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DIVERSIFICATION OF AQUACULTURAL PRACTICES BY INCORPORATION OF NATIVE SPECIES AND IMPLEMENTATION OF ALTERNATIVE SEX INVERSION TECHNIQUES

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Final Report

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ABSTRACT

This study sought to determine whether administration of steroids via bioencapsulation into live food is an efficient method for sex inversion of carnivorous species of fish in aquaculture. This technique may offer an alternative for sex reversing such species because the larvae strongly prefer live food compared to artificial diets. To determine whether steroids could accumulate in *Artemia*, nauplii were immersed in solutions containing 2,500 mg L^{-1} of either estradiol (E2) or trenbolone acetate (TA). Steroids were dissolved in ethanol (1 mg ml^{-1}) and then added to the water. Controls were immersed in water containing ethanol vehicle only. Each treatment consisted of three replicates. Water samples (50 ml) from glass jars containing *Artemia* nauplii were collected at 0, 2, 4, 6, 12, 16, 20, and 24 h. Nauplii were washed in nanopure water and dried, and samples were frozen (–20ºC) and preserved until processed. All samples were extracted using ether, and the concentration of steroids were determined by radioimmunoassay (E2) or High Performance Liquid Chromatography HPLC (TA). Immediately after addition of steroids, nauplii contained > 1,500 ng g^{-1} of E2 and > 2,000 ng g^{-1} of TA. The nauplii treated with E2 contained 5,500 ng g^{-1} at 2 h, remained at that level until 6 h, and then reached a concentration of 7,000 ng g^{-1} at 12 h, which remained until 24 h. A similar pattern was observed when TA was used; however, at 24 h concentrations declined to 5,000 ng g^{-1}. TA concentrations in *Artemia* nauplii enriched with highly unsaturated fatty acids (HUFA) ranged between 500 and 800 ng g^{-1}. Despite these large differences in steroid concentrations between HUFA enriched and non-enriched *Artemia*, both methods resulted in significant sex inversion of fish fry. Both Nile tilapia and mojarra castarrica fry were masculinized (> 90% males) after being fed TA-en

riched nauplii for 20 and 45 d, respectively. Tropical gars were also feminized (> 60% females) when fry were fed E2-enriched *Artemia* nauplii. It appears that bioencapsulation of steroids into *Artemia* nauplii is an efficient method for sex inversion of fish.

INTRODUCTION

In the tropics two approaches have been taken to utilize the large diversity of fish species for aquaculture purposes. The first is to use species with moderate growth rates that have high market values, and the second is the use of species with distinct sexual dimorphisms because there is a large demand for the attractively colored gender in aquarium stores. In southeastern Mexico, a growing number of fish producers have requested that alternative culture techniques, which involve native species, be developed. Since the early 1970s, the only species that has been available are Nile tilapia, (*Oreochromis niloticus*) and little effort has been devoted to develop options with local species. Worldwide, the administration of natural and synthetic steroids during early development of fish has been successfully used to induce sex inversion in several species (see reviews by Schreck, 1974; Hunter and Donaldson, 1983), and has become a common practice in the production of single sex populations to enhance productivity in the aquaculture industry. Protocols for masculinizing 47 species and feminizing 31 species have been developed (Pandian and Sheela, 1995). Among the steroids used, 17α-methyltestosterone (MT), trenbolone acetate (TA), and estradiol (E2) have proven effective for masculinizing (MT and TA) and feminizing (E2) fish.

At the Laboratory of Aquaculture at Universidad Juárez Autónoma de Tabasco (UJAT), the production of tropical gar (Atractosteus tropicus) fry has been successful over the last five years (Márquez-Couturier and Contreras-Sánchez, 2003). A previous study demonstrated that females reach larger sizes than males (Contreras-Sánchez and Alemán-Ramos, 1987), but little is known regarding the possible advantages of producing 100% female populations. The tropical gar is considered to be a delicacy in southern Mexico and is an integral part of local culture. It is analogous to the importance of salmon in the Pacific Northwest of the US. Gar are sold in every seafood restaurant in Tabasco, and fishing pressure has led to the listing of the species as "susceptible" (Contreras-Sánchez, 1990).

The mojarra castarrica (Cichlasoma urophthalmus) has been considered as one of the few native species that may perform as well as tilapia in aquacultural systems in terms of growth rates and achievable harvest size (Martínez-Palacios and Ross, 1994). As in tilapia, males grow faster and larger than females; however, little has been done concerning the production of 100% male populations, and the few studies that have been carried out resulted in less than 70% male populations (Hernández-Betancourt, 1988).

Possible alternatives to the problems encountered for sex inversion of new species of fish in aquaculture are the administration of steroids via bioencapsulation and immersions. The first technique is advantageous because larvae of the two species proposed for this study strongly prefer live food compared to artificial diets. This technique has been used for administering chemotherapeutics and nutrients to fish, shrimps, and prawns (Roque et al., 1998; Touraki et al., 1999) and is considered to be a good alternative method for incorporating hormones into manufactured feeds. The second alternative offers the possibility of feminizing or masculinizing the proposed species without the need of hormone-treated diets.

In order to determine the feasibility of sex inversion of native species of commercial importance, the bioencapsulation technique was validated using Nile tilapia (*O. niloticus*) as a model species. In this study we compared the most commonly used protocol for masculinization of tilapia (oral administration of MT) with either feeding of bioencapsulated TA or with immersions in TA. We also evaluated whether tropical gar fry could be feminized using bioencapsulated E2 or with immersions in this steroid.

METHODS AND MATERIALS

Study A: Enrichment and Detection of E2 and TA in *Artemia Nauplii*.

This experiment was conducted at the Laboratory of Aquaculture, UJAT. Two procedures for *Artemia* enrichment were evaluated:

1) Bioencapsulation via immersion in steroid solutions and
2) Bioencapsulation via immersion in solutions containing HUFA as recommended by Stewart et al. (2001).

Procedure 1

Bioencapsulation via immersion in steroid solutions consisted of three treatments:

1) *Artemia* nauplii immersed in 2,500 mg L^{-1} E2
2) *Artemia* nauplii immersed in 2,500 mg L^{-1}TA
3) *Artemia* nauplii immersed in ethanol vehicle

Artemia nauplii enrichment was conducted using artificial marine water that was prepared by using coarse salt (with no iodine) diluted in dechlorinated water until a concentration of 28‰ was reached. *Artemia* nauplii were immersed in solutions containing 2,500 mg L^{-1} of steroid at a density of 450 nauplii per ml. The steroid was dissolved in ethanol to a concentration of 1 mg ml^{-1} and then added to the water. Controls were immersed in water containing the ethanol vehicle only. Each treatment consisted of three replicates. Glass jars containing *Artemia* nauplii were thoroughly mixed, and samples were collected at 0, 2, 4, 6, 12, 16, 20, and 24 h. Nauplii were washed in nanopure water, and excess water was allowed to drain, and samples were stored in Whirl-Pak bags, frozen (–20ºC), and preserved until processing. All samples were lyophilized and shipped to Oregon State University for steroid determination.

All samples were extracted using ether, and the concentration of steroid was determined by RIA (E2) or HPLC (TA). The RIA methods followed the procedure outlined in Fitzpatrick et al. (1986) and Fitzpatrick et al. (1987) and the HPLC methods were outlined in Feist et al. (1990). Both protocols were modified as described in Contreras-Sánchez (2001). For E2 analyses, 0.1 g of dry *Artemia* nauplii were used, while 0.05 g of nauplii were used for detection of TA by HPLC. Extraction efficiencies for E2 and TA were determined (n = 5 for each). Extraction efficiencies were 45.1% for E2 and 51.5% for TA. Concentrations of E2 and TA in nauplii at the various sampling times were not compared statistically because of the limited sample size (n = 3 per date) and because the goal of the study was descriptive (presence/absence).

Procedure 2

Bioencapsulation via immersion in steroid solutions containing *Artemia* diet with HUFA (Ratio HUFA Enrich®, Salt Creek Co.). This procedure was evaluated because a previous study by Stewart et al. (2001) showed that *Artemia* nauplii could be enriched using high doses of steroids and HUFA. This trial consisted of three treatments:

1) *Artemia* nauplii immersed in a solution containing Ratio HUFA Enrich and 20,000mg L^{-1} E2
2) *Artemia* nauplii immersed in a solution containing Ratio HUFA Enrich and 20,000 mg L^{-1} TA
3) *Artemia* nauplii immersed in a solution containing Ratio HUFA Enrich and ethanol vehicle

Artemia nauplii enrichment was conducted using artificial marine water. *Artemia* nauplii were immersed in solutions containing 20,000 mg L^{-1} of steroid at a density of 450 nauplii per ml. The steroid was dissolved in ethanol to a concentration of 6.67 mg ml^{-1}. Controls were immersed in water containing HUFA and the ethanol vehicle. Ratio HUFA Enrich was thoroughly mixed in a blender for 1 minute with artificial marine water. The steroid was added to this solution and mixed again. The resulting emulsion was added to the enrichment glass jars and thoroughly mixed before the nauplii were added. All samples were obtained and processed in the same manner as described in Procedure 1.

Experiment B: Validation of Bioencapsulation by Masculinization of Nile Tilapia Fry

The goal of this experiment was to validate TA-enriched *Artemia* nauplii feeding as a masculinization method using Nile tilapia as a model species.

Nile tilapia fry were obtained from spawning ponds from the Laboratory of Aquaculture at UJAT. Fry were selected by grading with a 3-mm mesh, counted, and randomly assigned to experimental units (20 l buckets). Recirculating systems were built containing 12 buckets each. The system had an 80-l filter divided into three sections—a sediment trap, a granulated-charcoal bed, and a biological filter. Water was returned to the buckets using a 1/6 horsepower submersible pump. Two trials were run for this experiment. Treatments for trial 1 were as follows:

1) Fry fed bioencapsulated TA for 5 d
2) Fry fed bioencapsulated TA for 10 d
3) Fry fed bioencapsulated TA for 20 d
4) Fry fed bioencapsulated TA for 28 d
5) Fry fed control food (*Artemia* immersed in a solution containing ethanol only)

Treatments for trial 2 were as follows:

1) Fry fed bioencapsulated TA for 5 d
2) Fry fed bioencapsulated TA for 10 d
3) Fry fed bioencapsulated TA for 20 d
4) Fry fed bioencapsulated TA for 28 d
5) Fry fed bioencapsulated TA for 45 d
6) Fry fed bioencapsulated TA for 60 d
7) Fry fed control food (*Artemia* immersed in a solution containing ethanol only)

Each treatment consisted of three replicates with 100 (trial 1) or 50 (trial 2) fish each. Fry were fed *Artemia* five times a day. The *Artemia* enrichment protocol for this experiment used HUFA (Procedure 2) and 20 mg L^{-1} of steroid. The amount of *Artemia* eaten each day was estimated using a ratio based on nauplii consumption that allowed us to develop a feeding regime. Following treatment, fish were transferred to another recirculating system and feeding was changed to control food until the end of the experiment. At 90 to 100 days post-fertilization, a subsample of the tilapia in each experimental unit (30 fish, depending on survival) was killed with an overdose of anesthetic (MS-222) to determine if the treatment with TA resulted in masculinization. Sex ratios were determined by microscopic examination (10 and 40X) of gonads using squash preparations in Wright's stain (Humason, 1972). The weights of sampled fish were recorded at this time. Dissolved oxygen, pH, and temperature were monitored twice a week. To maintain water quality, 30% of the water in the system was exchanged twice a week.

Experiment C: Feminization of Tropical Gar Fry

The goal of this experiment was to determine if tropical gar fry could be feminized by feeding of E2-enriched *Artemia* nauplii or by immersions in E2 solutions. Tropical gar fry were obtained from spawning tanks from the Laboratory of Aquaculture at UJAT. Ten days after fertilization, fry were selected, counted, and randomly assigned to experimental units (5 l plastic containers) containing 4 l of water. Fry were stocked at a density of 20 fish l^{-1} (n = 80), averaging 18.3 mm (total length) and 28.4 mg (wet weight). Treatments were as follows:

1) Fry fed bioencapsulated E2 for 5 d starting at onset of feeding
2) Fry fed bioencapsulated E2 for 10 d starting at onset of feeding
3) Fry fed bioencapsulated E2 for 20 d starting at onset of feeding
4) Fry fed bioencapsulated E2 for 28 d starting at onset of feeding
5) Fry immersed for three consecutive d in 500 mg l^{-1} E2 for 3 h at 50% hatching time
6) Fry immersed for three consecutive d in 500 mg l^{-1} E2 for 6 h at 50% hatching time
7) Fry fed control food (*Artemia* immersed in a solution containing ethanol only)

Each treatment was triplicated. *Artemia* nauplii enrichment (Procedure 1) was conducted using artificial marine water. Nauplii were immersed in solutions containing 2,500 mg l^{-1} of steroid at a density of 450 nauplii per ml. The steroid was dissolved in ethanol (1 mg ml^{-1}) and then added to the water. Controls were immersed in water containing the ethanol vehicle only. Gar fry were fed with *Artemia* four times a day. The amount of *Artemia* used each day was estimated using a protocol proposed by Hernández-Vidal et al. (1997). This involved examination of stomach contents, which showed that gar ingest 50 nauplii at first feeding and 2,000 nauplii at day 30. Before feeding the gar, nauplii were washed with dechlorinated water and the excess water was allowed to drain. At the end of each treatment, fish were transferred to control food until the end of the experiment (28 d). After this, all fish were fed adult *Artemia* biomass to satiation for 15 d. On day 16, all fish began a conditioning feeding regime to train them

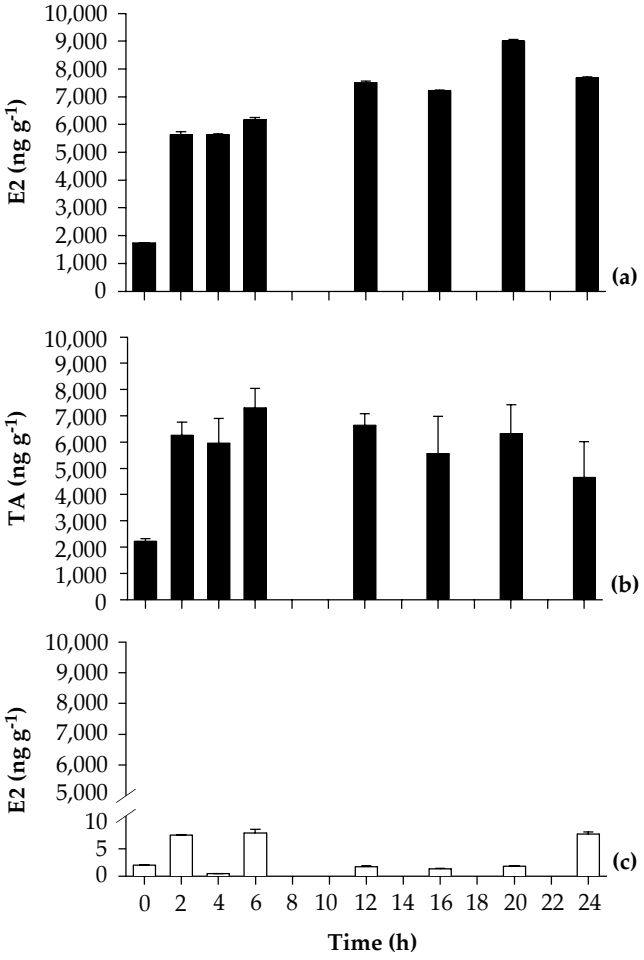

Figure 1. Mean concentration (± SE) of estradiol (a) and trenbolone acetate (b) in *Artemia* nauplii immersed in steroid. Mean concentration of estradiol (c) in *Artemia* treated with vehicle (ethanol). Each bar represents three replicates.

to eat pellet food. All fish received a mixture of adult *Artemia* biomass and 1.5-mm commercial trout food "El Pedregal" (Silver CupTM, Toluca, Mexico). After two weeks of conditioning, all fish received pellet food only. All fish were transferred to outdoor recirculating systems consisting of 24 100-l plastic tanks each. Fish were grown for 10 more months. At the end of the grow-out period, all fish in each experimental unit were killed with an overdose of anesthetic (MS-222) to determine if the treatments resulted in feminization. Due to difficulties determining the sex using the squash method, gonads were fixed in Bouin's solution and transferred to 70% EtOH solution after 24 h. Sex ratios were determined by microscopic examination (40 and 100X) of gonads that had been sectioned and stained with hematoxylin and eosin (Humason, 1972). The weights of sampled fish were recorded at this time. Dissolved oxygen, pH, and temperature were monitored twice a week. The efficacy of E2 treatments was compared between treatments with a Chi-square test.

Experiment D: Masculinization of Castarrica Fry

The goal of this experiment was to determine if castarrica fry could be masculinized using TA-enriched *Artemia* nauplii. Castarrica fry were obtained from spawning tanks from the Laboratory of Aquaculture at UJAT. First-feeding fry were selected, counted, and randomly assigned to experimental units (20 l buckets). Recirculating systems (described in Experiment B) were used. Each replicate hosted 50 fry initially. Treatments were as follows:

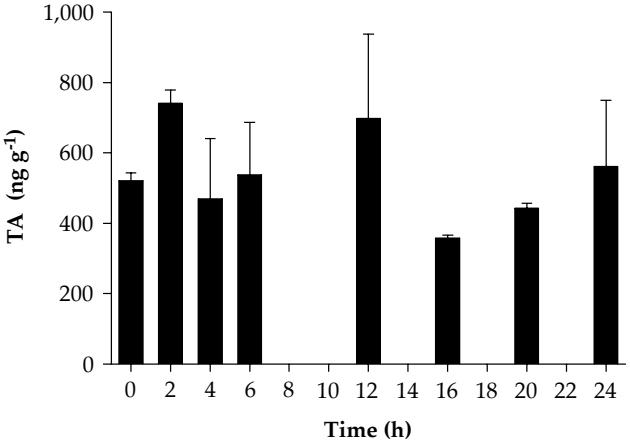

Figure 2. Mean concentration (± SE) of trenbolone acetate in *Artemia* nauplii immersed in steroid using diets enriched with HUFA. Each bar represents three replicates.

1) Fry fed bioencapsulated TA for 5 d
2) Fry fed bioencapsulated TA for 10 d
3) Fry fed bioencapsulated TA for 20 d
4) Fry fed bioencapsulated TA for 28 d
5) Fry fed bioencapsulated TA for 45 d
6) Fry fed bioencapsulated TA for 60 d
7) Fry fed control food (*Artemia* immersed in a solution containing ethanol only)

Feeding, sampling, and sex identification of castarricas were conducted as described for Experiment B.

RESULTS

Study A: Enrichment and Detection of E2 and TA in *Artemia* Nauplii:

Procedure 1

Immediately after addition of steroids, nauplii contained > 1,500 ng g^{-1} of E2 and > 2,000 ng g^{-1} of TA (Figures 1a and 1b, respectively). The nauplii treated with E2 contained 5,633 ng g^{-1} at 2 h and remained at that level until 4 h (Figure 1a). After 6 h, nauplii contained 6,176 ng g^{-1} and then reached a concentration of 7,497 ng g^{-1} at 12 h; this concentration remained relatively stable until 24 h. A similar pattern was observed when TA was used; however, the concentrations of TA reached were not as high as those of E2. Concentrations of TA in nau-plii reached 6,259 ng g^{-1} after 2 h of treatment and remained relatively stable between 5,561 ng g^{-1} (16 h) and 7,304 ng g^{-1} (6 h) (Figure 1b). At 24 h, concentrations declined to 4,650 ng g^{-1} (Figure 1b). Control *Artemia* showed slight background levels of E2 (Figure 1c), but no TA was detected by HPLC. These results indicate that bioaccumulation of steroids in nauplii occurs in less than two hours and remains stable for 24 h.

Procedure 2

The use of *Artemia* diets with HUFA sharply decreased the

amount of TA incorporated into the nauplii to approximately 10% of the amounts detected in Procedure 1 with no HUFA (Figure 2). Immediately after addition of the steroid, nauplii contained 520 ng g^{-1} of TA; this concentration remained relatively stable up to 24 h. At 4 h, levels of TA reached the highest concentration observed (741 ng g^{-1}).

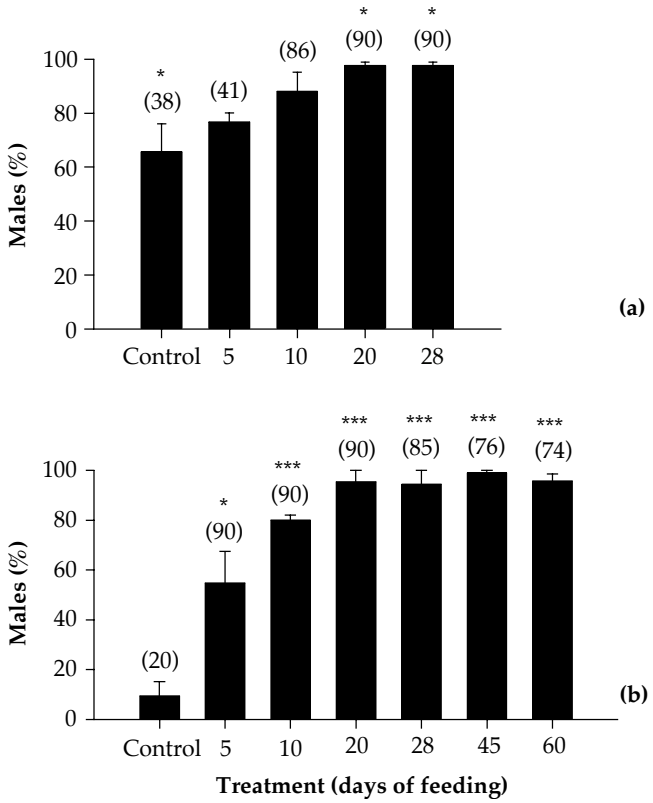

Figure 3. Masculinizing effects of feeding TA-enriched *Artemia* to Nile tilapia fry. Each treatment was triplicated. The first trial was run for 28 d (a) and the second trial for 60 d (b). The control group was fed *Artemia* nauplii with no steroid. The numbers of fish sampled are provided in parentheses. Asterisks indicate a significant difference from the control group.

Experiment B: Validation of Bioencapsulation by Masculinization of Nile Tilapia Fry

Nile tilapia fry were efficiently masculinized using TA-enriched *Artemia* nauplii despite the low levels of TA detected when HUFA was used in the enrichment solution. Highly significant masculinization (> 95% males) was obtained when fry were fed TA-enriched nauplii for 20 d or more. In the first trial (Figure 3a), controls had 66.0% males; feeding bioencapsulated TA for 5 d did not differ significantly from the control (76.7% males, $P > 0.05$), while feeding for 10 d significantly increased the percentage of males (88%, $P < 0.05$). The highest levels of masculinization were obtained with the treatments using TA-enriched nauplii for 20 and 28 d. These treatments provided similar levels of masculinization (97.7% males), which were significantly higher than those the controls ($P < 0.001$) (Figure 3a).

The second trial provided similar results although masculinization was more pronounced since the control group had only 9.3% males (Figure 3b). All treatments resulted in significant masculinization ($P < 0.01$). Fish fed bioencapsulated TA for 5 and 10 d had 54.7 and 80.0% males, respectively. All other treatments resulted in more than 94% males (95.3, 94.3, 99.0, and 95.7% males for 20, 28, 45, and 60 d of enriched feeding, respectively) (Figure 3b).

Survival in both treatments varied extensively, ranging from 13.3% in the worst case to 98% (Figures 4a and 4b, respectively). Occasional power failures during the night caused low oxygen values, resulting in high mortalities in some treatments. Dissolved oxygen, pH, and temperature averaged (± SD) 7.1 ± 2.8 mg l^{-1}, 7.4 ± 0.1, and 27.2 ± 0.3°C, respectively.

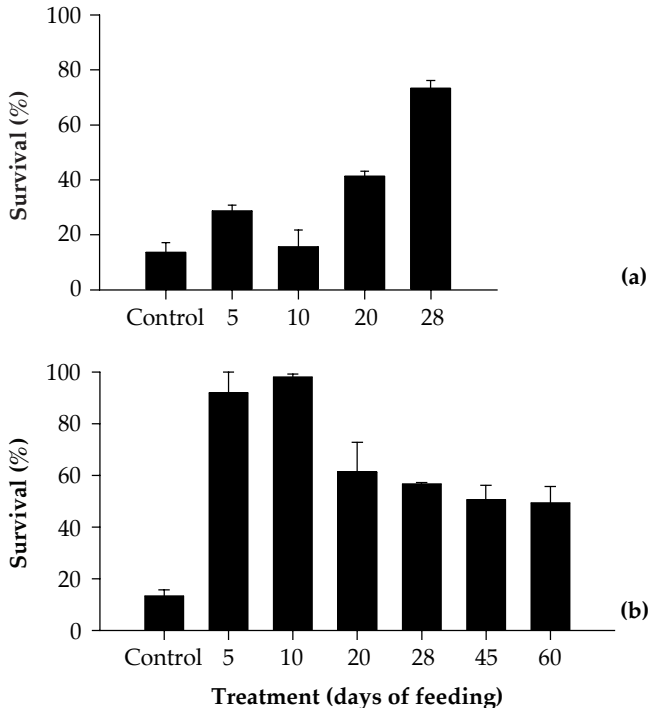

Figure 4. Mean percent survival (± SE) of Nile tilapia fed with TA-enriched *Artemia* nauplii for different lengths of time. (4a) first trial and (4b) second trial. Control groups (C) were fed *Artemia* nauplii with no steroid. Each treatment was triplicated.

Experiment C: Feminization of Tropical Gar Fry

Significant feminization of tropical gar was obtained when fish were fed for 20 and 28 d with E2-enriched *Artemia* nauplii ($P < 0.001$; Figure 5a). Control groups had 21.3% females (a condition that is similar to sex ratios we have observed in the field). Fish treated with E2-enriched *Artemia* nauplii for 5 and 10 d did not differ from the control groups. The group fed for 5 d had 8.7% females, while the group fed for 10 d had 15% females. When gar were fed for 20 d, the percentage of females reached 51.7%, while the group fed for 28 d reached 58.0%. Feminization did not occur when fry were immersed in E2 solutions for either 3 or 6 h (6.12 and 8.10% females, respectively).

Survival in all treatments was low and did not differ statistically ($P > 0.05$), ranging from 9.1% (group fed E2 for 10 d) to 28.3% (group fed E2 for 28 d) (Figure 5b). Some problems with water quality due to power failures may have been responsible for this high mortality. Dissolved oxygen, pH, and temperature

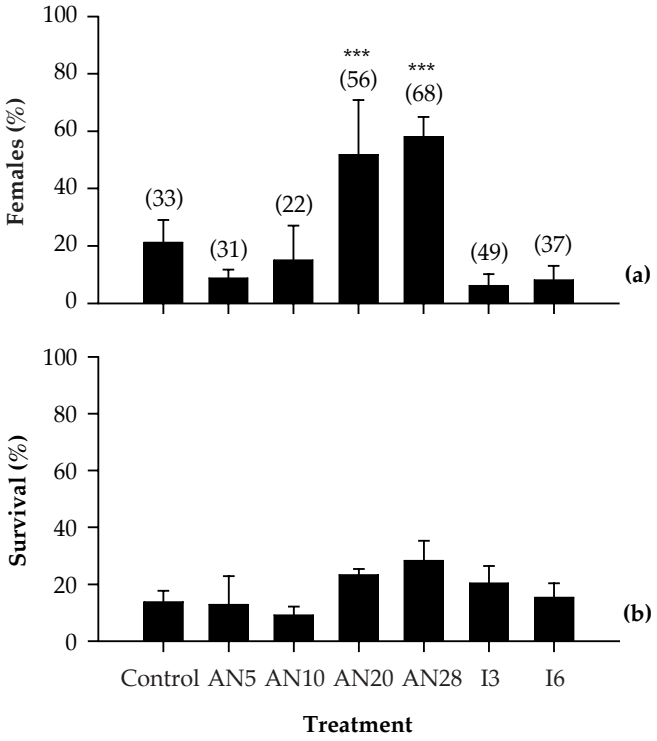

Figure 5. Feminization (a) and survival (b) of tropical gar fry fed with E2-enriched *Artemia* nauplii for 5, 10, 20, and 28 d (AN5, AN10, AN20, and AN28) and fry immersed in E2 solutions for 3 and 6 h during 3 consecutive days (I3 and I6). All treatments were triplicated. The control group was fed *Artemia* nauplii with no steroid. The numbers of fish sampled are provided in parentheses.

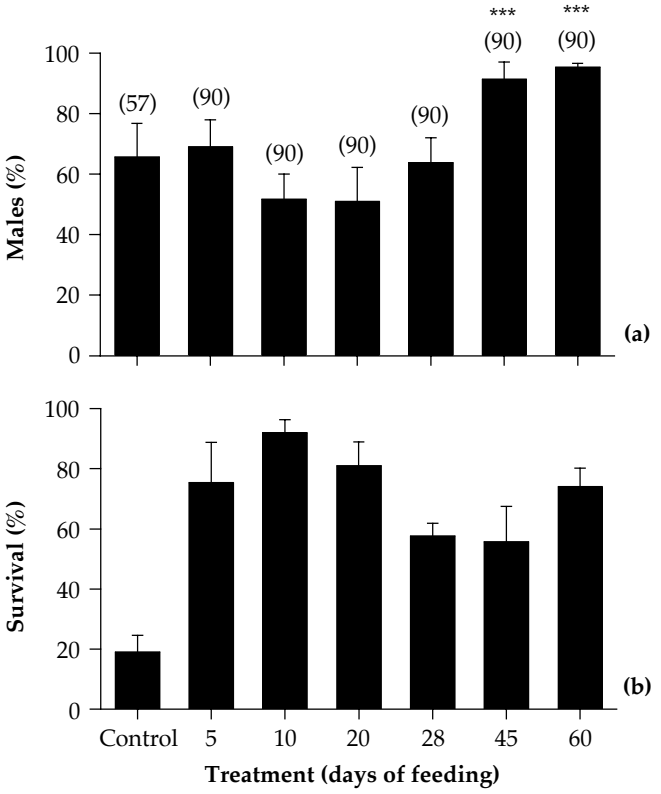

Figure 6. Masculinizing effects of feeding TA-enriched *Artemia* (a) and survival (b) on castarrica fry. Each treatment was triplicated. The control group was fed *Artemia* nauplii with no steroid. The numbers of fish sampled are provided in parentheses. Asterisks indicate a significant difference from the control group.

averaged (± SD) 5.3 ± 3.8 mg l^{-1}, 7.2 ± 0. 3, and 26.8 ± 1.1ºC, respectively.

Experiment D: Masculinization of Castarrica Fry

Castarrica fry were effectively masculinized with TA-enriched *Artemia* nauplii when fed for 45 d or more (Figure 6a). In this experiment controls had 65.7% males. Feeding bioencapsulated TA for 5, 10, and 28 d did not differ statistically from the control (70.0, 51.7, and 62.3% males, respectively). Feeding TA-enriched *Artemia* for 45 and 60 d significantly increased the percentage of males (91.3 and 95.3%, $P < 0.001$). Survival varied among treatments, being the lowest in the control group (19.2%). All other treatments ranged between 58 and 92% (Figure 6b). Dissolved oxygen, pH, and temperature averaged (± SD) 6.8 ± 0.5 mg l^{-1}, 7.2 ± 0.1, and 27.9 ± 0.5ºC, respectively.

DISCUSSION

High levels of E2 and TA (above 7,000 ng g^{-1}) were successfully incorporated into *Artemia* nauplii when HUFA was not used in the enrichment solution. To our surprise, much lower levels of TA were detected in *Artemia* when HUFA was added to the immersion solution. Despite these low levels of TA (approximately 700 ng g^{-1}), feeding of nauplii to both Nile tilapia and the native cichlid castarrica resulted in significant masculinization. Stewart et al. (2001) reported that high levels of both E2 and 17α-methyldihydrotestosterone were incorporated into *Artemia* when HUFA was used in the enrichment solution.

There are two explanations for this discrepancy. First, addition of HUFA could have reduced our ability to detect authentic TA in *Artemia*. This could have been due to decreased extraction efficiencies or inhibiting detection of TA by HPLC. Secondly, the low levels of TA that we found are accurate (binding of the steroid to the HUFA may have prevented its bioaccumulation in the nauplii), but the steroid is present in sufficient quantities to masculinize fish. Further experiments will need to be conducted to determine which scenario is occurring.

Our results indicate that administration of steroids via bioencapsulation is an efficient method for sex inversion of fish species. This technique offers an advantage for sex reversing carnivorous species because larvae strongly prefer live food compared to artificial diets. We were able to validate that steroid-enriched *Artemia* nauplii is a reliable method for sex inversion of fish by using Nile tilapia as a model species. High levels of masculinization (higher than 90% males) were achieved when tilapia were fed TA-enriched nauplii for 20 d. These results are encouraging since tilapia are usually fed MT-impregnated food for 28 d (Phelps and Popma, 2000). Our results indicate that masculinization is feasible using steroid-enriched *Artemia* nauplii.

Interestingly, masculinization of castarrica fry required more days of feeding with TA-enriched nauplii than for tilapia (45 versus 20 d of feeding, respectively). Similar results were found at the Laboratory of Aquaculture at UJAT when castarrica were fed MT-treated food (Real-Ehuan, pers. Comm., 2003).

Because growth rates of tilapia are much higher than those of castaricca, it is possible that the period of gonadal differentiation in castarrica occurs at a later period in development or takes longer to proceed than in tilapia. Despite the conclusiveness of our results, more research is needed to determine if this technique can be optimized using smaller amounts of steroid in the enrichment protocol.

We were able to feminize tropical gar fry using E2-enriched *Artemia* nauplii for 20 and 28 d. We are currently studying the gonadal development of this species to determine the specific time at which gonadal differentiation occurs. This information will be very valuable since higher feminization rates may be accomplished by feeding steroid-treated diets during this time. Our preliminary results suggest that gar undergo gonadal differentiation during the first month of life. This is similar to the results reported by Piferrer and Donaldson (1993) for coho salmon (Oncorhynchus kisutch), where the developmental period when fish are susceptible to sex inversion is between 21 to 28 d after hatching. The very low incidence of females in the control groups is apparently a normal condition for tropical gar. Márquez-Couturier and Contreras-Sánchez (2003) reported similar sex ratios for wild populations of this species. We are planning to determine if feminized populations of gar have higher growth rates than do normal populations with large proportions of males.

Feminization of gars via immersion in steroid solutions was not successful. This may reflect that the window of opportunity for feminization of gar differs from other species. We have demonstrated in previous studies (Contreras-Sánchez, 2001) that immersions of Nile tilapia fry may result in high masculinization rates; however, this method showed a large variability in its success, which is probably related to the rate at which each batch of fish developed.

CONCLUSIONS

The validation of sex inversion of fishes using steroid-enriched *Artemia* nauplii represents a keystone for the production of monosex populations of carnivorous species of fish. The successful masculinization of tilapia and castarrica and the feminization of tropical gar in this study open a window of opportunity for the culture of monosex populations of carnivorous species that have different growth rates between sexes. If either sex has a higher growth rate than the other, the use of steroid-enriched *Artemia* nauplii could provide a reliable method for obtaining monosex populations.

Local farmers in Mexico have consistently requested that native species be incorporated into aquacultural practices. Therefore, the use of alternative methods for promoting successful culture provides a step ahead in the development of sustainable aquaculture. More research is needed to determine if this technique can be optimized and used to produce monosex populations of other important species.

ANTICIPATED BENEFITS

The incorporation of the anabolic steroids trenbolone acetate and estradiol in *Artemia* nauplii provides an alternative method for sex inversion of carnivorous species of fish. The successful sex inversion of tilapia, castarrica and gar offers new opportunities for the aquaculture industry in southeastern Mexico. The workshops conducted at Villahermosa, Teapa, and Rio Playa in Tabasco were well received by farmers, students, and technicians that work in aquaculture in the region. We are currently assessing the use of this new technique for other native species such as the cichlids tenhuayaca (*Petenia splendida*) and paleta (*C. synspillum*).

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