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Comparative study of population density of *Brachionus plicatilis* (Mueller, 1786), fed with different microalgae and Selco® (HUFA), cultured in laboratory at 10gL^{-1} salinity.

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ABSTRACT

The rotifer *Brachionus plicatilis* is used like live food in larval stages aquaculture feeding larval stages of crustaceans and fishes, so it is essential to have continuously laboratory mass production of this organism. Present study use three microalgae (*Chlorella vulgaris*, *Sphaerocistys* sp. and *Haematococus pluvialis*), each diet enriched with 10 mL of Selco® solution enriched with fatty acids (HUFA) and a combination of them. The culture salinity was 10gL^{-1} concentration; temperature was controlled at 23 ±2°C, light and continuous aeration. Culture tests were made in 200L cylinders with 160L of water. Every third day, density of organisms were counted during 3 months. The inoculation density was 1.6 million rotifers (10 org/mL). Rotifer density reached between diets were 4.8 x 10^6 to 5.9 x 10^6 L organisms/160 culture medium, with an organisms produced by female of 6.90-8.93; a generation time between 21-31 days; and reproduction rate of 0.070 and 0.089, meanwhile combined diet (three microalgae + Selco reached 35x10^6 org/160L, a production of 59.70 organisms per individual; generation time of 33 days and a growth rate of 0.120. Although each microalgae+Selco diet cover the requirements of size and nutritional composition, the combined diet complete the nutritional requirements as well as the fatty acid supplement, allow higher feed digestibility, better survival and higher rates of reproduction and growth of these filter feeders.

Keywords: Rotifers, HUFA, production, life table

INTRODUCTION

In order to meet the food demands of different aquatic organisms commercially high demanded for aquaculture, *Brachionus plicatilis* shown advantages for their high nutritional value for aquatic consumers; easily isolation and cultured in high concentrations to use it in the development of marine fishes and crustaceans hatcheries (Rose 1998). The rotifer *Brachionus plicatilis* located in the class Aschelminta, order Monogononta, superfamily Brachionoidea (Castellanos 1999), is a microinvertebrate crustacean representing smaller metazoans, pseudocelomata, unsegmented which have global distribution and found in aquatic and semi-terrestrial habitat, sessile or free swimmers forms, who have three anatomical characteristics that identify them: corona, lorica and mastax (Romero 2008).

Nogardy (1993), cited by Romero (2008) cited that rotifers have higher reproductive rates among metazoans and are able to occupied quickly biotopes and reach 30% of the total biomass of plankton. Rotifer reproduction can be sexual and parthenogenesis type, depending culture medium environmental conditions and food type and concentration. The sexual stage involves male presence for a short period such as under laboratory conditions.

Rotifers are not selective filter which feed bacteria and appropriate size organisms like microalgae *Chlorella vulgaris*, *Sphaerocistys* sp. and *Haematococus pluvialis*, which supply proteins and pigments (Cunningham 1999).

The rotifer *Brachionus plicatilis* has been considered in the past 20 years, as one of the most suitable food aquaculture resource, for their easy

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cultivation, little size, nutritional quality and eurythermal and euryhaline capacity (Vallejo, 1993).

MATERIAL AND METHODS

Food preparation

To feed *Brachionus plicatilis* organism's three freshwater microalgae were cultivated (*Chlorella vulgaris*, *Sphaerocistys* sp. and *Haematococus pluvialis*) and enriched solution with HUFA (Selco®). Each three days, 6L of microalgae culture medium were added to rotifer culture medium with 20mL of Selco® solution.

The fertilized used to growth microalgae were Urea (Growgreen product 1 Kg/4L of tap water; 5mL/20mL culture medium) and Triple 17 (N:P:K 17%; 100 g/500mL of tap water; 10 mL/20L culture medium). These quantities were added when microalgae were inoculated at 20 L plastic beakers or 50% of culture medium was changed. The Selco® solution is a emulsified commercial product rich with HUFA fatty acids.

Brachionus plicatilis culture medium

The rotifer culture was made in 200L plastic beakers filled with 160L at $10gL^{-1}$ salinity water. Temperature condition was 25±2°C, pH 7-8 and illumination and aeration were maintained continuously during all experiments. Plastic beakers were inoculated with 10 org/mL concentration and feed with the microalgae+Selco® diets and combined diet+Selco® every three days during three months experiment.

Organisms sampling

Every three days, 10L of rotifer culture medium were filtered and collected biomass was placed in 80mL glass container. Later, with a micropipette 10 samples of 0.1 mL were taken and counted with an optical microscope AO ZX40. The values obtained were extrapolated to 160L.

Life table

Organisms' density values were obtained each third day and processed with a Life Table program to obtain Reproduction rate (Ro), generation time (Tc) and intrinsic growth rate (r).

RESULTS

The rotifer population density values taken each third day were shown in Table 1. It can observe that in all diet experiments, growth rate is constant until day sixty, founded the greatest growth value. The combined diet with three microalgae+Selco reaches their maximum density value three days early (57 day). All diets density values decay until 90 day. Only the combined diet with three microalgae+Selco remains 50% density value respect their maximum value.

The density value for each microalgae+Selco diet maintain a range of 30-37 org/mL; the combined three microalgae+Selco diet reaches 178 org/mL (35,035,480 ±3,340 organisms).

The best production rate (Ro) was shown in combined three microalgae+Selco diet with 59.70 org/female; a generation time (Tc) of 34 days and a growth rate of 0.120. The lowest values were shown with *C. vulgaris* diet (Ro= 6.90; Tc=22 days; r=0.089) (Tabla 2).

In Fig.1 were shown the growth tendency curves from each diet. The best relation (R^2) to explain the growth density from each diet was a polynomial curve.

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Table 1. Sampling means values (±S.D.) of population density from each experimental diet.

Sampling day	*Chlorella vulgaris* + Selco	*Haematococus pluvialis* + Selco	*Sphaerocistys* sp. + Selco	Combined three microalgae + Selco
0	1 600 000	1 600 000	1 600 000	1 600 000
	±5 874	±2 838	±3 088	±3 636
3	658 474	704 407	648 965	699 254
	±4 906	±4 332	±4 524	±3 137
6	680 133	667 377	632 244	690 963
	±4 856	±5 906	±2 840	±4 803
9	676 426	619 839	627 803	697 317
	±4 810	±5 130	±5 967	±5 163
12	1 737 985	1 672 782	1 033 978	3 754 093
	±3 632	±3 965	±3 051	±3 463
15	2 968 758	2 276 786	1 317 812	458 8230
	±4 574	±2 178	±2 819	±3 760
18	3 013 837	2 534 434	2 778 208	4 821 405
	±4 023	±2 209	±2 509	±5443
21	3 439 417	2 715 073	2 961 820	15 879 376
	±2 788	±2 148	±3 040	±5 386
24	3 978 474	3 953 985	3 883 932	16 824 928
	±3 269	±3 297	±5 603	±5 184
27	3 777 899	3 804 125	3 971 762	17 893 742
	±4 885	±5 173	±3 722	±4 080
30	3 719 169	3 771 174	3 801 872	16 774 835
	±5 187	±4 044	±4 869	±3 221
33	3 694 203	3 788 233	3 799 798	16 032 308
	±5 586	±5 807	±2 112	±5 529
36	3 923 672	3 879 698	3 951 109	18 079 738
	±4 825	±5 307	±3 647	±4 218
39	3 946 515	3 941 131	4 084 988	20 096 138
	±2 885	±4 811	±5 503	±4 467
42	4 079 142	4 826 338	4 293 264	2 5304 010
	±5 783	±2 171	±3 329	±3 311
45	4 660 767	4 948 670	5 076 811	2 859 6010
	±2 165	±3 579	±4 335	±5 616

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Table 1. Sampling means values (±S.D.) of rotifer population density (Continue….).

Sampling day	*Chlorella vulgaris* + Selco	*Haematococus pluvialis* + Selco	*Sphaerocistys* sp. + Selco	Combined three microalgae + Selco
48	4 489 547	4 929 909	5 139 894	30 599 420
	±2 931	±3 552	±2 369	±4 900
51	4 640 063	5 087 224	5 235 417	31 132 130
	±3 170	±4 745	±3 546	±2 083
54	4 634 476	5 112 674	5 254 757	32 341 460
	±3 797	±5 175	±2 576	±5 028
57	4 711 831	5 138 016	5 677 971	35 035 480
	±5 309	±3 948	±2 823	±3 340
60	4 834 798	4 926 186	5 962 693	28 378 030
	±2 414	±2 378	±4 393	±2 498
63	4 766 203	2 248 871	4 882 404	26 395 547
	±2 605	±2 466	±5 259	±4 726
66	4 436 294	1 277 971	2 239 759	25 119 991
	±4 584	±5 920	±5 075	±3 071
69	3 724 800	1 280 208	2 778 951	28 910 515
	±4 874	±5 185	±2 951	±3 381
72	2 946 154	1 389 099	1 858 555	29 116 040
	±4 319	±5 494	±3 415	±5 986
75	1 564 487	933 949	712 110	28 743 376
	±2 610	±5 858	±3 667	±3 673
78	686 021	875 559	651 583	28 033 172
	±2 925	±2 678	±2 460	±3 321
81	572 441	874 069	507 890	28 215 304
	±2 518	±3 721	±5 565	±2 682
84	450 122	647 750	444 112	26 428 366
	±2 199	±3 738	±2 732	±4 426
87	263 781	159 209	685 80	16 067 728
	±3 945	±3 750	±3 647	±2232
90	87 136	94 453	455 99	14 512 104
	±2 748	±2 476	±5 573	±6 000

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Table 2. Production rate (Ro), generation time (Tc) and growth rate (r) of rotifers fed with four experimental diets.

Diet provided	Production rate (Ro) $\sum l_x m_x$	Generation time (Tc) $\sum x l_x m_x / R_o$	Growth rate (r) $\log_e R_o / T_c$
Chlorella **+ Selco**	6.90	21.64	0.089
Haematococus **+ Selco**	7.14	23.42	0.084
Sphaerocistys **+ Selco**	8.93	31.03	0.070
Combined three microalgae + Selco	59.70	33.89	0.120

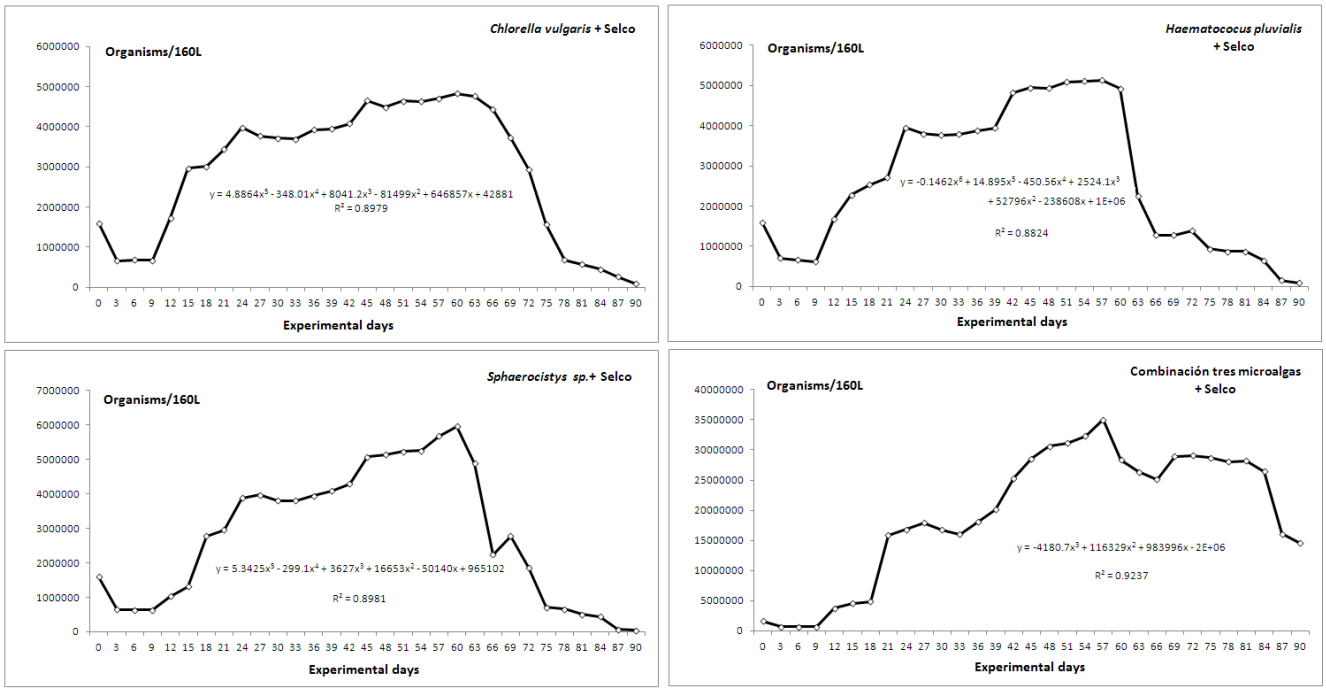

Fig.1. Tendency growth rate curves of rotifer density fed with four experimental diets (microalgae+Selco).

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DISCUSSION

Several studies were made comparing growth density of different rotifer strains fed with different diets. Rotifer intensive cultures medium of *B. plicatilis* can be made using microalgae or combined diets like this research which use three separately microalgae or combined diet, incorporating in all of them a commercial enriching solution (Selco ® HUFA). Perez (1989) and Espinosa (2007) in their researches obtained best results combining *Tetraselmis* sp. microalgae and baking yeast enriched with cod-liver oil, to increase their fatty acid content compared to a conventional diet, because the higher energy obtained by intake food increase the reproduction, growth and maintenance increasing the population density.

Larios (2001), mentioned that with *B. calyciflorus* and *B. patulus* fed with a green alga (*Chlorella vulgaris*) and yeast (*Saccharomyces cerevisiae*) combined diet (50:50) shown better density results. That´s why *C. vulgaris* is one of the most frequently microalgae used as food. It´s has a properly size (1-5 nm), allowing rotifers consume them more efficiently (high filtration rate) and better digestibility as well as increasing the amount of amino acid and protein assimilating in these organisms (Espinosa 2007). Benitez (2006), suggesting that numerical and functional response showed in *B. plicatilis* populations culture medium, is the result, not only of the size of feed used, but also their nutritional value composition. These two conditions were maintained during all experiment.

In density curves figure from each experimental diet, was observed high mortality between 9-10 sampling days of experiment. This period of time, was taken by organisms to acclimatization to salinity, food and culture conditions (Sosa 2007). This author cited that growth rate of these euryhaline organisms capacity, in addition to their generalist food intake (low ability to discriminate the food), allowing them to adapt quickly to environmental conditions medium, and their feed supplied (live food: microalgae; inert food: Selco).

A determinate factor to growth rotifers was water temperature (Pavón 1993), which study rotifer culture at 25, 27 and 29ºC. The better density result was 27°C culture medium. Hirayama and Kusano (1972) cited in Coll (1991) and Pérez (1989), shown that optimal temperature range was 25-27°C, with a 7.7-8.2 pH range. These two factors increase the quantity of eggs per female and consequently the growth density in culture medium. This temperature and pH conditions were maintained in this research and that´s why 30 to 37 org/mL range was observed in separately microalgae+Selco diet, at difference with combined microalgae+Selco diet with 178 org/mL concentration.

In all diet experiments the growth rate ranges were 0,089-0,120. These density values were shown the maximum density value who the container can maintain. After the plastic beaker reach that density concentration, the density values begin to fall down until 90 experiment day. This kind of survival curve is type I when organisms dye at advanced age categories (Valverde 2005). This can be explained due the possibility to controlling physical, chemical and nutritional factors in laboratory conditions during all experiment.

We can conclude that in semi intensive culture system at laboratory, plus be care with physical and chemical parameters, it´s necessary take care about food incorporation, because it´s not only size the mainly factor who need to be considered, also quantity and nutritional value. But it´s important to considered the combined of different microalgae resources with these characteristics and enrich the diet with fatty acids (HUFA), chemical component who don´t found in high levels in green microalgae and be inoculated or incorporated in a lipid solution rich in these fatty acids, which not only cover the nutritional value from rotifers, but improved better feed digestibility, survival, reproduction and growth rate of these little filter feeders (Wallace 2006).

CONCLUSSION

The combination of three green microalgae, with the appropriate size and density concentration, as well as addition of rich fatty acid (HUFA) solution, allows a better population density and reproduction of *B. plicatilis* in laboratory culture medium conditions.

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