Induced Spawning and Larval Rearing of the "Chame" *Dormitator latifrons* in Laboratory Conditions

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ABSTRACT

The proposed research is focused on the production of chame (Pacific Fat Sleeper) juveniles for aquaculture and research purposes. Spawning was achieved using several delivery techniques for LHRHa. A series of experiments over two years have been conducted, during year 1 (2010) a trial was conducted using recently caught broodstock and for the year 2 (2011) a similar trial was carried out using breeders both males and females, with a whole year in captivity with the following treatments: control group (0.9% saline solution), Desgly^{10}-Ala^6 LHRHa, Ovaprim® or a single implant of 75 µg Ovaplant®. Spawning results showed 80-100% success within 24h-48h for the Ovaplant group, 25-50% for LHRHa but 0% for Ovaprim within 48-72h for both years. Obtained data describes as oocyte size as 300 µm and a relative fecundity of 50,000 to 80,000 cells per gram. All LHRHa delivery treatments were effective to induce spermiation in volumes from 0.5 to 10 ml per male; however several males released sperm naturally up to 1 ml throughout the reproductive season. Data indicates that sperm activation time is close to 4 minutes, and overall concentration is within the range of 1 to $2X10^9$ cells per milliliter. Increased sperm motility is achieved after predilution on a 1:10-1:40 ratio in Ringer´s solution. As optimal salinity values, both for fertilization and egg incubation, our results indicate that there is no sperm activation above 5‰ of salinity. Data was recorded for optimal incubation salinity, as initially no hatching was observed above 15‰ salinity, although fertilized eggs can be transferred directly to salinities between 0-12‰ with no negative impact on hatching rate, total length (µm) yolk sack diameter and lipid drop size among salinity treatments. Extensive data has been recorded on incubation time at 26-28°C as 14 hours, hatching size of larvae (1-1.4 mm), time for yolk absorption (26-32 hours post fertilization hpf) and eye and mouth appearance time (30-40 hpf). Several feeding trials were conducted to initiate exogenous feeding; during year 1, feeding trails included live fresh water microalgae, dry powered microalgae, yeast and probiotics, and filtrated water from rotifer and tilapia rearing tanks and biofloc either as a single feed or a combination of 2 or 3 feeding treatments at a larval density

of 0.25-0.5 larvae per ml. For year two, we conducted a series of feeding trial using enriched rotifers or dry feeds, either at 0‰ or daily increments of 1.5‰ or fixed salinities within 2-8 ‰. Larvae from all trials on year one survived for only 6 days post fertilization at rearing temperature, with 100% mortality afterwards. However, last results for year 2 indicate that the use of either artificial plankton or an artemia substitute (2-8‰ rearing salinity) had values within 20-50% survival by day 6; although no survival was recorded after utilization of enriched rotifers. As preliminary conclusion, there is an apparent feeding on organic particulate matter for early larval stages as digestive track develops and matures for later consumption of live feeds such as rotifers and other zooplankton organisms, fact to be later validated as continued experiments for future research.

INTRODUCTION

Chame (*Dormitator latifrons*) (Fig. 1) also known as puyeque, popoyote, chalaco, Fat sleeper among other is a species of special interest for aquaculture. At present, medium-scale commercial aquaculture in Ecuador, as well as initial experiences of chame culture in Mexico, are conducted with wild caught juvenile fish. There is also interest in this fish in Nicaragua, where freshwater fishes such as tilapia are currently fetching higher prices than cultured shrimp. Therefore, the goals of this work are focused on the production of juveniles under laboratory conditions and minimize the dependency on wild fish supply. Available information indicates that in Ecuador, chame aquaculture has continuously decreased over the last eight years due to the shortage of juvenile fish since controlled propagation has not been achieved. Research in this area was largely abandoned over ten years ago, but most available references are available through a series of books, thesis and non-published reports from several institutions in Ecuador, with information on basic biology (Bonifaz *et al* 1985) and culture trials (Ecocostas, 2006) as well as reported production statistics of 1500 MT per year (FAO, 2009). For Mexico, there is a steadily demand on the central and the southern Pacific Coast. Also, as surveyed by the authors, there are already fish farmers interested in acquiring laboratory produced juveniles for commercial aquaculture in Oaxaca State, as a few preliminary growth-out trials have been conducted (Castro-Rivera *et al*, 2005). In addition, the species is not considered for protection under Mexican laws, and controlled juvenile production will provide a considerable benefit for the diversification of fish culture in Mexico. The main goals of this project are the following: 1) attempt hormonally induced reproduction by outlining the viability of the utilization of newer spawning techniques; 2) fertilization and egg incubation at different salinities to evaluate hatching success; 3) establish the viability of the initiation of exogenous feeding throughout a series of trials using both live and dry food as potential starter diets. This reports details the advances on the control of induced reproduction both in males and females, relevant details on the reproductive and early stages biology of the species and the results of the of larval rearing in laboratory conditions.

MATERIALS AND METHODS

Objective 1: Induced spawning and spermiation using synthetic analogues of luteinizing hormone releasing hormone (LHRHa).

Adult fish with a minimum size of 10 cm total length were collected within a 60 mile radius of Mazatlán Sinaloa, México and later transported and acclimated in FACIMAR-UAS facilities (23°12′57" N; 106°25′31" W). Fish were fed with a combination of 60% floating pellets (32% protein 8% lipids) and 40% sinking pellets (35% protein 10% lipid) (PURINA®). Fish of both genders were identified by the differences on the genital papilla and tagged using PIT-Tags (Passive Integrated Transponder tag, Biomark®) and potential breeders with visible signs of gonad maturation such as swollen abdomen, significant individual weight gain and changes in coloration on males and females, both in the papilla and the abdomen (Bonifaz *et al*, 1985; Estuardo

Campoverde, pers. comm.) were separated and monitored; however gonad biopsies were not possible due to the significantly reduced size of the pore at the papilla.

A series of experiments were conducted over two years. For year 1 (2010) 16 females were divided into the following groups: control group (0.5 ml/kg 0.9% saline solution), Desgly[10]-Ala[6] LHRHa injected at 40 µg/kg (priming dosage) and 80 µg/kg (resolving dose), or injections of Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®)., for year two (2011) 20 females divided into the following groups: control group (0.5 ml/kg 0.9% saline solution), Desgly[10]-Ala[6] LHRHa injected at 40 µg/kg (priming dosage) and 80 µg/kg (resolving dose), 2 injections of Ovaprim®, 0.5 ml/kg as priming dose and 1.0 ml/kg as resolving dose or a single implant 75 µg (Ovaplant®). In all cases, variables evaluated were spawning efficiency per group (%), oocyte size (um), and relative and total fecundity.

For males the conducted trials were as follows: for year 1, 16 males were divided into a control group (0.5 ml/kg 0.9% saline solution), LHRHa injected at 40 µg/kg, Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®); For year 2, 20 fish were used for an experiment was carried out using a control group (0.5 ml/kg 0.9% saline solution), either 40 or 80µg/kg Desgly[10]-Ala[6] LHRHa or Ovaprim® at 0.5 ml/kg at 5 fish per treatment. Studied variables for sperm quality were collected sperm volume, motility percent, and activation time and sperm concentration per treatment.

Objective 2. Effect of water salinity on fertilization, egg incubation and hatching success.
An protocol for sperm activation and fertilization as well as hatching success in terms of water salinity was conducted as follows: sperm samples were pre-diluted in ringer´s solution at several dilution ratios (1:1-1:40) (Arias-Rodriguez L. UJAT-Tabasco, per. comm.) as sperm viscosity was too high to allow effective activation with direct dilution in activation media (10 µm filtered, UV sterilized water). Once pre-dilution was completed, again 50-100 µl of Ringer´s diluted sperm samples were activated in 900 µl of activation media (0, 5, 15, 25, 35, 45, 55 and 65 ‰) to establish best activation conditions as water salinity value.

During year 1, once spawns were achieved, water salinity incubation conditions were estimated by placing 1000-1500 fertilized eggs in 1 l containers with 10 µm filtered, UV sterilized water at 0, 5, 15, 25, 35, 45, 55 and 65 ‰ with three replicas per salinity. Survival (%) per salinity and total length and morphological characteristics of larvae at hatching and thereafter were observed using digital image analysis with Motic Image Plus 2.0 software (Fig. 1).

For year 2, as continued study for optimal larval incubation and hatching conditions, 200 fertilized eggs were placed by triplicate in water previously adjusted to 0, 2, 4, 6, 8, 10 and 12 ‰ for later evaluation of hatching rate (%), total length yolk size diameter and oil drop size at hatching, again using digital image analysis with Motic Image Plus 2.0 software (Fig. 1), this procedure was repeated for several trials.

Objective 3: Effect of water salinity, food type and availability on larval survival and growth after yolk sack absorption.
For year 1, several trials were conducted using the following feeds: 50-80,000 cell/ml of *Chlorella* and *Scenedesmus* as freshwater phytoplankton, 50µm filtrated water (5-10 ml/day) from a rotifer *Brachionus rotundiformis* rearing tank, micropowdered *Spirulina* <20 µm (Mackay Marine®) (3 mg/l), Algamac 3000 1 m/lg , yeast 3 mg/l, microparticulate MPz < 70µm (Mackay Marine®) 1 mg/l, Epicin G2 (probiotic) as bacterial feed and biofilm promoter (3 mg/l), 60 l tanks with 15-20 ml/l of bioflocs, natural biofilm using shade cloth strips as substrate, and 35 µm filtrated Green-water originated in a 250 L tilapia bioflocs rearing tanks; for the trials, either a single or a combination of 2 or 3 treatments with four replicates per dietary treatment at a larval density of 0.25-0.5 larvae per ml, either on 1 l containers or at 50 l tanks. Alternatively, similar trials were

conducted considering an increment of 1.5 ‰ salinity per day using a combination of 2 or 3 of the previously mentioned feeds. Here, larvae from experiments were measured for survival, length increments, gut content (Rocha *et al.* 2008), and modified Fulton´s condition index (Sarnowski and Jezierska 2007), as well as fixation of larvae from several treatments in 10% buffered formalin for 24h and preserved in 75% ethanol afterwards. Several larvae were embedded in Historesin (Leica) and stained with Harris hematoxilin and eosin for optical analysis at the Aquaculture Center, UNESP Brazil.

For year 2, a new series of dry feeds as well as enriched rotifers were used in several feeding trials as follows: a first experiment using Artificial plankton 50 um particle size (Argent®) at 2 mg/l, ArteMac 0 (Aquafauna Bio-Marine Inc.®) at 4.5 mg/l, Encapsulon 0 rotifer substitute (30 um) 10 mg/l (Argent®) and Encapsulon 1 (50 um, Argent®) 20 mg/l; a second experiment using live rotifers as follows: control group (unenriched rotifers), enriched rotifers with Culture HUFA (Salt Creek Inc®), enriched rotifers with Protein HUFA (Salt Creek Inc®) and rotifers enriched with Algamac Protein Plus (Aquafauna Bio-Marine Inc.®) at a equal concentration of 5-8 rotifers per ml for all treatments, twice a day; both experiments were conducted at a rearing salinity of 0‰ in 1 l semi-clear plastic containers. Afterwards, three more experiments were conducted, with 2 trials using the same feeding treatments mentioned above (both live and dry feeds) but considering a gradual 1.5‰ salinity increment per replicate per day, starting with replicate 1 on the first day, both replicates 1 and 2 for the second day, etc. in 3 l clear plastic containers. At last, another experiment was conducted using a combination of 50,000 cels/ml of NANNO 3600 microalgae concentrate (Reed Mariculture Inc.) and 3 mg/l of Epicin (Epicore®) for all experimental units, using each one of the feeding treatments listed for the first experiment, with four replicates per feeding groups, each one at a different salinity of 2, 4, 6 and 8‰ in 1 l semi-clear plastic containers. For all cases, survival was estimated 6 days post hatching, and larvae from each feeding treatment and salinity were fixated either on 4% paraformaldehyde or Karnovski´s fixative for later histochemical analysis. Also, digital images of apparent digestive activity were obtained with a phase contrast optical microscope.

Objective 4. Studies on the utilization of artificial diets for weaning of chame larvae

Once larval feeding activity is observed, larvae will be fed with live rotifers *Brachionus plicatilis* and *Artemia* nauplii in a sequence as initial food and then 7 or 14 day old larvae/juveniles will be weaned to formulated diets as follows: (1) a commercial diet, (2) an experimental casein-gelatin based diet with maca meal as attractant, (3) an experimental diet based on freeze-dried preparation of fish muscle, (4) freshly hatched brine shrimp nauplii. Both experimental diets will be formulated based on our previous experience and will be isonitrogenous (protein requirement: 55% for most larval fish, Dabrowski 1986). At the end of the experiment, growth performance will be evaluated in terms of final individual body weight, survival (%), specific growth rate (SGR, %) and weight gain (%). Fish from each dietary treatment will also be sampled for proximate body analysis (water, protein, lipid, ash) if the size at the termination of the rearing period will permit (at least 0.5 g individual weight).

RESULTS

Objective 1.

For year 1, spawning results showed 80-100% success within 24h-48h for the Ovaplant group, 25-50% for LHRHa but 0% for Ovaprim within 48-72h. Obtained data describes as oocyte size as 300±50 μm and a relative fecundity of 80,000 to 100,000 cells per gram (Table 1). All LHRHa delivery treatments were effective to induce spermiation in volumes from 0.5 to 10 ml per male (LHRHa injected at 40 or 80 µg/kg, Ovaprim® at 0.5 ml/kg or a single implant 75 µg (Ovaplant®); however several males released sperm naturally up to 1 ml throughout the reproductive season

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(Table 3). Obtained data indicates that sperm activation time is close to 4 minutes, and overall concentration is within the range of 1 to $2X10^9$ cells per milliliter (Table 3). Sperm activation is highly improved after predilution on a 1:10-1:40 ratio in Ringer´s solution. No spermatocrit values were recorded as chame sperm viscosity probed to high in undiluted sperm.

For year 2, using one year captive broodstock, reproductive parameters did not change significantly from those observed on year 1. Again females from the Ovaplant group did spawn within 24-48 hours of implantation and females from the LHRHa treatment spawned 24-48 hours after injections, both at 80% efficiency (n=5 per treatment). Relative fecundity was close to 800,000 cells per female, with no natural spawns observed (Table 2). As sperm quality, there was an apparent reduction on released amounts per treatment, as well as reduction of 20% on activation values and 50-60% total activation time as well as estimated sperm concentration values in 30-40% (Table 4)

Objective 2.

As optimal salinity values, both for fertilization and egg incubation, our results indicate that there is no sperm activation above 5‰ of salinity. Data was recorded for optimal incubation salinity, as initially no hatching was observed above 15‰ salinity, although fertilized eggs can be transferred directly to salinities between 0-12‰ with no negative impact on hatching rate, total length (μm) yolk sack diameter and lipid drop among salinity treatments (Fig. 3). Therefore all fertilization procedures were conducted in 1 μm filtered, UV sterilized fresh water. The values of hatching size, yolk sack and oil drop diameter were not affected by rearing salinity on values of 0, 2, 4, 6, 8, 10 or 12 ‰ as observed in several trials (n=3). As example, for trial 1 values for total length at hatching were 1541.1±73.5 μm, yolk sack diameter 146.6±13.2 μm and oil drop 81.4±10.3 μm (Fig. 3). It is important to mention that larvae were photographed also at 3 and 5 days post hatching with interesting results as unfed larvae survival, given that most remaining larvae were found at salinities of 2, 4, 6 and 8 ‰.

As morphological description, eggs are transparent and spherical with a 300 μm average diameter and have an adhesive layer (Fig. 4a). Hatching occurs at 14-17 hours at 26°C, initial larvae length is close to 1288.2±137.2 μm, yolk sack diameter is around 171.2±10.6 μm with a single oil drop, no eyes or mouth are visible and show a vertical floating position with no active movement (Fig. 4b). At 24 h (1 day posthatching DPH), yolk sack diameter reduces to 137.1±8.3 μm, eyes are perceptible, with no pigmentation and digestive tract is noticeable (Fig. 4c). Mouth opening occurs at 2 DPH, ayes are well pigmented and digestive tract structures become more discernable (intestine, vestigial anus); yolk sack diameters is significantly smaller 93.8±10.7 μm (Fig. 4d). At 3 DPH, yolk sack is fully consumed and oral movements are perceptible and digestive tract has an evident circumvolution and pigmentation (Fig. 4e). Anus fully opens at 4 DPH, and some other internal structures are visible (i.e. liver) and body pigmentation increases considerably (Fig 4f).

Objective 3

Several feeding trials were conducted to attempt initiation of exogenous feeding. For year 1, larvae from all trials survived for only 5-7 days post fertilization at rearing temperature (26-29°C), with 100% mortality afterwards. Observed data both on length increment or condition index, were not significant different among diets or rearing salinity, figures 5a and 5b exemplify observed values for these two variables for larvae reared in a salinity gradient. For intestinal content, a few larvae showed particles inside the digestive track, particularly when feed in a combination of Algamac plus yeast or spirulina (Fig 6a). As mentioned a few larvae were processed for histological analysis (Fig. 9 and 10) with a few noticeable structures such as remnants of yolk inside the peritoneal cavity (Fig. 9) and the presence of an apparent digestive gland (pancreas) 6 days after hatching, although further analysis will be conducted for histochemistry on newly obtained larvae as continued studies for a preliminary depiction for enzymatic activity on early stages of chame larvae.

For year two, the inclusion of fresh water rotifers as live feed (non and bioenriched) as well as a new series of artificial diets, either in fresh water, salinity gradient of fixed salinities (2, 4, 6 and 8 ‰) did not showed different results in larvae survival, although as described in Figure 7, there was still a significant number of surviving larvae in two of four treatments, Artificial plankton and Artemac in combination with 50,000 cells/ml NANNNO 3600 and 3 mg/l of Epicin probiotic. At this moment the same trial is been replicated to substantiate this particular finding. Phase contrast microscopy analysis of live larvae fed with these two treatments allowed to observed large quantities of particles inside the digestive tract (Fig. 6b).

Objective 4
As no larvae has been successfully until the point of live feed consumption or beyond 7 days post hatching, no results were obtained for this particular objective; although at present moment feeding trails are still in progress.

DISCUSSION

The present report describes significant findings on the advancement of Chame broodstock and gamete management as well as egg incubation conditions as proposed in objectives 1 and 2. However, even that larvae survival has not been achieved beyond 8 days post hatching; there are important findings that will possibly allow to success in further studies.

Objective 1.
Induced spawning techniques are a reliable tool for laboratory condition reproduction of many fish species of relevance for aquaculture. As observed for both years, synthetic analogs of gonadotropin releasing factors such as GnRHa and LHRHa either by injection or implantation, did allow gamete release for both genders as successfully proven in many other species such as bullseye puffer both for males (Rodriguez M. de O., 2001) and females (Duncan *et al*, 2003), salmonids (Zohar and Mylonas, 2001), barramundi *Lates calcarifer* (García, 1989), stripped seabass *D. labrax* (Fornies *et al.,* 2001). Implants have an advantage as the continued and prolonged release of hormones is stipulated as 50% within 2-3 hours and the remaining hormone over 8-10 hours (Crim *et al*, 1988), being very advantageous on Chame induced reproduction. The product Ovaprim is a valid tool for induced reproduction in many fish species mostly catfish (Sahoo *et al*, 2007) and ornamental fish (Yanong *et al*, 2009); however for chame positive results were only observed for males as increased spermiation but no oocyte release in females; whereas dosages of 0.3-0.7 ml/kg had induce spawns in spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) (Kather Haniffa and Sridhar, 2002).

Purified gonadotropins such as hCG were not considered, as previous unplublished reports and personal communication form researchers from Ecuador mentioned dosages as high as 10,000 IU per fish, when in most cases required dosages are significantly lower (10 times) as reported for leopard grouper *Mycteroperca rosasea* (Gracia-Lopez *et al*, 2004) as well as potential immunoreactivity on treated fish, reducing or eliminating the effectiveness of the treatment (Patiño, 1997). Despite this information, other fish from the Eleotridae family do respond to spawning induction both with Ovaprim and HcG (Chorulon®) treatments as evaluated in *Gobiomorus dormitor* were 1500-3000 IU of HcG induced gamete release (Harris *et al*, 2011). Nevertheless we consider spawning induction with LHRHa either by injection or implantation more effective for Chame.

Reduced spermiation is a recurrent problem on males in captivity; nevertheless is not apparent in newly-capture Chame, although it increases when breeders, have been in captivity for over one year as observed in our experiment. The main difference was the reduction on several sperm quality

parameters such as released volume, motility, time of activity and concentration from year 1 to year 2; and yet sustaining the relevance of the use of LHRHa as a valid tool to induce sperm release in Chame with good quality as described for many other fish species (Zohar and Mylonas, 2001)

Objective 2.

Chame biology is quite interesting as salinity appears to play a major role on reproduction. Our findings indicate a low salinity tolerance both for sperm activation and fertilization, although both are closely related, hatching can occur at higher salinity values with no negative effect on larvae characteristics. Initially this factor was not considered relevant as larvae have been found in many environments; both brackish (Navarro-Rodriguez *et al*, 2006) and sea water (Franco-Gordo *et al*, 2002); thus reproductive biology of Chame occurs mostly in freshwater. It is not uncommon to find this type of reproductive performance in estuarine fishes, were fertilization occurs more efficiently at low salinities but hatching is not affected by the salinity gradient for egg incubation, as reported for *Fundulus heteroclitus* with better fertilization values around or below 15‰ and high hatching .percentage between 10-30‰ (Bush and Weis, 1983). Therefore the main mechanism for sperm activation in Chame is an osmotic change due to environmental conditions within 0-5‰, subsequently affecting fertilization.

Incubation salinity can significantly reduce larval survival and increase deformities as observed in *Anguilla japonica* (Okamoto *et al*, 2009). On a first trial (year 1) we observed zero % hatching at salinity values above 15‰; therefore for year 2 we conducted a second trial at reduced salinities, no negative effect was observed on hatching rates with values close to 95% in most cases, some fish despite of being present mostly in marine environments need a lower salinity value for early development such as *Takifugu obscures*, where preferred incubation salinities are between 0-8 ‰ (Yang and Cheng, 2006). Other fish, Australian bass *Macquaria novemaculeata*, have a similar behavior as Chame where adults are mostly present in freshwater; however, when eggs are incubated on salinities between 5-35‰ there in hatching on salinities below 25‰ (Van Der Wal, 1985).

There are a series of studies that mention the potential effects of rearing salinity on larvae size or yolk sack utilization. At selected salinities for year 2, Chame larvae did not show any differences on larval length, yolk sack and oil drop diameter, indicating no negative effect of such incubation salinities; on the other hand, the snapper *Pagrus auratus* (Steward Fielder *et al*, 2005) and yellowtail *Caranx mate* (Santerre 1973) exhibit reduced sizes and differences on yolk sack utilization in high salinities salinities (>35‰), although hatching was not affected by this factor. Todd (1975) described hatching lengths smaller than those observed in our study for Chame larvae as 0.8-0.9 mm, whereas Gaudé *et al* (2010) described hatching sizes of 1.13±0.01 mm for *Dormitator maculatus*; however both studies were conducted at temperatures of 23 or 13°C respectively, a 4-14°C difference with the present work. The last study also describes that yolk sack is not fully consumed until 9 dph at a rearing temperature of 13°C and 4.4‰ salinity. Chame larval length at hatching corresponds to values described for bigmouth sleeper *G. dormitor* at 1.0-1.5 mm but with significantly smaller yolk sack (Harris *et al*, 2011)

Objective 3.

Fish larvae first feeding after yolk sack depletion is essential for growth and survival. As observed in our study low survival rates are not uncommon at this particular stage (Dou *et al*, 2000), however zero survival observed in most conducted trials in this study by day 6-7 post hatching, can also be related to species resilience as reported for unfed sargus (*Diplodus sargus*) as larvae survived for up to 10 days (Darias *et al*, 2003), thus is quite possible that Chame larvae were not able to utilize offered feeds.

Observations of particle content in larvae digestive tracts, both for year 1 or 2 do not warranty digestion of such particles, probably due to poor enzymatic activity and a probable dependence of exogenous enzymes commonly present in live feeds (Cahu and Zambonino, 2001), however the lack of consumption of offered freshwater rotifers (year 2) and other zooplankton sources (year 1) do not provide enough evidence of the contribution of exogenous enzymes to ensure food utilization. Nevertheless, there is evidence of the feasibility of rearing larvae as small as Chame larvae, considering the evidence provided by Mata *et al*, (2004) where grunt Orthopristis ruber with an hatching size of 1.35±0.15 mm were successfully reared with a combination of *Isochrysis* (microalgae), *Brachionus plicatilis* and *Apocyclops distant* copepodids.

For year 1, we observed a perceptible preference for micropowdered spirulina particle ingestion, but still with no apparent utilization, while other fish larvae can be reared with this microalgae almost as successfully as when rotifers are used in knifefish *Chitala chitala* (Sarkar *et al*, 2006). Also, both for year 1 and 2, the use of probiotics or yeast did have as purpose to estimulate digestive activity, growth and metabolism as food utilization (Vine, 2006; Getasuope, 2007), but with no relevant results for year 1. On the other hand, as promising results the combination of microalgae concentrate, probiotic and artificial feeds, with some significant survival up to 6 dph during the last conducted experiment provides some tentative course to continue other similar feeding trials for the remaining of 2011 Chame reproductive season. Addition of microalgae can potentially improve larvae survival by providing nutrients (Naas *et al*, 1996) or visual contrast, attractants and probiotics (Olsen *et al*, 2000).

Conclusion

More research is needed to achieve Chame larval rearing, as at this moment particle ingestion of several kinds of artificial feeds has been assured after macroscopical analysis, both on fixated and live larvae. Also more research is required into obtaining a suitable live feed prey or preys along with the simultaneous evaluation of a wider selection of artificial feeds.

REFERENCES

Bonifaz, N; Campos, M; Castelo, R; 1985. El Chame, una nueva fuente de alimentación e ingresos. Fundación Ciencia. 173 pp.

Bush, C.P. Weis, J.S. 1983. Effect of salinity on fertilization success in two populations of F*undulus heteroclitus*. Biol. Bull. 164:406-417.

Cahu, C., Zambonino, J. 2001. Substitution of live food by formulated diets in marine fish larvae. *Aquaculture,* 200: 161-180.

Castro-Rivera, R., Aguilar-Benítez, G.,. Hernández- Girón, J. P. 2005. Conversión alimenticia en engordas puras y mixtas de Popoyote (*Dormitator latifrons* Richardson) en estanques de cemento. Revista AquaTIC 23:45-52.

Crim LW, Sherwood NM, Wilson CE. 1988. Sustained hormone release. II. Effectiveness of LHRH analog (LHRHa) administration by either single time injection or cholesterol pellet implantation on plasma gonadotropin levels in a bioassay model fish, the juvenile rainbow trout. Aquaculture 74: 87-95.

Darias, M., Pacual, E., Cárdenas, S., y Yúfera, M. 2003. Metabolismo energético de huevos y larvas sin alimentar del sargo (*Diplodus sargus*) en cultivo. IX Congreso Nacional de Acuacultura. Cadiz 2003.

Dou, S.Z., Seikai, T. y Tsukamoto, K., 2000. Cannibalism in Japanese flounder juveniles, *Paralichthys olivaceus*, reared under controlled conditions. Aquaculture 182:149–159.

Duncan, N.J, G. A. Rodriguez M de O, D. Alok, Y. Zohar. 2003. Effects of controlled delivery and acute injections of LHRHa on bullseye puffer fish (*Sphoeroides annulatus*) spawning. Aquaculture 218, 625-635.

EcoCostas. 2006. El Cultivo de Chame (*Dormitator latifrons*) en el Estuario del Río Cojimíes. Proyecto para la Conservación y Desarrollo del Estuario de Cojimíes (Sustainable Coastal Communities and Ecosystem Project -SUCCESS). Pp. 24.

FAO, Fishstat Plus. 2009. Aquaculture production 1950-2009. [18 de Abril 2011]. URL:http://www.fao.org/fishery/statistics/software/fishstat

Fornies MA, Mananos E, Carrillo M, Rocha A, Laureau S, Mylonas CC, Zohar Y, Zanuy S. 2001. Spawning induction of individual European sea bass females (*Dicentrarchus labrax*) using different GnRHa delivery systems. Aquaculture 202: 221-234.

Franco-Gordo, C., Godinez-Dominguez, E., Suarez-Morales, E. 2002. Larval fish assemblages in water off the central Pacific coast of Mexico. Journal of Plankton Research 24:775-784.

Gatasoupe, F.J. 2007. Live yeasts in the gut: Natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture, 267: 20-30.

Gaudé, A., Brown, C., Green, C. 2010. Awakening the sleeper: bringing *Dormitator maculatus* into full market potential as a premier marine bait for Louisiana. Abstract Aquaculture 2010. San Diego Californa, USA.

Gracia-Lopez, V., Rodriguez-Romero, J., Perez-Ramirez, J. 2004. Inducción del desove con HCG y desarrollo embrionario y de larvas de la cabrilla sardinera *Mycteroperca rosasea* (Streets, 1877). Ciencias Marinas, 30:279-284.

Harris, N., Wesley Neal, J., Perschbacher, P.W., Mace C.E., Muñoz-Hincapíe, M. 2011. Notes on hatchery spawning methods for bigmouth sleeper *Gobiomorus dormitor*. Aquaculture Research. 42:1145-1152.

Kather Haniffa, M.A., Sridhar, S. 2002. Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadotropin and synthetic hormone (ovaprim). Veterinarski Arch 72:51-56.

Mata, E., Rosas, J., Velasquez, A., Cabrera, T. 2004. Hormonal induction to spawning and larval descritption of the grunt *Orthopristis ruber* Cuvier (Pisces: Haemulidae) . Revista de Biología Marina y Oceanografía 39:21-29.

Naas, K., Huse, I. y Iglesias, J. 1996. Illumination in first feeding tanks for marine fish larvae. Aquacultural Engineering, 15:291-300.

Navarro-Rodríguez, M.C., González-Guevara, L. F., Flores-Vargas, R., González-Ruelas, M. E. Carrillo-González, F. M. 2006. Composición y variabilidad del ictioplancton de la laguna El Quelele, Nayarit, México Ichthyoplankton composition and variability of the laguna El Quelele, Nayarit, Mexico. Revista de Biología Marina y Oceanografía 41: 35 – 43.

Okamoto T, Kurokawa T, Gen K, Murashita K, Nomura K, Kim SH, Matsubara H, Ohta H, Tanaka H (2009), Influence of salinity on morphological deformities in cultured larvae of Japanese eel, *Anguilla japonica*, at completion of yolk resorption. Aquaculture, 293: 113-118.

Olsen, A.I., Olsen, Y., Attramadal, Y., Christie, K., Birkbeck, T.H., Skjermo, J. y Vadstein, O. 2000. Effect of short term feeding of microalgae on the bacterial flora associated with juvenile *Artemia franciscana*. Aquaculture. 190: 11-25.

Patino, R., 1997. Manipulations of the reproductive system of fishes by means of exogenous chemicals. The Progressive Fish-Culturist. 59:18-128.

Rocha, R.J., Ribeiro L., Costa R., Dinis M.T. 2008. Does the presence of microalgae influence fish larvae prey capture? Aquaculture Research 39: 362-369

Rodriguez M de O, G.A. 2001. Evaluación de la calidad del esperma del botete Diana *Sphoeroides annulatus* (Jenyns, 1834) en condiciones de cautiverio y bajo inducción hormonal con LHRHa. Masters degree Thesis. CIAD, A.C. Mazatlán Sin.

Sahoo, S.K., Giri, S.S., chandra, S., Sahu, A.K. 2007. Effect of Ovaprim doses and latency periods on induced spawning of *Clarias batrachus*: observation on larval deformity. Indian Journal of Experimental Biology, 45:920-922.

Santerre M.T. 1976. Effects of temperature and salinity on the eggs and early larvae of *Caranx mate* (Cuv. & Valenc.) (pisces: carangidae) in Hawaii Journal of Experimental Marine Biology and Ecology, 21:51-68.

Sarkar, U.K., Lakra, W.S., Deepak, P.K., Negi, R.S., Paul, S.K. y Srivastava, A. 2006. Performance of different types of diets on experimental larval rearing of endangered *Chitala chitala* (Hamilton) in recirculatory system. Aquaculture, 261: 141-150.

Sarnowski, P., Jezierska, B. 2007. A new coefficient for evaluation of condition of fish larvae. Electronic Journal of Ichthyology. 2:69-76.

Steward Fielder D., Bardsley William J., Geoff L. Allan, Patricia M. (2005). The effects of salinity and temperature on growth and survival of Australian snapper, *Pagrus auratus* larvae. Aquaculture, 250: 201-214.

Todd, E.S. 1975. Vertical movement and development of the prolarvae of the eleotrid fish, *Dormitator latifrons*. Copeia 3: 564-568.

Van Der Wal, T. 1985. Effects of temperature and salinity on the hatch rate and survival of australian bass (*Macquaria novemaculeata*) eggs and yolk-sac larvae. Aquaculture, 47: 239-244.

Vine, N.G., Leukes, W.D. y Kaiser, H. 2006. Probiotics in marine larviculture. FEMS *Microbiol Rev*. 30: 404–27.

Yang Z, Y. Chen. 2006. Salinity tolerance of embryos of obscure puffer *Takifugu obscures*. Aquaculture, 253: 393-397.

Yanong, R.P.E, Martinez, C., Watson, C.A. 2009. Use of Ovarpim in Ornamental Fish Aquuaculture. University of Florida IFAS Extension. FA161, 8 pp.

Zohar, Y. y C. Mylonas. 2001. Endocrine manipulations of spawning in culture fish: from hormones to genes. Aquaculture, 197: 99-136.

Figure 1. Chame *Dormitator latifrons* (Photo by Gustavo Rodriguez)

Figure 2. New hatched larvae scanned with digital analysis tools
(Motic Image Plus 2.0 software) (note: Scale bars not accurate)
(Photo by Eva Medina)

Table 1. Estimated values of spawning females for all experimental treatments within the experiment for year 1 (2010).

Variable	Control	LHRHa	Ovaprim	Ovaplant
Weight (g)	393.3±185.1	486.15±205.2	388.9±151.6	388.6±216.1
#of fish per treatment	4	4	4	4
% of spawning fish	25%	50%	0%	100%*
Relative fecundity (cell g^{-1})	83000[ç]	59000 [ç]	n/a	50000±10000**
Oocyte diameter (μm)	392.6±51.8 [ç]	327.7±18.5 [ç]	n/a	353.8±106.6**

*n=4 **Pooled from 4 females
[ç]n=1 female

Table 2. Estimated values of spawning females for all experimental treatments within the experiment for year 2 (2011).

Variable	Control	Ovaprim	LHRHa	Ovaplant
Weight (g)	438.6±153.58	467±242.49	526.8±171.32	539.4±229.73
# of fish per treatment	5	5	5	5
% spawning fish	0%	0%	80%	80%
Relative fecundity (cell g^{-1})	n/a	n/a	50598.3± 5134.35	54310.3± 1639.4
Total fecundity per fish	n/a	n/a	691841± 637549	428541 ± 275455
Oocyte diameter (μm)	n/a	n/a	273.33± 0.10	273.55±1.51

Table 3. Estimated values of sperm quality for all experimental treatments for year 1 (2010)

Variable	Control	LHRHa (40μg kg^{-1})	Ovaprim	Ovaplant
Weight (g)	622.7±54.7	434.7±139.4	538.75±187.4	540.6±202.1
# of spermiating fish	3	4	3	4
Mean volume ml	0.5	2.3	4.3	8.2
Motility (%)	93.3±11.5	83.3±11.5	80.0±26.4	93.3±5.77
Activation time (min)	4:24±0:22	4:57±1:91	2:47±1:37	2:90±1:02
Concentration(cell ml^{-1})	1.96E+09± 1.29E+09	2.29E+09± 7.8E+08	1.26E+09± 2.37E+08	2.31E+09± 7.84E+08

Table 4. Estimated values of sperm quality for all experimental treatments for year 2 (2011)

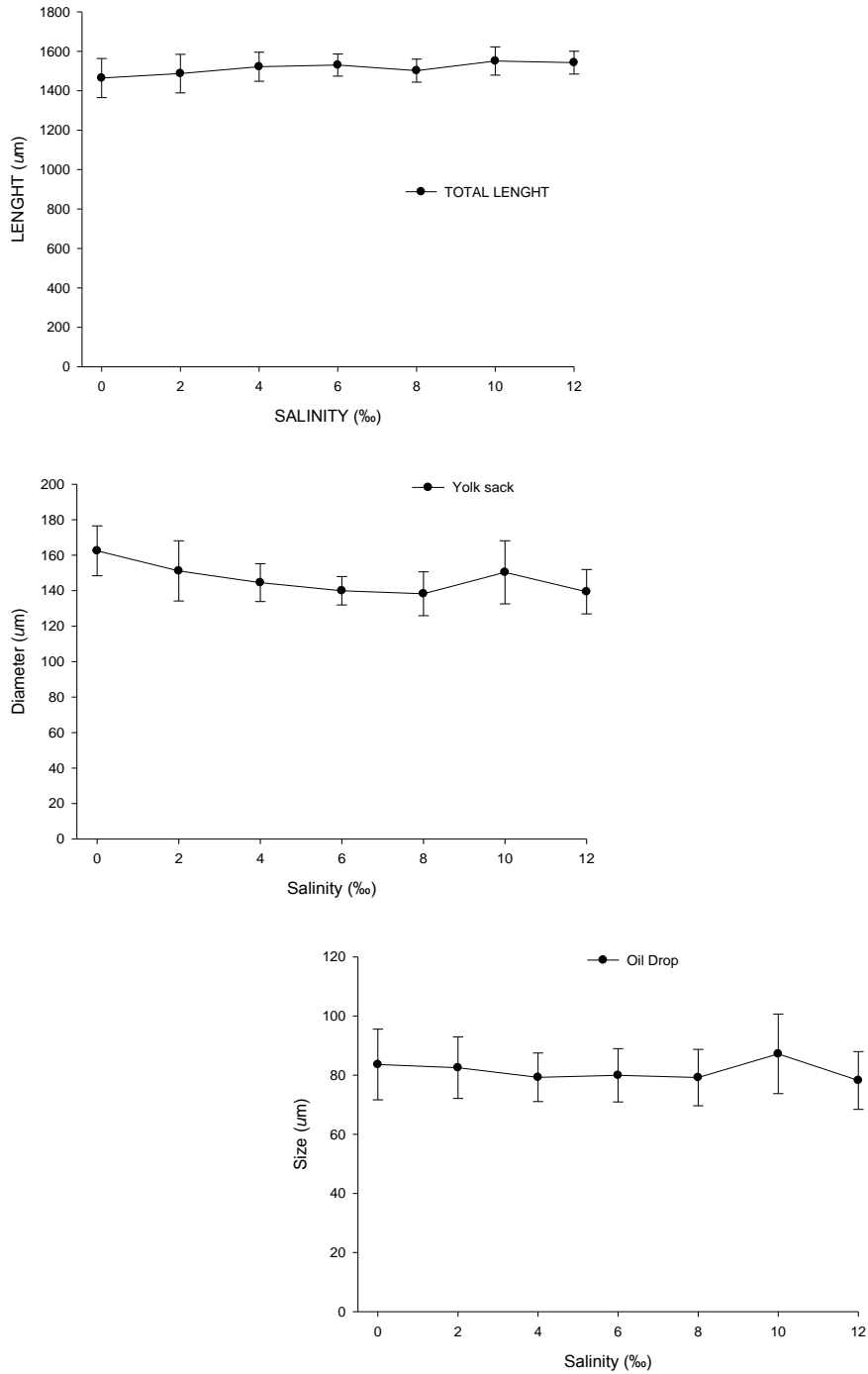| Variable	Control	Ovaprim	LHRHa (40μg kg^{-1})	LHRHa (80μg kg^{-1})
Weight (g)	560.2 ± 65.14	554.6 ± 122.65	550.8±205.06	737.4±204.4
# Spermiating fish	5	5	5	5
Released volume (ml)	0.25±0.2	0.28±0.3	0.77±0.6	4.24±7.4
Motility %	60 ± 14.14	66 ± 30	76 ± 24	62 ± 0.27
Activation time (min)	1:63± 0:41	02:16± 1:15	1:47 ± 0.41	0:83±0:45
Concentration (cell ml^{-1})	1.04E+09± 6.93E+08	1.11E+09± 3.90E+08	1.52E+09± 8.25E+08	1.65E+09± 4.65E+08

Figure 3. Observed values for hatching length (a), yolk sac (b) and oil drop (c) diameter in chame larvae incubated in salinities between 0-12 ‰, 12-16 hours post hatching.
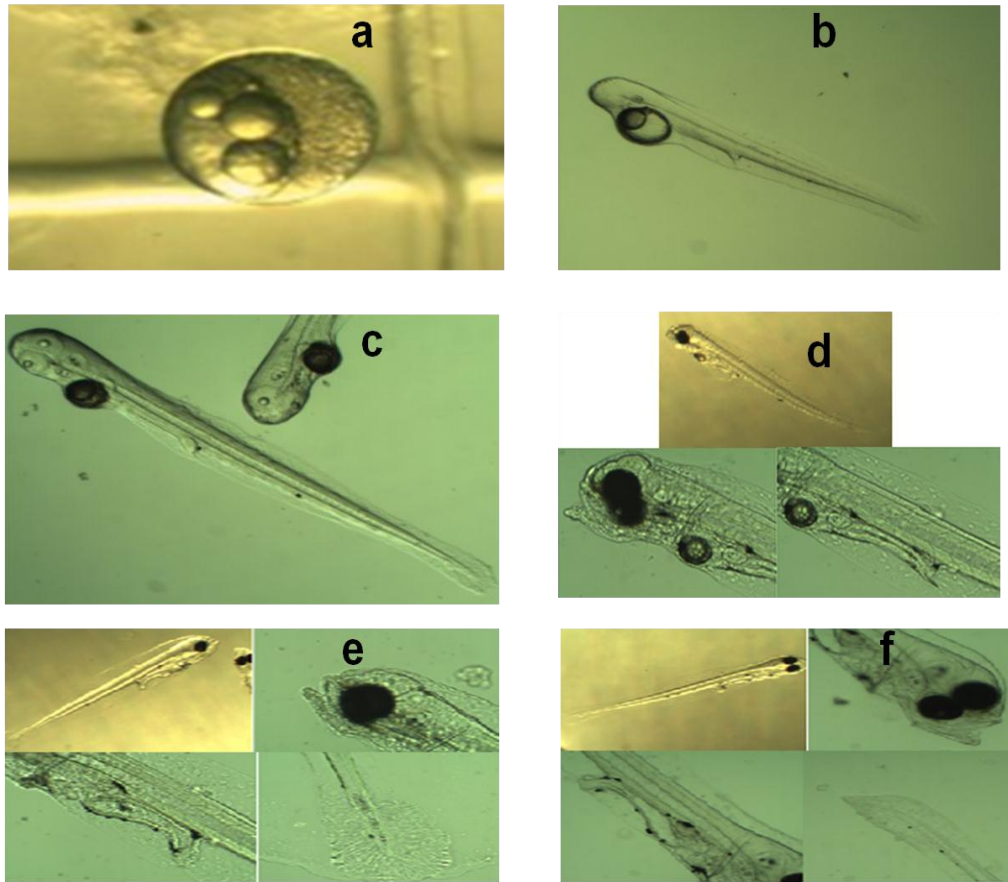
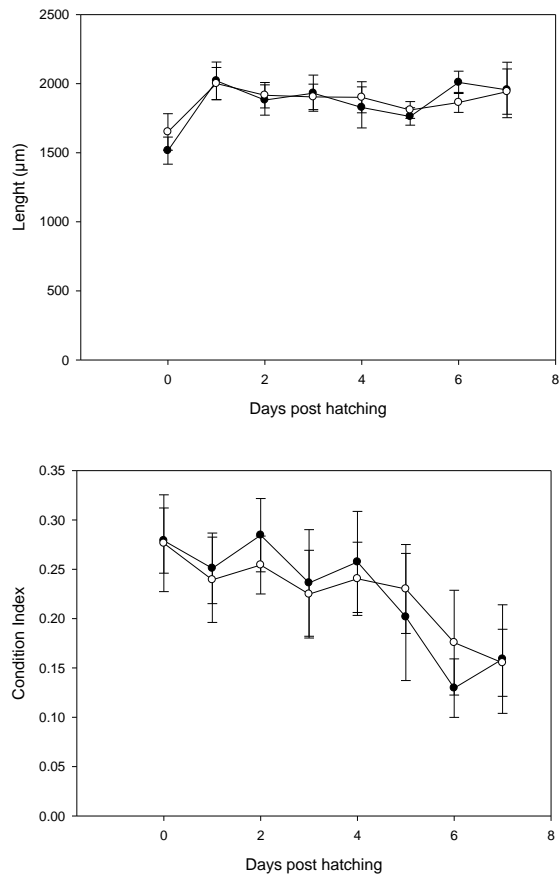Figure 4. Early morphological development of chame larvae at 26-28°C. (40-400x)

Figure 5. Length increment (µm) and progression of condition index (CI) of larvae reared at 1.5 ‰ increments from 1dph (○) or from 2 dph (●).
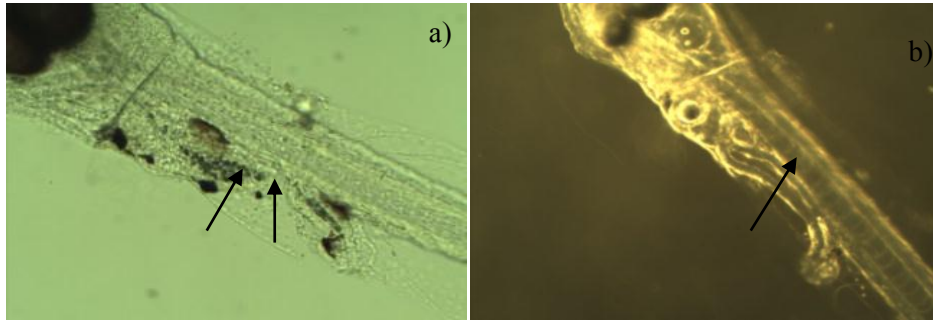
Figure 6. Observed digestive tract content (→) observed after staining Year 1 (a) or phase contrast microscopy Year 2 (400x).
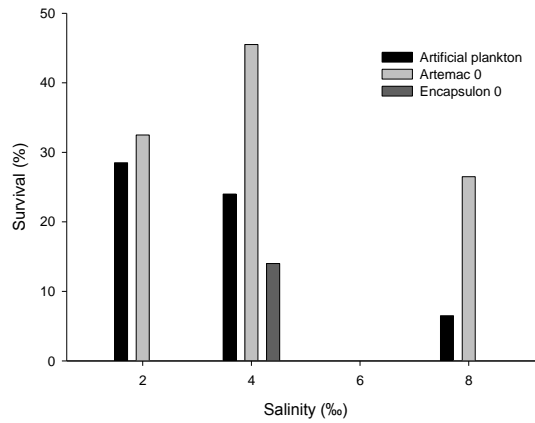

Figure 7. Chame larvae survival (%) observed during last feeding experiment at day 6 after initiation at each rearing salinity per treatment, n=200 larvae per container
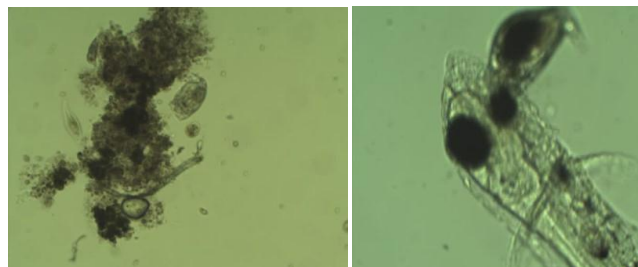

Figure 8. Biofloc produced for Chame larvae rearing and predation by present rotifers on bioflocs.
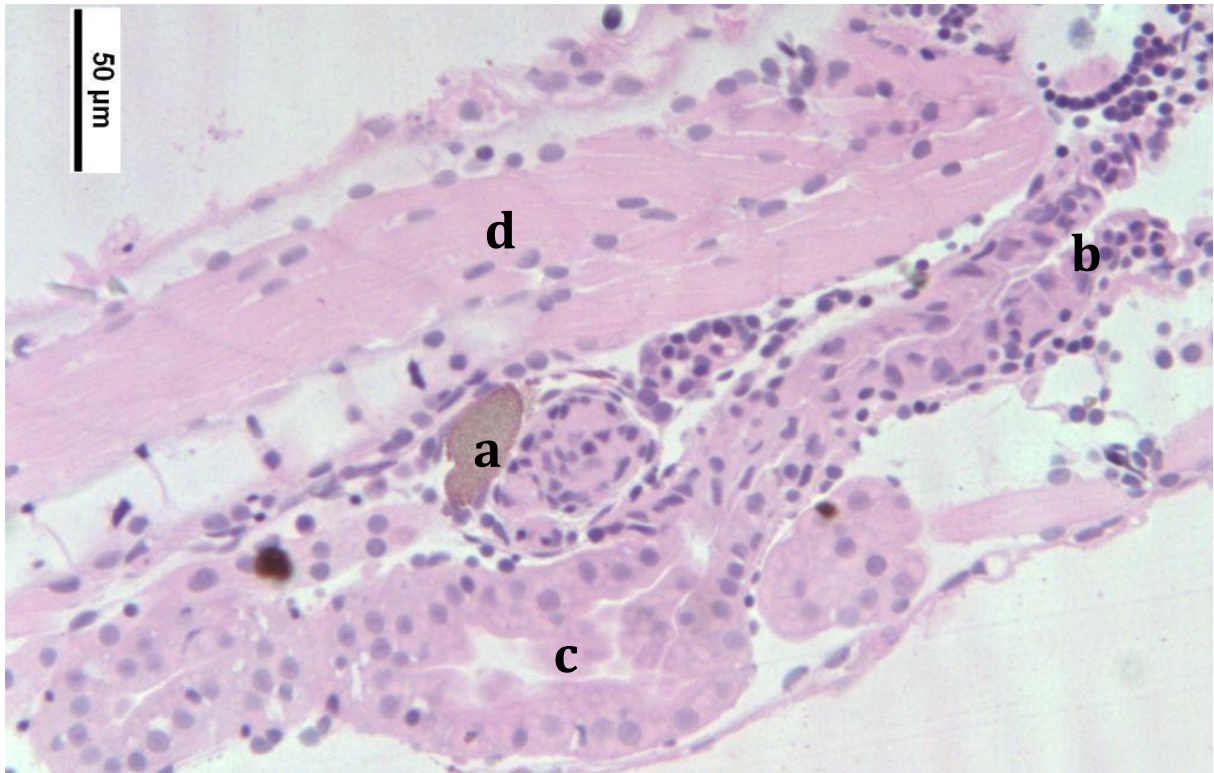
Figure 9. Chame larvae samples embedded in historesin and stained with Harris hematoxilin and eosin, observed structures are yolk sack residues (a), esophagus (b), stomach (c), muscle fibers (d) (400x).

Figure 10. Chame larvae samples embedded in historesin and stained with Harris hematoxilin and eosin, observed structures are mouth (a), jaw (b) eye (c), pancreas (d), stomach (e), muscle fibers (f) (400x).