



# AQUACULTURE CRSP 22<sup>ND</sup> ANNUAL TECHNICAL REPORT

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## TRAINING LOCAL FARMERS ON SAFE HANDLING OF STEROIDS AND MASCULINIZATION TECHNIQUES IN CENTRAL AMERICA

*Eleventh Work Plan, Applied Technology and Extension Methodologies Research 1 (11ATER1)  
Final Report*

Carl B. Schreck  
Oregon Cooperative Fishery Research Unit  
Department of Fisheries and Wildlife  
Oregon State University  
Corvallis, Oregon

Guillermo R. Giannico and Grant W. Feist  
Department of Fisheries and Wildlife  
Oregon State University  
Corvallis, Oregon

Wilfrido M. Contreras-Sánchez, Bernardita Campos-Campos, Ulises Hernández-Vidal, and Eunice Perez-Sánchez  
Laboratorio de Acuicultura  
División Académica de Ciencias Biológicas  
Universidad Juárez Autónoma de Tabasco  
Villahermosa, Tabasco, México

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### ABSTRACT

The need to deliver recently generated information and technological packages to the immediate users (students, extension agents, and farmers) is fundamental for aquaculture development. Training workshops are one way to achieve these goals. Through workshops, researchers can obtain feedback information from farmers and identify problems that may compromise advances in the field of interest. Developing new techniques, such as methods for sex reversal or production of clean effluents, would be futile unless the information that is generated is transferred to people conducting aquacultural activities. This is especially difficult in Mexico and Central America because information is not readily accessible. Workshops conducted in Mexico under CRSP support have already impacted tilapia culture in Tabasco and Chiapas and most farmers are growing sex-reversed tilapias—this activity was not conducted until only a few years ago. To complement research for the production of clean sex-inversion techniques, we implemented three regional workshops and one local workshop on safe handling of steroids and masculinization techniques in Mexico. We have also generated printed and electronic materials for safe handling of steroids and masculinization techniques.

### INTRODUCTION

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. Among the techniques developed, oral administration of steroids via feeding has become the most commonly used. In tilapia culture, the production of all-male populations through treatment of fry with 17 $\alpha$ -methyltestosterone (MT) impregnated

food has become the most widely used procedure. Other readily available anabolic steroids (such as fluoxymesterone) are also used by farmers who exercise little or no precaution concerning exposure to the compounds. Despite the success of this masculinizing technique, significant “leakage” of MT into the pond environment may occur from uneaten or unmetabolized food. This leakage poses a risk of unintended exposure to anabolic steroids by hatchery workers as well as fish or other non-target aquatic organisms. Furthermore, in some countries, pond sediments are dredged and sometimes used to prepare soil for crop production, thereby spreading the risk of exposure to

MT to terrestrial systems and to other aquatic systems (Contreras-Sánchez, 2001).

Despite the wide use of MT for masculinizing tilapia in aquacultural facilities, few efforts have been devoted to eliminate this steroid from farm effluents. Recently, several institutions in the US have combined efforts to provide information needed by the FDA to gain MT use approval for aquaculture (Green and Teichert-Coddington, 2000). These efforts are focusing on maintaining low levels of MT in the water, instead of eliminating it completely. The problems associated with contamination of water and sediments are further compounded by the many effects related to bioaccumulation and the transfer of the contaminants and their metabolites through the food web (Kime, 1998). Therefore, it is important to promote the safe use of MT and other steroids in aquacultural facilities by incorporating preventive measures such as filtration, biodegradation, or photodegradation of the steroid and its metabolites. Aquaculture systems worldwide have been responsible for severe environmental degradation. Producing clean farm effluents through environmentally sound technology (such as charcoal filtration or photodegradation) may be a means of reducing negative impacts on the environment.

Developing new techniques for production of clean effluents would be futile unless the information that is generated is transferred to people conducting aquacultural activities. This is especially difficult in Mexico and Central America because information is not readily accessible. Workshops conducted in Mexico under CRSP support have already impacted tilapia culture in Tabasco and Chiapas and most farmers are growing sex-reversed tilapias—this activity was not conducted until only a few years ago. To complement research for the production of clean sex-inversion techniques, we developed three regional workshops and one local workshop on safe handling of steroids and masculinization techniques in Mexico. We have also generated printed and electronic materials for safe handling of steroids and masculinization techniques.

## METHODS AND MATERIALS

Training activities have been undertaken at different levels. Graduate and undergraduate students received hands-on training at the aquaculture laboratory at UJAT. Extension agents, researchers and students from the southeastern, Central and Central-Pacific regions attended regional workshops on safe handling of steroids and masculinization techniques in Villahermosa Tabasco, Mexico City, and Mazatlán Sinaloa, respectively. Recently, UJAT offered an extensive workshop to the State Center for Training, where a section on fish sex inversion and safe handling of steroids was included. Farmers, researchers, extension

agents, and students attended this workshop. Printed and electronic materials for safe handling of steroids and masculinization techniques were generated and distributed during training activities. Carl Schreck, Grant Feist, Guillermo Giannico, and Wilfrido Contreras constructed a PowerPoint® presentation to be used in workshops. This presentation included information on safe handling of steroids, diagrams of filtration systems and videos.

In December 2003, Oregon State University Principal Investigators Guillermo Giannico and Grant Feist visited UJAT to organize and participate in the first regional workshop. During this visit materials were revised and workshop sessions were planned. Workshops were implemented as follows:

First regional workshop: Safe Handling of Steroids and Clean Technologies in Aquaculture. 8–10 December 2003. División Académica de Ciencias Biológicas, (DACBiol-UJAT), Villahermosa, Tabasco, Mexico.

Second regional workshop: Safe Handling of Steroids and Clean Technologies in Aquaculture. 19–20 February 2004. Universidad Autónoma Metropolitana campus Xochimilco (UAM-X), Mexico City, Mexico.

Third regional workshop: Safe Handling of Steroids and Clean Technologies in Aquaculture. 23–24 February 2004. Centro de Investigaciones en Alimentación y Desarrollo (CIAD), Mazatlán, Sinaloa, Mexico.

Local workshop for Tabasco Government: Sex Reversal and Safe Handling of Steroids. 28 April–1 May 2004. División Académica de Ciencias Biológicas, (DACBiol-UJAT), Villahermosa, Tabasco, Mexico.

## RESULTS

### Training of Graduate and Undergraduate Students

In the aquaculture laboratory, 22 students have received hands-on training on safe handling of steroids and use of clean technologies to eliminate steroids from sex-inversion systems. Training consists of reviewing the theory behind sex inversion and safe handling of steroids. All students that work at the laboratory have to go through this training in order to perform activities related to sex inversion. To reinforce this training, students attend workshops implemented at UJAT regarding this topic.

In February 2004, Bernardita Campos-Campos defended her masters degree thesis entitled “Evaluation of a continuous filtration system with activated charcoal for the elimination of the synthetic steroid 17 $\alpha$ -Methyltestosterone from an intensive Nile tilapia masculinization tank.” Bernardita has become a

strong advocate for the use of clean technologies in masculinization systems and a main speaker at our workshops.

### Regional Workshops

First regional workshop: Safe Handling of Steroids and Clean Technologies in Aquaculture. 8–10 December 2003. División Académica de Ciencias Biológicas, (DACBiol-UJAT), Villahermosa, Tabasco, Mexico.

There were 33 participants at the workshop, fifteen of which were students from UJAT. Six of the participants were indigenous farmers of the “Chontal” ethnic group and three represented the rural farm “Rio Playa.” Five of the attendees were either owners or workers of private farms. Two participants were municipal extension agents, two worked at the state tilapia farm and one was a professor from UJAT.

Mario Fernández-Perez gave the morning lecture on the first day. It was concerned with producing suitable tilapia broodstock for fry production and followed the general outline from a recent pamphlet that he has produced titled “Formacion del banco de reproductores de tilapia.” The lecture covered basic principles of reproduction in tilapia, how to transport fish, identify sex, spawn and rear fry. He explained how to select broodstock (based on length and condition factor) to obtain a line of fish that would generate high quality fry. The practicum in the afternoon was dedicated to examining stages of the reproductive cycle in tilapia, identifying the sex of fish, and determining fecundity, gonadosomatic index and condition factor. This included both external observation and dissection. From this, participants were shown how to calculate fry production based on broodstock characteristics and how to select for the most robust fish.

During the second day of the workshop, two presentations were given in the morning, one by Wilfrido Contreras-Sánchez and the other by Ulises Hernandez-Vidal. Wilfrido discussed sex differentiation in fish including both hormonal and genetic components. He also described how other species of fish besides tilapia (including species native to Mexico) could be sex reversed by hormones. Both feminizing and masculinizing techniques were covered. Dose and timing of hormone administration was shown for several species. The use of steroids incorporated into live food was presented as a way to sex reverse carnivorous fishes. Ulises gave a detailed presentation on the methodology for sex reversing tilapia using steroid treated food. He included temperature and duration of treatment as important components in the process. Sorting and grading of fry to select fish at the appropriate stage for sex reversal was documented. The methodology to incorporate steroids into food was presented, as well

as safety precautions when using hormones. In the afternoon practicum, participants were shown how to grade, sort, and count fry to be masculinized. They were also shown how to make hormone laced food and informed of safe handling practices when working with steroids. Finally the filtration systems used to treat water for nitrogenous wastes and for removing steroids was introduced.

During day three, two presentations were given in the morning, one by Wilfrido Contreras-Sánchez and the other by Guillermo Giannico. Wilfrido gave an overview of steroid hormones and discussed how steroids can accumulate in water and sediments following sex reversal of fry. He also discussed the effects of residual steroids on other fish and why it is important to eliminate them. Guillermo gave a presentation on the safe handling of steroids and how to eliminate them from aquaculture systems. He described the dangers of steroid exposure for both humans and the environment. He then gave a detailed account of the components for a filtration system to remove steroids and nitrogenous waste including how to construct them. Use of solar light or UV sterilizers was discussed as another method to remove steroids from effluent. Finally, safe handling of steroids and methods to clean work areas after their use was presented. In the afternoon practicum, participants were shown the components of the filtration system. They were also taught how to separate masculinized fry into hapas. The remainder of the afternoon was spent outlining the different systems for grow-out and how to feed the fish. Extensive systems, intensive systems in ponds, and super intensive systems for masculinization were all discussed. Methods for feeding fish included, fertilizing the pond, the use of natural foods, adjusting the amount and size of food with growth of fry, and effects of environmental parameters.

Second regional workshop: Safe Handling of Steroids and Clean Technologies in Aquaculture. 19–20 February 2004. Universidad Autónoma Metropolitana campus Xochimilco (UAM-X). Mexico City, Mexico.

All materials used at the first regional workshop were used to elaborate PowerPoint® presentations. Bernardita Campos-Campos and Eunice Perez-Sánchez organized presentations and were the main speakers of the 2nd workshop. Four more speakers were invited: Héctor Salgado-Zamora (Laboratorio de Química Orgánica del Instituto Politécnico Nacional). Ing. David Martínez-Espinosa. Biól. Eduardo Maya-Peña and Biól. Samuel Marañón-Herrera (Laboratorio de Sistemas Acuícolas, UAM-X.). Salgado gave a lecture on chemical structure of steroids. David talked about aquaculture extension experiences in the central State of Morelos, and Samuel and Eduardo gave two presentations on masculinization of ornamental fishes using steroids. A total of eleven participants attended the workshop. Ten were students

from five different Universities: Instituto Politecnico Nacional, Universidad Nacional Autónoma de México, and Universidad Autónoma Metropolitana campus Xochimilco from México City; Universidad del Mar, from the State of Oaxaca (Pacific coast); and Universidad Autónoma de Nayarit, from the State of Nayarit (Pacific coast). One attendee was a researcher from the Ecological Park Xochimilco, located in Mexico City. Researchers and students from UAM-X requested UJAT to repeat this workshop during the second semester of 2004.

Third regional workshop: Safe Handling of Steroids and Clean Technologies in Aquaculture. 23-24 February 2004. Centro de Investigaciones en Alimentación y Desarrollo (CIAD). Mazatlán, Sinaloa, Mexico.

For this workshop Bernardita Campos-Campos and Eunice Perez-Sánchez organized presentations and were the main speakers. They used all the materials presented at the first regional workshop using PowerPoint® presentations. A total of ten participants attended the workshop. Six attendees were from the Centro de Investigación en Alimentación y Desarrollo, Mazatlán Sinaloa (CIAD), one student from UJAT, one extension agent from the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Mazatlán Sinaloa, one municipal extensión agent from Mazatlán, and one researcher from the Programa de Desarrollo Comunitario.

Local workshop for Tabasco Government: Sex Reversal and Safe Handling of Steroids. 28 April–1 May 2004. División Académica de Ciencias Biológicas, (DACBiol-UJAT), Villahermosa, Tabasco, Mexico.

This training activity was not proposed for the Eleventh Work Plan; however, UJAT offered State officers a training program with an option to obtain a diploma for an update on aquaculture practices. The diploma can be obtained after participants attend 11 workshops, one of which is our sex reversal and safe handling of steroids presentation. This workshop was conducted by Wilfrido M. Contreras Sánchez, Bernardita Campos-Campos, Arlette Hernández-Franyutti, and Ulises Hernández-Vidal. All contents were similar to those used at the first regional workshop. Thirty-one people participated in the workshop, twelve were students from UJAT, Universidad del Mar (Oaxaca) and Universidad del Carmen (Campeche), eight were extension agents from the State Secretariat for the Development of Agriculture Cattle Raising and Fisheries, five were aquaculture consultants, two were fish farmers, two were researchers, and two were aquaculture technicians from the State tilapia farm.

Printed and Electronic Materials

A manual on sex inversion techniques, safe handling of steroids and treatment methods for effluents was produced. This manual is currently in press and will be available in both printed and electronic versions.

The following is the outline for the manual:

- I. Biology of fishes used in aquaculture in Central America
  - Growth and feeding
  - Reproduction
  - Diseases
- II. Culture Systems
  - General aspects
  - Fry production
  - Onset of feeding
  - Grow-out
  - Pond culture / cage culture
- III. Control of sex
  - Hand sorting
  - Sex inversion with steroids
  - Oral administration
  - Immersion
  - Hybridization
  - Production of YY males
- IV. Safe handling of steroids
- IV. Incorporation of steroids into food
- VI. Working with water containing steroids
- VII. Treatment of effluent water
  - Charcoal filtration
  - Photodegradation

The website is currently being built and will soon be open to the public. It will be maintained at the UJAT server at no cost. Posters regarding safe handling of steroids will be printed during the second year of the proposal and distributed to farms that use steroids for sex inversion.

## DISCUSSION

UJAT has become a major training institution in southeastern Mexico. The laboratory of aquaculture has taken the lead on clean technologies for aquaculture and safe handling of steroids. Students, researchers, extension agents, private farmers, and rural farmers have been trained on these important topics for the development of clean technologies. The interest from farmers and extension agents is increasing and there is a growing sense of awareness about the potential risks that mishandling steroids pose and the precautions that farmers must take to prevent accidents. All workshops have provided feedback from the attendees that improved the quality of our training material which will be on line by the end of 2004.

**ANTICIPATED BENEFITS**

We have educated students, researchers, extension agents, technicians, and farmers on safe and effective sex inversion techniques in three regions of Mexico (Southeast, Central, and Pacific). The commitment of these personnel is to share the information obtained from us and to train additional producers. We have a request for providing a new workshop on sex inversion and safe handling of steroids in Mexico City. This workshop will be conducted by November–December 2004. Our manual will be disseminated shortly to high school and university students as well as the personnel that work at farms that use steroids in their facilities.

### REGIONAL INTEGRATION

We exchanged information with Daniel Meyer, at the Pan-American Agricultural School and we plan for more collaboration. Technician Franklin S. Martinez Toscano a member of the aquaculture program from the Department of Biology, Escuela Agrícola Panamericana Zamorano, Tegucigalpa, Honduras visited UJAT for a hands-on training on MT elimination from masculinization systems using charcoal filtration and sex identification techniques. Franklin also attended the snook workshop conducted at UJAT during 27–30 April 2004.

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