Rev. Biol. Trop., 44(3)/45(1): 565-572, 1996-1997

Two experimental assays to produce biomass of *Artemia franciscana* (Anostraca)

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(Rec. 9-II-1995. Rev. 19-VII-1995. Acep. 6-V-1996)

Abstract: Two experimental assays were done to calculate the biomass of *Artemia franciscana* produced in 15 and 13 days, respectively. **Experimental assay 1)** three sets of three aquaria had 20 l of brackish water (20 ‰) and 6 nauplius/ml each. One had dry *Spirulina* as food source, the other two sets had fertilizers: chicken manure and "fosforin", and live *Tetraselmis* as food source. The pH varied: 7.3-8.0, temperature (T °C) varied: 25.5 - 33.0, and oxygen (O_2 mg/l) varied: 4.5 - 6.0 The obtained furcal length was from 6.0 mm to 9.5 mm. The mortality rate was between 95.7% and 98.9%. Biomass was between 13.0 g and 36.2 g in 20 l. **Experimental assay 2)** three sets of three concrete tanks had 120 l of brackish water (20 ‰) and 1 nauplius/ml each. They had the same food source as essay (1), except that *Tetraselmis* was no used. The pH was: 7.5 - 9.0, T °C: 25.5 - 35.0, and O_2 mg/l: 0.5 - 3.4. Furcal length was 8.7 mm to 10.1 mm. The mortality rate was 89.7% - 96.5%. Biomass was 41.9 g - 86.5 g in 120 l. Values higher than 0.624 mg/l of ammonia were lethal. *Spirulina*, as a food item, gave the best results.

Key words: *Artemia franciscana*, biomass production, fertilizers, Mexico.

An advantage of using *Artemia* (nauplius and/or adults) as a food item is that it satisfies most of the nutritional requirements for several organisms. There is no artificial balanced food that can be compared favorably. Kinne (1977) quoted that more than 85 % of marine species had been cultured. This was due to *Artemia* nauplius used as food item complemented with other substances. One of the goals of producing huge quantities of *Artemia* is to feed organisms in culture.

D'Amelio and Santulli (1988) mentioned that high yields of *Artemia* biomass and cysts could eventually be achieved. This can be done by seasonal inoculation of other *Artemia* species in solar saltworks, where endemic *Artemia* strains are present. Royan *et al*. (1992) fertilized a pond of 0.22 *ha* so that it could be used as a salt pans condenser unit with inorganic salts and strains of the San Francisco Bay nauplii. These nauplii were inoculated within a period of 5 months, and a production of 20.6 kg dry cyst/*ha* was achieved.

Bridgeford et al. (1988) added brine shrimp cysts to a culture of *Chlorella* and dried sewage effluent. Rapid growth followed after 13-16 days. Those with 28.3 g fertilizer yielded the highest wet-weight *Artemia* biomass. During growth, the bacterial forming colony (units/ml) and the *Chlorella* density dropped due to brine shrimp removed. This suggested that the microbial flora may be an important food base in these communities.

Producing large quantities of *Artemia* can be very expensive if it is fed with a special food item such as dry *Spirulina*. However, if fertilizers are used, production can be extremely cheap. The aim of this paper is to compare the *Artemia* biomass production. First by feeding it with dry *Spirulina*, and using organic and inorganic fertilizers and compare *Artemia* production.

TABLE 1

Experiment assay 1, shows pH, T(°C) *and* O_2 *(mg/l) variations*

Aquaria	1			2			3		
	pH	T°C	O_2	pH	T°C	O_2	pH	T°C	O_2
Days									
1	7.6	31	6	7.5	31	6	7.5	33	6
2	7.6	30	6	7.5	31	6	7.5	33	6
3	7.5	32	6	7.5	32	6	7.6	30	6
4	7.4	30	6	7.4	30	6	7.5	29	6
5	7.5	28	6	7.4	28	6	7.5	28	6
6	7.5	28	6	7.5	28	6	7.6	31	6
7	7.5	33	6	7.5	33	6	7.6	30	6
8	7.5	28	4.5	7.5	28	4.5	7.5	32	4.5
9	7.5	30	4.5	7.3	30	4.5	7.5	30	4.5
10	7.5	29	4.5	7.5	29	4.5	7.4	28	4.5
11	8	26	4.5	7.5	26	4.5	7.5	28	4.5
12	8	26	4.5	7.5	26	4.5	7.7	26	4.5
13	8	25.5	4.5	8	25.5	4.5	7.9	25.5	4.5
14	8	28	4.5	8	28	4.5	8	28	4.5
15	8	28	4.5	7.9	28	4.5	8	28	4.5

This experimental work was carried out in the "Instituto Tecnologico del Mar", Boca del Rio, Veracruz. Two experimental essays, one with three sets of three 20 l aquaria, and the other with three sets of three 120 l cement tanks of 20 ‰ salinity, were developed as follows:

Assay 1: In 15 days, nine aquaria under laboratory conditions with artificial aereation. The density of *A. franciscana* was of 6 nauplius/ml. *Set A*: three aquaria was given 1 g/day of dissolved dry *Spirulina*. The water was changed every two days. *Set B*: three aquaria were first fertilized with 40 g "fosforin" (inorganic)(see appendix), and *Set C*: three aquaria were fertilized with 40 g chicken manure (organic) per aquarium. The *Artemia* in set B and set C was given daily 1/2 l of the culture of *Tetraselmis* sp (density of 3.0 x 10 exp 4 cel/ml). After the second, it was given 1 l daily. The organic sediments were extracted by siphoning, daily.

Assay 2: In 13 days nine cement tanks were set under natural conditions, covered with plastic so as to be protected from the rain. These tanks contained *A. franciscana* with a density of 1 nauplius/ml. *Set A*: The *A. franciscana* of three cement tanks was fed with 2 g/day of dissolved dry *Spirulina*. *Set B*: three other cement tanks were fertilized with 240 g of "fosforin" (inorganic), and *Set C*: the three last cement tanks were fertilized with 240 g of chicken manure (organic). This was done by introducing a fine mesh bag in the bottom, and allowing it to dissolve. The environmental parameters were measured daily, and the water in the tank was stirred by hand to avoid stratification.

Every day at noon, the pH was measured with a Merck indicator paper 0-14. The temperature (°C) was measured with a Griffin -10 to 100 °C thermometer. The oxygen mg/l was measured using the Winkler method. And, once a week, ammonium was measured using the Fenol- Hypoclorite method, quoted by Contreras (1980).

Every two days, furcal length measurements were taken. These were measured from the head to the furcal branch (furcal length). A sample of 25 adult *Artemia* of each aquarium and cement tanks was used. This was done with a Zeiss microscope with a micrometric ruler. The total wet weight (biomass) was registered at the end of the experiment. A Stanton D 40 T - 1000 g scale was used.

The mortality rate was calculated as follows: the final biomass from each aquarium and cement tank, was divided by the mean individual weight (0.007-0.01g). This result was then divided by the initial seeded number of nauplii (120,000) and subtracted from 1.0. Even though some adults weighed more than 0.01 g and some less than 0.007 g, these two ranged weights were needed to calculate the number of adults of the biomass.

Experimental assay 1) In 15 days, table 1 and figure 1 shows both the environmental and the biological parameters. The pH varied from 7.3 to 8.0. The highest values of pH were found towards the end ofthe experiment, perhaps due to the increase of ammonium. Temperature decreased from the beginning of the experiment. It ranged from 33.0 °C to 25.5 °C. The oxygen decreased mainly because the air flow diminished. It varied from 6.0 mg/l to 4.5 mg/l.

Bacteria and protozoa that grew forming part of the food source because there were no strict culture controlled conditions, were not possible to measure.

A. franciscana grew 6.0 mm (**set B**) and 9.5 mm (**set A**) in furcal length (figure 1). Calculated mortality rate varied first from 97.0 % (**set A**) and 98.9 % (**set B**) were the individual weight was 0.01 g. Then the mortality rate varied from 95.7 (**set A**) to 98.4 (**set B**) were the individual weight was 0.007 g. The biomass obtained in 15 days was 13.0-13.5 g / 20 l (**set B**), 19.5-20.2 g/20 l (**set C**), 32.87-36.16 g / 20 l (**set A**) (table 1). All the juvenile *A. franciscana* organisms of the aquaria with "fosforin" and the one with *Spirulina*, died during the first week (table 3). Concentrations of 0.680 mg/l and 0.624 mg/l of ammonium were registered respectively.

Experimental assay 2) In 13 days, table 2 and figure 2 shows that the pH varied between 7.5 and 9.0. The tanks fertilized with chicken manure (**set C**) have higher pH values than the ones fed with *Spirulina*. This is possible, because chichen manure has higher ammonium concentrations. The temperature varied from 25.5°C to 35.0°C. The highest values were found towards the end of the experiment. The oxygen had a tendecy to decrease due to an increase of

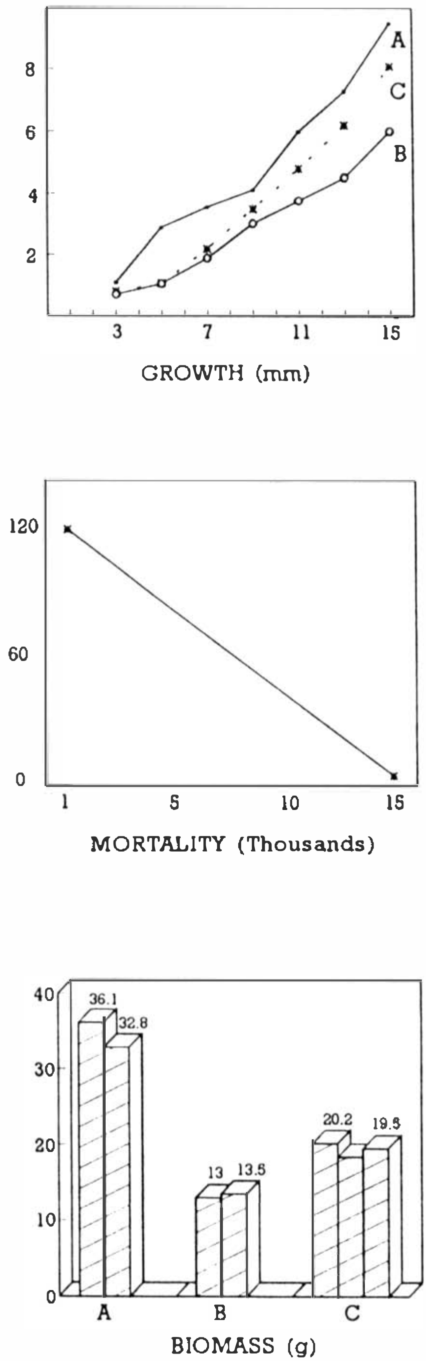

Fig. 1. Experiment assay 1: furcal length growth, mortality rate in 15 days and the total biomass variation in three sets of three aquaria. A with *Spirulina*, *B* with "fosforin" and *C* chicken manure with *Tetraselmis*.

TABLE 2

Experiment assay 2, shows pH, T(°C) and O_2 (mg/l) variations

Cement Tanks	1			2			3		
	pH	T °C	O_2	pH	T °C	O_2	pH	T °C	O_2
Days									
1	7.5	29	2.8	—	—	—	8.5	30	2.7
2	7.5	28	2.8	—	—	—	8.5	29	2.4
3	7.5	28	2.2	—	—	—	8.5	28	2.8
4	8.5	28	1.1	—	—	—	9	25.5	1.5
5	7.5	28	2.3	—	—	—	8	28	3.4
6	8	25	2	—	—	—	9	25	2.9
7	8	27	1.6	—	—	—	8.5	27	2.3
8	7.5	27.5	1.8	—	—	—	8.5	26.5	2.6
9	8	27	1.9	—	—	—	8.5	27.5	2
10	8	35	1.3	—	—	—	9	35	1.8
11	8.5	30.5	0.5	—	—	—	8.5	35	0.6
12	8	31	0.9	—	—	—	8.5	31	0.7
13	8	30	1.2	—	—	—	8.5	32	0.9

temperature. The highest value was 3.4 mg/l (**set C**) and lowest value was 0.5 mg/l (**set C** and **set A**).

Chlorella sp and *Pseudomonas* sp. grew forming part of the food source.

A. franciscana grew 8.7 mm (**set C**) and 10.1 mm (**set A**) in furcal length (figure 2). The mortality rate varied first from 92.8 % (**set A**) and 96.5 % (**set C**) where the individual weight was 0.01 g. Then the mortality rate varied from 89.7 % (**set A**) to 95.0 % (**set C**) where the individual weight was 0.007 g. The biomass obtained in thirteen days was 41.9 g - 47.8 g / 120 l (**set C**) and 53.4 g - 86.5 g / 120 l (**set A**) (table 2). All the *Artemia* died (**set B**).

In the cement tanks with "fosforin", (**set B**) all the juvenile *A. franciscana* organisms died after the first week. The organisms in the tank with chicken manure and in the tanks with *Spirulina*, also died (table 4). Concentrations above 2.0 mg/l of ammonium were registered.

It is clear from both experiments, that biomass production could be higher, and that the natural mortality was the problem. If the mortality rate was reduced 10%, *Artemia* biomass could reach, as much as 1 to 6 kg per m^3, under both tank and aquarium conditions, respectively.

Figure 3 shows an average variation of environmental parameters of 24 h measuremets after a week of the three sets of cement tanks: one with A) Spirulina, one with B) "fosforin" and one with C) chicken manure. The temperature was high (above 37 oC) at 14:00 hrs, and less than 28 oC at 07:00 hrs. The pH was high (9) at 11:00 hrs and low (7) at 07:00 hrs. However in B) it varied between 8 and 9, perhaps due to calhydra and limestone ingredients. The O2 mg/l that was considered low, oscillated above 0.5 at 07:00 hrs and 1.6 at 14:00 hrs.

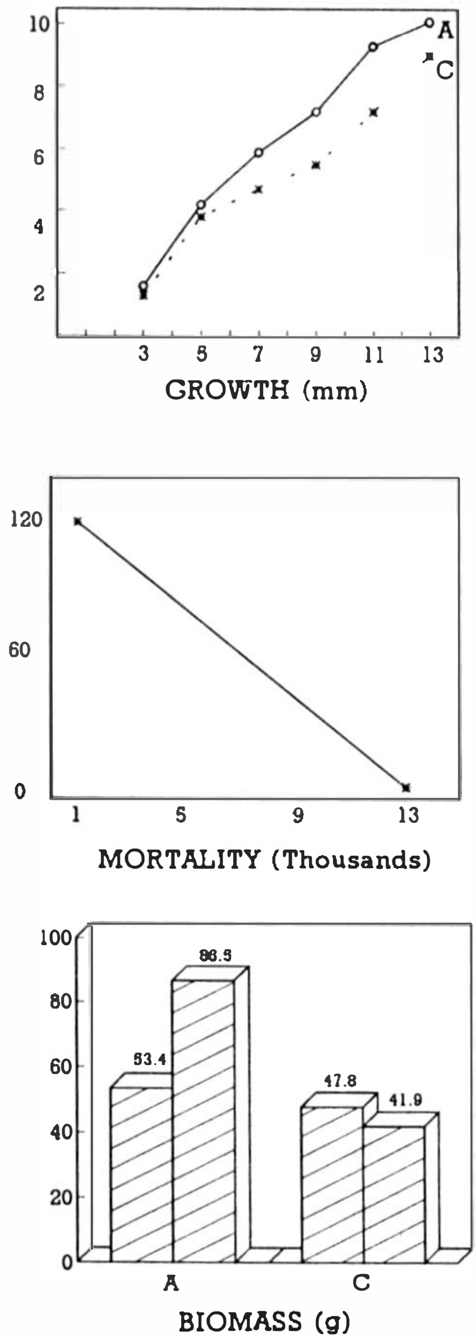

Fig 2. Experiment assay 2: furcal length growth, mortality rate in 13 days and the total biomass variation in three sets of three cement tanks. A with *Spirulina*, and C fertilized with "fosforin" and chicken manure, respectively.

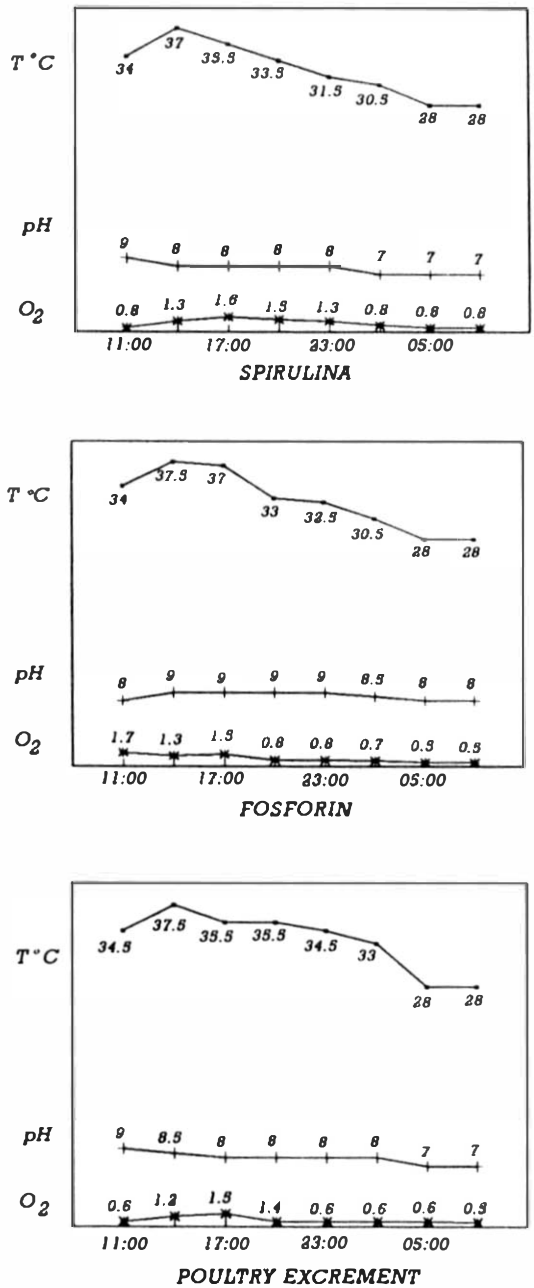

Fig. 3. 24 hrs average measurements of pH, T(°C), and O_2 (mg/l) from the three sets of three cement tanks. A with Spirulina), B and C fertilized with "fosforin" and chicken manure, respectively.

TABLE 3

Experiment assay 1: 120,000 nauplii of Artemia adults were introduced and fed in three different ways. The final number, biomass(g), and mortality(%) are shown. The mortality rate was taken from the obtained individual weigths of 0.01 g (1) and 0.007 (2) after 15 days of the three sets of three 20 l aquaria

Aquaria		1			2			3		
Set	Diet	Final	Biom. (g)	%M	Final	Biom. (g)	%M	Final	Biom. (g)	%M
A)	Spirulina	1) 3,616 2) 5,165	36.16	97.0 95.7	3,287 4,695	32.87	97.3 96.0	—	—	—
B)	Inorganic fertiliz.	1) 1,300 2) 1,857	13.0	98.9 98.4	1,350 1,928	13.5	98.9 98.4	—	—	—
C)	Inorganic fertiliz.	1) 2,020 2) 2,885	20.2	98.3 97.6	1,840 2,628	18.4	98.5 97.8	1,950 2,785	19.5	98.4 97.6

TABLE 4

Experiment assay 2: 120,000 nauplii of Artemia adults were introduced and fed in three different ways. The final number, biomass(g), and mortality(%) are shown. The mortality rate was taken from the obtained individual weights of 0.01 g (1) and 0.007 g (2) after 13 days of the three sets of three 120 l cement tank

Cement tanks		1			2			3		
Set	Diet	Final	Biom. (g)	%M	Final	Biom. (g)	%M	Final	Biom. (g)	%M
A)	Spirulina	1) 5,340 2) 7,628	53.4	95.5 93.6	—	—	—	8,650 12,357	86.5	92.8 89.7
B)	Inorganic fertiliz.	—	—	—	—	—	—	—	—	—
C)	Inorganic fertiliz.	—	—	—	4,780 6,828	47.8	96.0 94.3	4,190 5,986	41.9	96.5 95.0

DISCUSSION

Artemia is able to feed on any kind of organisms no more than 50 µm in size such as phytoplankton, protozoa, bacteria, or yeast. *Artemia* is not a selective filtrator. Buck (1981) was able to culture many kinds of such organisms using organic and inorganic fertilizers, through different aquacultural systems. This is the reason why organic and inorganic fertilizers were used in the present study.

Schroeder (1978) mentioned that *Artemia* also ingest fine particles of fertilizers, even though they have low nutritional value. Kerns and Roelots (1977) mentioned that bacteria and protozoans, which grow where there are fertilizers, obtain a high nutritional value.

Sorgeloos (1982) obtained more than ten thousand *Artemia* adults per litre and more than 10 g of *Artemia*/m^2 daily, using organic fertilizer (chicken manure) and inorganic fertilizer. The results of this paper show that in 15 - 13 days up to 20.2 g in 20 l and 47.9 g in 120 l, were obtained using chicken manure.

The temperature under this specific condition varied between 25 °C - 33 °C which according to Amat (1985) a good growth should have been obtained.

Castro and Gallardo (1983) mentioned that an optimum pH oscilates between 7.5 - 8.5. If it is lower than this, *Artemia* growth diminishes or even stops. But, in this study pH values varied between 7.3 and 8.0 without any growth problem.

The tolerance of toxic ammonium for crustacean larvae is 0.15 ppm (Heins, 1988). In three cement tanks of this study, where all *Artemia* organisms died, ammonium levels were above 2.0 mg/l.

De los Santos *et al* (1980) stated that the production of cysts from 15 to 30 kg/ha and *Artemia* biomass from 500 to 1000 kg/ha were obtained in an extense reservoir in Philippines.

Contreras and Hinojosa (1989) mentioned that 5.26 kg of *A. franciscana* biomass per m^3 in 20 days could be obtained. This could be done using 8 kg of rice bran sifted through a 74 um mesh sieve. Pillay (1990) reported that after two weeks of culturing *Artemia*, larvae reached an average length of 8 mm, yielding a wet weight of around 5 kg/m^3. In this study the furcal length was from 6 to 9.5 mm and every 15 days from 1 to 6 kg of *A.franciscana* per m^3 could be produced.

Ortega-Salas and Martinez (1987) reported for *A. franciscana*, in the Yavaros area, an oxygen variation from 0.1 to 4.0 ml/l, and temperature from 22 °C to 42 °C. The cysts for this study came from this area where the oxygen varied from 0.5 to 3.4 mg/l, and the temperature from 25 °C to 35 °C.

If we extrapolate, **Essay** 1 could produce 650 g - 1,810 g per m^3, and **Essay 2**: could produce 349 g - 720 g per m^3. From both experiments biomass production could be higher, however mortality was the problem. Reducing the mortality by 10% and extrapolating, 1 to 6 kg per m^3 of biomass could be produced under tank and aquaria conditions.

Finally, 1) Production of *Artemia* biomass gave the best results under aquaria conditions. 2) Chicken manure used as fertilizer gave good production of *Artemia* biomass, at low cost. 3) Dry *Spirulina*, used as food source produced greater biomass, however it is expensive. 4) "Fosforin" used as a fertilizer produced high concentrations of algae. Although its use should be very well controlled to avoid massive mortalities of *Artemia*.

AKNOWLEDGMENTS

To Maria de los Angeles Herrera for checking some of the materials.

RESUMEN

Se realizaron dos ensayos experimentales para calcular la biomasa de *Artemia franciscana* producida en 15 y 13 días, respectivamente. *Ensayo 1*) Se instalaron tres módulos de tres acuarios cada uno con 20 l de agua salobre (20 ‰), y seis nauplios/ml; a uno de los módulos se le proporcionó *Spirulina* seca como alimento. En los otros módulos, uno con gallinaza y el otro con "fosforin" (ver apéndice), se les proporcionó *Tetraselmis* viva como alimento. El pH varió de 7.3-8.0, la temperatura en grados centígrados (T°C) fue de 25.5-33.0, y el oxígeno (O_2) mg/l de 4.5-6.0. La longitud furcal midió entre 6.0 y 9.5 mm. La tasa de mortalidad se calculó entre 89.7% y 96.5%. La biomasa varió entre 13.0 g y 36.2 g por cada 20 l. *Ensayo 2*) Se instalaron tres módulos de tres tanques de concreto con 120 l de agua salobre (20 ‰) y un nauplio/ml. Se alimentaron como en el ensayo 1, excepto que no se usó *Tetraselmis* sp. El pH varió de 7.5-9.0, la T°C de 25.5-35.0, y el O_2 mg/l de 0.5-3.4. La longitud furcal midió entre 8.7 y 10.1 mm. La tasa de mortalidad se calculó entre 89.7% y 96.5%. La biomasa varió en-

tre 41.9 y 86.5 g en 120 l. Los valores mayores a 0.624 mg/l de amonio fueron mortales. *Spirulina* como alimento dio los mejores resultados.

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APPENDIX:

"FOSFORIN" COMPOSITION

UREA	150 g
17-TRIPLE (P - K - N) nutrients	125 g
GROUND OYSTER SHELLS	150 g
LIME FROM A WATER RESERVOIR	25 g
ASHES (TREE LEAVES)	25 g
CALHYDRA $Ca(OH)_2$	25 g