Full Length Research Paper

Enhancing rotifer mass culture through algal diversity

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Outdoor cultures of *Brachionus quadridentatus* raised in 10 m^3 concrete tanks fed on co-cultured three algal species (*Chlorella vulgaris*, *Eudorina elegans* and *Scenedesmus quadricauda*) feed showed statistically significant differences in population biomass growth. Growth of *B. quadridentatus* feeding on *S. quadricauda* was significantly greater than when fed on *C. vulgaris.* No significant differences in growth were observed between *Brachionus* cultures fed on *S. quadricauda* and *E. elegans* and between those fed on *C. vulgaris* and *E. elegans.* Daily *B. quadridentatus* population biomass increase was highest with *S. quadricauda*, followed by *E. elegans* and lastly *C. vulgaris. B. quadridentatus* needed approximately 20, 48 and 63 h, respectively, with *C. vulgaris*, *S. quadricauda* and *E. elegans*, to double its population.

Key words: Zooplankton, algae, culture, nutrient.

INTRODUCTION

Mass production of catfish under controlled conditions depends on the provision of live plankton food for early fry and larval stages. The importance of live food in fry and larval rearing has been reported (Ovie et al., 1993; Ajah and Holzlöhner, 1996; Ajah, 1997, 1998; Hagiwara et al., 1997, 2007). The successful production of finfish or shellfish such as shrimps and crabs not only requires a starter diet such as *Lepadella ovalis* (L-type (93.6 × 70.3 μm^2) or S-type (70.3 × 35.1 μm^2) (Ajah and Ajah, in press), but also a second diet such as *B. quadridentatus* (432.9 × 128.7 μm^2) and a third diet, for example, *Asplanchna* (685 × 356 μm^2) (Ajah, 2008). A number of authors have emphasized the importance of adequate supply of rotifer cultures with the appropriate nutritional quality, for the survival of larvae cultured in marine hatcheries (Hirata, 1980; Kafuku and Ikonoune, 1983; Watanabe et al., 1983; Lubzens, 1987). Previous authors like Ovie et al. (1993); Adeyemo et al. (1994) and Ajah (1997, 1998) have established the advantages of fre-shwater plankton over *Artemia nauplii*, a salt- water Ano-straca. It is best to culture freshwater fish with freshwater live foods (Ovie et al., 1993; Ajah, 1997). *B. quadric-dentatus,* a rotiferan, could be a suitable alternative to *A. nauplii*, being of equivalent size to imported *A. nauplii*. Another reason for preferring *B. quadridentatus* is that, it occurred quite frequently and in large numbers in ponds at the University of Calabar fish farm. Earlier, Lubzens et

al. (1990) stated that rotifers (*Brachionus plicatilis*) cons-titute a major, and in most cases the only food source for larval stages of several organisms raised in marine aquaculture including fish and various invertebrates.

The culture of at least 10 species of marine rotifers including *B. plicatilis*, *Brachionus rotundiformis*, *Brachionus pterodinoides*, *Brachionus satanicus* and *Hexarthra jenkinae* is well documented (Gatesoupe and Luquet 1981; Lubzens, 1987; Lubzens et al., 1989, 1990; Hampton and Starkweather, 1998). Gatesoupe and Luquet (1981) noted that there could be nutritional differences in the value of rotifers as food for larvae based on the feeding conditions of the rotifers themselves. Watanabe et al. (1979), found that *Chlorella*-fed and yeast-fed rotifers differed in their proximate compositions. Ajah (1998) found increased growth and survivorship of *Heterobranchus longifilis* larvae when fed on enriched zooplankton.

Earlier, Fujita (1979) indicated the importance of long chain ω-3 polyunsaturated fatty acids in rotifers as food for Red Sea bream larvae, and found a further improvement in the dietary value of yeast cultured rotifers by secondary culture with marine *Chlorella* for 6 h. *Senecio quadridentatus* has been found to thrive better in freshwater cultures than *B. plicatilis* (Ajah, 1995).

One advantage of *B. quadridentatus* over *B. plicatilis* is Its low failure rate during culture. *B. quadridentatus*

culture was maintained continuously for two years irrespective of seasons with little fluctuation in population size (Ajah, 1995) . The main difference between the dry and wet season blooms is in the initiation of blooms which brought about a doubling of the initial nutrients for the first two days during the wet season (Ajah, 1995).

Brachionus calyciflorus, a freshwater rotifer was successfully fed on two types of algae, *Scenedesmus obliquus* and *Chlorella* sp. (Kennari et al., 2008).

In this study, freshwater food items (*Chlorella vulgaris*, *Eudorina elegans* and *Scenedesmus quadricauda*) that might be more suitable, acceptable, cheap, readily available and capable of yielding higher population densities of *B. quadridentatus* a freshwater rotifera were investigated.

MATERIALS AND METHODS

Three hard bottomed 10 m^3 circular concrete tanks with central drainage pipes and five airlift pipes, arranged in an anticlockwise pattern to keep the plankton culture afloat were used for the outdoor culture of the rotifer. Axenic monocultures of micro algae (*C. vulgaris* Beij. *var. vulgaris* Fott, (2 - 12 μm), *S. quadricauda* (Turp.) Bréb (12 - 15 μm) and *E. elegans* Ehr. (95 - 105 μm) as well as pure monocultures of *B. quadridentatus* (Hermann, 1783), were cultured in the laboratory of the Hatchery Complex of the Institute of Oceanography, University of Calabar, Calabar, Nigeria, using both the "Gelose" and "Dilution" isolation methods (Harder, 1917; McVey and Moore, 1983). The algae samples were collected from the fish ponds of the Institute using a plastic container and inoculated into the algal medium using Pasteur pipette. Axenic algal cultures were produced under highly hygienic laboratory conditions using autoclaving and inhibitory bacterial growth precursors such as 0.005 - 0.01% hypochlorite solution, UV radiation and antibiotics (Ajah, 1995). Species were identified using Ward and Whipple (1959), Bold and Wynne (1978) and Jeje and Fernando (1986).

Zooplankton culture

Two 36-watt daylight fluorescent tubes were suspended 60 cm above a series of 5 - 60 L aquaria to illuminate a wooden cupboard of 120 × 60 × 80 cm^3 (0.6 m^3). Room temperature was kept at 28 ± 1°C and aeration using two 1.5 hp pumps was continuous. The pure zooplankton cultures received 50 mgl^{-1} of baker's yeast (*Saccharomyces cerevisiae*) or 1.75 mgl^{-1} of inorganic fertilizer (N: P: K) (20:10:10) every other day to release the macronutrients needed for zooplankton growth, whereas 1.0 ml of nutrient salt solution A and 0.1 ml of solution C (Laing and Ayala, 1990) were administered every other day to the pure algal cultures (Ajah, 1995).

Outdoor cultures

Fertilization of outdoor cultures was made possible using pig manure supplied regularly by 20 weaned pigs that were fed daily on 10% body weight with pig mash (Akpan and Okafor, 1997). The chemical composition of samples of pig manure was determined and found to contain the essential nutrient elements for algal growth. One (1.0) kg of pig manure was introduced into each outdoor tank during the dry season and 2 kg during the wet season for the first two consecutive days. Thereafter, 1 kg per tank was administered every three days and every other day, for the dry and

the wet seasons, respectively. 50 ml of each axenic algal monoculture, namely, *C. vulgaris*, *S. quadricauda* and *E. elegans*, from the laboratory, were inoculated into the respective tanks at cell densities of 2.6 × 10^6, 1.68 × 10^6 and 0.12 × 10^6 cells/ml. Five litres each of 4 ind ml^{-1} from pure *B. quadridentatus* cultures (Ajah, 1995) were introduced into each 10 m^3 tank. The feeding duration of *B. quadridentatus* with each of the three algae lasted for an average of 18 days (range 15 - 21 days).

Each culture was repeated three times during both the dry season (November to March/ April) and wet months (May/ June to October). The batch culture trials continued for a period of three years amounting to a total of eighteen trials, nine per season (Ajah, 1995, 1997, 1998).

Cell counts

A 1 l plastic funnel was used to scoop samples from the thoroughly mixed plankton cultures. The samples filtered through a 56 μm-plankton sieve to collect and retain both phyto- and zooplankton. Four cell counts of 1 ml each using a haemocytometer and four zooplankton counts using a one ml counting chamber (model: AJAH001) (Ajah, 1995) were carried out every day or every two days, and average values were recorded.

Physicochemical parameters

The physical and chemical factors were monitored throughout the experimental period. Dissolved oxygen in the pond was determined using Lectron 5509 DO meter and temperature was read using standard thermometer. Chlorine was assessed by the chlorosity method of Rump and Krist (1988). Nitrite (NO_2^--N) by the diazotization (spectrophotometric) method; nitrate (NO_3^-N) by the cadmium reduction/ diazotization method, ammonium (NH_4^+-N) level by the Nesslerization (spectrophotometric) method, phosphate by the molybdenum blue method (spectrophotometric) (Parsons et al., 1984), conductivity was read using a HACH 3000 spectrophotometer and turbidity by secchi disc.

Single classification analysis of variance (ANOVA) was used following Sokal and Rohlf (1981) to compare means of population density of zooplankton counts from the replicates. Correlation coefficients between *B. quadridentatus* and each primary producer as well as the coefficients of determination were also calculated using SPSS. Descriptive statistics were used to compare the means. The intrinsic rate of natural increase (r) and the population doubling time in days (t_D) were calculated as follows:

$r = (\ln N_t - \ln N_0) /t = D'/t$ or $2.3026(\log N_t - N_0)/t$ (James and Dias, 1984).

Where:

D' = The dilution time in days.
$N_t = N_0.e^{rt}$ (James and Dias, 1984).
N_t = Final number of individuals.
N_0. = Initial number of individuals, and t = time in days   .
e (exponent) = 2.7183.
r = The intrinsic rate of natural increase.
t_D = 0.6931/r (James and Dias, 1984).
t_D = Doubling time of the population in days.

RESULTS

C. vulgaris, *E. elegans* and *S. quadricauda* showed statistically significant ($_{2,115}F3.27$, p < 0.05) differences in

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Table 1. Daily mean values and summary of the three replicates showing some growth characteristics of *Brachionus quadridentatus* while feeding on the three algae.

	Chlorella vulgaris			*Scenedesmus quadricauda*			*Eudorina elegans*		
Date	r	t_D (time in days)	N_t (ind/L)	r	t_D (time in days)	N_t (ind/L)	r	t_D (time in days)	N_t (ind/L)
1									
4	0.207	3.508	3,000	0.414	1.801	4,000	0.496	1.435	3,000
5	0.433	1.611	15,000	0.504	1.423	18,000	0.638	1.094	8,000
8	0.326	2.129	31,000	0.381	1.835	41,000	0.371	1.369	11,000
9	0.312	2.368	50,000	0.424	1.637	65,000	0.433	1.071	32,000
10	0.284	3.484	62,000	0.291	2.414	70,000	0.286	1.615	46,000
11	0.375	1.848	123,000	0.360	1.936	153,000	0.272	2.422	93,000
12	0.373	1.860	143,000	0.354	1.948	160,000	0.096	5.722	104,000
15	0.310	2.234	124,000	0.158	4.888	155,000	0.119	5.802	118,000
16	0.279	2.488	88,000	0.207	3.366	125,000	0.319	2.169	127,000
17	0.257	2.707	63,000	0.140	4.978	94,000	0.189	3.660	98,000
19	0.198	3.500	55,000	0.155	4.472	77,000	0.170	4.111	55,000
22	0.216	0.482	25,000	0.108	9.02	46,000	0.123	5.724	36,000
Biomass indiv. $\times 10^6/10\ m^3$/day	366.92			489.52			289.05		
Peak densities(ind./L)	102,000			104,000			176,000		
Av. pop. (N_t) (ind/day)	65,167 ± 45622			72,000 ± 51464			60,916 ± 44971		
Av. rate of natural increase (r) l/day)	0.149 ± 0.01			0.276 ± 0.01			0.286 ± 0.01		
Av. Doubling time of pop. (t_D) in days.	0.84 ± 0.89			2.04 ± 0.77			2.61 ± 0.38		
R^2 b/w *Brachionus* and alga density	0.166, p < 0.05			0.044, p > 0.05			0.162, p > 0.05		

population biomass growth and development. *B. quadridentatus'* growth using *S. quadricauda* was significantly ($F_{1,76}$6.804, P 0.01) greater than when fed on *C. vulgaris.* There were no significant ($F_{1,77}$0.330, P 0.05; $F_{1,76}$3.499, P 0.05, respectively) growth differen-ces between *B. quadridentatus*-fed, *S. quadricauda* and *E. elegans*, and, between *B. quadridentatus* fed on *C. vulgaris* and *E. elegans.* Correlation coefficients between *B. quadridentatus* densities fed on *C. vulgaris* and *E. elegans*, *E. elegans* and *S. quadricauda* and *S. quadri- cauda* and *E. elegans* were 0.454, 0.413 and 0.618, respectively.

The population biomass of *B. quadridentatus* increased quite rapidly with *S. quadricauda*, followed by *E. elegans* and lastly *C. vulgaris* (Table 1). High average population densities of *B. quadridentatus* achieved with *C. vulgaris*, *E. elegans* and *S. quadricauda* are shown in Table 1. *B. quadridentatus* showed extremely high rates of natural increase, with correspondingly very short population doubling times (t_D) with *S. quadricauda* (Table 1). The intrinsic rate of natural increase (r) and doubling time in days (t_D) for *B. quadridentatus* using *E. elegans*, and *C. vulgaris* cultures are equally represented in Table 1.

The overall daily mean population growth (N_t) was 65,167 ± 45622, 60,916 ± 44971 and 72,000 ± 51464 for *C. vulgaris*, *E. elegans* and *S. quadricauda*, respectively. However, based on the three replicates, *B. quadridentatus* needed less time, on the average 0.84 days, to double its population when fed on *C. vulgaris*, followed by 2.04 and 2.61 days, respectively, was required by *S. quadricauda* and *E. elegans* to double their populations (Table 1). More stable and higher growth rates were obtained using *S. quadricauda* and *E. elegans* compared to *C. vulgaris*. The chemical composition of the swine manure was: NH_4^+-N = 178.3 mgl^{-1}, SO_4 = 85.44 mgl^{-1}, SiO_2 = 85.2 mgl^{-1}, NO_2^--N (below detectable level), NO_3^-

Figure chart (Wet season / Dry season):

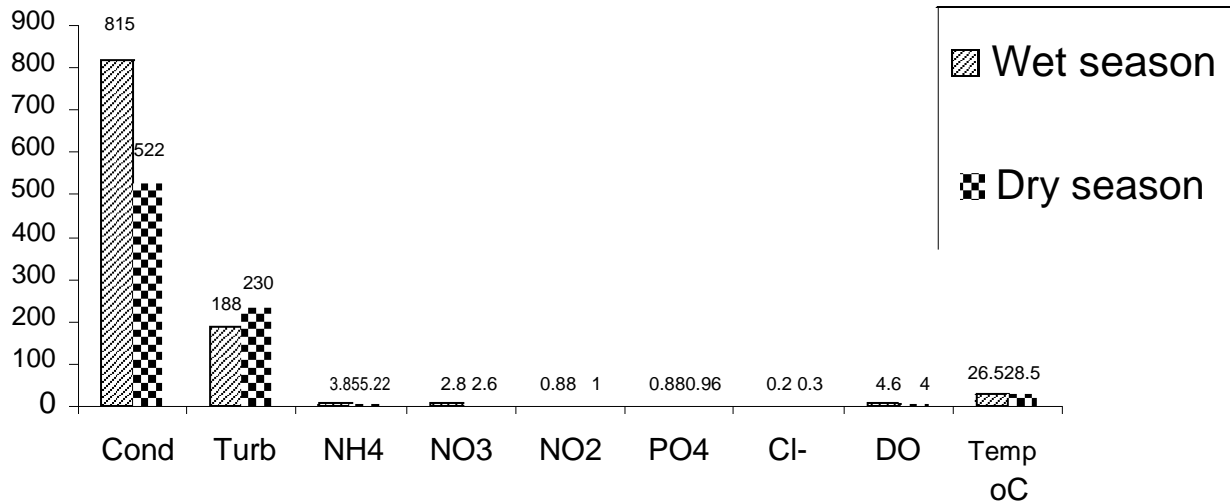|	Cond	Turb	NH4	NO3	NO2	PO4	Cl-	DO	Temp oC
Wet season	815	188	3.85	2.8	0.88	0.88	0.2	4.6	26.5
Dry season	522	230	5.22	2.6	1	0.96	0.3	4	28.5

Figure 1. Showing seasonal mean physicochemical factors and nutrient contents obtained during the culture of *Brachionus quadridentatus* in earth pond.

N = 25.3 mgl-1, PO43--P = 0.64 mgl-1, N = 28.7 ppm/22.7%, P = 0.20 ppm/0.66%, K = 12.5 ppm/31.96% and moisture content 62.6%. The percentages represent total available nitrogen, phosphorus, potassium and moisture content in the sample. The mean values and ranges of the physicochemical parameters and nutrient loads of the culture environments are shown in Figure 1. Temperature ranged from 26.5°C to 29.4°C with average of 27.74 ± 0.108°C. Dissolved oxygen levels ranged from 2.7 - 5.4 mgl^{-1} with a mean of 4.238 ± 0.126 mgl^{-1}. Mean conductivity values in the culture tank were 642.567 ± 142 s/cm ranging from 153.7 - 921 s/cm. The turbidity of the culture system ranged from 109 - 279 FTU with a mean of 218.607 ± 32 FTU. The NO$_3^-$-N level range from 1.081 to 3.050 mgl^{-1} with a mean of 2.048 ± 0.549 mgl^{-1} while the phosphate levels in the tanks were 0.150 to 1.016 mgl^{-1} with a mean of 0.647 ± 0.149 mgl^{-1}.

DISCUSSION

The physicochemical factors and nutrient load were within the acceptable limits required for the proper management of earth ponds (Wedemeyer et al., 1976; Post, 1987; Swift, 1988; Agarwal, 1994; Biswas, 1996, Ajah, 2008). Physicochemical values in this report that fell within the optima required for plankton growth included amongst others temperature (IFAS Circular, 1951), DO (Banerjea, 1967), the electrolyte conductivity, pH and the DO (Ajah, 1995, 2008), turbidity (Ajah, 1995, 2008), nitrate (Sachidanandamurthy and Yajurvedi, 2004) and phosphate (Sawyer, 1947; Screenivasan, 1965).

B. quadridentatus due to its extremely high fecundity doubled its population in only 0.149 days and had a t$_D$ of 0.84, giving it a comparative advantage over *B. plicatilis* that had r = 0.84, t$_D$ = 1.212 obtained with

Nannochloropsis sp. (Ahmad et al., 1991). This puts *B. quadridentatus* as a more promising alternative food source to *Artemia*.

The greater preference for *S. quadricauda* and *E. elegans* to *C. vulgaris* by *B. quadridentatus* could be explained in terms of the voracious nature and size of *Brachionus* as well as its choice of food item. Kennari et al. (2008) rather observed that *B. calyciflorus* had better growth and fatty acid content when fed on *Chlorella sp.* compared with *S. obliquus.* Investigations revealed that phytoplankton abundance was controlled by zooplankton abundance and not fertilization as in a hard water pond (Sierp, 2001), whereas in soft bottom ponds such as ours both zooplankton abundance and fertilization controlled phytoplankton abundance. Ajah (2008) earlier reported a greater preference of *Asplanchna priodonta* for *E. elegans* which confirms the need for larger prey by larger predators.

The initial population growth of *Keratella cochlearis* was significantly affected by the feeding schedule as well as the presence of competitors, while that of *Daphnia* was affected by neither factor. Population densities of both species tended to increase as the frequency of food addition increased (MacIsaac and Gilbert, 1991). Xi et al. (2002) found out variations in population growth, body size and egg size of *B. calyciflorus* while feeding on different algae. Fabiola et al. (2005) equally observed differences in population growth of rotifers and cladocerans when raised on algal diets supplemented with yeast. Mean population growth rate (*r*) of 0.61 and 0.44 were obtained for rotifer fed with *Chlorella* sp. and *S. obliquus* , respectively (kennari et al., 2008) which are lower than values obtained in this report using three algae, *C. vulgaris* (0.149), *S. quadricauda* (0.276) and *E. elegans* (0.286).

Conover and Mayzaud (1984) made the following

generalizations regarding selective grazing: (1) At low concentrations of food, feeding tends to be spread over a wide range of particle sizes, (2) Although "tracking" or selection of biomass peaks as a strategy to facilitate capture of rapidly reproducing, and presumably more nutritious phytoplankton (Poulet and Chanut, 1975), and (3) If the particle has a "suitable" shape and is not chemically distasteful, only then do zooplankton tend to select bigger objects. Points 2 and 3 above agree with the findings made here. My investigation showed that there was an almost equal attraction for the various food items administered, proving that none of the food items had unpleasant odour or taste to *B. quadridentatus*, nor did any possess an awkward shape.

In conclusion, prey size, shape, and motion are implicated as probable causes for the slight differences in feeding behaviour. *B. quadridentatus* generally preferred the bigger prey like *E. elegans*, or medium size prey, but with some flagella-like structure as in *S. quadricauda* as well as prey in constant motion such as *E. elegans.*

The cilia of *B. quadridentatus* were capable of generating waves of current for trapping its prey. Distance was not a barrier for capturing prey, due to its fast motion. *B. quadridentatus* could easily be grown on three algal live diets such as *C. vulgaris*, *E. elegans and S. quadricauda.*

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