TOPIC AREA:
INDIGENOUS SPECIES DEVELOPMENT

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DEVELOPING FEEDS FOR LARVAL DORMITATOR LATIFRONS (CHAME)

Indigenous Species Development/Experiment/09IND10UH

FINAL INVESTIGATION REPORT

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ABSTRACT

Chame (*Dormitator latifrons*) is a fish species with significant aquaculture potential. In Ecuador, there have been many years of commercial aquaculture and in México; producers have the expectation to include it as a viable option to produce fish, along with several marine fish species, due to sanitary problems with shrimp culture. In both countries, particularly in Ecuador, upscaling to larger scale commercial aquaculture depends on the achievement of laboratory juvenile production. As to this moment all reported aquaculture in Ecuador relies solely on wild fish. For the last three years our research team has accomplished several objectives regarding the controlled reproduction in captivity of both wild and in-lab adult fish. Other advances have been made in determining best fertilization and incubation conditions. Secondly, many larval rearing trials have been conducted, using both live and dry feeds, but given chame´s morphological features at hatching, significantly small zooplankton and dry feed particles are required. For the present work plan, we focused on the identification of a suitable live prey by isolation, identification and culture from natural environments; to be used either as only food source or in combination with dry feeds, as well as a few trials of pond rearing conditions. Our results identified some specific rotifer genus, which comply with a lorica length under 90 microns (chame´s mouth gap size), and are present at different salinities (*Proales* and *Proalides* sp. to mention a few); several feeding trials either using *Proalides* as single food source (with or without bioenrichment) along with dry feeds were carried out. Although no survival was observed beyond 6-7 days post hatching, significant consumption of offered rotifers was observed. Also, a shrimp and artificial pond rearing trials were conducted, with similar results with regards to survival. As final conclusions, there is still an initial signal, given by food type or composition missing on the tested feeding protocols, to ensure enzymatic activity and therefore chame larval survival, once exogenous feeding starts.

INTRODUCTION

One of the main limiting factors for fish larval rearing is to offer a suitable zooplanktonic prey that contains all the needed nutrients for a particular species, being among the most important amino acids and fatty acids (Garcia-Ortega et al., 2003). Rotifers are an important source of live feed for larviculture. However, most commonly used species have not been appropriate for many fish, due to their body size when compared to fish larvae mouth gap (Wullur et al., 2011). Thus, research focused on live feed

massive production is essential for fish commercial production at several levels. Diversification of aquaculture relies on the controlled propagation of a particular species of interest. For fish, larvae exhibit many particularities especially at the early ontogeny stages, when focusing on the physiological ability to start exogenous feeding, as previously described for chame (Rodriguez M de O et al., 2012). Many approaches to offer a suitable pray or feed are described in the literature; a particular emphasis is made on the composition and proximal quantity and quality to guaranty a significant amount of both protein and fatty acids by means of zooplankton bioenrichment techniques (Garrido et al., 2013). Also, the utilization of both pre and probiotics are described as a relevant factor to ensure, maturation of digestive tract using bacteria as initial feed or as a contribution to intestinal flora for supplemental enzymatic activity in larval gut (Zink et al., 2013). Nevertheless, both aspects are of more significance as to increase survival rates once larval rearing has taken place (Lihnsteiner et al., 2012), as well as to reduce the time for weaning to dry diets (García-Ortega, 2009). Other approaches include the co-feeding of both dry and live feeds, the later obtained from sources such as mesocosm (Cunha et al., 2013), biofloc (Wilson, 2012), and broodstock rearing tanks, among others.

From our previous work, we have identified two major constrains to achieve chame larval rearing. The first key aspect is related to the identification of a suitable prey of chame larvae due to its small mouth gap (approximately 90 microns), as previously used rotifer strains with lorica size above 120-150 microns are too large for ingestion (Rodriguez et al., 2012). Second, we must identify the initial factor or factors for optimal composition or type of initial feed, either alone or in combination (mixed feeding). Also the possibility to offer a wider variety of potential food sources either as phyto- or zooplankton by means of pond rearing (Ludwic, 1999), that potentially offers a significant diversity of organisms in a similar way to chame natural environments; where salinity might play a major role as described by McDowall (2009) given chame´s amphidromy conditions as part of its life cycle.

Therefore, we focused on the identification, isolation and culture of microzooplankton (smaller than 100 microns) from several locations within a 150 km radius of Mazatlán Sin., where chame juveniles have been gathered for the last 4 years, after collection of sediment from these locations. Also, such sediment is a reservoir of other many planktonic resting stages, therefore suitable to recreate natural conditions in a pond; offering an alternative technique for fish larval rearing. Following Ludwig (1999) recommendations for fish larvae pond rearing, in a tank in a research facility, rotifers encysted in the soil will hatch in the tanks and feed on the various phytoplankton blooming from previous fertilization. Unlike most marine larviculture practices where axenic zooplankton cultures are used, we will purposefully culture numerous rotifer species simultaneously to simulate the successful strategies used in pond culture situations. For the later, salinity is a key element to obtain a wider diversity of organisms from these sediments; hence, the effect of several salinities (10, 15, 20 and 25 ppt) on the abundance and specific composition of emerging rotifers from resting eggs found on pond sediment was investigated, showing a significant effect on specific composition and rotifer abundance. Resting eggs from observed species were produced after initial culture, and cyst production was achieved and subsequently stored using synthetic sponges for later use as initial inoculation for massive rotifer production. Such generated information was used according to the originally proposed research objectives as follows:

1. Artificial pond rearing of chame larvae
2. Use of microzooplankton (<50 microns) as initial feed
3. Use of highly digestible artificial microdiets
4. Mixed feeding programs

MATERIAL AND METHODS

Chame Broodstock

3,000 Chame juveniles (1-4 g individual weight) were collected in Hacienda el Tamarindo, Rosario Sinaloa (23°01´57´´N; 105°48´26" W) during Sep-Nov 2012. Later on Dec 2012, fish were placed in twelve $2m^3$ polyethylene plastic tanks previously used for tilapia/pangasius biofloc culture, using the biofloc as natural food for overwintering purposes, until March 2013. A second set of 250 chame adults (15 cm minimal length) were collected at the commercial shrimp farm "Acuícola San Jorge" (23°09´10.54´´N; 106°18´22.84" W) in early September 2013. Later on, chame from the first lot were transferred to $9m^3$ elevated plastic liner tanks, to complete grow-out and gonad maturation. Potential breeders from both groups were monitored starting mid-September for maturity and pre-selected for hormonal induction.

Larvae production

Sexually mature chame broodstock were managed according to pre-established hormone application procedures outlined in Rodriguez M de O *et al*, (2012). Females were induced using a single implant of 75 µg LHRHa (Ovaplant, Syndel®) and males were injected a single 40 µg/kg LHRHa dosage. A modification on female spawning was carried out for females towards the end of the reproductive season (Mid October-Late November) with 3 consecutive 100-150 µg/kg LHRHa intramuscular injections, with a 20-35% success rate in 5-8 fish groups.
Once both males and females released gametes, artificial fertilization was performed at 0 ppt salinity, using UV sterilized water, previously filtered to 5 µm and with activated charcoal. Oocytes were placed at a ratio of 5 g of spawn (80,000 cell/g proximately) with 1-3 ml of sperm from 2-4 males per liter. Fertilization was carried out for 3-4 minutes and eggs were washed twice with clean water. Thereafter, eggs were placed in McDonald jars with significant aeration for 14-18 hours until all viable eggs hatched. Larvae were transferred to holding tanks at 0 ppt, counted and distributed into experimental rearing tanks.

Pond rearing of chame larvae

Natural pond. A shrimp pond (0.3 ha) located in Estación Dimas, San Ignacio Sinaloa México (23°42´57´´N, 106°48´58´´O) was facilitated by Mr. Fernando Osuna Palacios. For preparation, 30 kg/Ha was added for 7 days to the pond, and previously filled with water at 15 ppt salinity, passed through a 250 micron screen. Later on, 75,000 (2 dph) chame larvae were stocked and monitored after 10 days to evaluate larvae survival (Fig. 1).

Outdoor artificial ponds. Two $2m^3$ polyethylene plastic tanks were setup at FACIMAR-UAS for a similar larval rearing trials (Fig. 2). Each tank was prepared at two different salinities, 0 and 15 ppt, using both fresh water and salt water screened with a 100 bag filter and fertilized continuously for 12 days with a mixture of wheat bran, urea and phosphoric acid. Each pond was stocked with 3 kg of sediment from specific locations, later on 18,000 larvae were stocked within 5 days after tank setup. Water quality as pH, DO and T° were monitored daily. Several rotifer monitoring surveys were performed during a 15 day period.

Laboratory artificial pond (Experiment 1). Sponges impregnated with rotifers cysts produced from previous trials (see procedure below), were placed in 21 clear polyethylene plastic tanks (20 l total volume) prepared at different salinities (0, 4, 12, 19, 28, 35 ppt) with three replicates per salinity, using both UV sterilized filtered water to adjust salinity. Each tank was fertilized with urea, wheat bran and phosphoric acid (1.6, 20 and 0.6 ppm respectively) (Fig. 3) and subjected to 24 light cycle, two sponges about 10x10x8 cm were stocked on each replicate. Tanks were monitored for rotifer emerging within 5

days after initiation, for later adding of 100/l chame larvae 1 dph. Thereafter, 2 ml of RotiGrow® were added daily to each tank.

Production of micro rotifers (<50-100 microns) as initial feed

Wild rotifers were produced using the same procedure as described above for artificial ponds. In specific, collected as latency stages from soil samples originated from different locations. Organisms from the Brachionus genus and other brackish and saline environments were produced from sediments collected at the commercial fish farm "Acuicola San Jorge" located nearby the Mazatlán international airport (23°09´10.54´´N; 106°18´22.84" W) directly from several ponds an used to inoculate tanks at 10, 15, 20 and 25 ppt salinity. Rotifer diversity was monitored for over 20 days at each salinity. Rotifers produced from these soil samples were induced to produce cysts, by placing 3-5 liters from production tanks in clear plastic boxes with the addition of synthetic polyurethane sponges, directly exposed to sunlight to allow water evaporation (Fig. 4).

Fresh water and other low salinity inhabitant rotifers were obtained from sediments collected in several temporary water reservoirs at the Rincon del Verde, Escuinapa Sinaloa community, 80 km south of Mazatlán (22°53'44.07"N, 105°48'57.47"W). For these rotifers, tanks were setup and the desired rotifers (less than 100 microns) only appeared once the rain season started, after 45 days of initial inoculation. Among the observed microrotifers was the genus *Proalides* sp.; this particular rotifer was selected due to its morphological characteristic (@50-70 microns lorica length, Fig. 5). Several attempts were carried out for massive culture of one of the identified and isolated rotifers using artificial feeds such Rotigrow®, Algamac®, commercial baker´s yeast and live microalgae (*Chlorella* and *Scenedesmus* sp) without success. Thus, as an alternative, given that this rotifer was present at very high concentrations in the chame broodstock holding tanks, green water was transferred to other set of tanks and used as production units, concentrating present rotifers using 55, 35 and 11 μm sieves prior to larval feeding trial.

Mixed feeding schedules evaluation.
According to the originally proposed activities on the present work plan, several experiments were conducted (Fig. 6), using as starting point the micro rotifers produced as described above in combination with the following inert diets and other elements:

- Algamac 3000 (Aquafauna Bio Marine®) **(A)**
- Algamac protein plus (Aquafauna Bio Marine®)
- *RotiGrow*® (Reed Mariculture Inc) **(R)**
- Centrifugated fractions of Rotigrow® **(RC)**
- Green water[a]. **(GW)**
- Probiotic mix[b]. **(Pro-M)**
- Epicin G2 **(P1).**
- Epicin Hatcheries **(P2).**
- Protein HUFA rotifer enrichment product (Salt Creek®) **(PH)**
- Herbal Tea[c] **(Tea)**
- Molasses **(M)**
- Epilite Z (70 micron liquid microparticulated diet) (Epicore BioNetworks Inc.)
- Forze Vit forte[d] **(Vit mix).**

[a]Green water: mixture 1:1 of 55 micron sieved water from Chame broodstock rearing tanks plus tilapia biofloc water supplemented with molasses as carbon source.

[b]Probiotic Mix: 5 g of Epicin G2, Epicin Hatcheries and Epicin 3W (Epicore BioNetworks Inc.) was prepared. 0.2 g were dissolved in 100 ml filtered water and 1 g of molasses was incorporated prior to addition to larval rearing tanks.

[c]Herbal tea: Infusion Kundalini® *(Althaea officinalis, Crataegus pubescens, Cocolmeca smilax, Matricaria recutita*).
[d]Forze Vit forted infant vitamin and mineral supplement (Bee royal jelly, PUFA´s, glutamic acid, B vitamin complex, L-lysine).

Experiment 2: As an initial trial for the rotifer *Proalides*, either as a single feed or in combination with the above-mentioned inert diets. 100 chame larvae were stocked in 1 liter containers previously filled with UV sterilized 5 µm filtered fresh water, and assigned to 7 experimental treatments, with three replicates each, as follows (Fig. 1): No food as control (CON), green water (200 ml)+protein Hufa 1 ppm+rotifer (GW/PH/R), centrifuged Rotigrow+rotifers (RC/R), rotifer (R), green water+epicin G2+molasses+rotifer (GW/P1/M/R), green water+epicin hatcheries+molasses+rotifer (GW/P2/M/R) and green water+rotifers (GW/R), with 15% water exchange rate on a daily basis, rotifers were added in corresponding treatments at a daily ratio of 3 org/ml. Simultaneously, the same experimental treatment layout was carried out, including a 1 ppt salinity increment per day as part of the 15% water exchange procedure. Daily rotifer counts and larvae presence per container were conducted.

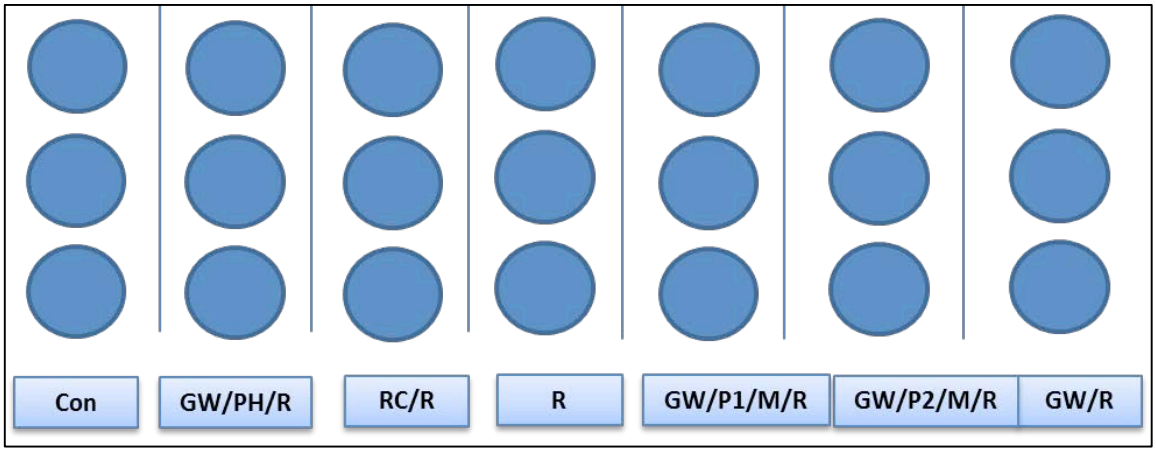

Figure 1*. Treatment layout for experiment 2.*

Experiment 3: Following Cunha et al. (2013) and Wilson (2012) procedures that combined different live food sources such as green water or biofloc in combination with bioenriched rotifers, this experiment was performed considering all these factors, along with the inclusion of a waterborne source of enzymatic co-factors (vitamin mixture) and the addition of a probiotic source in the water. Four major treatments with different levels, either as presence or absence of green water (mesocosm) and bioenriched rotifers with one of two different products were outlined (Fig. 2). Such treatments correspond to the following arrangement: A: Algamac 3000; A/P: algamac 3000 + probiotic mix; A/P-M: algamac 3000 + probiotic mix + molasses and P-M: probiotic mix + molasses. All products were added twice to rearing containers (10 L clear plastic containers with constant aeration) using manufacturer´s guidelines and rotifers were added daily after 1 hour of bioenrichment with either Algamac Protein Plus or Protein HUFA, at a minimum of 10-11 rotifers per ml, at 3 dph of larvae and counted daily, until larvae were no longer observable. Prior to initiation of feeding, 250 2 dph larvae were stocked at an initial volume of 2 L of UV sterilized 5 µm filtered fresh water; for green water tanks, 250 ml of green water were added to complete 2 L. Total container volume was increased on a daily basis with either 250 ml of fresh water or green water as corresponding from experimental layout (Fig. 2). As additional management, 4 tanks per treatment were supplemented with 0.15 ml of liquid vitamin mix.

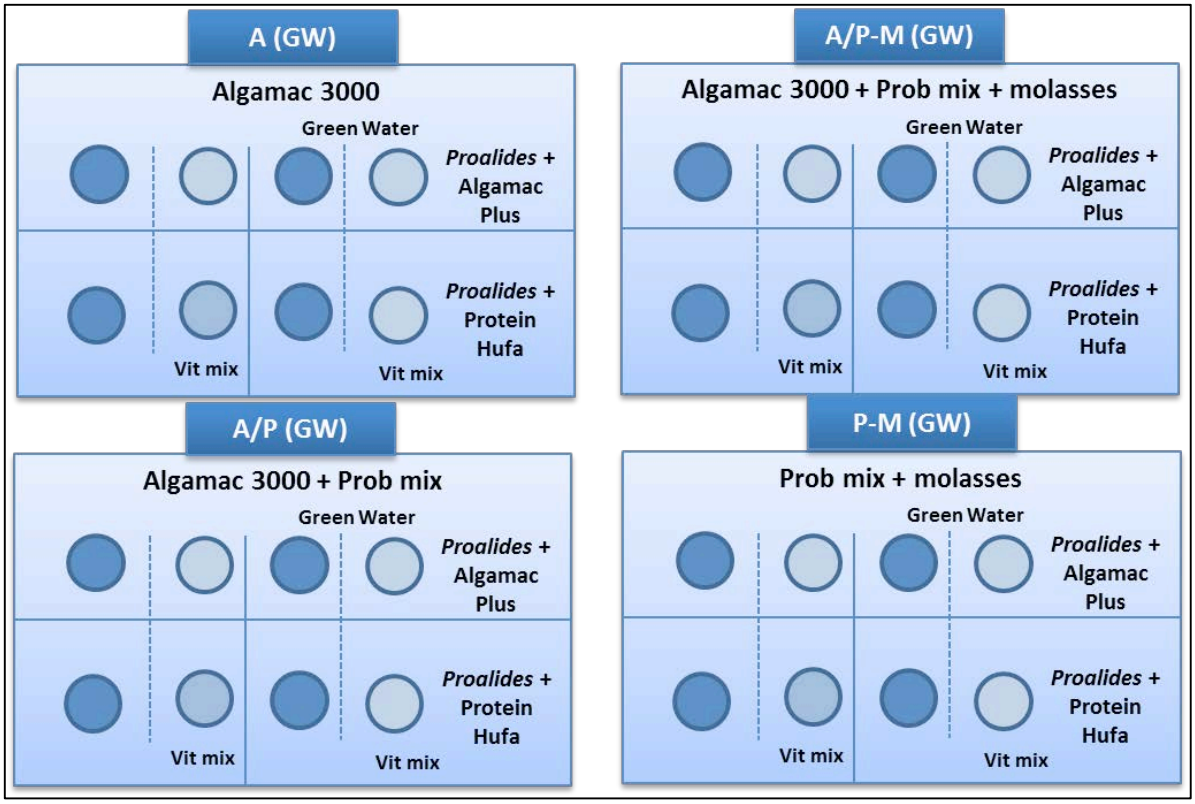

***Figure 2**. Experimental treatments layout for experiment 3.*

Experiment 4: A higher density enriched rotifer feeding trial was carried out, once again, in combination of either green water, probiotics, molasses and a bag of a commercially available herbal tea (as potential sources of feeding stimulants and/or other factors) according to the layout described in the following diagram (Fig. 3). 20 L clear plastic tanks with constant aeration were filled with either 5 L of UV sterilized 5 um filtered fresh water or 35 um sieved green water, were stocked with 250 larvae/L. Bioenriched rotifers (Algamac Protein Plus) were added daily, twice a day, days 3 and 4 dph at 4 (am) and 8 (pm) rotifers per ml, and from day 5 dph adding numbers were 10 (am) and 20 (pm) rotifers per ml, respectively at morning (9 am) and afternoon (pm), probiotic mix and/or molasses were added daily. Tanks volume was increased daily adding 2 L of water, in a 1:1 ration fresh water (FW)/salt water (SW). FW was added directly to tanks and SW was added by constant dripping to rearing tanks. Rotifers were counted daily in the morning, until larvae were no longer visible.

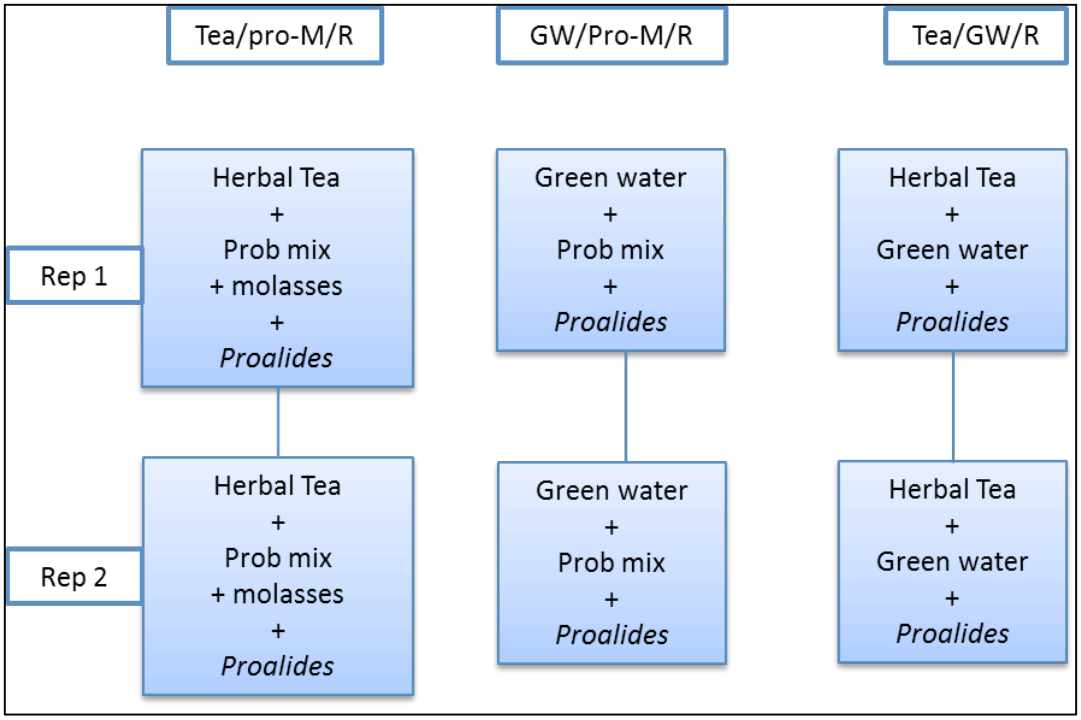

***Figure 3**. Experimental treatments layout for experiment 3.*

Extra activities: Identification and isolation of potentially probiotic bacteria from juvenile chame intestinal tract.

Chame juveniles (49.8±0.1 g) were dissected for full digestive tract removal, placed in sterile saline solution and shipped to the Faculty of Medicine of the Universidad Autónoma del Estado de Morelos (UAEM, Cuernavaca Morelos) for isolation and culture of probiotic bacteria. Intestinal segments were homogenized and consecutive dilutions in 0.89% saline solution were conducted and incubated in MRS Agar (each dilution). Samples were incubated for 24h at 37±2 °C. Each plated was counted for colony forming units, and positive dilutions were used for reseeding; thereafter, positive plate bacteria were used for catalase and gram staining for further identification. Probiotic activity was measured by means of bile salts tolerance at different pH values and API CHL and API 50 CH biochemical tests were carried out for taxonomical identification.

RESULTS

Pond rearing trial: No presence of post-larval fish was observed after 10 days, when final observations took place. Water samples (15 ppt salinity 30.5°C temperature, secchi depth 22 cm) were taken for microscopic observation to FACIMAR-UAS at moment of stocking and 3 days later, showing a significant number of copepod nauplii, copepodits and larger stages, diatoms and large *Brachionus* sp. rotifers at the moment of larvae seeding in pond, A significant number of mussels invaded the pond by day 6, removing most of primary productivity by day 8-9, resulting in transparent water; although pond was fertilized two more times (Nutrilake) and extra water added to avoid a sudden increment of salinity at days 4 and 8.

Artificial pond trial: For the outdoor tank, daily observation of 1l water samples indicated that larvae were still present at 5 dph at low salinity (0 ppt), whereas live larvae were observed only until 3-4 dph at 15 ppt. Low salinity tanks, in all cases, reached lower secchi values sooner (3-5) days at 26-30°C than higher salinity tanks. Predominant phytoplankton was different as color of rearing water, with a high presence of both *Chlorella* and *Scenedesmus* sp.; being diatoms such as *Chaetoceros* sp among others free living

microalgae. A relevant observation was made with regards to rotifer species' natural succession in the artificial ponds, given those smaller rotifers, either fresh water (*Proalides*) or brackish water (*Proales*) can be observed only when larger organisms (rotifers) are no longer present at high counts in the tanks. Therefore, blooming tanks might not offer the desired small rotifer composition and density, appropriate for chame larvae rearing. Other pertinent observations made, indicate some degree of synergy between the presence of specific types of microalgae, as *Proales* eggs adhere to an unidentified filamentous algae, and *Proalides* apparently consume another colony forming algae (*Eudorina*-like sp). This algae can be extensively found in broodstock holding tanks and be eventually used its culture.

For the indoor (laboratory) artificial ponds; although no segregation by species was made for daily counts, observed rotifers were high within 6 days after initial inoculation with sponges and daily fertilization; particularly for brackish salinities (between 10-20 ppt) as described in table xx, being as high as 100 rotifers per ml (Table 1). Although fertilization was discontinued when larvae were placed in experimental tanks, Rotimac® was used as supplemental feed for both rotifers and larvae. Larvae were no longer visible in tanks within 5 days after stocking, despite high rotifer counts, in a similar fashion to both natural and artificial pond rearing trials (Fig. 15).

Production of micro-rotifers: Further trials to taxonomical and morphologically identify emerging rotifer species and the effect of incubation salinity of collected sediment was conducted. Among the main rotifer genus identified at salinities above 10 ppt were *Brachionus, Lecane, Proales,* and *Colurella* (Fig. 10) being the last three rotifers with many desired characteristics for further utilization as initial live feed for fish larviculture; in particular, *Proales* and *Colurella* showing sizes under 90±20µm (Fig. 11). Rotifer counts revealed significant differences in specific diversity in relation to water salinity and time after inoculation (Fig. 12). For fresh water trials, main genus' included *Filina* and *Proalides* as well as small size *Brachionus angularis* with similar size characteristics (Fig. 11).

Mixed feeding trials: As mentioned, many feeding trials were carried out. Survival did not improve in number of days until larvae were no longer visible in rearing vessels, as in past rearing seasons larvae did survive until 7 days. Although other variables are being studied to validate the magnitude of the effect of actual rotifer consumption as observed on larvae fed with *Proalides* (Fig. 13), such as modified Fulton´s condition index still under analysis, we observed different survival values as total days among the executed experiments (Fig. 14). At first glance, we observed a longer life span on larvae feed on a mixed food regime (Exp, 2, 3 and 4) as overall number of days stretched to at least 6 days, than on the in-lab artificial pond trial. Also, we observed different life durations within experiment for trials 2, 3 and 4 (Fig. 16 and 17), where probiotic, to rearing water either as a single type or in a mixture addition, allowed to observed larvae presence for 1-2 extra days (Fig 16 and 17). The use of an Herbal tea on rearing water also increased larval life span as no differences were observed between trails (Fig. 18).

An interesting finding was the actual reduction values of rotifer density per experimental unit, providing an idea of an apparent consumption of the *Proalides* rotifer by chame larvae (Table 2, 3, 4 and 5). At this moment, with still pending digital analysis of chame larvae to establish potential condition index differences both between experiments and within treatments, the favorable ingestion of this rotifer provides relevant information on finally finding a suitable prey for chame larvae for low salinity trails, and further research using *Proales* at higher salinity conditions.

Probiotic bacteria identification: Tests conducted on juvenile chame digestive tracts allowed the identification of several gram-positive bacterial strains of the *Lactobacillus* genus and *Lauconostoc*. With mild to high tolerance to bile salts as well as low pH values (3 and 4), with pending results for negative catalase activity evaluation by means of API tests.

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Table 1. *Number of emerging rotifers of several genus observed per salinity treatment in laboratory condition artificial pond trial using sponges as cyst reservoirs (rotifers per ml) after stocking newly hatched chame larvae (experiment 1).*

Salinity (ppt)						
dph	0	4	12	19	28	35
1	2	12	116	72	65	78
2						
3	3	16	102	54	43	66
4	3	25	98	50	49	55

Table 2. *Initial rotifer count in 1 l containers (2 dph) and morning rotifer count in following days (prior to daily stocking 3 rotifers/ml) in experiment 2 at 0 ppt conditions.*

Treatment							
dph	Con	GW/PH/R	RC/R	R	GW/P1/M/R	GW/P2/M/R	GW/R
1							
2		3	3	3	3	3	3
3		3	1	1	1	1	3
4		4	4	4	3	3	4
5			4		5	5	
6							
Rotifers per ml counted the day after feeding.							

Table 3. *Initial rotifer count in 1 l containers (2 dph) and morning rotifer count in following days (prior to daily stocking 3 rotifers/ml) in experiment 2 with salinity gradient (1 ppt increase per day).*

Treatment							
dph	Con	GW/PH/R	RC/R	R	GW/P1/M/R	GW/P2/M/R	GW/R
1							
2							
3		3	3	3	3	3	3
4		5	4	3	2	2	2
5			5	4	2	2	2
6							

Table 4. *Proalides sp. rotifer apparent consumption of chame larvae, describing both observed (O) and added (A) rotifers per day per ml of rearing tank at each treatment within experiment 3.*

Treatment																
dph	A		A/GW		A/P-M		A/P-M/GW		A/P		A/P/GW		P-M		P-M/GW	
	O	A	O	A	O	A	O	A	O	A	O	A	O	A	O	A
1																
2																
3		11		11		11		11		11		11		11		11
4	6	11	8	11	6	11	6	11	6	11	8	11	10	11	8	11
5					8	11	10	11			10	11	10	11		

Table 5. *Proalides sp. rotifer apparent consumption of chame larvae, describing both observed (O) and added (A) rotifers per day per ml of rearing tank at each treatment within experiment 3.*

Treatment						
	Tea/GW/R		Tea/Pro/R		Tea/Pro-M/R	
dph	O (am)	A (am-pm)	O (am)	A (am-pm)	O (am)	A (am-pm)
1						
2		4-8		4-8		4-8
3	2	4-8	3	4-8	2	4-8
4	4	10-20	5	10-20	5	10-20
5	10	10-20	8	10-20	10	10-20
6	12		10		10	

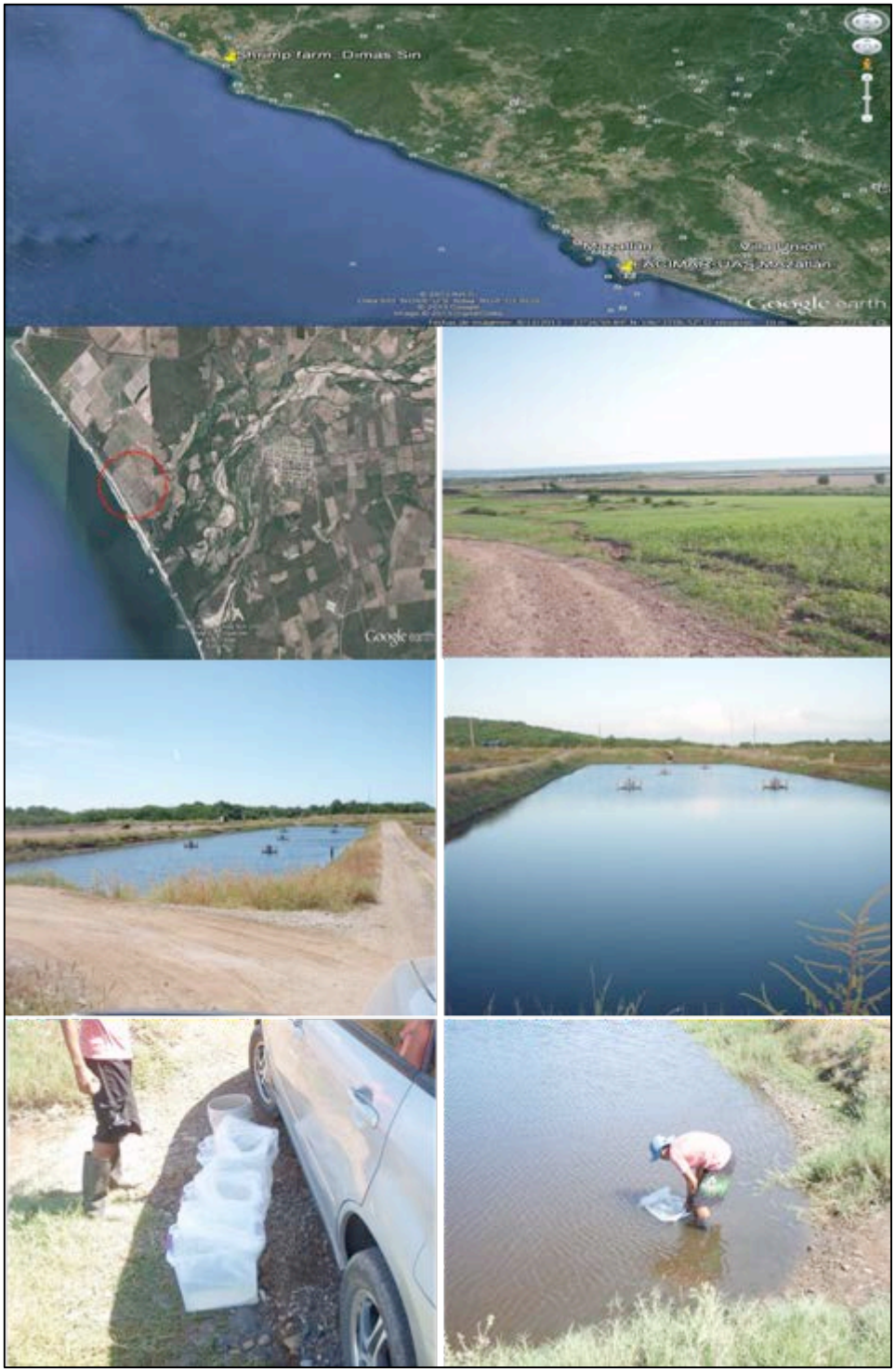

Figure 4. *Location of shrimp farm pond (Estación Dimas Sin. México) and 2 dph chame larvae stocking for pond rearing trial.*

Figure 5. *Artificial ponds for chame larvae rearing trials and wild rotifer production.*

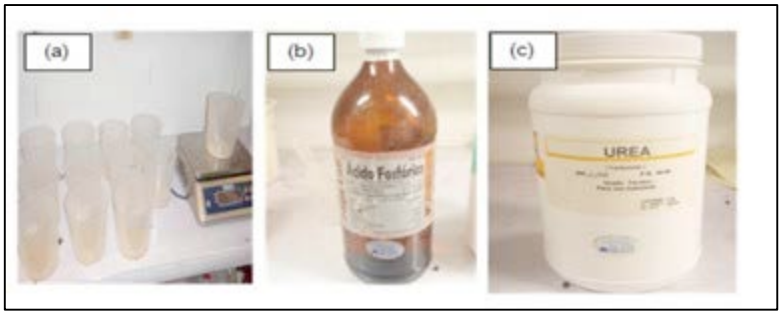

Figure 6. *Organic and inorganic fertilizer used for artificial ponds. A) wheat bran 20 ppm, b) phosphoric acid 0.6 ppm and c) urea 1.6 ppm.*

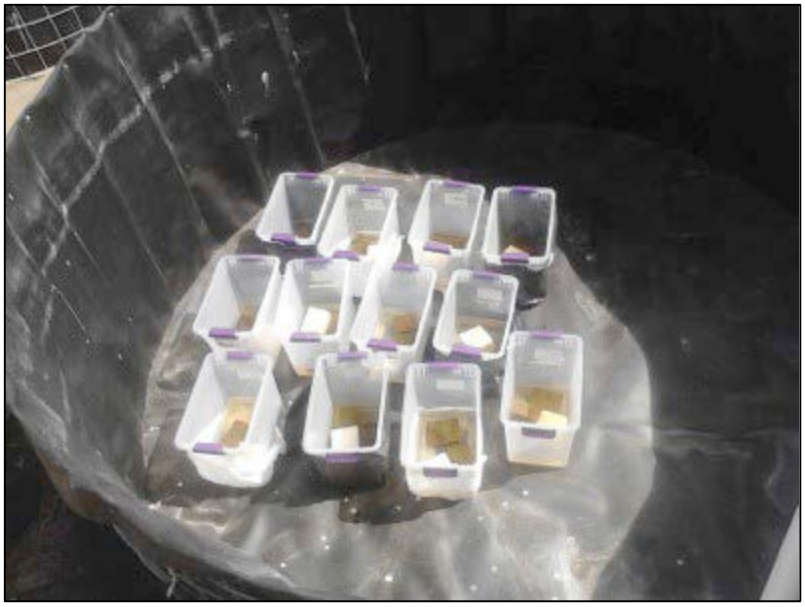

Figure 7. *Rotifer cysts production with collecting sponges.*

Figure 8. *Proalides sp rotifer, produced in fresh water from collected sediments. (Left: in-lab photography 400x magnification; Right: high resolution pic with size bar for demonstrative purposes (source:http://www.plingfactory.de/Science/Atlas/KennkartenTiere/Rotifers/Epiphanidae/im/Liliferotocha121-8.jpg).*

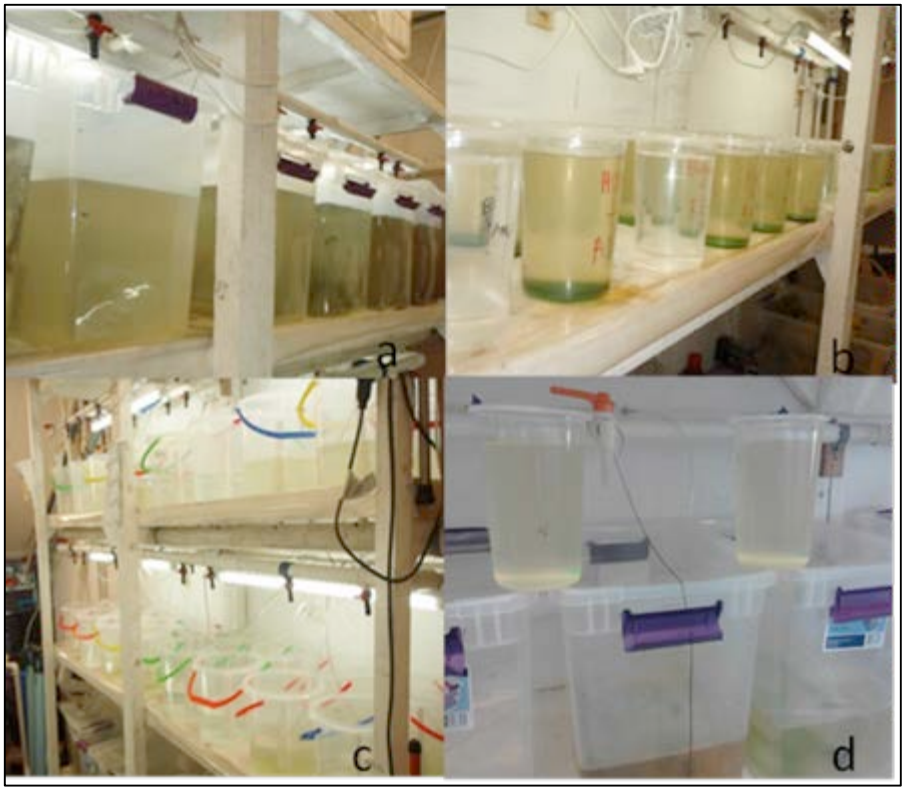

Figure 9. *Mixed feeding experiments tank layout for chame larvae rearing. a) experiment 1, b) experiment 2, c) experiment 3 and d) experiment 4.*

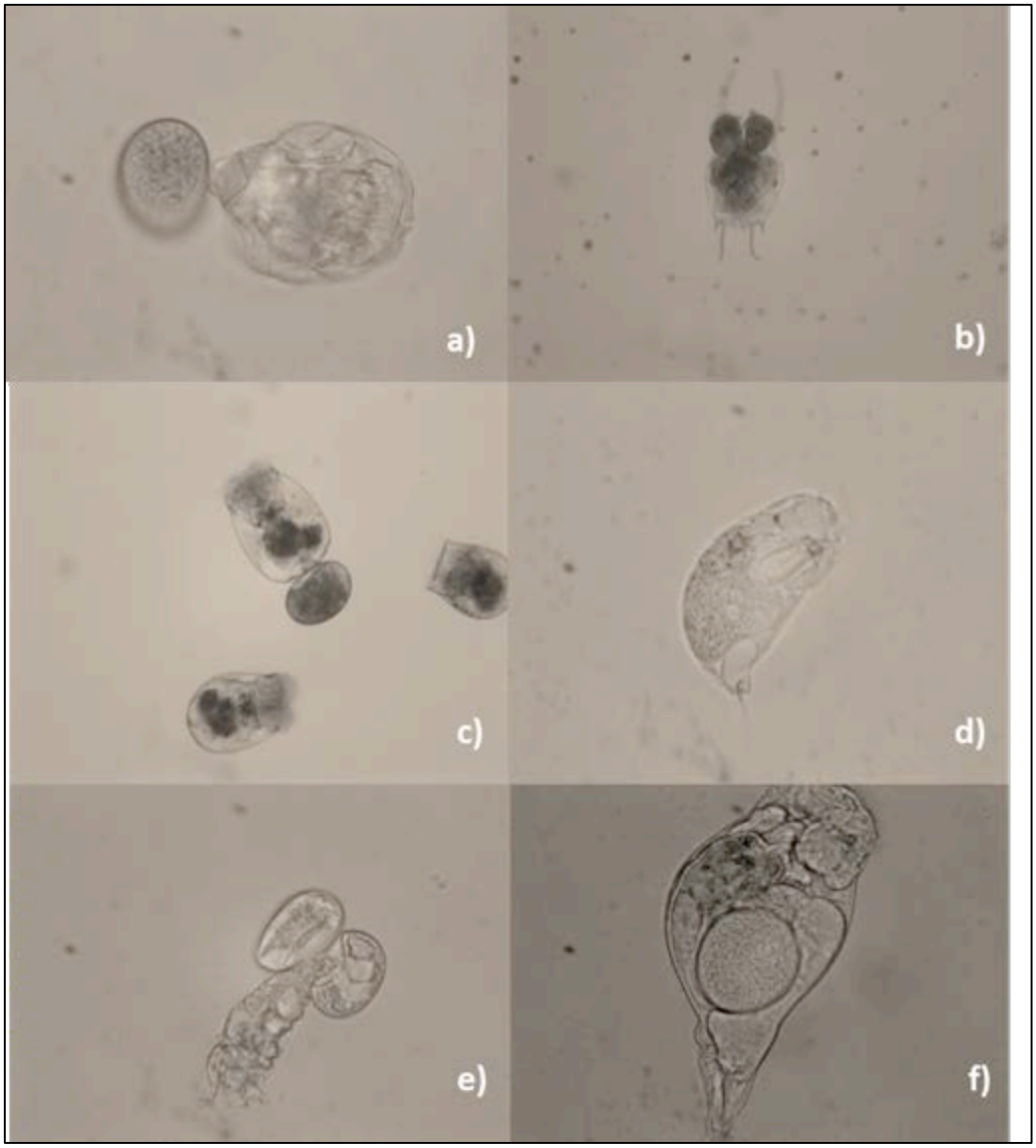

Figure 10. *Sample of wild rotifers produced from artificial ponds at different salinities. a) Brachionus angularis, b) Brachionus falcatus, c) Brachioonus plicatilis, d) Colurella sp., e) Filina longiseta, e) Proalides tentaculus, f) Proales sp.*

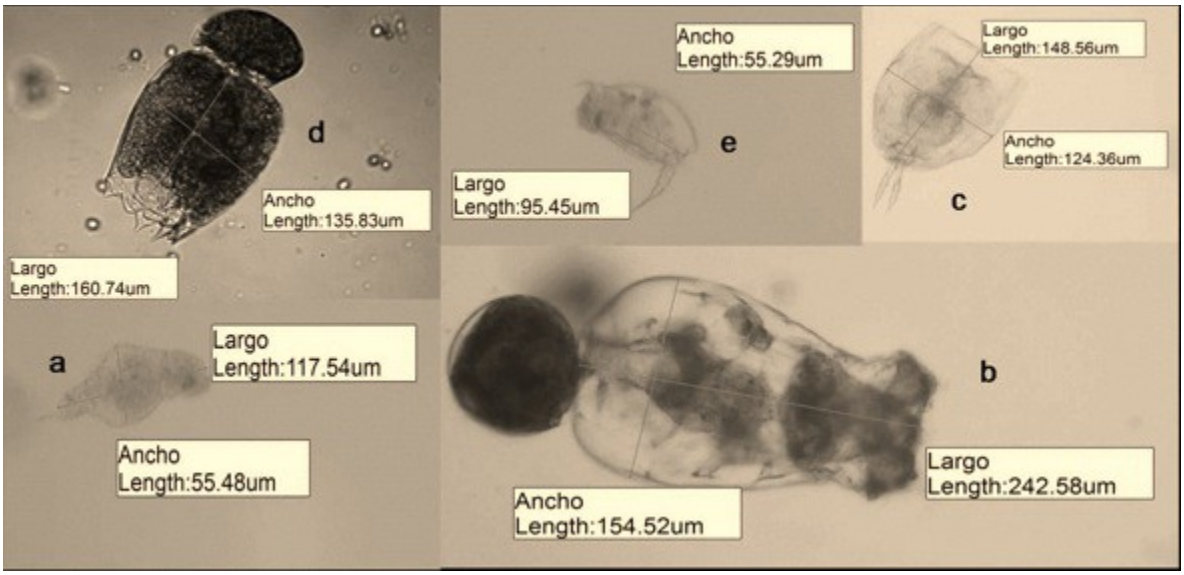

Figure 11. *Morphological characteristics of emerging rotifers produced at different salinities: a) Proales sp., b) y d) Brachionus sp., c) Lecane sp. y e) Colurella sp.*

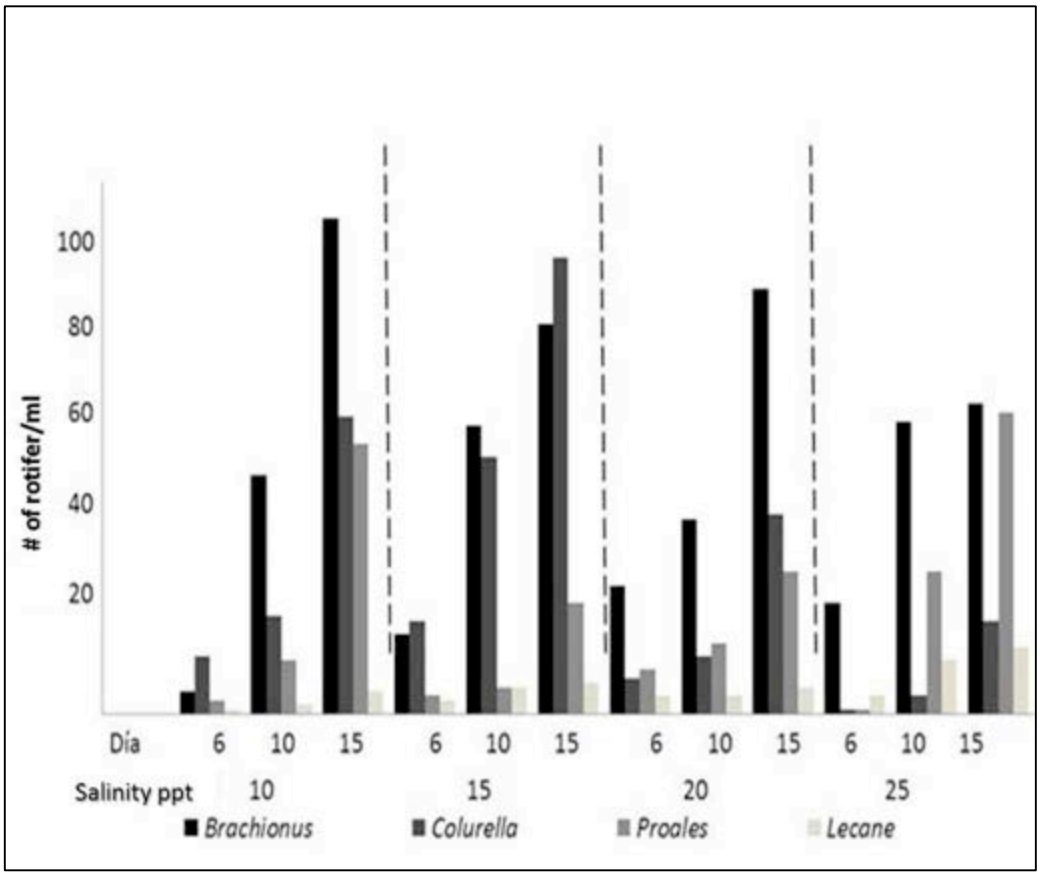

Figure 12. *Specific abundance of emerging rotifers (densities rotifer/ml) at four salinities (10, 15, 20 and 25 ppt) at different times after initial fertilization (6, 10 and 15 days) in artificial ponds using sediments from a commercial shrimp farm.*

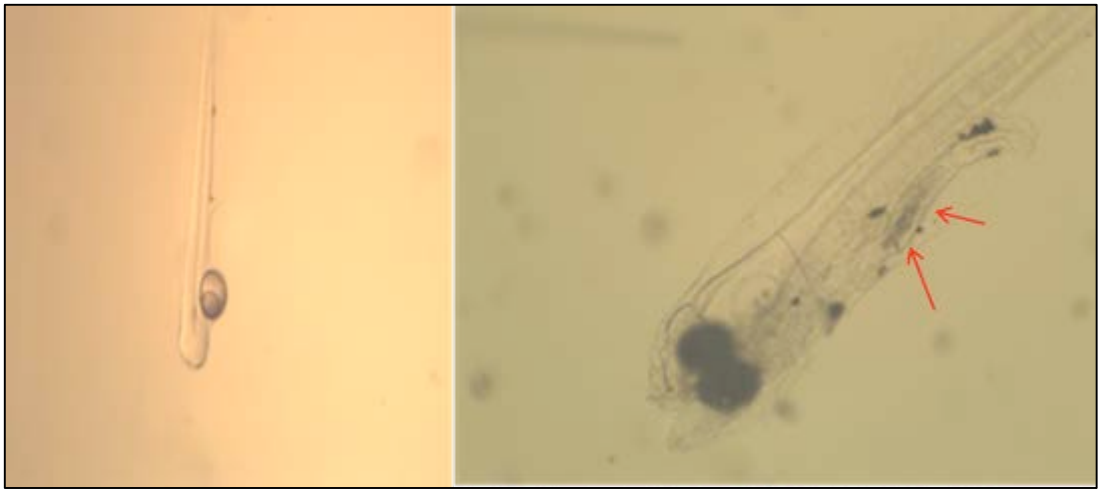

Figure 13. *Newly hatched larvae (left) and 5 dph chame larvae fed with rotifer Proalides and Algamac 3000® (right). Arrow indicates gut content*

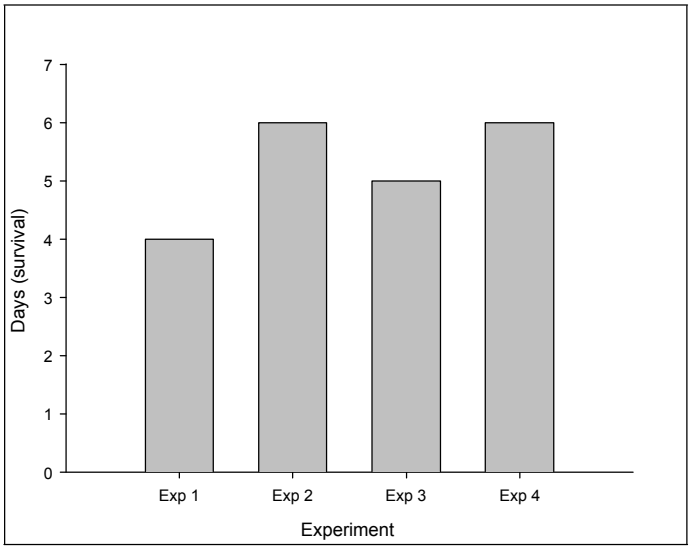

Figure 14. *Overall survival values (days) observed within different chame larvae rearing trials.*

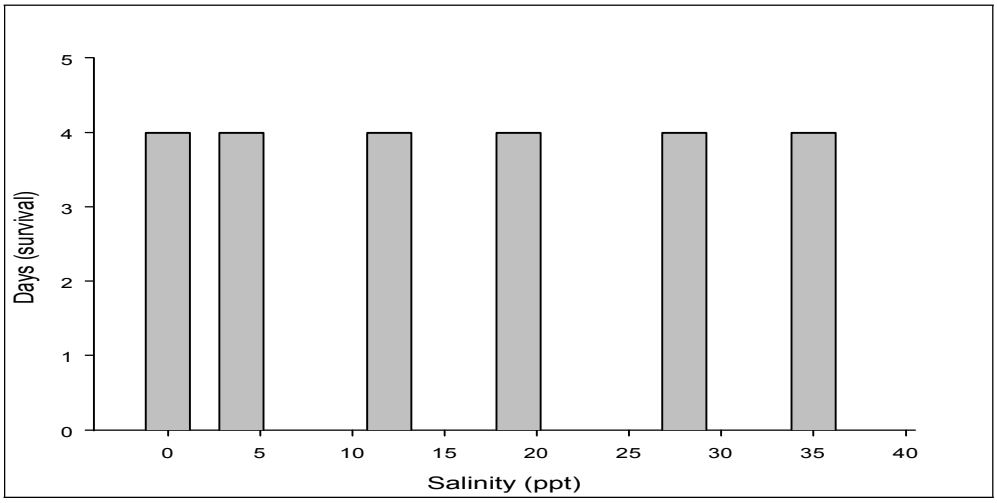

Figure 15. *Overall chame larval survival in days (live larvae presence) in experiment 1, using sponges as rotifer reservoir at different salinities.*

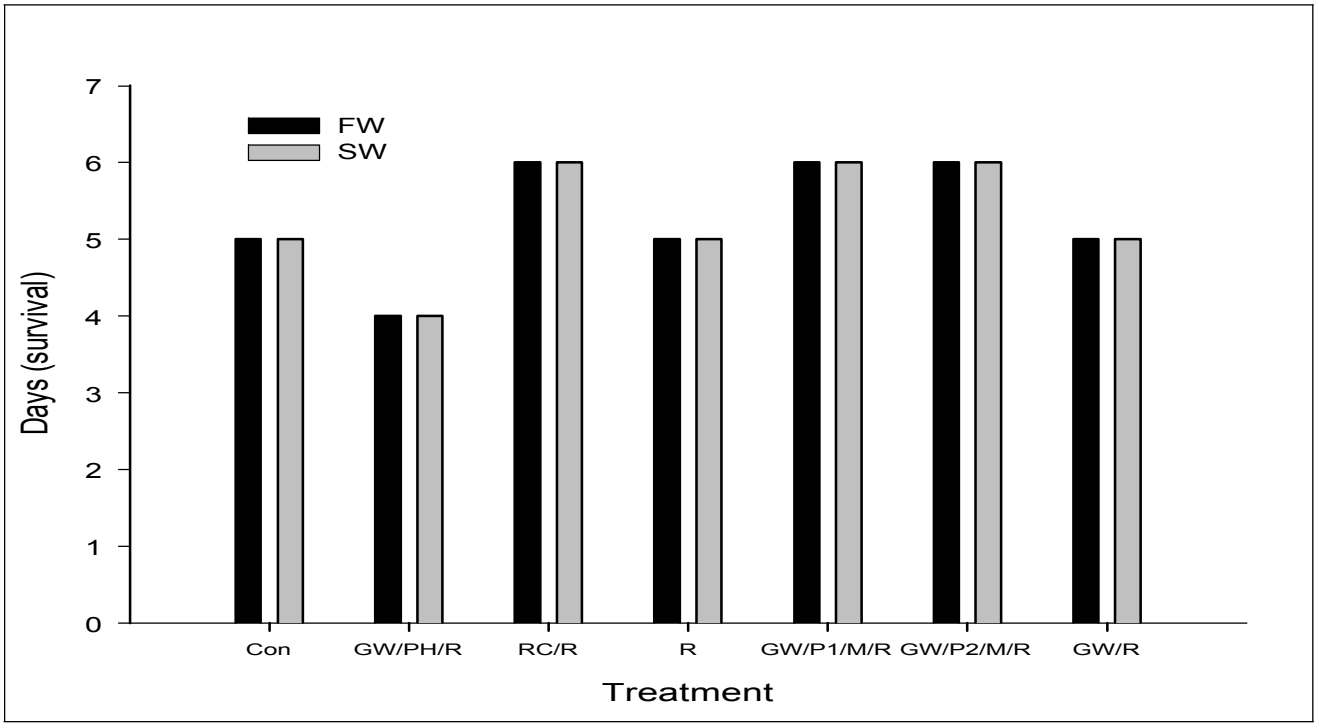

Figure 16. *Overall chame larval survival in days (live larvae presence) during experiment 2, in fresh water (black) or 1 ppt per day salinity increment (gray)*

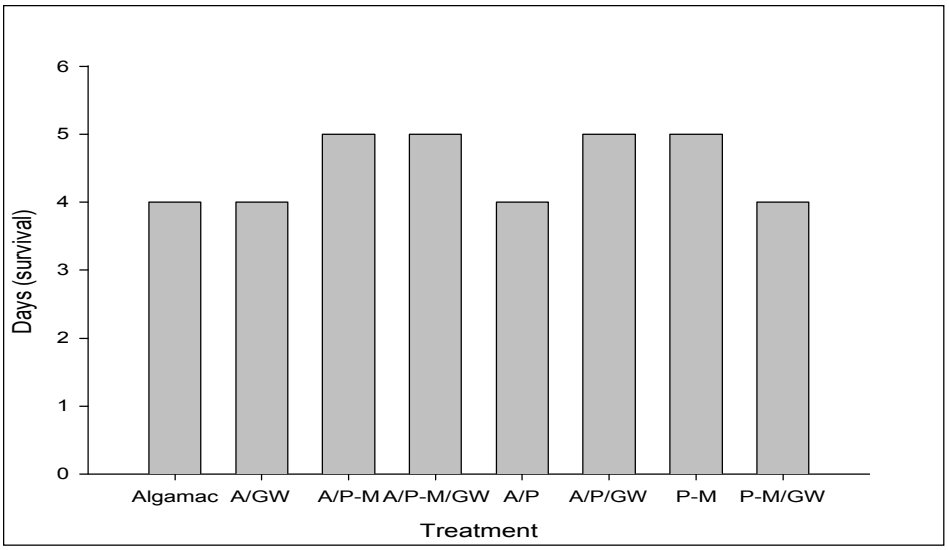

Figure 17. *Overall chame larval survival in days (live larvae observance) in experiment 3.*

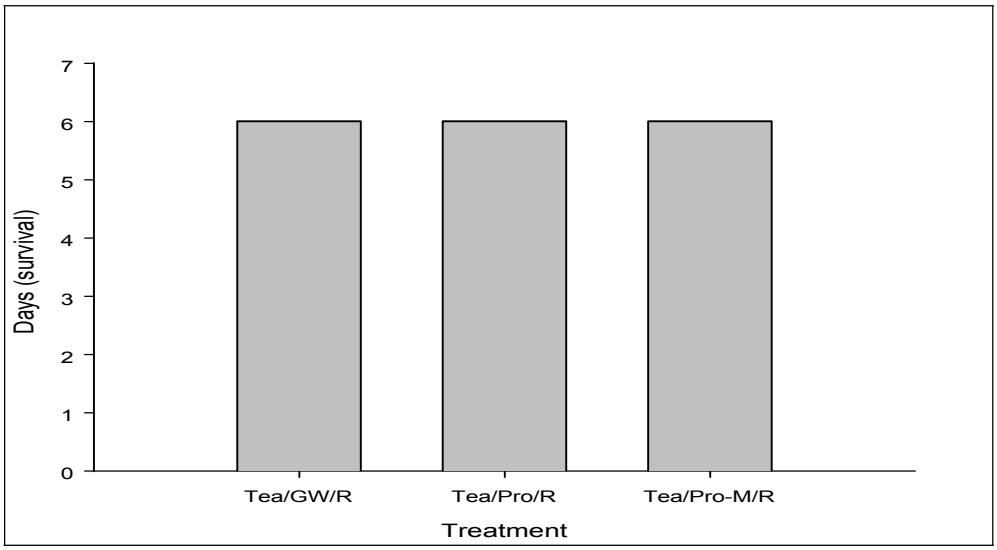

Figure 18. *Overall chame larval survival in days (live larvae observance) in experiment 4.*

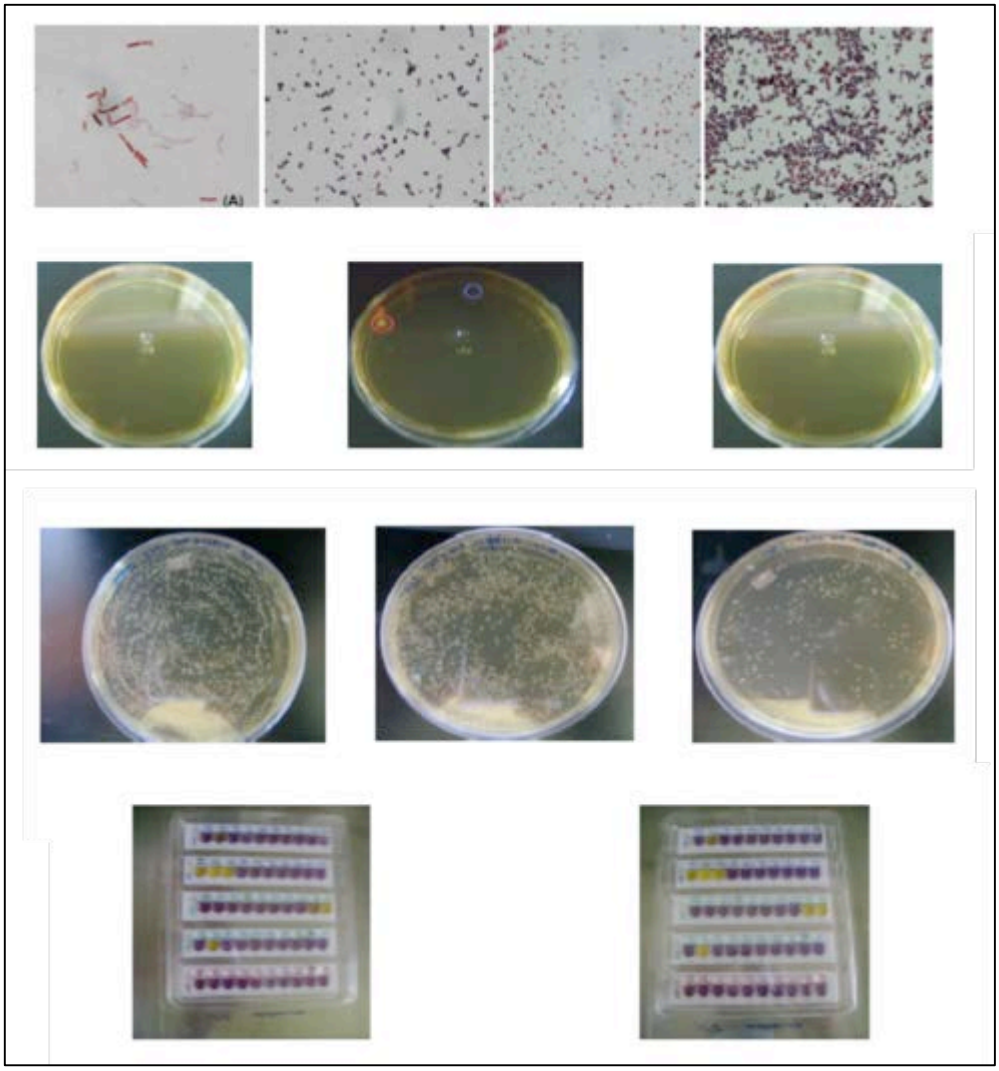

Figure 19. *Probiotic caracterization of isolated bacteria from Chame intestinal tract. From top to bottom: Gram staining, bile salt tolerance, pH resistance and API test for biochemical bacteral identification*