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Population density comparison of copepods Order Cyclopida Burmeister, 1834 fed with microalgae and dry yeast in 200 L plastic beakers.

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ABSTRACT

Copepods are the first food link between primary producers (microalgaes) and secondary consumers (fishes) that is why they are considered as an important food source in larvicultura industry, principally in marine fish's aquaculture, where they proved to be an excellent food in larval stages. That's why, this experiment was made to determine the population density of copepod cyclopoid in a triplicate culture system in 200 L plastic beakers, at 25 ± 2°C controlled temperature and pH (7-8), with continuous light and aeration. Each cylinder began with 700 org 100 mL^{-1} density. Every third day, the organisms were fed with 600 mL of microalgae at 500 $x10^3$ cell mL^{-1}. The microalgae diets were *C. vulgaris*, *H. pluvialis*, *Sphaerocystis* sp. and combined diet (200 mL from each microalgae culture). Every third day, a 100 mL sample was taken from 200 L beakers and 10 aliquots were taken to determine population density. The *H. pluvialis* and *Shpaerocystis* sp. diets were able to cultivate only during 33 days while combined diet and *C. vulgaris* diets during 45 culture days. The *C. vulgaris* diet reached densities of > 6000 org 100 mL^{-1}; while the lowest density was found in combined diet with a 300-900 org 100 mL^{-1} range, even though density was constant throughout the experiment. Tendency growth curves formulas were a fourth degree polynomial. The knowledge of the effect of several species of unicellular microalgae on copepods cyclopoid organisms as live food can generate criteria for the better selection of them according to their easy obtainment, easy culture technique in laboratory and facility to produce it in high densities to use it in fish or crustacean larviculture.

Key words: copepods, microalgae, population density, growth tendency curves.

INTRODUCTION

One of productive activities that have grown in industry is aquaculture. This biotechnology has shown an annual growth of 10% for human consumption (White et al. 2004). For this growth, it has been important technological development in food production provided for fishes, crustaceans or mollusks that have commercial importance, but especially fingerlings and larvae of these aquatic organisms, to which living or inert (flaked or pelletized) diets are supplied as food. With respect to inert diets, deficiencies remain in their physical properties, such as stability in water, buoyancy and flavor. This is not the case of live food organisms, which have mobility and odor that is attractive to predators for their easy capture and, can contain or can be modified their nutrient quantity and quality that are essential for optimal growth of aquatic species in aquaculture (Castro et al. 2003).

The first stages of many marine or freshwater fishes and crustacean larvae do not have a well-developed digestive system and organisms used as food like copepod cyclopoids, pass faster through intestine and better digested than other organisms. Their typical zigzag movements are an important visual stimulus for these aquatic organisms, which prefer them to rotifers (Lavens and Sorgeloos 1996; Ruiz et al. 2012).

Copepods generally have high protein content (44-52%) and good amino acid profile, with exception of methionine and histidine and high concentrations of essential fatty acids, which depends on the type of consumed microalgae in

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copepods diet (Lavens and Sorgeloos 1996). Copepods lifespan is complex, before reaching adult stage, this organisms can pass through six nauplii stages (characteristic larval crustacean stage) and five juvenile stages (copepodites) (Morales and Perez 2012). Females have longer lifespan (50-55 days) than males (17-22 days). The copepodites development period in females was 15-16 days, and in males 10-11 days (García et al. 2007).

Copepods are generally aquatic organisms, mostly marine, and have cosmopolitan distribution (Calbet and Landry 2004). Their easily adapt to survive in different habitats, but many species have narrow tolerance ranges to environmental conditions (Suarez 2000). Copepods feed on unicellular organisms such as microalgae and ciliates (Calbet and Landry 2004), therefore this diet is employed in copepods cultures (Lavens and Sorgeloos 1996).

Therefore, it is important to determine potential massive culture at laboratory level, because in those systems environmental conditions can controlled and can supply them with high concentration diets of microalgae to use them for fingerlings and crustacean larvae in aquaculture ponds. In this study, three microalgae were used like live diets: *Chlorella vulgaris*, *Haematococcus pluvialis* and *Sphaerocystis* sp, and a combine diet (three microalgae equally concentration). To achieve copepods nutritional requirements, dry yeast was used as carbohydrates and vitamin supplement.

MATERIAL AND METHODS

Organisms supply. Organisms were obtained from a sample of fish farming pond water of Cuemanco Aquaculture Biological Research Center. The water sample was filtered through a 0.1 mm mesh sieve and organisms were inoculate in 20 L plastic beaker and fed with 600 mL of microalgaes (three experimental microalgae). Temperature maintained to 25 ±2°C, pH 7-8, with continuous light and aeration, during two weeks to acclimate. After that, all organisms were collected by a 250 µm mesh sieve and placed in 2 L vessel. From these vessels, 5 mL samples were taken to separate copepods by micromanipulation. The organisms were put in 4 L plastic beakers at same environmental and food conditions to increase density for putting them in 200 L plastic beakers and began experiment.

Microalgae culture. The three unicellular green microalgae used for experimental test were *C. vulgaris, H. pluvialis* and *Sphaerocystis* sp. that

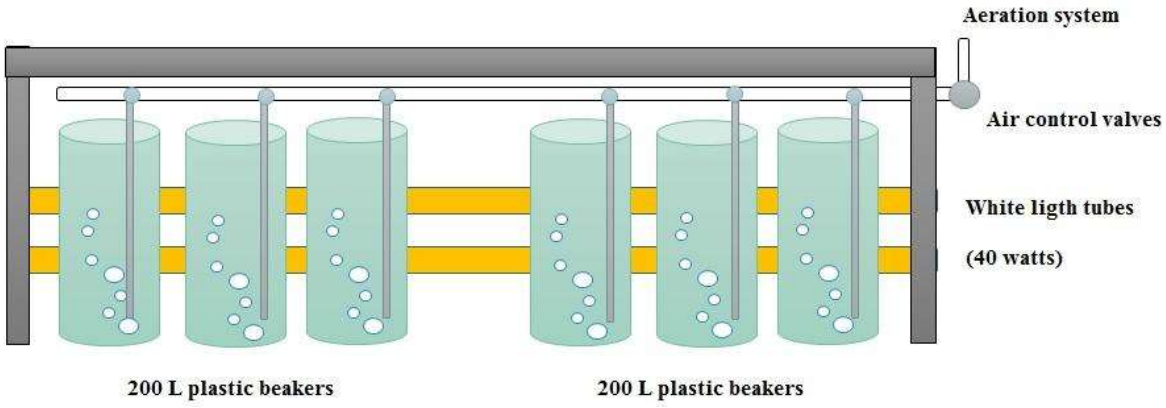

Fig.1. Culture system used to produce massive copepods biomass in laboratory.

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were maintained separately in 20 L plastic beakers with 19 L of pre-filtered water, which was deionized and dechlorinated. The microalgae culture medium was fertilized with 10 mL of Triple 17 (50 g 500 mL^{-1}) and 5 mL of inorganic urea (1 Kg 4 L^{-1} water). Each week, the microalgae concentration in plastic beakers was maintained at 500 x 10^3 cells mL^{-1}.

Experimental system. Copepods were put in 200 L plastic beakers (triplicate) at an initial density of 700 org 100 mL^{-1}, 25 ±2°C temperature medium, pH 7-9, and continuous light and aeration (Fig.1). Four experimental diets were used: 1) *C. vulgaris*, 2) *H. pluvialis*, 3) *Sphaerocystis* sp. and 4) combined diet (three microalgaes at same concentration). Every third day, from each diet, 600 mL were put in experimental beakers, at a concentration of 500 x 10^3 cells mL^{-1}. Combined diet was prepared with 200 mL from each microalgae culture. Copepods maintained on those conditions until all organisms died.

Sampling and data processing. Every third day, a 100 mL sample was taken from each experimental diet. From this sample, 10 aliquots taken of 2 mL to count all the copepods, using an Olympus stereomicroscope and determine the population density of culture vessels. Density values were capture in Excel 2010 database and processed to determine the mean value (±S.D.). To determine significant differences between samples diet and between diets densities values a one-way ANOVA test was performed ($P < 0.05$) through statistical program SYSTAT 13. Finding significant differences, a performed multiple mean test by Tukey technique. In addition, tendency growth density curves for each experimental diet was determined.

RESULTS

Table 1 shows the mean values (± S.D.) of copepods population densities at four experimental diets. Better results were shown with *C. vulgaris* diet, because after 45 culture days, the other experimental diets decrease density, but this diet increase density until reach 6,161 ± 36 org 100 mL^{-1}. Diets with *H. pluvialis* and *Sphaerocystis* sp. decrease completely 12 days before other two diets. Combined experimental diet has the lowest density with 950 ± 44 org 100 mL^{-1}.

ANOVA analysis between samples from same diet, respect each experimental diet, shown in Fig.2.

In Fig. 3, growth tendency curves of density population from each experimental diet were shown. The four curves are polynomial, but two of them are fourth grade (*H. pluvialis* and *Sphaerocystis* sp.), while other two diets (*C. vulgaris* and combined diet) are third degrees.

DISCUSSION

Copepods culture density at laboratory conditions shows great variety information, because it depends on the type and quality food supplied and copepod type feeding form. This is why the increase percentage density of culture mediums in this experiment obtained different values, density increase 134% in combined diet, 224% in *H. pluvialis*, 243% for *Sphaerocystis* sp. and *C. vulgaris* with 880%. It is obvious that variation in density of different diets is obtained by microalgae nutritional quality, as well as their cell cover was easily to digest, because in all four diets, a growth is observed and this cyclopoid copepod was consider herbivorous (Fryer 2014).

DeMott (1986), note that copepods select food according particle size and not for taste or hardness. These organisms have more ability to discriminate particles unlike cladocerans, by using chemoreceptors located in antennae and mouthparts structures for this activity. Copepods do not produce water currents to trap particles or algae, but use mechanical or chemical receptors that allow them to find and obtain the available food in culture medium. It also mentions that in copepods cultures fed with microalgae, these organisms stop eating inert food; but especially when diatom microalgae was supply as food.

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Table 1. Mean values (±S.D.) of copepods population density per sample day for each experimental diet.

Sample culture day	Experimental diet			
	Haematococcus pluvialis	*Sphaerocystis sp.*	*Chlorella vulgaris*	Combined
0	700	700	700	700
	±58	±57	±58	±53
3	1,061	887	799[a]	714[a]
	±41	±56	±20	±49
6	1,238[a]	1,217[a]	836	682
	±46	±57	±22	±37
9	1,314	1,330	815	688
	±46	±41	±24	±20
12	1,356	1,232	763[a]	721[a]
	±58	±59	±51	±26
15	1,405	1,129	708	771
	±32	±36	±49	±46
18	1,471	1,137	679	829
	±38	±33	±27	±52
21	1,541	1,286	702	885
	±31	±46	±58	±44
24	1,571	1,522	807	928
	±34	±49	±46	±23
27	1,493	1,701	1,022	950
	±28	±37	±41	±44
30	1,207	1,594	1,373	939
	±23	±22	±53	±33
33	591	885[a]	1,889	887[a]
	±59	±46	±35	±43
36	0	0	2,598	782
			±28	±42
39	0	0	3,528	617
			±30	±21
42	0	0	4,706	379
			±27	±29
45	0	0	6,161	61
			±36	±15

Same letters in a row shown no significant differences (P>0.05) between diets per sample day.

DeMott (1988) also mentions that copepods catch and eat better, when food particles (live or inert) have 6µm diameter because it is easier to manipulate it. Leising and Franks (2000) indicate that copepods eyes are underdeveloped and that is why chemo or mechanic reception is very important, especially when food form clusters, which facilitates their location and ingest.

Kang and Poulet (2000) worked with *Calanus helogandicus* fed with diatoms and dinoflagellates, they founded cannibalism, especially in copepods egg stage, when microalgae or inert diet concentration were not adequate. The copepods need to ingest highly nutritional and energetic food and eggs have this

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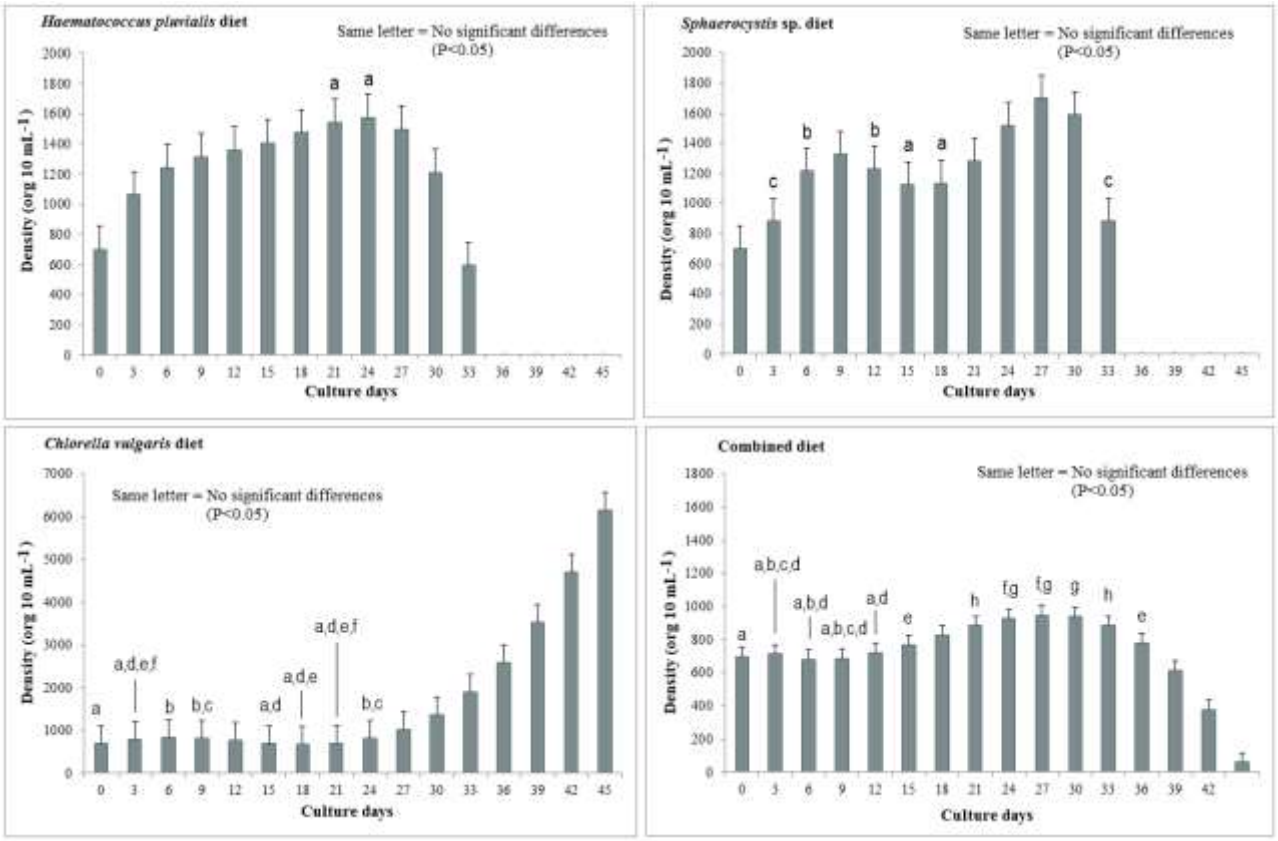

Fig. 2. Density variance analyses per sample from each experimental diet.

feature. Regarding calanoid copepods, they do not have a selective ingestion between microalgae and inert diet, if these diets have optimal concentration in culture water medium. Several omnivores' calanoid copepods become carnivores when phytoplankton concentration decreases in culture medium. That is why cultured copepod food (live or inert) must keep between 10^3-10^5 cells mL^{-1} concentration. In this experiment, the microalgae concentration was maintained in 5 $x10^5$ cells mL^{-1}).

Peterson (2001) and Castro-Longoria (2003) mention in their studies that marine copepods grow faster than those in freshwater, temperature and food concentration need to maintain in optimal ranges, regardless body size copepod specie. High mortalities in copepods culture medium due to low food concentration and their poor available energy content, which were necessary to growth copepods and possible to achieve egg production. These findings confirm with what was found in *C. vulgaris* diet in this experiment (adequate concentration and high energy levels), allowing to increase population density beyond 45 culture days.

Calliari and Tiselius (2005) who worked with *Acartia clausi* fed with *Thalassiosira weissflogii, Rhodomonas* sp. and *Tetraselmis* sp. found in their copepods cultures that is better to use a microalgae mix than microalgae monocultures, because their quality as food and energy content increased significantly and consequently can obtain better growth and reproduction in copepods culture. This disagrees

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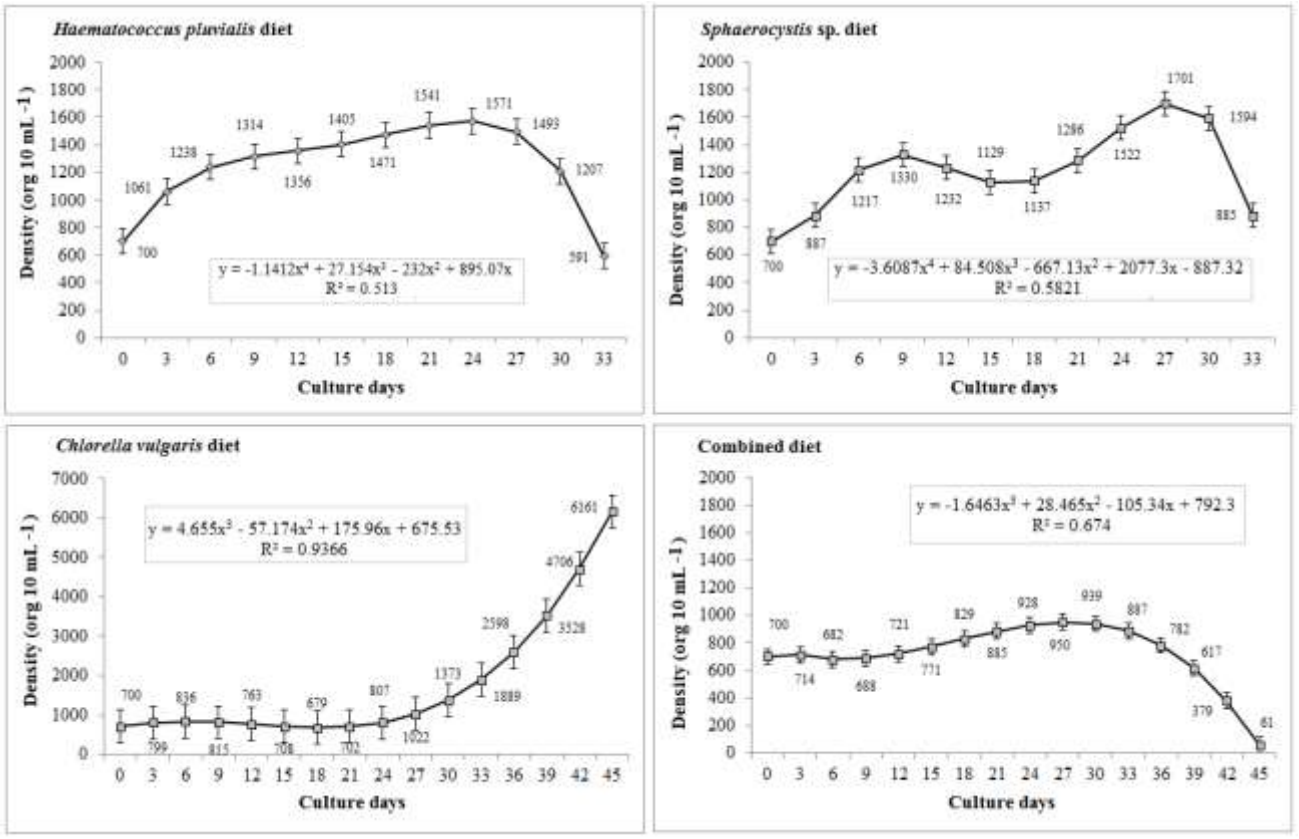

Fig. 3. Tendency growth curves of copepods population density from each experimental diet.

with this study because unialgal diets (*H. pluvialis; Sphaerocystis* sp. and *C. vulgaris*) have better density result than combined diet.

Vengadeshperumal et al. (2010) who worked with calanoid copepod fed with three microalgae: *Chlorella* sp., *Isochrysis galbana* and *Nannochloropisis salina*, obtained total densities of 41,603 org L^{-1}, corresponding 6,196 org L^{-1} adults. In this study were obtained tenfold (61,160 org L^{-1}). These density concentrations are possible when culture mediums have constant oxygen saturation. Santhaman et al. (2011) obtained densities between 6,232 to 8,569 org L^{-1} adults, fed with marine *Chlorella* sp. (3 x10^5 cells mL^{-1}), values below this study (9,390 to 61,160 org L^{-1}), but difference is at microalgae concentration with 5 x 10^6 cells mL^{-1}.

Finally, it is important consider the type of food supplied, because this organisms are not 100% herbivores, they have a tendency of being carnivorous, especially when food concentration (microalgae or inert diet) do not have optimal concentrations, nutritional quality and higher energy content to cover copepods requirements for growth and reproduction. That is why besides microalgae food source a small rotifer (<6 µm) or first stages of daphnias have to be supply. It is necessary to be careful with rotifer and daphnias concentration, because they need to be used as food and not like competitors for microalgae or inert food in culture medium (Fryer 2014). This author also mentions that *Chlorella* sp. has in

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their cellulose cell walls a short carbohydrates chain, allowing be easily ingested by copepods and use it like nutrient or energy available to all copepod body. Also recommends the supply of diatoms rich in lipids and carbohydrates, covering together with green microalgae, rotifers and nauplii daphnias the nutritional and energy requirements needed for optimal growth and reproduction in copepods laboratory cultures level.

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