenesdesmus* between the first and sixth day of observation were 5 and 7 respectively. Between day 7 and 15, nine 9 casts were produced by chironomids that were fed *Scenedesmus* while 7casts were produced by chironomids fed with yeast. Between the sixth and twentieth day of observation, 10 casts were observed in the treatment fed *Scenedesmus* and 8 casts were observed in the treatment fed yeast. 0n the twenty first day of observation 22 casts were produced from the chironomids fed with *Scenedesmus* while 21 casts were produced from the chironomids fed with yeast, making the total number of casts produced by *Scenedesmus* and yeast fed chironomids to be 48 and 41 respectively as described in Figure 3.

Mortality of Larvae

Although no death was recorded for the first 6 days of observation, 1 was recorded between day 7 and 15 for the larvae fed yeast. No death was however recorded from the larvae fed with *Scenedesmus* between day 1and 15. 0n the twentieth day of observation, 3 and 2 deaths were recorded for larvae fed *Scenedesmus* and yeast respectively while on the twenty first day, 5 and 2 deaths were recorded for *Scenedesmus* and yeast respectively. Hence, total number of mortality recorded for both yeast and *Scenedesmus* during the 3 wks of observation was 5 and 8 respectively as seen in Figure 4.

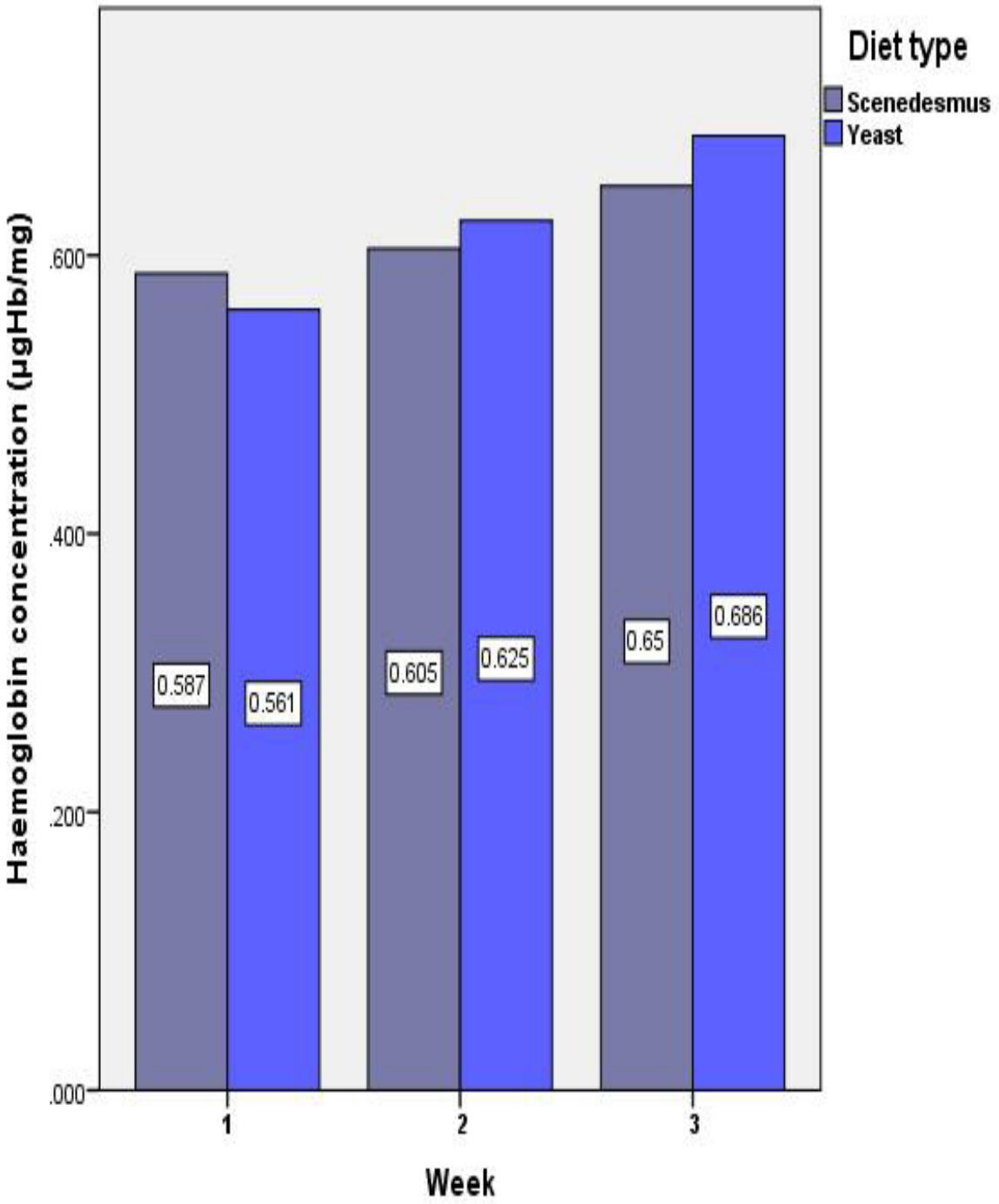

Figure 1: Mean hemoglobin concentration of chironomids fed yeast and *Scenedesmus* separately for 3 weeks.

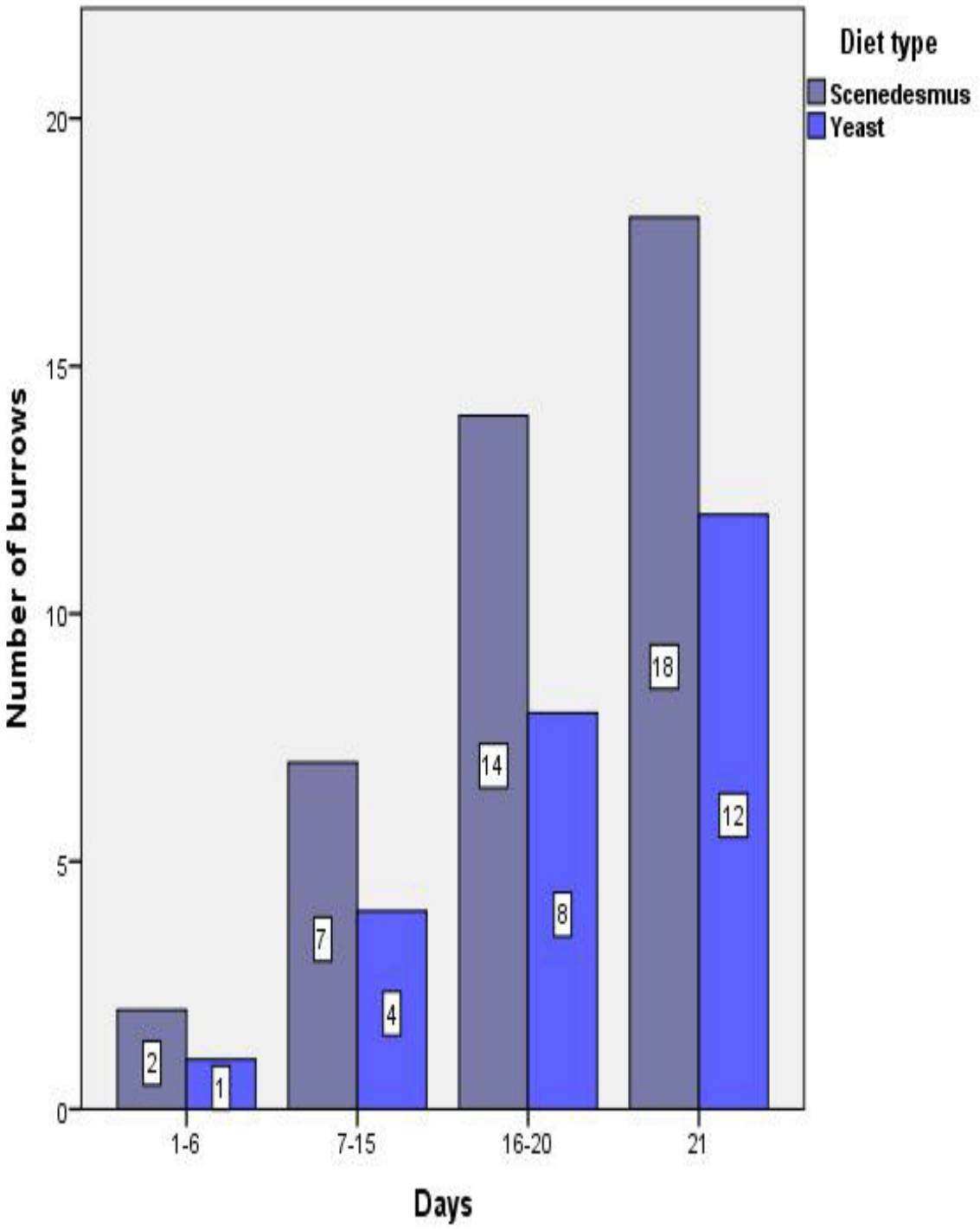

Figure 2: Number of burrows by chironomid larvae fed separately with yeast and *Scenedesmus* feed for 3 weeks.

Fly Emergence

During the first 15 days of observation, none of the larvae from both treatments (*Scenedesmus* and yeast) emerged as adult larvae (chironomid fly) but between day 16 and 20, 2 flies each emerged from the *Scenedesmus* and yeast fed larvae. On the day 21 of observation, 5 and 8 flies emerged from the *Scenedesmus* and yeast treatments respectively. The total number of flies that emerged at the end of the 3 weeks experiment were7 and 10 for *Scenedesmus* and yeast respectively.

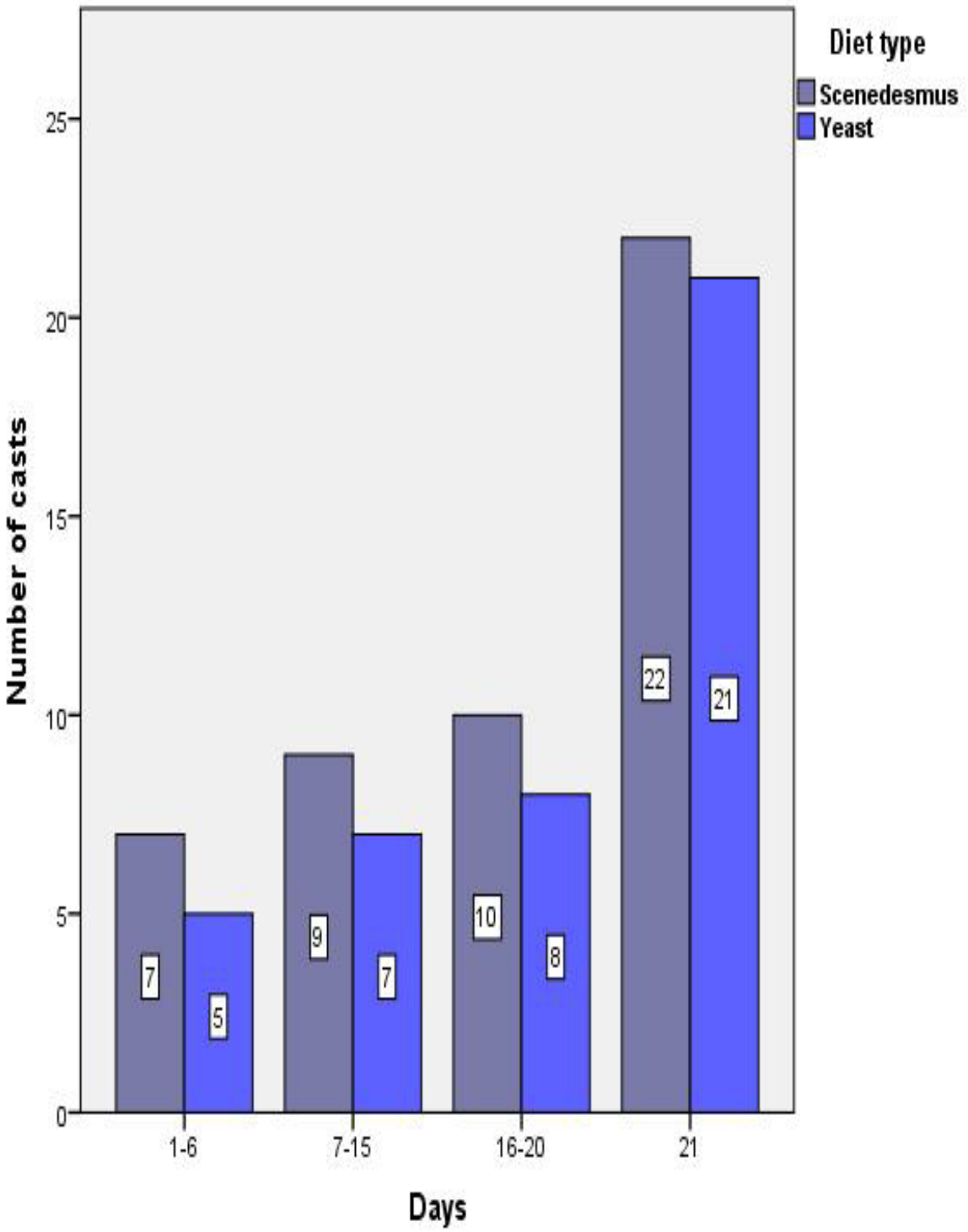

Figure 3: Number of casts made by chironomid larvae fed separately with yeast and *Scenedesmus* for 3 weeks.

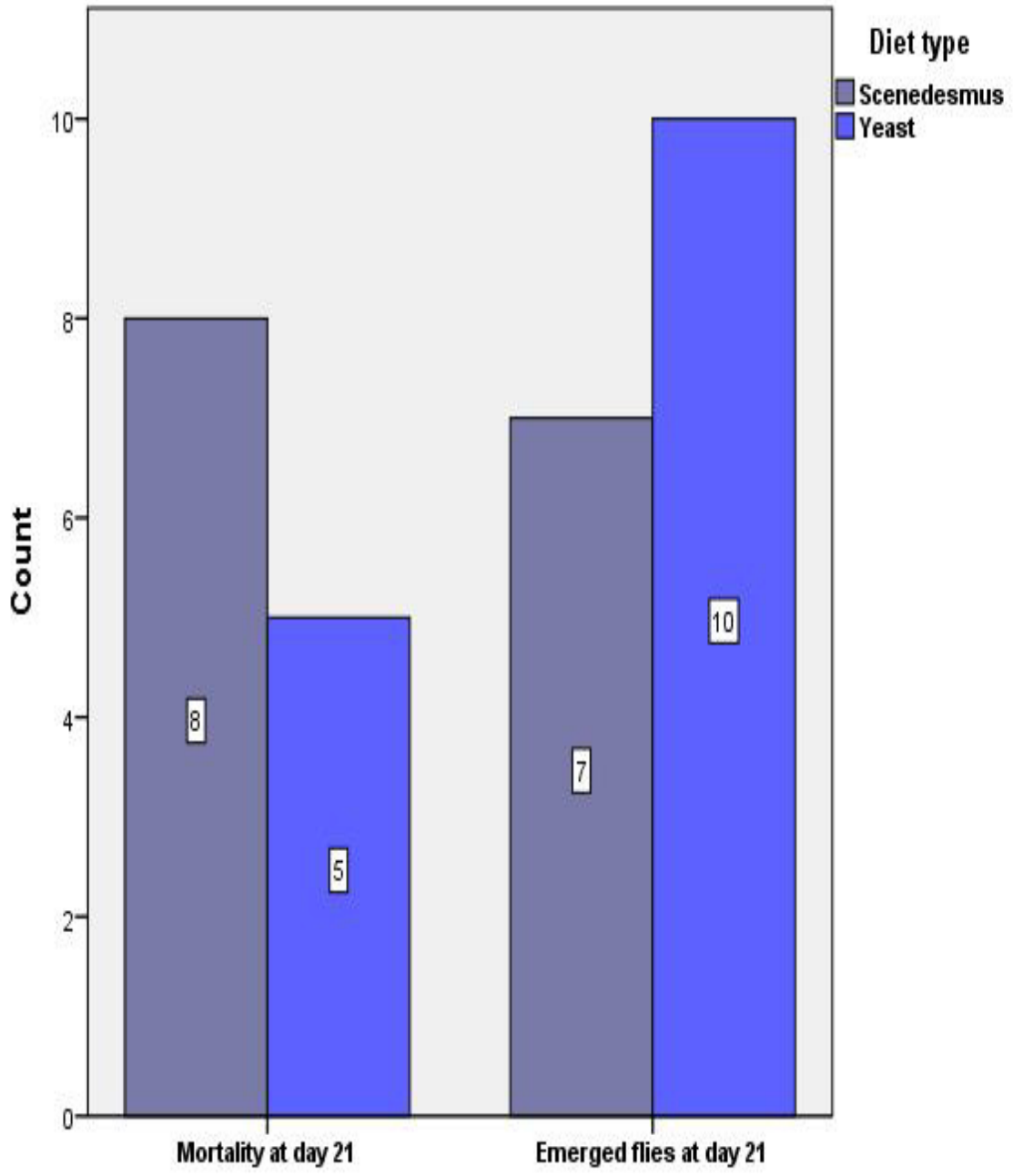

Figure 4: Number of deaths and fly emergence recorded for chironomids fed with yeast and *Scenedesmus* after 3 weeks

Discussion

In this study, Yeast and *Scenedesmus* were used as diets in the culture of chironomid larvae although there are different kinds of diets that can be used for Chironomid feeding; freshwater microalgae (*Isochrysis* sp., *Chlorella* sp), yeast and many other artificial diets. Habashy (2005) reared chironomid larvae in the laboratory and fed them three different types of diets which were Tetramin Flaked commercially available fish food, algae (*Scenedesmus sp*.) and baker's yeast.

Chironomids that were fed with *Scenedesmus* had 2.13±0.09µg mean weight during the first week of observation which was lower than those fed with yeast that had mean weight of 2.42±0.11µg even though weight range of 2.00-2.10µg was estimated for both treatments at the beginning of the experiment. The final mean weight of larvae fed with *Scenedesmus* was 4.09±0.16µg while those fed with yeast had 4.36±0.21µg. ANOVA revealed

significant difference between both feeds (P<0.05), this is in accordance with the findings of Habashy, (2005) who reported a mean weight of 7.47µg and 6.83µg for chironomids fed with yeast and *Scenedesmus* respectively on the first week of observation as well as a final mean weight of 31.03µg and 27.08µg for yeast and *Scenedesmus* treatments respectively.

The mean length of chironomid larvae fed with *Scenedesmus* and yeast during the first week of observation was 2.36±0.06mm and 2.77±0.12mm respectively at first week of observation. This is similar to the report of Habashy (2005) where length of chironomid at first week of observation for yeast and *Scenedesmus* feeds were 2.90mm and 2.81mm respectively. Final mean length of 3.16±0.20mm and 3.75±0.17mm were recorded for *Scenedesmus* and yeast respectively in this study and interestingly, this is also in line with the results of Habashy (2005) in which final mean length for chironomids fed with yeast and was 7.03mm and 6.28mm respectively. There was significant difference between the yeast and *Scenedesmus* feeds for the three weeks of observation (P<0.05).

The Hemoglobin counts of chironomids were determined and those fed with yeast had higher hemoglobin content. For the 3weeks exposure, chironomids that fed on yeast initially had average hemoglobin count of 0.562 and a final average hemoglobin count of 0.684 while those that were fed *Scenedesmus* had initial average hemoglobin count of 0.587 and a final average hemoglobin count of 0.649. Hence, confirming the hypothesis that yeast is more suitable for chironomid culture. Chironomids fed with yeast had higher hemoglobin count than those that were fed with *Scenedesmus*.

Chironomids are known to burrow into the soil when conditions are not very favorable. There was continuous burrowing as weeks passed. Chironomids fed yeast had initial average burrows of 1 and final of 25 while those fed *Scenedesmus* had initial average burrows of 7 and final average of 41. Chironomids fed with *Scenedesmus* had higher number of burrowed individuals compared to those fed yeast. Since burrowing is a phenomenon whereby organisms, in this case chironomids go into the sediment from surface water when the conditions of the water is no longer conducive for them, one can easily say that the conditions of surface water in the yeast treatment was better and more suitable hence less number of chironomids burrowed.

Mortality seems to increase with increase in number of days. Initially there was no mortality but with increasing number of days, mortality started increasing. Total average mortality of chironomids fed with *Scenedesmus* was 8 and those fed with yeast was 5. Therefore chironomids that fed on yeast had lower mortality.

Normally, chironomids are known to make casts prior to their emergence as adults in the form of flies. The average total casts made by chironomids fed with *Scenedesmus* were 12 and that of chironomids fed with yeast were 9 but surprisingly more flies were observed from the cages containing the chironomids that were fed yeast even though they had less number of chironomids casts unlike the *Scenedesmus* treatment which had more number of casts yet less number of flies. This exposes the suitability of yeast for chironomid culture in relation to *Scenedesmus*.

In general, the chironomids fed with yeast had higher length, weight and hemoglobin content indicating better growth possibly because they are 50% protein and a rich source of vitamin B1, B2, Niacin and Folic acid which are easily digestible while those fed with *Scenedesmu*s had lower weight, length and hemoglobin count indicating lower growth. Also, Chironomids fed with yeast as discussed earlier had lower average total mortality compared to those fed with *Scenedesmus*. This tells us that the yeast treatment had a better surviving environment therefore fewer deaths were recorded. Chironomid casts are made by chironomids prior to fly emergence. Even though the chironomids fed with *Scenedesmus* had higher number of casts, those fed with yeast had more no of flies. Yeast is therefore more suitable in this aspect for chironomid culture unlike *Scenedesmus* which had more number of casts yet less flies emerged.

Conclusion

The comparison of the response of Chironomid larvae fed with yeast and *Scenedesmus* to growth as well as biological parameters clearly shows that yeast is a better feed for chironomid culture.

Recommendation

This research work was based on just two feeds; it is therefore recommended that more feeds should be used and their effects should be compared together as this will help to identify which feed is best among wide varieties of feeds.

Also, a multigenerational research should be carried out on chironomid larvae as they are important component of the ecosystem.

References

Ciborowski JJH, Corkum LD (2003) Sediment-zoobenthos interactions. In: *Evaluating Ecosystem Results of PCB Control Measures within the Detroit River–Western Lake Erie Basin,* Appendix 9: 78-82. United Earth Fund.

Cummins KW, Klug MJ (1979) Feeding ecology on stream invertebrates.*Ann. Ver. Ecol. Syst*., *10*: 147-172.

Epler JH (2001) *Identification manual for the larval Chironomidae (Diptera) of North and South Carolina.* Pp. 27 Source: http://www.esb.enr.state.nc.us/BAUwww/Chironomid.htm

Farhad R, Nassrin M, Habib S, Ahmad B, Mohammad M, Ahad S (2011) Academic Journal of Entomology 4 (2): 41-46,

Habashy MM (2005) Culture of Chironomidae larvae (Insecta-Diptera Chironomidae) under different feeding systems. Egyptian Journal of Aquatic Research 31(2):403-418.

IntPanis L, Goddeeris B, Verheyen R (1996) "On the relationship between vertical microdistribution and adaptations to oxygen stress in littoral Chironomidae (Diptera)".*Hydrobiologia*318: 61–67. doi:10.1007/BF00014132.

IntPanis L, Goddeeris B, Verheyen, R (1994b) The hemoglobin concentration of Chironomus cf. plumosus L. (Diptera: Chironomidae) larvae from two lentic habitats . Netherlands Journal of Aquatic Ecology (in press).

Tentori L, Salvati A (1981) Hemoglobinometry in Human Blood.In:Antonini, E., Rossi-Bernardi, L. and Chiancone. E. (eds), Methodsin Enzymology, Hemoglobins. Academic Press 76: 707-715.

Van-Kampen, E, Zijlstra W (1961) Standardization of hemoglobinometryII. The hemoglobincyanide method. Clinicachimicaacta 6: 538-544.

Volkman ET, Pangle KL, Rajchel DL, Sutton TM (2004) Hatchery performance attributes of juvenile lake sturgeon fed two natural food types. North American J. Aquac., 66: 105-112.

Vos JH, Ooijevaar MAG, Postma JE, Admiraal W (2000) Interaction between food availability and food quality during growth of early instar chironomid larvae. Journal of North American Benthological Society. 19:(1), 158-168.

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