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ISSN 0792 - 156X

PUBLISHER:
Israeli Journal of Aquaculture - BAMIGDEH -
Kibbutz Ein Hamifratz, Mobile Post 25210,
ISRAEL
Phone: + 972 52 3965809
http://siamb.org.il

Population Growth of the Freshwater Cladoceran, *Diaphanosoma excisum*, Fed Different Densities of the Alga, *Scenedesmus acuminatus*

Solomon I. Ovie[1*] and Stella O. Ovie[2]

[1] *Limnology Program, National Institute for Freshwater Fisheries Research, PMB 6006, New Bussa, Niger State, Nigeria*

[2] *Fish Nutrition Program, National Institute for Freshwater Fisheries Research, PMB 6006, New Bussa, Niger State, Nigeria*

(Received 28.8.07, Accepted 25.10.07)

Key words: population growth, zooplankton, *Diaphanosoma excisum*, feeding, alga, *Scenedesmus acuminatus*

Abstract

The freshwater cladoceran, *Diaphanosoma excisum* Sars 1885, was fed the micro-alga *Scenedesmus acuminatus*, at one of six densities (0.5, 1.0, 1.5, 2.0, 3.0, or 4.0 x 10^6 cells/ml) in 40-l glass aquaria. The *D. excisum* density increased as the *Scenedesmus* density increased to 1.5 x 10^6 cells/ml, in which it peaked at 7345 individuals per liter. Population growth was inhibited at higher algae densities. The percent of egg-bearing females and the number of eggs per egg-bearing female followed a similar pattern. Production of approximately 7000 individuals per liter was encouraging from a mass production point of view and indicates that *S. acuminatus* is suitable for use as a live starter feed for *D. excisum* which, in turn, is a live feed for larvae of induced-breeding in fish hatcheries.

Introduction

Feed and feeding regimes are crucial considerations in studies of the growth of aquatic organisms. The quantity and quality of micro-algae are major factors that affect the rate of zooplankton consumption, assimilation, and development in culture (Goulden et al., 1982; James and Abu Reseq, 1988; Ovie and Egborge, 2002; Savas and Guclu, 2006). Increased food concentration and quality lower the age at maturity and, consequently, enhance the rate of egg production and number of offspring per female (Duncan, 1989; Dumont et al., 1995; Ovie and Egborge, 2002). A number of zooplankton species are raised in small scale and mass cultures for fish larvae. One of the most frequently used freshwater micro-alga used to produce zooplankton is *Scenedesmus* (De Pauw and Persoone, 1988). This alga, alone or in combination with other species, has been used in

* Corresponding author. E-mail: soloovie@yahoo.com

the culture and maintenance of *Daphnia* (Goulden et al., 1982) *Brachionus rubens* (Peters, 1987), *Anuraeopsis fissa* (Dumont et al., 1995), and *Moina micrura* (Ovie and Egborge, 2002).

Several zooplankton species are mass-cultured for rearing fish larvae at the fish hatchery of the National Institute for Freshwater Fisheries Research (NIFFR) in New Bussa, Nigeria. *Scenedesmus acuminatus* is the principal algae used as food for the zooplankton. Often, the zooplankton population rapidly crashes when the algae density is high (Ovie and Egborge, 2002). Thus, this research was designed to determine the ideal *Scenedesmus* level for optimum and sustained culture of various freshwater zooplankton used for fish larviculture.

Diaphanosoma excisum, a common freshwater zooplankton, has great potential as a starter diet for larvae of commercial carnivorous fish species such as *Clarias anguillaris, Heterobranchus bidorsalis, H. longifilis*, and their hybrids (Ovie et al., 1993; Adeyemo et. al., 1994). In this study, *D. excisum* were fed different densities of *S. acuminatus* to determine the optimum feeding density for growth of the zooplankton.

Materials and Methods

The choice of six algae densities (0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 x 10^6 cells/ml), was based on an earlier study in which *Moina micrura* was the tested organism (Ovie and Egborge, 2002). Each density was serially diluted from a concentrated stock of the alga, harvested, and stored at the exponential growth phase. The required density was computed according to N = X(V/Y), where N = volume of inoculum, X = desired algal density, V = volume of culture vessel, and Y = density of algal stock (Escritor and Javellana, 1981; Ovie et al., 1993; Ovie and Egborge, 2002).

Each density was carefully measured using a measuring cylinder and inoculated into a 40-l glass aquarium in triplicate. The aquaria were inoculated with laboratory-raised *D. excisum* at an initial density of 200 animals/l (Escritor and Javellana, 1981; Ovie and Egborge, 2002). The *D. excisum* inoculum consisted of egg-bearing females, non-egg-bearing females, and neonates. Cultures were aerated to prevent stratification (settling) of algal cells and to remove volatile metabolites (Heisig, 1979; Ovie and Egborge, 2002).

The densities of the algae and zooplankton populations were estimated daily using a hemocytometer and a Sedgewick-Rafter counting chamber, respectively. The population density of *D. excisum* was estimated using two aliquot sub-samples of 0.5-1 ml culture, taken immediately after aeration was switched off. The samples were fixed in sugar-frosted 5% formalin to prevent egg ballooning (Prepas, 1978). Algae densities were kept constant by adding fresh exponentially growing *Scenedesmus* cells.

The daily algae feed requirements were computed as: feed/ml to be added = V(desired-actual/algal count-desired), where desired = desired feeding density (i.e., 0.5-4.0 x 10^6 cell/ml), actual = actual algae count in culture, algal count = algae count in stock, and V = culture volume (Escritor and Javellana, 1981).

After an equivalent volume of culture medium was removed from the tank, the daily algae requirement was added by filtering it through a 50-µm standard nylon zooplankton net as described by Ovie et al. (1993) and Ovie and Egborge (2002). Trapped *D. excisum* individuals were returned to the culture immediately to prevent stress and possible death.

Data on egg-bearing females and number of eggs per egg-bearing female were collected daily and analyzed by filling a Sedge-Wick rafter zooplankton chamber (1-ml capacity) with the culture sample after mild agitation of the sample bottle to disperse organisms. Adult individuals were counted (total count) under a compound binocular microscope. The percent of egg-bearing females were computed from the total count while the number of eggs per egg-bearing female was computed from the first fifty egg-bearing females encountered in the sample.

Dissolved oxygen (DO), pH, and temperature were routinely taken by standard methods (APHA, 1980).

The experiment was terminated when the population growth of the *D. excisum* dropped. One way analysis of variance (ANOVA) was computed using SPSS at a significance level of $p<0.05$.

Results

Temperature, pH, and DO were fairly similar in all cultures, ranging 29.6-32.5°C, 6.8-7.6, and 6.4-7.2 mg/l. Due to rapid development of parthenogenetic females, the density of *D. excisum* rapidly increased and peaked on day 4 or 5 (Table 1). The highest density was obtained when the *Scenedesmus* level was 1.5 x 10^6 cells/ml. The only significantly different treatments were the 0.5 and 1.5 x 10^6 cells/ml treatments.

The percentage of egg-bearing females and number of eggs per egg-bearing female followed a similar pattern, i.e., the highest values for both were obtained in the algae density of 1.5 x 10^6 cells/ml (Tables 2, 3). The percentage of egg-bearing females significantly differed among all treatments except treatments 0.5, 1.0, and 3.0, and treatments 1.5 and 2.0. The number of eggs per egg-bearing female significantly differed between treatments 0.5 and 2.0, 0.5 and 4.0, 1.0 and 3.0, 1.0 and 4.0, 1.5 and 3.0, 1.5 and 4.0, 2.0 and 3.0, 2.0 and 4.0, and 3.0 and 4.0.

In general, the number of eggs per female ranged 2.0-4.5. Population growth, percentage of egg-bearing females, and number of eggs per egg-bearing females were similar in treatments 1.5 and 2.0.

Discussion

The decline in *D. excisum* production above the algal density of 1.5 x 10^6 cells/ml indicated that high densities of micro-alga inhibit zooplankton population growth, just as low *Scenedesmus* densities result in sub-optimal levels of *D. excisum*. The insignificant difference between the 1.5 and 2.0 treatments in population increase, percent egg-bearing females, and number of eggs per egg-bearing female indicate that these levels are optimal for *D. excisum* culture.

Several reasons have been suggested for the inhibitory properties of algae feed at high densities. Peters (1987) and Ivleva (1973) showed that too high algae densities result in overfeeding, obstruction of the filtration apparatus, and suffocation to death of animals. Hirata (1979) maintained that high micro-algae feed density often leads to a population decrease as a result of the fouling effect of accumulated feces and uneaten food. Heisig (1979) attributed such inhibition to a temporary depression of dissolved oxygen mainly caused

Table 1. Growth performance of *D. excisum* fed different algal densities of Sc*enedesmus.*

	Algae density (x 10^6)					
Day	*0.5*	*1.0*	*1.5*	*2.0*	*3.0*	*4.0*
0	200±0.00	200±0.00	200±0.00	200±0.00	200±0.00	200±0.00
1	375±185	415±200	600±245	502±352	385±146	350±150
2	680±270	875±450	1650±320	1300±408	960±250	865±215
3	1565±425	3560±400	5123±375	4956±380	2560±202	2240±300
4	2050±480	4920±450	7345±480	6438±475	3765±300	3500±245
5	2842±560	4070±380	6012±395	5820±350	3850±285	3450±375
6	2650±390	3965±345	5040±420	4020±220	3624±325	3400±280
7	2500±450	3750±400	4900±380	3856±300	3450±402	3260±400
8	1860±490	2600±450	3800±435	2920±425	2860±360	260±295

Table 2. Percentage of egg-bearing females of *Diaphanosoma excisum* fed different algal densities of *Scenedesmus*.

	Algae density (x 10^6)					
Day	*0.5*	*1.0*	*1.5*	*2.0*	*3.0*	*4.0*
0	5.3±0.60	5.3±0.60	5.3±0.60	5.3±0.60	5.3±0.60	5.3±0.60
1	6.2±0.53	6.2±0.35	9.3±1.20	7.8±0.95	6.5±0.40	4.8±0.5
2	6.5±0.58	7.35±0.40	11.8±.90	10.2±1.00	6.3±0.60	4.3±0.8
3	8.9±0.90	7.40±0.53	15.6±1.80	13.6±0.90	8.5±0.80	5.7±0.40
4	7.60±0.84	8.26±1.20	17.6±1.45	12.4±0.72	5.0±0.56	5.3±0.50
5	10.30±0.90	7.45±0.80	16.7±1.10	13.5±1.30	8.5±0.40	8.4±1.20
6	6.5±0.45	8.50±0.50	14.3±0.80	13.6±1.80	7.6±0.72	6.0±0.70
7	9.19±0.75	8.8±0.90	13.3±1.30	11.2±0.90	6.8±1.00	5.2±0.83
8	7.5±0.50	8.25±0.75	12±0.75	11.8±0.85	7.2±1.50	4.8±0.73

Table 3. Number of eggs per egg-bearing female of *D. excisum* fed different algal densities of *Scenedesmus.*

	Algae density (x 10^6)					
Day	*0.5*	*1.0*	*1.5*	*2.0*	*3.0*	*4.0*
0	2.5±0.32	2.6±0.20	3.2±0.50	3.0±0.30	2.8±0.25	2.3±0.50
1	3.0±0.20	3.2±0.33	3.0±0.60	3.2±0.45	2.2±0.30	2.5±0.22
2	3.2±0.40	3.0±0.32	3.5±0.50	3.6±0.30	2.8±0.25	2.8±0.35
3	3.5±0.50	3.8±0.40	4.5±0.70	4.0±0.35	3.2±0.60	3.0±0.26
4	3.4±0.70	3.5±0.28	4.0±0.43	3.8±0.38	3.5±0.40	2.3±0.20
5	3.0±0.50	3.6±0.33	3.5±0.34	3.5±0.35	2.6±0.35	2.0±0.25
6	3.0±0.60	8.5±0.50	14.3±0.80	13.6±1.80	7.6±0.72	6.0±0.70
7	3.0±0.60	3.2±0.20	3.0±0.30	3.2±0.25	2.5±0.28	2.2±0.30
8	2.2±0.60	2.7±0.34	2.8±0.40	3.0±0.03	2.7±0.50	2.3±0.40

Values are means and standard deviations of three replicates.

by the intense algae respiration of high densities. Too high a concentration of algae caused mortality of rotifers by choking the corona and resulting in decomposition of toxic products and secretions by the algae (Sarma and Rao, 1990). A population of *Moina micrura* was similarly inhibited when fed high densities of *Scenedesmus* (Ovie and Egborge, 2002) and filtration and ingestion rates of *Brachionus plicatilis* dropped in high algae feed densities (Yufera and Pascual, 1985).

The inhibitory effect of *S. acuminatus* on the population growth of *D. excisum* could be due to a combination of these factors. However, continuous aeration of the cultures ensured that dissolved oxygen and pH were

adequate and that volatile metabolites were removed. For example, excess ammonia, inimical to zooplankton growth, is known to accumulate in cultures without aeration (Pagano et al., 2000). At low algae densities, the lower population development may have been due to limited food as observed by Sarma and Rao (1990), Pagano et al. (2000), and Ovie and Egborge (2002). In general, the decline in population growth in all treatments after its peak is attributed to crowding, which regulates population increases (Sarma and Rao, 1990; Bonou and Saint-Jean, 1998; Pagano et al., 2000; Ovie and Ebgorge, 2002).

Comparative data on the growth of *D. excisum* reared on a single species of algae is scanty. Pagano et al. (2000) reared *D. excisum* on phytoplanktons consisting of eleven species of Chlorophycea (including *Scenedesmus acuminatus* and *S. obtusus*), two species of Cyanophycea, and one diatom, and obtained a maximum density of 1200 individuals/l. Therefore, the peak production in our study, approximately 7,000 individuals/l, can be considered satisfactory and the microalga *S. acuminatus*, maintained at the optimal density of 1.5-2.0 x 10^6 cells/ml is confirmed as a suitable diet for the culture of the freshwater cladoceran*, D. excisum*.

Acknowledgements

We are grateful to the Federal Ministry of Agriculture through the National Institute for Freshwater Fisheries Research, New Bussa, for providing funds for the Project on Natural Live Fish Food Culture for fingerling production.

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