EVALUATION OF THE LIVE ATTENUATED VACCINE AQUAVAC ESC® AND

THE EFFECTS OF A PRIMARY NURSERY PHASE ON THE PRODUCTION

OF CHANNEL CATFISH FINGERLING IN EARTHEN PONDS

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

_____

Abel Carrias

Certificate of Approval:

_____	_____
Covadonga R. Arias Assistant Professor Fisheries and Allied Aquacultures	Jeffery S. Terhune, Chair Assistant Professor Fisheries and Allied Aquacultures
_____	_____
William H. Daniels Associate Professor Fisheries and Allied Aquacultures	Jesse A. Chappell Assistant Professor Fisheries and Allied Aquacultures

_____

Stephen L. McFarland
Dean
Graduate School

EVALUATION OF THE LIVE ATTENUATED VACCINE AQUAVAC ESC® AND THE EFFECTS OF A PRIMARY NURSERY PHASE ON THE PRODUCTION OF CHANNEL CATFISH FINGERLING IN EARTHEN PONDS

Abel Carrias

A Thesis

Submitted to

the Graduate School of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
December 16, 2005

EVALUATION OF THE LIVE ATTENUATED VACCINE AQUAVAC ESC® AND THE EFFECTS OF A PRIMARY NURSERY PHASE ON THE PRODUCTION OF CHANNEL CATFISH FINGERLING IN EARTHEN PONDS

Abel Carrias

Permission is herewith granted to Auburn University to make copies of this thesis at its discretion, upon the request of individuals or institutions and at their expense. The author reserves all publication rights.

_____
Signature of Author

_____
Date

VITA

Abel Antonio Carrias, son of Abel Carrias and Marina Carrias, was born in Cayo, Belize, on May 16, 1975. He attended high school at Sacred Heart in San Ignacio, Belize, and graduated in 1993. In 1996, he graduated with an associate degree in general agriculture from the College of Agriculture in Central Farm, Belize C.A. He entered college at Auburn University in January, 2000, and earned a Bachelor of Science degree in Fisheries Science in December 2003. In January 2004, he entered graduate school at Auburn University to pursue a Master of Science degree. He married Lily Y. Melendez, daughter of Elias Melendez and Maria-Elsie Melendez, on June 17, 2000, and had a son Melvin Ottoniel on November 28, 2000.

THESIS ABSTRACT

EVALUATION OF THE LIVE ATTENUATED VACCINE AQUAVAC ESC® AND THE EFFECTS OF A PRIMARY NURSERY PHASE ON THE PRODUCTION OF CHANNEL CATFISH FINGERLING IN EARTHEN PONDS

Abel Carrias

Master of Science, December 16, 2005
(B.S., Auburn University, 2003)

62 Typed Pages

Directed by Dr. Jeffery S. Terhune

The evaluation of the live attenuated vaccine AQUAVAC ESC® in field conditions as well as the use of a primary nursery phase were studied to determine if better production and survival of fingerlings were obtained. This was followed by a laboratory study to evaluate the vaccine under better controlled environment.

Twenty-five 0.04-ha earthen ponds were stocked with channel catfish fry at a rate of 247,000 fry/ha and grown for five months in one of five treatments: (1) 10-d post-hatch (PH) fry were sham vaccinated and stocked into ponds; (2) 10-d PH fry were vaccinated and stocked into ponds; (3) 10-d PH fry were sham vaccinated, kept in a primary nursery phase for 22 d and then stocked into earthen ponds; (4) 10-d PH fry were vaccinated, kept in a primary nursery phase for 22 d and then stocked into ponds; (5) 10-d PH fry were sham vaccinated, kept in a primary nursery phase for 22 d, vaccinated at that time and then stocked into ponds.

Fingerling mean standing crop ranged from 4,716 kg/ha in treatment 2 to 8,112 kg/ha in treatment 5. A significant difference by treatment occurred only between treatment 2 (4,716 Kg/ha) and treatments 3, 4, and 5 (6,653, 6,910, 8,112 kg/ha, respectively). Individual fish mean weight ranged from 38.8 g in treatment 2 to 40.9 g in treatment 5, and feed conversion ratios (FCR) ranged from 1.15 in treatment 5 to 1.51 in treatment 2. No significant differences, in average weight and FCR were observed between any of the treatments. Mean survival ranged from 47.5 % in treatment 2 to 73.4 % in treatment 5. Significant differences were observed between treatment 2 (47.5 %) and the other three treatments as well as between treatment 3 (60.7 %) and treatment 5 (73.4 %). Mean observed mortality ranged from 1.5 % in treatment 5 to 6.8 % in treatment 2, no significant differences were observed between any of the treatments.

In the laboratory study, mortality ranged from 8 % in treatment 4 to 19.9 % in treatment 2. No statistical differences were observed between any of the treatments.

The proper evaluation of the vaccine was difficult because the presence of mixed infections of ESC and Columnaris disease made it impossible to determine which of the two diseases was responsible for the mortality of fish collected during a documented disease outbreak. A primary nursery phase to hold catfish fry for 32 days before stocking into ponds can be very useful in increasing fingerling yield and survival. Higher survival and final standing crop obtained from the nursery system can reduce space and other resources needed to produce a target quantity of fingerlings hence enabling producers to utilize the space and resources in other more profitable ventures.

ACKNOWLEDGMENT

The author would like to thank Dr. Jeff Terhune for making possible the opportunity to conduct research at Auburn University and for his valuable lessons and guidance in the development and implementation of this project. He would like to thank Mr. Christopher Sayles for his friendship, advice and help throughout the study and to Dr. Edzar VanZanten for his help in analyzing the data collected. Special thanks are also given to the staff at the North Auburn University Fisheries Center, especially to Mr. Esau Arana and Ms. Karen Veverica for their help to conduct research at the Fisheries Station. Love and gratitude are expressed to his wife Lily and son Melvin for their understanding and care in the years of study. The author would like to dedicate this work to his grandfather who did not live to see the completion of this project but was the source of his strength in times of difficulties.

Style manual or journal used: Journal of the World Aquaculture Society

Computer software used: Microsoft Word 2003, Microsoft Excel 2003, SAS 9.1.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

The commercial catfish industry is the largest aquaculture industry in the United States of America with sales of 480 million dollars in 2004 and production acreage of 58,681 hectares (USDA 2005). The primary producing states are Mississippi, Alabama, Arkansas and Louisiana, which account for 95 % of the sales. Although raising food-fish accounts for most of the sales and production area, rearing of fry and fingerlings occupied 8,701 hectares and totaled 22.2 million dollars in sales (USDA 2005).

The production of catfish fingerlings has traditionally been carried out in combination with egg production, fry production, and food-fish production in many of the catfish farms in the southeast. Recently, a survey showed that in Mississippi, Alabama, Louisiana and Arkansas 95 % of the producers raised food-size fish, 14.2 % bred catfish, 12.8 % operated hatcheries and 29.9 % raised fry to fingerlings (USDA 2005). Fingerling producers typically raise fingerlings for stocking into their own food-fish production ponds, to sell to other food-fish producers, or to a lesser extend, to be used in the recreational fishery industry. In 1983, most producers devoted part or all of their farm resources to producing fingerlings. In Alabama alone, this included 59 producers **(**Hebicha 1984**)**. Presently, the majority of farms in Alabama depend on an outside source for fingerlings to use in their food-fish production phase. The relative scarcity of ground water supply is the major factor that prevents catfish farmers in

Alabama from producing fingerlings on a large-scale level like those fingerling producers in Arkansas and Mississippi **(**Bailey et al. 1996).

Although most fingerlings that supply food-fish farms in Alabama come from other areas, vertical integration of farms in Alabama that produce fry, fingerlings and food-fish is not uncommon. Hebicha (1984) observed, however, that inefficient production methods and high fingerling production costs can lead to decreased returns to resources used in production in a vertically integrated farm. This can result in either a shift in these resources to other uses that are more profitable or to an emphasis in increasing production efficiency of fingerlings. Better production efficiency of fingerlings increases the profits of a farm not only by increasing revenue through sales but by allowing for better allocation of resources in production activities that maximize the net return of the farm (Hebicha 1984). For example, increasing survival of fingerlings would mean that less fry have to be initially stocked and less pond area and water have to be used to meet the required number of fingerlings needed for a production cycle of the farm. This means that the brood fish maintenance, pond area, water, labor, time and other efforts that would be needed to produce the required number of fingerlings in systems with low survival can otherwise be used for other farm diversification activities to maximize net returns of the farm.

Primary Nursery phase

From the early days of intensive catfish fingerling production, researchers and farmers have experimented with different ways to raise catfish fingerlings. People have used cages, earthen ponds, metal or concrete raceways and various tank systems (Meyer et al. 1973). The method that was eventually widely accepted was to stock 7-14 d old fry

directly into earthen ponds at densities ranging from 250,000 to 375,000/ha and growing them typically for 120-150 d (Brown and Gratzek 1980). In this system fry are typically held in rearing troughs for 7-14 d after hatching during which time the fry utilize their yolk reserve and are then fed complete, high protein diets until they are stocked into ponds and grown to 7 to 20 cm fingerling fish in 5 to 10 months (Tucker and Robinson 1990). This method, with refinements in stocking densities, feed quality, and better management, is presently the most widely practiced management technique in most catfish farms. Survival and yield utilizing this method have been variable. A survey done in Alabama of twenty two channel catfish producers showed that fingerling survival using this method on average is around 56 % (Hatch et al. 1987). Tucker and Robinson (1990) mentioned that 80 % survival in individual ponds is considered good when utilizing this method.

Many studies done over the past years have been geared towards better understanding and refining the established method of catfish fingerling production. The improvement in areas such as feeds has led to better understanding and better management resources available for the production of catfish fingerlings using earthen ponds. Dupree et al. (1970) looked at ways to improve production through the use of better feed types. The authors experimented with three different complete diets (feed A, feed B and Auburn No. 2) and obtained yields of 1,074, 1,085 and 1,398 kg/ha and feed conversions of 1.7, 2.2 and 2.2, respectively. The survival rates obtained were 79, 77 and 91 percent. These production values were obtained using a growing period of 120 days and stocking 0.07 g fry at a density of 222,378/ha in earthen ponds. McGinty (1980) conducted studies that evaluated the effects of stocking density. The author stocked fry

in ponds at densities of 85,500 and 100,000 fry/ha and obtained yields of 830 kg/ha and 1,053 kg/ha respectively. In this experiment, 1.4 g fry that were initially grown for 60 d were stocked and further cultured for 91 days with the use of supplemental feed. Silva de Gomez (1990) studied feeding practices when she stocked 0.04 g fry at a density of 378,600 fry/ha and grew them for 30 days. Feed was applied either under or not under a feeding shelter. Net yield of 229 kg/ha, survival of 36 %, and FCR of 1.3 for fry fed under shelter and net yield of 353 kg/ha, survival of 60 %, and FCR of 0.8 for fry that were not fed under shelter were reported. Carpenter (2001) stocked 7 d PH fry at a stocking density of 300,000/ha directly into ponds and obtained 5 % survival during a culture period of 119 days. The author mentioned that the low survival was due to disease problems during the first month of fry growth. Even with advancements in technology and the different refinements to the fingerling production systems, the result still remains the same; the production efficiency of the present method is extremely variable and has a lot of room for improvement.

The variable and low production indicators obtained from using the established method of culturing catfish fingerlings has been attributed to several factors. These factors include difficulty in managing disease outbreaks and making accurate assessment of losses, difficulty in controlling predacious insects and other organisms, fry not readily accepting or locating artificial feed, wide fluctuations of growth environmental parameters and variability in natural food items (zooplankton densities). Steinbach (1977) and Carpenter (2001) observed that most of this inputs occurred during the early culture period of the fish.

Several studies have been conducted to investigate ways to increase survival of 7-14 d old fry that are stocked directly into ponds. Snow (1962) compared different methods of rearing catfish fingerlings and observed that survival appeared to be closely related to the amount of early care given to fry. The use of oils and insecticides has been widely studied to control predacious insects (Bryan and Allen 1969; Alsagoff 1981; Piper et al. 1982; Tucker and Robinson 1990). To manage feeding practices better and to increase the chances of the fry locating feed in their early feeding stages, Silva de Gomez (1990) utilized 1m x 1.5 m floating rubber mats as feed shelters. Feed was applied in the same area of the pond, in front of the shelters throughout the experiment. Rodgers (1994) did a study of the distribution patterns of channel catfish in nursery ponds during their first 38 days. One of the author's objectives was to understand the distribution and habitat utilization of fry to make better management decisions about feeding. The effort to reduce mortality by giving young fry an early advantage to survive is very hard when working with ponds, especially large ponds (4.5 to 7.1 surface ha) that are common in commercial operations. Factors such as pond size, water turbidity and uncontrollable environmental parameters make it difficult to manage fry during their early life stages (Carpenter 2001).

Other production methods need to be used to increase production efficiency of catfish fingerling operations. The use of a primary nursery phase offers the advantages of holding fry at high densities for longer periods of time in a more controlled rearing facility to allow for better management to enhance production efficiency. In this production scheme, factors that negatively affect fry during the initial 30 days of their life can be controlled more efficiently. By concentrating fry in small volumes of water in

nursery containers, faster diagnosis and treatment of disease outbreaks can occur and mortalities in fish this size can be more easily observed (Bernardez 1995). A primary nursery phase also can potentially allow fry to be in better water quality conditions because water exchanges are more manageable and are possible. In a primary nursery phase, predation and competition can be reduced or eliminated and access to feed can be made much easier to fry. Furthermore, holding fry in a primary nursery phase allows fry to be vaccinated at an older age and investigate its effects, a topic that has been of interest to researchers.

One relatively inexpensive and simple method that can be used to culture catfish fingerlings through a primary nursery phase is the use of tanks. Stickney et al. (1972) was one of the first to show that it is possible to grow catfish fingerlings at high densities in tanks. Steinbach (1977) further evaluated this method by utilizing 208 L drums with 0.32 l/sec water flow and stocking 5-7 day old fry at stocking rates ranging from 250 to 2,000 per tank. After a growth period of 30 days, he reported survival rates ranging from 86 to 92 % and feed conversions ranging from 2.66 to 2.76. Most commercial attempts to utilize this system in the early days were unsuccessful due to nutritional problems (Lock 1973). With the improvements in the quality of feeds and better management techniques, it is quite possible that the full impact of utilizing a nursery phase in the production of channel catfish fingerlings should be thoroughly evaluated.

Vaccination

Enteric septicemia of catfish (ESC) is an infectious disease caused by a rod-shape, gram-negative bacteria known as *Edwardsiella ictaluri* (Hawke 1979). ESC can manifest itself in an acute or chronic form (Hawke 1979). The acute form of ESC develops 4-6

days after infection and the chronic form develops 3-4 weeks after infection (Klesius 1992). In the early stages of infection, gross signs of ESC-infected fish include pale gills, exophthalmia, enlarged abdomens and, at later stages, hemorrhages and ulcers most commonly around the flanks and back of the fish. In its chronic stage, ESC-infected fish exhibit an open lesion on the top of the head commonly known as hole-in-the-head (Hawke 1979). Behavioral clinical signs include slow, erratic, spiral swimming at the surface of the water commonly known as "whirling" and in some cases affected fish also may hang in the water column with its head up and tail down (Hawke et al. 1981). The pathogenesis of ESC is not well understood but laboratory studies suggest that the olfactory sac and the brain are first to be infected when the pathogen enters via the olfactory bulb and the intestines is first to be infected when it enters via the gut (Newton et al. 1989). ESC is a seasonal disease and occurs most frequently when water temperatures are between 18 and 28^{o}C (Plumb 1988). ESC can affect catfish of all ages (Klesius 1992) but predominantly affects young of the year fingerlings (Francis-Floyd et al. 1987).

ESC is one of the most devastating bacterial diseases affecting the culture of channel catfish. The high mortality rates and the fast onset in the spring and fall make it a major health threat to the catfish industry (MacMillan 1985). From 1996 to 2000, ESC along with *Flavobacterium columnare* infections represented 60 % of the cases submitted at the Thad Cochran National Diagnostics Laboratory Culture Center in Stoneville, Mississippi (Wagner et al. 2002). Fry losses were attributed to ESC on 52 % of farms surveyed in the 4 major catfish producing states (USDA 1997). The annual economic

loss due to ESC has been reported to be as high as $60 million (Klesius and Shoemaker 1999).

Due to its economic importance, ESC has received the attention of many researchers and several studies have been carried out with or developing new ways to treat or to reduce the losses due to the disease. One of the first suggestions to reduce losses due to ESC was through the use of hybrids (channel x blue catfish). Hybrid catfish reportedly has higher resistance to ESC (Yant et al. 1975; Smitherman et al. 1983; Wolters et al. 1996). Bosworth et al. (1998) conducted an experiment using channel catfish *Ictalurus punctatus* (Rafinesque), blue catfish *Ictalurus furcatus* (Lesueur) and their reciprocal F1 hybrids to investigate resistance against ESC. The results, based on survival and antibody levels, showed no statistical difference in resistance of the F1 hybrids when compared to channel catfish and blue catfish, however, they showed a trend in mortality and antibody levels lowest in blue catfish, intermediate in hybrids and highest in channel catfish.

Antibacterial drugs have also been used to treat *E. ictaluri* infections. Romet® is a drug approved by the Food and Drug Administration (FDA) agency. Aquaflor® or Florfenicol® is being used in experimental trials in the United States and has been determined to be safe to treat ESC-infected channel catfish in other parts of the world (Gaikowski et al. 2003). Romet® has shown to be very effective in its original formulation (162 mg Romet® premix/kg of feed) but unpalatable at this concentration (Wilson and Poe 1989). Wise and Johnson (1998) in a study used a more palatable formulation of 40.5 mg of Romet-30 premix/kg feed and concluded that feeding Romet-medicated feed for five consecutive days was not an effective treatment. The use of non-

medicated feed every other or every third day was equally as effective for reducing *E. ictaluri* associated mortalities as feeding Romet-medicated feed on a daily basis. He also mentioned that prolonged consumption of Romet-medicated feed may suppress antibody production. Also, treatment using Romet® can only be effective if administered early in the epizootic while the majority of the affected population is actively feeding. This small window can easily be missed (Petrie-Hanson and Ainsworth 1999). The repeated use of antibiotics can also lead to antibiotic-resistant bacterial strains (Petrie-Hanson and Ainsworth 1999). The approval of any other available or any new antibiotic to treat ESC is very difficult in the US due to the regulations of the FDA and further complicated by the fact that *E. ictaluri* is naturally resistant to 41 out of 71 of the available antibiotics for use in animals (Stock and Wiedemann 2001).

The claims by farmers that withholding, or not feeding, during an ESC outbreak reduces ESC-related mortality was investigated by Wise and Johnson (1998). They reported that feeding every second or third day during an ESC outbreak resulted in reduction of ESC-associated mortality similar to that obtained from daily feeding of Romet-medicated feed and that completely withholding feed resulted in the highest survival. In a laboratory study Lim and Klesius (2003), however, contrary to what Wise and Johnson reported, found that feeding every day and every other day resulted in fish having higher macrophage chemotaxis in response to an *E. ictaluri* exo-antigen and thus more resistant to ESC than those not fed at all throughout the disease outbreak epizode. In this study, however, fish were not fed on the day of challenge which may have impacted results.

The use of commercial vaccines, in particular bacterins, to treat bacterial diseases in the aquaculture industry has been utilized for many years in the salmonid industry to treat against bacteria such as *Vibrio anguillarum* and *Yersenia rukeri* (Bush 1981). The interest of commercial biological companies to produce a vaccine for the catfish industry against ESC was created when economic loss due to ESC was estimated to be reaching $20-30 million yearly (Plumb and Vinitantharat, 1993). Vaccine development against ESC has been aided by the fact that *E. ictaluri* is thought to be a very homogeneous species. Shotts et al. (1985) biochemically characterized 119 isolates of *E. ictaluri* and found that the bacterium shows a high degree of homogeneity. Plumb and Vinitnantharat (1989) compared 40 isolates of *E. ictaluri* from different geographic locations and found that the 40 isolates were very similar biochemically and serologically. More recently, Arias et al. (2003) reported that not enough resolution was provided to be able to discriminate among *E. ictaluri* isolates when PCR-based typing methods were utilized. However, Klesius and Shoemaker (1997) carried out an experiment to investigate if immunizing with one isolate of *E. ictaluri* would provide protection against different isolates of *E. ictaluri.* Their results showed that differences do exist between E. *ictaluri* isolates in their ability to induce protective immunity against ESC but that protection was obtained against several isolates that were used to challenge.

One of the first reported vaccines used against *E. ictaluri* was a commercially-prepared killed bacterin (Plumb and Vinitnantharat 1993). A study using a killed bacterin showed that vaccination by immersion followed by an oral booster administered through the feed resulted in catfish having higher agglutinating antibody titers and lower mortality for the vaccinated group after they were challenged with a water-borne exposure to *E.*

ictaluri (Plumb and Vinitnantharat 1993). Many other studies of the humoral response of channel catfish to killed bacterins have also been reported with varying results (Plumb et al. 1994; Thune et al. 1994; Thune et al. 1997; Petrie-Hanson and Ainsworth 1999). Lawrence et al. (1997) claims that reported success in this kind of study is hard to evaluate because it is difficult to control natural exposure to *E. ictaluri* which can result in positive antibody titers in non-vaccinated fish. Overall, favorable results using killed vaccines have not been consistent and the use of the vaccine has not been well accepted by the catfish industry (Hawke et al. 1998).

Klesius (1992) reported that bacterins only partially protect against ESC because they only stimulate antibody immunity but no cell-mediated immunity. He suggests that an avirulent live vaccine has the best potential to stimulate both antibody and cell-mediated immunity which is the ideal response to immunization. One of the first live attenuated vaccines used against *E. ictaluri* was an adenine-auxotrophic strain of *E. ictaluri* constructed from a mutated *PurA* gene (Lawrence et al. 1997). This vaccine is commonly known as LSU-E2. LSU-E2 when administered by immersion persisted in the host fish for at least 48 h and provided significant protection of channel catfish against ESC (Lawrence et al. 1997). The authors suggested that LSU-E2 had potential as a live vaccine for use by commercial catfish producers.

In 1999, the development of a modified live *E. ictaluri* vaccine (RE-33) was reported (Klesius and Shoemaker 1999). This vaccine showed to be efficacious in channel catfish as young as 7 days post hatch in laboratory studies (Shoemaker et al. 1999). In both laboratory and in field experiments when vaccinating 60 d PH catfish group with RE-33 at a dose of at least $1x10^7$ cfu/ml resulted in lower cumulative

mortality than the non-vaccinated group. However, when using a dose of $1x10^6$ cfu/ml, some protection was shown on the laboratory study but failed to show protection to fish under field conditions (Wise et al. 2000). Wise and Terhune (2001) investigated the relationship between vaccine dose and the efficacy of RE-33 and found that using higher doses resulted in lower mortality of fry when challenged with a virulent *E. ictaluri* isolate. They also were able to recover RE-33 from a greater percentage of fry that were vaccinated at higher dose compared to fry vaccinated at a lower dose.

The RE-33 vaccine was modified and licensed under the name of AQUAVAC-ESC® and is now being marketed as a vaccine against ESC by Intervet, Inc. (Shoemaker et al. 2002). A study in which RE-33 as well as AQUAVAC-ESC® was used to vaccinate eyed channel catfish eggs resulted in less mortality for both RE-33 and AQUAVAC-ESC® as compared to their non-vaccinated controls (Shoemaker et al. 2002). The experiment showed that it was safe and efficacious to use AQUAVAC-ESC® in eyed channel catfish eggs following single vaccination.

INTRODUCTION

The growth of the channel catfish (*Ictalurus punctatus*) industry has prompted the increase in demand for fingerlings. Production of catfish fingerlings in the four major catfish fingerling producing states (Mississippi, Alabama, Arkansas and Louisiana) has ranged from 1.3 to over 1.8 billion in the years between 1996 and 2005 (USDA 2005). In 2005, 1.45 billion fingerlings were produced in a total of 8,701 surface hectares of water totaling 22.2 million dollars in sales.

The current management practices used to produce catfish fingerlings involves the stocking of 7 to 14-d old fry directly into earthen ponds and growing them up to 7 to 20 cm long in 5 to 10 months. This method has resulted in variable production and low survival of fingerlings (Carpenter 2001). Fry stocked directly into ponds experience high levels of mortality during the first 30 days. The high levels of mortality during this period has been associated with factors such as predation by insects, adverse and wide fluctuations in environmental conditions in ponds, and the inability of fry to readily accept or locate artificial feed or lack suitable natural food items.

Many studies have been geared towards reducing fry mortality during their early life stages. Management practices of eliminating predators and feeding inside shelters have been used to improve the survival of catfish fingerlings in ponds during their first 30 days (Piper et al. 1982; Silva de Gomez 1990).

A possible management scheme that could allow control over factors mentioned above as the causes of heavy mortality during the first 30 days is to keep the fry in a primary nursery system before being stocked into ponds.

The disease enteric septicemia of catfish (ESC) is of importance to the catfish industry due to high levels of economic loss (Klesius and Shoemaker 1999). The causative agent of ESC is the bacterium *Edwardsiella ictaluri* (Hawke et al. 1981). ESC can affect catfish of all ages but predominantly affects young-of- the-year fish (Francis-Floyd et al. 1987). In the past, the catfish industry has used the antibiotics Romet® (ormethoprim-sulfamethoxine) and Terramycin® to treat ESC. Both Romet® and Terramycin® are administered in the feed and can be very effective in controlling the disease, if given early in the epizootic while the majority of the affected population is actively feeding. However, this narrow window can easily be missed (Petrie-Hanson and Ainsworth 1999).

Recently, a live attenuated vaccine (AQUAVAC-ESC®, Intervet, Inc., Millsboro, Delaware) was developed and is being marketed as a vaccine against ESC (Shoemaker et al. 2002). This vaccine has shown potential in reducing losses due to ESC in laboratory conditions.

The objectives of this study were to incorporate as a management strategy the use of a live attenuated vaccine AQUAVAC-ESC® against *E. ictaluri* in conjunction with the use of a primary nursery phase in the production of channel catfish fingerlings. Of special interest was the evaluation of the efficacy of the vaccine in pond conditions, the benefits of vaccination of older fry, and also the effects that holding fry in a primary nursery phase has on subsequent production characteristics of catfish fingerlings grown

in earthen ponds. The specific questions to be answered from this study were: 1) does holding fry for 32 days in a primary nursery phase results in better production characteristics of fingerlings subsequently grown in ponds than fingerlings stocked directly into ponds at 10-d PH? 2) does the use of the AQUAVAC-ESC® vaccine result in better production characteristics (better mean survival, feed conversion ratios (FCR), individual fish weight and final standing crop) and does it reduce mortalities associated with ESC? and 3) does vaccinating fry at an older age (32-d PH) results in better production characteristics and less mortalities associated with ESC than vaccinating at 10-d PH?

MATERIALS AND METHODS

On June 6, 2004 approximately 270,000 channel catfish fry *Ictalurus punctatus* (NWAC 103 strain) were obtained from a local commercial producer and transported to the North Auburn Fisheries Research Unit, Auburn, Alabama. The fry were 3 d post-hatch (PH) when received and were divided into four aluminum troughs and reared for 7 days before being assigned to a treatment and stocked. In the troughs, after absorption of their yolk sac fry were fed a 50 % crude protein fry starter (AquaMax 5D00, Purina Mills, inc., St. Louis, MO, USA) utilizing a belt feeder. Oxygen was supplied using a ½-hp air blower (Sweetwater model S-31, Aquatic Eco-systems, Apopka, Florida), water flow was kept at about 4 l/min, and Calcium chloride was continuously added through the use of a 4.73 l/h peristolic pump (Chem-Tech series 100, Viking Pump Inc., Cedar Falls, Iowa) to maintain water hardness at a minimum of 80 ppm. Troughs were siphoned on a daily basis to remove uneaten food and dead fry.

A randomized incomplete block design was utilized in the experiment with five treatments of five replicates per treatment. Treatments 1 and 2 involved the sham vaccination and vaccination, respectively, of 10-d old fry, after which the fry were stocked into earthen ponds. In treatments 3 and 4, 10-d old fry were sham vaccinated and vaccinated respectively, kept in nursery tanks for twenty two days and then stocked into

ponds. Fry in treatment 5 were sham vaccinated at 10 days of age, kept in nursery tanks for twenty two days before being vaccinated, and then stocked into ponds.

At 10-d PH, average fry weight was determined by weighing and counting three samples of 250 fry/sample from each rearing trough and used to calculate the number of fry stocked into treatment rearing units. Vaccination of fry was carried out according to the recommendation of the manufacturer for use on channel catfish fry. Briefly, vaccine vials containing avirulent, live vaccine culture in a lyophilized form were reconstituted with 40 ml distilled water, combined into one sterile container and left undisturbed for at least 15 minutes before use. Prior to use, a sample of the vaccine was collected to verify final vaccination dosage. For each replicate in the 10-d old fry vaccinated treatment (treatments 2 and 4), approximately 10,000 fry were placed into 1.8 L of aerated water and 23.5 ml of reconstituted vaccine added. After 2 minutes, an additional 1.89 L of water was added to the bucket and the fry were left in the vaccine bath for 30 minutes before being stocked into the appropriate rearing unit. Fry for treatments 1, 3 and 5 were sham-vaccinated as above minus the addition of the vaccine.

Twenty-five 0.04-ha experimental ponds, with an average depth of approximately 1.0 m were used in this experiment. The ponds were drained and rotenone (1.0 ppm) was applied to the remaining puddles 4 weeks prior to stocking. Diquat (Reward®) at a rate of 13.87 l/ha and copper sulfate at a rate of 112 kg/ha were utilized to eliminate emergent weeds. Two weeks prior to stocking, designated ponds were filled with water and fertilized with liquid fertilizer (10-30-0) at a rate of 12.4 l/ha and with cottonseed meal at a rate of 500 kg/ha. A combination of liquid limestone (Cal-Flo®) at a rate of 23.4 l/ha and agricultural limestone at a rate of 3,368 kg/ha was utilized to raise water hardness

and alkalinity to 60 ppm in each pond. Diesel fuel was added to the ponds at a rate of 9.35 l/ha to control aquatic insect populations.

Fry in treatment 1 and 2 were stocked directly into ponds on June 13, 2004 with approximately 247,000 fry/ha. Fry from treatments 3-5 were stocked twenty two days later after nursing. Approximately 10-20 % of the fry in the rearing troughs died because of a parasitic infestation with *Trichodina* sp. Due to these mortalities, three of the ponds in treatment 1 were not initially stocked along with the other ponds. Additional fry were obtained from another source at 5 days PH and then used to stock the three remaining ponds within this treatment with fry 10 d of age.

Primary Nursery phase

Fry from treatments 3, 4 and 5 were stocked into 1,134-L round fiberglass tanks on June 13, 2004, and grown for 22 days. With the use of a submersible deep well pump water from a well was supplied to the nursery tanks. Flow valves regulated the flow at approximately 9 l/min. Hardness of the water inside the tanks was maintained at a minimum of 80 mg/l by continuous addition of calcium chloride utilizing a peristolic pump (Chem-Tech, series 100, Viking Pump Inc, Cedar Falls, Iowa). Aeration was supplied to each of the 15 tanks with the use of a ½-hp blower (Sweetwater model S-31, Aquatic Eco-systems, Apopka, Florida).

A 50 % protein fry powder (Purina Aquamax) and later fingerling starter (Purina AquaMax 00, 100 and 200) trout feed were used to feed fry during the tank nursery phase. Feeding was done manually 5 times a day at 3.5 hour intervals with the first feeding occurring at 0500 h and the last feeding of the day at 2230 h. Uneaten feed and

waste material were siphoned out of the tanks on a weekly basis and dead fry were removed on a daily basis throughout the nursery phase.

Dissolved oxygen and temperature were monitored on a daily basis with a YSI dissolved oxygen meter (YSI model 55, YSI Inc., San Diego, California). Water hardness, alkalinity, pH, and ammonia were monitored on a weekly basis with a water quality test kit (Hach® model FF-2, Hach Company, Loveland, Colorado).

On July 5, 2004, the fry from treatments 3, 4 and 5 were removed from the nursery tanks, weighed, and stocked into experimental ponds. The average weight of three samples containing 50 fish each were used to estimate the number of fry required for each pond. At this time, fry from treatment 5 (32-d PH) were vaccinated according to manufacturer's directions. Once all groups of fry were stocked into earthen ponds, these fish were fed and water quality from the pond monitored as described below.

Water Quality Management

Dissolved oxygen (DO), and temperature were recorded twice daily in the morning and afternoon at 0600 and 1800 hrs, respectively, using a YSI dissolved oxygen meter (YSI model 55, YSI Inc., San Diego, California). Emergency aeration was provided when evening DO patterns indicated that morning DO levels might drop below 3 mg/l. Water quality parameters (total alkalinity, total hardness, nitrogen ammonia, pH, nitrite nitrogen and chlorides) were measured using a Hach® test kit initially on a weekly basis for the first six weeks and then every two weeks for the remaining growing period. Water was added to the ponds periodically throughout the experiment to replace that which was lost through evaporation and seepage. On August 13, 2004, another treatment

of granular copper sulfate at a dosage of 112 kg/ha and Diquat (Reward®) at 469 fluid oz/ha was administered to control excessive algae in particular *Chara* sp and *Pithophora* sp.

Feeding

Treatments 1 and 2 were fed by hand three times during the day with Purina AquaMax (50 % protein) trout feed (AquMax 100, weeks 1 and 2; AquaMax 200, week 3; AquaMax 300, weeks 3, 4 and 5). Once all treatments were stocked into ponds and once all fish were observed feeding, they were fed to satiation, but never exceeding 134 kg/ha/day. At week six, the fish were introduced to a 36 % protein floating catfish feed (Southern States, Inc.) and fed with it for the remainder of the growing season. Fish were fed three times a day initially for the first month and then readjusted to two times when the fish in all treatments were observed feeding on the surface of the water. Finally, after the third month and for the remainder of the study the fish were fed once a day.

Fingerling fish were harvested out of the ponds beginning on November 15, 2004 until November 18, 2004 after five months of growth. All fish harvested were weighed in bulk on a standard industrial bench scale (0.001 lb readability). A total number of fingerlings per pond were calculated based on the average weight of three samples of 250 fish each.

Disease Management

Dead fish from all ponds were collected on a daily basis and enumerated throughout the disease outbreak. Moribund fish exhibiting abnormal behavior or disease-related clinical signs in, particular those of ESC or columnaris were necropsied. Samples from the liver, spleen and trunk kidney were plated on brain heart infusion (BHI) agar

media and Hsu-shotts agar media. Biochemical tests were run on isolated bacterial colonies to confirm any pathogens present in the fish and etiology. Presumptive tests based on colony morphology, indole and cytochrome oxidase were performed throughout the disease episode to keep tract of the presence, in particular of ESC during the disease outbreak. Once any pond had mortalities of fish exhibiting ESC-related clinical signs, all ponds of the corresponding treatment fish were fed every other day (Wise and Johnson 1998) until mortalities due to the outbreak episode stopped.

Laboratory Challenge

To have better control of environmental parameters and to better evaluate the vaccine, fish were removed from the ponds and challenged in a laboratory setting with *E. ictaluri*. Fifty fish from each pond of treatments 2, 3, 4 and 5 were removed and transported to the Fish Disease Laboratory located at the North Auburn Fisheries Research Station on September 30, 2004. The fish from each pond were equally distributed and stocked into two replicate 30-L aquaria supplied with continuous aeration and continuous water flow at a rate of 0.5 l/min. Water temperature was mantained at 28^{o}C prior to the fingerlings being challenged and at 26^{o}C after challenge. Fish were fed daily with a 36 % protein trout fingerling feed (Purina AquaMax 300). Uneaten feed and waste products were siphoned from aquaria on a daily basis.

On October 12, 2004, ten fish were removed from each aquarium and blood samples were extracted from the caudal vein into Vacutainer® blood collecting tubes. Samples were allowed to clot overnight at 5°C. The samples were then centrifuged to collect the serum and the serum samples were stored at - 80°C for later use. Antibody analysis was carried out on the samples to test for the presence of antibodies against ESC

in each of the treatments based on the methods of Conrath (1972). Briefly, the antigen was prepared by growing an *E. ictaluri* culture to early stationary phase (24 h) and killing it with formalin (37 % formaldehyde) at a rate of 0.5 % of culture volume. The bacterial cells were collected by centrifugation and washed three times with PBS containing 0.2 % Tween 80. The collected cells were then diluted with PBS containing 0.05 % Tween 80 and standardized to an optical density at 540 nm between 0.7 and 0.8. Into each well of a 96-well microtiter plate, 25 µL of PBS was placed followed by 25 µL of serum placed into the first well. Serial dilutions (log 2) were made for all samples. The serum dilutions were then overlaid with 50 µL of *E. ictaluri* antigen. Two negative and two positive serum samples were used as controls. The plates were placed in humidor overnight and evaluated the following day.

The remaining fifteen fish in each aquarium were challenged with a virulent *E. ictaluri* isolate by immersion with 1x 10^7 CFU/ml on October 22, 2004. The culture used to challenge the fish was grown on brain-heart infusion (BHI) broth for 18 h at 30^{o}C and was inoculated with a pure *E. ictaluri* isolate (S97-773) that had been kept at – 80^{o}C. Prior to challenge the water flow in each aquaria was stopped, the culture of virulent *E. ictaluri* was added and after 1 h the water flow was resumed. On the day of challenge, fish were not fed. Moribund fish were collected, necropsied, and biochemical tests were done to identify the pathogens present on the fish throughout a documented disease outbreak.

Data Analysis

The mixed procedure (Wolfinger et al. 1991) was used to make contrast of treatments in the pond study. The model used in the analysis included the blocking effect

by pond location of the experimental design. The variables compared were mean survival, intermediate survival, final standing crop, individual fish weight, and FCR. For the laboratory study, an analysis of variance was used to determine differences in mortality between treatments of interest. All statistical analyses were carried out using SAS (version 9.1, SAS Institute, Cary, North Carolina). Significant differences were considered at $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Water Quality

Water quality data for the pond study are presented in Table 1. TAN ranged from 0.71 in treatment 1 to 0.88 to treatment 5, pH ranged from 7.33 in treatment 5 to 7.52 in treatment 3, and nitrite ranged from 0.03 mg/L in treatment 5 to 0.11 mg/L in treatment 2. No statistical differences were observed among treatments in any of the water quality parameters. Water quality parameters during the study were within known acceptable levels for culture of channel catfish (Tucker and Robinson 1990).

Primary Nursery Phase

Mean standing crop after a 22-d growing period in the primary nursery phase ranged from 4.67 to 5.15 kg/m^3, survival ranged from 95 to 100 % and individual fish mean weight ranged from 0.60 g to 0.65 g (Table 2). No significant differences were observed among treatments.

Pond Production

Treatment 1 was eliminated from any statistical analysis because three replicate ponds stocked at the later date within that treatment had both blue catfish and channel catfish in them that came along with the second batch of fry. The blue catfish were detected prior to harvest while sampling to observe for growth uniformity. Blue catfish

have been reported to be more resistant to ESC (Dunham et al. 1994) and also could have potentially interfered with the transmission of the disease within those ponds.

Production characteristics for the pond study are presented in Table 3 and Table 4. Mean fingerling standing crop ranged from 4,716 kg/ha in treatment 2 (10-d vacc/no nursery) to 8,112 kg/ha in treatment 5 (32-d vacc/nursery) (Table 3). A significant difference by treatment occurred only between treatment 2 and treatments 3, 4, and 5. Individual fish mean weight (Table 3) ranged from 38.8 g in treatment 2 to 40.9 g in treatment 5, but no significant treatment differences were observed. Feed conversion ratios ranged from 1.15 in treatment 5 to 1.51 in treatment 2; however, no significant differences were observed among the treatments (Table 3). Mean survival ranged from 47.5 % in treatment 2 to 73.4 % in treatment 5 (Table 4). Significant differences were observed between treatment 2 and the other three treatments as well as between treatments 3 and 5. Observed mortality (total # of fish collected during a disease outbreak) ranged from 1.5 % in treatment 5 to 6.8 % in treatment 2 (Table 4). However, no significant differences were observed between any of the treatments.

Laboratory Study

Once the fish were moved into the laboratory, no antibody titers were observed from titration of serum from samples collected prior to challenge (data not shown). Percent mortality in aquaria after challenge (Figure 2) ranged from 8 % in treatment 4 (10-d vacc/nursery) to 19.9 % in treatment 2 (10-d vacc/no nursery). No statistical differences were observed ($P = 0.34$) among any of the treatments.

Mixed infections of ESC and columnaris are given as percentage of the total number of cases necropsied for the detection of bacterial pathogens. All dead fish were

collected; however, only sixty nine were suitable for necropsy. Mixed infection percentages (Figure 3) ranged from 10.34 % in treatment 2 (10-d vacc/no nursery) to 20.68 % in treatment 2 (sham vacc/nursery).

Effects of Primary Nursery Phase

Comparison of treatment 2 (10-d vacc/no nursery) and treatment 4 (10-d vacc/nursery) allows the evaluation of stocking 10 d old fry directly into earthen ponds versus stocking of fry that are kept in a nursery phase before being stocked into earthen ponds. Standing crop (4,716 kg/ha) and survival (47.5 %) for treatment 2 were significantly lower than the yield (6,910 kg/ha) and survival (67.5 %) for treatment 4. In treatment 2, FCR (1.51) was observed to be higher than that of treatment 4 (1.28) although statistically the model showed no significant differences between any of the 4 treatments ($P = 0.12$). No difference was seen between the individual fish mean weight for treatment 2 (38.8 g) and for treatment 4 (39.2 g).

The comparison of production results of this study are greater than those obtained in similar studies for fry both directly stocked and those held for a period of time in a primary nursery phase before being stocked into earthen ponds. Morrison et al. (1995) compared holding fry for 27 d in raceways before stocking into ponds versus direct pond stocking for fry at densities of 200,000 fry/ha grown for 150 d. The authors reported yields of 3,672 kg/ha and 3,317 kg/ha with an average weight per fingerling of 21.4 g and 19.7 g, and overall survival of 95.2 % and 84.1 % respectively. Carpenter (2001) reported yields of 1,475 kg/ha and 812 kg/ha, final survivals of 35 % and 5 %, and average weight per fingerling of 56.39 g and 17.39 g in a study in which he compared, respectively, holding fry for 29 d in in-pond raceways before stocking into ponds vs.

direct pond stocking and then growing them for 119 d. Seven day old fry were stocked at 296,400/ha for direct stocking and 247,000 fry/ha for the nursery group. Carpenter (2001) observed statistical difference at the $\alpha = 0.05$ level for survival and individual fish weight and mentioned that his results were affected by very high mortalities due to diseases (columnaris and ESC) and parasite infestations (*Capriniana*) occurring during the first month of the growth cycle.

It is very difficult to compare the results of this study with other similar studies because there are differences between studies such as stocking density, length of growing period, quality of feed utilized, management techniques, and other aspects that vary from one study to another. For example, McGinty (1980) showed that stocking density had an effect on yield and average fish weight. The author reported that fry stocked at 85,000/ha and 100,000/ha and reared up to 1.4 g/fry in primary nursery ponds resulted in yield and average fish weight respectively of 830 kg/ha and 12.1 g/fish and 1,053 kg/ha and 8.7 g/fish.

The economic benefits of a primary nursery phase are equally hard to evaluate because of the same reasons mentioned above and in particular the type of technology used and the level of management utilized. Carpenter (2001) did an economic analysis and showed net returns per hectare of $4,306 and minus $4,003 respectively, for fingerlings grown in in-pond raceways (before stocking into ponds), and on-land raceways (before stocking into ponds). That study showed that even though the two systems were treated alike with the only differences being the technology utilized in each of the systems, in particular the way of pumping water, increased net returns were obtained when using in-pond raceways but money was lost when using on-land raceways.

Early observed response to feed from fry in the primary nursery tanks (6-d post stocked) as compared to the response in the directly stocked ponds (28-d post stocked) suggests that initially (at least during the first 28 days) more efficient management of the slow sinking artificial feed used was obtained by the fry in the nursery tanks. Immediately after stocking, fry in the primary nursery tanks were fed only what they were observed to eat. However, fry stocked in ponds at 10 d were fed (16.8 kg/ha/day) regardless of whether they ate all the feed or not since it was not possible to observe them feeding. Similar feeding practice is performed on research and commercial farms alike so that the fry have a better chance to locate and eat artificial feed in the early stages of the production cycle. Although the FCR in our study was not statistically different the observed difference could partially be explained by either the fry locating most of the feed offered to treatment 2 during the first 28 days or effectively utilized some of the artificial feed along with the natural food available in the ponds. If it is due to the latter reason, it can be assumed that a portion of the feed was wasted and yielded increased costs.

Individual fish mean weight of both the directly stocked fry and the fry that underwent a primary nursery phase were not different in this study. No difference in weight of individual fish is possible because fish were fed to satiation. Competition for feed in fish from treatment 2 (lower survival) potentially resulted in fish that grew bigger while fish in treatment 4 (higher survival) did not grow to their full potential. Alsagoff (1981) made a similar observation. The author observed that two groups of ponds that had different standing crops resulted in similar fingerling size.

An important factor not evaluated to its full extent in this study is the claim that the use of a primary nursery phase can enable culturist to make timely adjustments for early fry mortality and reduce unaccounted fish losses (Morrison et al. 1995). In this study, the fry mortality for treatments 3, 4, and 5 during the primary nursery phase was insignificant (Table 2). This shows that the use of the nursery phase results in little fry mortality during the first 30 days or during the time at which high mortalities have been reported when stocking fry directly into ponds (Steinbach 1977, Morrison et al. 1995). Mortality data during the first 30 days in directly stocked ponds was not gathered in this study. However, using treatment 4 as an example and taking into consideration that little mortality occurred during the first 30 days, adding the mortality documented during the disease outbreak (2.9 %) with the final survival (67.5 %), 29.6 % of the fry were unaccounted for or fry that died from the point of harvest from the nursery phase until the time of harvesting from the ponds. Out of this 29.6 % unaccounted fish mortality, some fish may have been eaten by birds and some dead fish were not documented because submergent weeds prevented them from floating and being observed. Treatment 2 resulted in 45.7 % of fingerlings unaccounted for at the point of harvest. This is in accordance with what other studies in the past have reported. Carpenter (2001) reports that directly stocked 7-d old fry into ponds had 24 % mortality of fry occurring during the first 29 days. Tamassia (1993) in a similar study reported 17 % mortality during the first 30 days. Because there is not any other obvious sources for the unaccounted fish losses and the average final weight of individual fish being similar, the data in this study suggest that some mortality most likely occurred during the first 30 days in the directly stocked

ponds and that an insignificant amount occurred in the fry that underwent the nursery phase (Table 2).

Net returns at the point of fingerling harvest is probably the best way to evaluate the use of a primary nursery phase for a farm that only produces fingerlings. However, for vertically integrated farms that produce fingerlings for stocking into their own ponds, other factors such as reducing pond space need to be taken into consideration. In this experiment, (20 %) higher survival and (2,194 kg/ha) higher final standing crop, were obtained for the group that underwent a primary nursery phase. Increasing these production parameters on commercial farm could not only translate into potentially more revenue but could save production resources and space that may be used in more profitable ventures (Hebicha 1984) or other business diversification.

Fingerlings are generally priced by length on average 7.5 ¢/15cm fingerling, the bigger the fingerling the better the price obtained for those fingerlings. The study however shows that the monetary benefits of using the primary nursery system may not come from better prices due to bigger fingerlings but from more fingerlings produced.

Effects of Vaccination

The vaccine dosage used to vaccinate fry was 1 x 10^7 Cfu/ml. The first mortalities of fingerlings expressing clinical signs of ESC were observed on October 8, 2004, ESC was diagnosed for the first time at the Southeastern Cooperative Fish Disease Laboratory at Auburn University on October 11th. Mortalities were observed on ponds across all treatments on October 11th. The disease episode ran from October 8th, when the average daily temperature was 22^{o}C to November 4th when the temperature fell below

17.5^{o}C (Figure 1). Fish exhibiting columnaris-related signs were first seen on October 15th and was confirmed at the fish disease laboratory on October 18th.

Comparison of results in treatments 3 (Sham vacc/nursery), 4 (10-d vacc/nursery) and 5 (32-d vacc/nursery) allowed the evaluation of the vaccine. Comparison of treatment 3 with treatment 4 evaluated the benefits of vaccinating fry at 10 d of age over not vaccinating at all. Comparison of treatment 3 with treatment 5 evaluated the benefits of vaccinating at 32 d of age over not vaccinating while comparing treatment 4 with treatment 5 allowed evaluation of the benefits of vaccinating older fry (32 d PH) over vaccinating younger fry (10 d PH). Effects of vaccination were evaluated by the observed mortality percentage (number of dead fish collected during an ESC outbreak) and the survival of fingerlings in each treatment at the end of the study. Mean standing crop, individual fish mean weight and FCR were also taken into consideration as an indication of vaccination benefits. The same parameters were evaluated in the laboratory part of the study.

No statistical differences were obtained in observed mortality (67.5 % and 60.7 %) and survival (2.9 % and 4.7 %) respectively, for the treatment vaccinated at 10 d PH and the sham-vaccinated treatment (Table 4). No statistical differences were also observed in standing crop; individual fish mean weight and FCR (Table 3). Comparing treatment 4 against treatment 5 showed no statistical differences in any of the production parameters when vaccinating 10 days post-hatch fry versus vaccinating fry at 32 days post-hatch (Table 3, Table 4). However, comparing treatment 3 against treatment 5 suggests that their might be some benefits of vaccinating fry at 32 d of age over not vaccinating fry at all. Significantly higher survival was obtained for the 32-d

vacc/nursery treatment (73.4 %) than for the sham vacc/nursery treatment (60.7 %). No statistical differences were observed in standing crop, observed mortality, individual fish mean weight and FCR (Table 3, Table 4).

Past studies have shown that there may be benefits to vaccinating catfish fingerlings based on laboratory findings and limited field studies. Shoemaker et al. (1999) in a laboratory study reported that 12-day PH fry vaccinated by immersion for 2 minutes with a dosage between 5 x 10^5 and 1 x 10^6 CFU/mL and challenged with a virulent ESC strain resulted in lower mortality (33.3 %) than that of a non vaccinated group (78.7 %). Wise et al. (2000) in a laboratory study vaccinated 72-d old fry at a dose of 1 x 10^6 for 2 minutes and after a challenge with a virulent ESC isolate reported a lower percent mortality (58.5) for the vaccinated group as compared to the non-vaccinated group (77.5 %). In a second component of the same study, however, when 21-d post vaccinated fry under field conditions were challenged by exposure to an *E. ictaluri* epizootic occurring in a commercial catfish pond resulted in no protection, as shown by similar percent mortalities.

In this study, no benefits to vaccinating at 10-d PH were observed. Wise et al. (2000) did a study using the RE-33 isolate (an *E. ictaluri* rifampicin-mutant isolate) both in the laboratory and in the field and reported that other diseases, in particular *F. columnare*, may have confounded the results especially in the field study. The results of this study, in particular the observed mortality and percent survival may have been confounded as well by *F. columnare* infection that occurred during the same time frame that the ESC outbreak occurred. It was practically impossible to determine which of the two pathogens from the mixed infection was responsible for the death of every fish that

was collected. Also, birds and weeds interfered with the collection of all the fish that died during the disease outbreak.

The laboratory study, however, allowed the determination of percentage of mixed infections from a sample of the total amount of dead fish collected (Figure 3). ESC and columnaris mixed infections for the non-vaccinated group represented (17.2 %) of the total number of cases biochemically diagnosed as compared to the vaccinated group which represented 13.8 %. The late outbreak of the disease, October 8th, at daily average temperatures around 22° C, which is at the lower end of the temperature range for ESC, and the falling of temperature below 17.5° C could likely have interrupted the infection process thereby not allowing the evaluation of the full impact of the vaccine. Francis-Floyd et al. (1987) inoculated catfish fingerlings with *E. ictaluri* suspensions at different temperatures (17, 21, 23, 25, 28 and 32^{o}C) and observed differences in mortality between *E. ictaluri*- infected fish and control fish at 23, 25 and 28^{o}C but not at 17, 21 and 32^{o}C. The authors concluded that a given fish may be susceptible to ESC infection at any of these temperatures; however, the entire population may be at less risk when the temperatures are not between 23 and 28^{o}C.

The short infection period also did not allow the full implementation of restrictive feeding as a management tool to control ESC associated mortalities, a practice that is implemented widely in the catfish industry in the Mississippi delta (Wise and Johnson 1998). Producers are reporting that they are able to feed more to vaccinated groups of fish and thus are able to obtain larger fingerlings that command a higher price. In this study, the average fish weights obtained from both the vaccinated group and non vaccinated group were similar. The management strategy of restrictive feeding was

implemented but because of the quick spread of the disease across all treatments and the short duration of the disease outbreak, differences in individual fish average weight and length were not seen in this study. Furthermore, the disease outbreak occurred late in the season when the water temperatures were cool and the fish in all the ponds were generally not eating very much.

Vaccination of catfish fry at an older age has been suggested to have better efficacy. Petrie-Hanson and Ainsworth (1999) reported that the acquired humoral immune response of catfish increases as the age and the average size of the fish increases. Wise and Terhune (2001) mentioned that fry that were vaccinated at 12 d of age (post hatch) with RE-33 showed the development of a limited but persistent infection for up to 12 d past vaccination, which in theory could have allowed enough time for the proper development of components necessary for a functional immune response. The results of treatments 4 and 5 (Table 3, Table 4) indicate slight improvements in production indicators when vaccinating at an older age, but not enough to be statistically different. However, when comparing treatment 3 with treatment 5 shows that significantly higher survival is obtained when vaccinating at an older age. Shoemaker et al. (1999) vaccinated with RE-33 at 12, 14, 16 and 31 d PH in a laboratory study and reported no difference in vaccinating at older ages. However, even though not statistically different the data showed slight increase in the relative percent survival (RPS) for treatments vaccinated at the older age. Again, in this study, it is possible that the removal of the variability introduced by the mixed infection would result in a totally different outcome.

Data from both the pond and laboratory portions of this study are inconclusive in regards to vaccination. Although a significant difference was not observed between non

vaccinated and 10-d vaccinated/nursery treatment, there was a significant difference observed between the control and 32-d vaccinated treatment. Reports from the catfish industry indicate that overall survival of fry vaccinated at 7 – 10 d PH may range from 5 – 12 % higher than vaccinated groups and may or may not be significantly different (K. Schuster, Intervet, Inc., Personal communication). The 7 % increase in survival obtained in this study is about the breakeven point in recovering the cost of vaccination (K. Schuster, Intervet Inc, personal communication).

Although statistical differences may not be observed, especially when comparing small numbers of ponds, economic differences may exist. Mixed ESC and columnaris infection made it difficult to evaluate the full impact of the vaccine. The late outbreak of the disease and the low temperatures may have interrupted the infection process resulting in data that is not representative of what a normal ESC infection process is really like.

Table 1: Mean ± SE water quality values measured in ponds used to grow channel catfish fingerlings from June 13, 2004 to November 18, 2004. Significance between treatments ($P < 0.05$) is indicated by different letters within the same column.

Treatment	TAN	pH	NO_2 (mg/L)
1 (Sham vacc/no nursery)[1]	0.71	7.45	0.06
2 (10-d vacc/no nursery)	0.74 ± 0.13[a]	7.35 ± 0.06[a]	0.11 ± 0.04[a]
3 (Sham vacc/nursery)	0.77 ± 0.08[a]	7.52 ± 0.10[a]	0.02 ± 0.01[a]
4 (10-d vacc/nursery)	0.81 ± 0.11[a]	7.45 ± 0.12[a]	0.03 ± 0.01[a]
5 (32-d vacc/nursery)	0.88 ± 0.12[a]	7.33 ± 0.06[a]	0.03 ± 0.01[a]
P-value	0.77	0.51	0.2

[1] Treatment 1 = Treatment that was eliminated from statistical analysis and is presented for information purposes only.

Table 2: Mean individual fish weight, standing crop and survival for channel catfish fry that were either sham vaccinated or vaccinated and kept in primary nursery tanks for 22 d. Values are means of five replicates ± SE. Significance between treatments ($P < 0.05$) is indicated by different letters within the same column.

Treatment	Ave. Weight (g)	Standing Crop (Kg/m^3)	Survival (%)
3 (Sham vaccinated)	0.60 ± 0.02^a	4.76 ± 0.16^a	95 ± 1.84^a
4 (10-d vaccinated)	0.64 ± 0.01^a	4.79 ± 0.11^a	96 ± 3.09^a
5 (Sham vacc/32-d vaccinated)	0.65 ± 0.01^a	5.15 ± 0.09^a	100 ± 3.15^a

Table 3: Standing crop, individual fish weight, and feed conversion ratios (FCR) for channel catfish fry that were either sham vaccinated or vaccinated and then stocked either directly into ponds or into primary nursery tanks for 22 d and then into ponds for grow out to the fingerling stage. Values are mean ± SE. Significance between treatments ($P < 0.05$) is indicated by different letters within the same column.

Treatment	Standing Crop (Kg/ha)	Ave. Weight (g)	FCR
1 (Sham vacc/no nursery)[1]	4,923	33.87	1.9
2 (10-d vacc/no nursery)	4,716 ± 804[a]	38.8 ± 6.5[a]	1.51 ± 0.09[a]
3 (Sham vacc/nursery)	6,653 ± 921[b]	40.8 ± 3.4[a]	1.38 ± 0.16[a]
4 (10-d vacc/nursery)	6,910 ± 999[b]	39.2 ± 3.8[a]	1.28 ± 0.06[a]
5 (32-d vacc/nursery)	8,112 ± 402[b]	40.9 ± 0.4[a]	1.15 ± 0.04[a]
Pooled SEM	1267	6.35	0.14
P-value	0.01	0.94	0.13

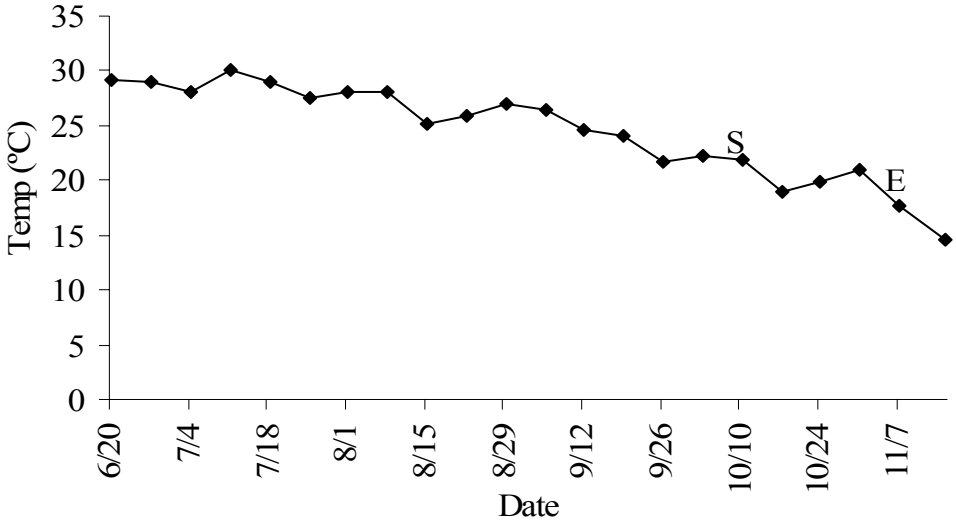
[1] Treatment 1 = Treatment that was eliminated from statistical analysis and is presented for information purposes only.

Table 4: Observed mortality and survival for channel catfish fry that were either sham vaccinated or vaccinated and stocked either directly into ponds or into primary nursery tanks for 22d and then into ponds for grow out to fingerling stage. Values are mean ± SE. Significance between treatments ($P < 0.05$) is indicated by different letters within the same column.

Treatment	Observed Mortality (%)	Survival (%)
1 (Sham vacc/no nursery)[1]	4.6	51
2 (10-d vacc/no nursery)	6.8 ± 3.9^{a}	47.5 ± 3.8^{a}
3 (sham vacc/nursery)	4.7 ± 2.6^{a}	60.7 ± 6.2^{b}
4 (10-d vacc/nursery)	2.9 ± 0.9^{a}	$67.5 \pm 2.7^{b,c}$
5 (32-d vacc/nursery)	1.5 ± 0.5^{a}	73.4 ± 3.4^{c}
Pooled SEM	2.43	4.31
P-value	0.4	0.003

[1] Treatment 1 = Treatment that was eliminated from statistical analysis and is presented for information purposes only.

Figure 1: Weekly mean temperatures of ponds used to grow channel catfish fingerlings from June 13, 2004 to November 18, 2004.



[S]Starting date of a documented ESC/columnaris disease outbreak.

[E]Ending date of the disease outbreak

Figure 2: Efficacy of AQUAVAC-ESC® in channel catfish (*Ictalurus punctatus)* stocked either directly into ponds or into primary nursery tanks for 22d and then into ponds for grow-out to fingerling stage. Fish were either sham vaccinated or vaccinated in pond setting and then challenged in laboratory setting with *E. ictaluri* (AL-93-75) post vaccination. Data are presented as means ± SE. Values with different letters are significantly different from each other at $P < 0.05$.

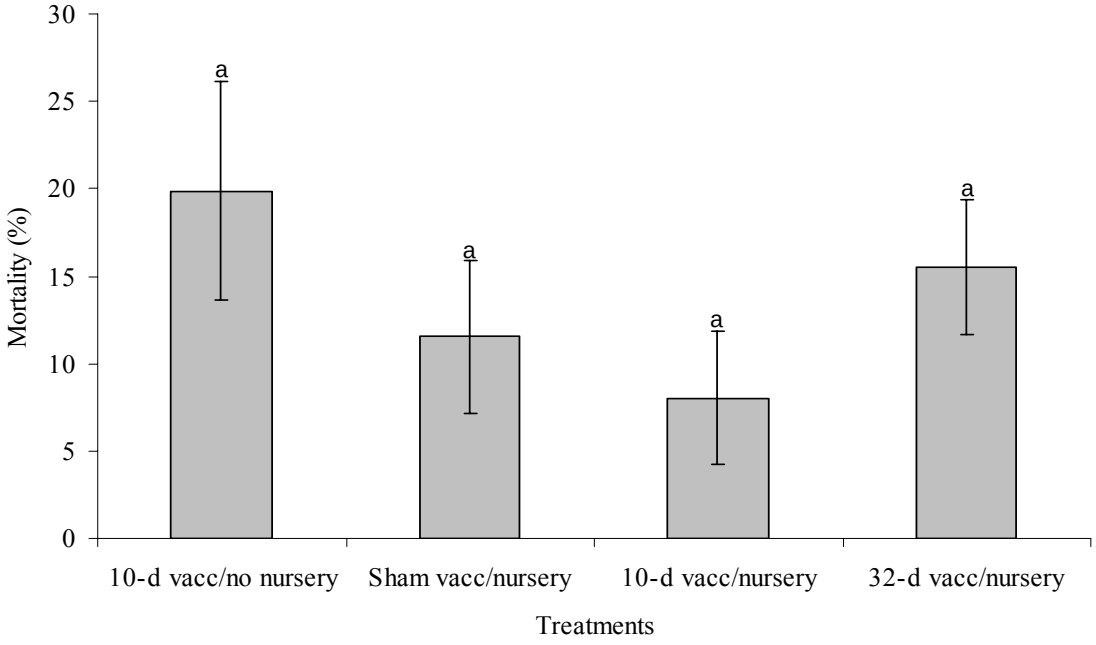
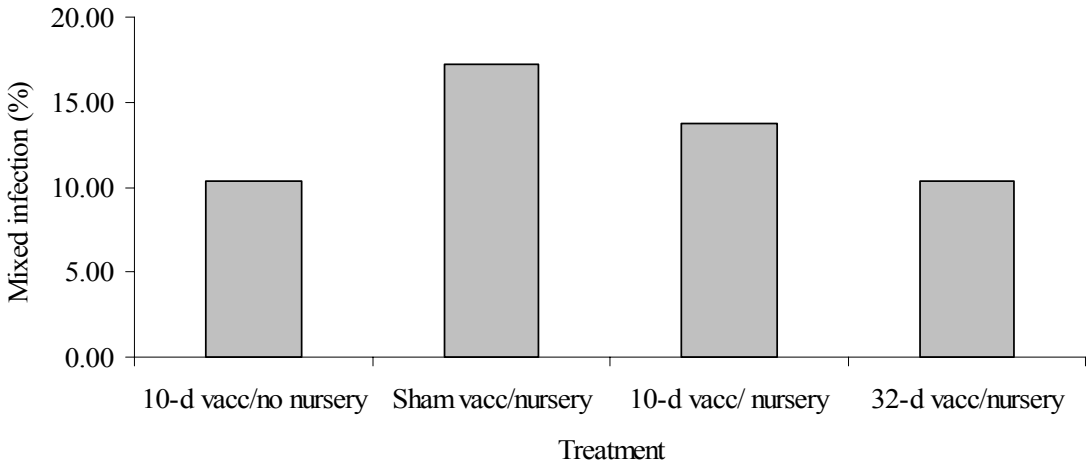


Figure 3: Percentage of cases exhibiting mixed infections of enteric septicemia of catfish (ESC) and columnaris disease after a challenge with a virulent ESC isolate under laboratory conditions.



SUMMARY

The primary nursery system used in this study increased the final standing crop and overall survival of catfish fingerlings. An increase in standing crop and especially in survival can allow producers to utilize pond space and production inputs more efficiently. The much better survival in the group that underwent the nursery phase was potentially related to the ease of monitoring the fry more closely in the smaller more controlled environment. Fry in the tanks responded to artificial feed much quicker, were fed more efficiently, kept in good water quality conditions, and not affected by predation during the first 30 days of their early fragile life. Having an idea of the mortality during the first 30 days can allow producers to make timely adjustments for early fry mortality and hence make better management decisions with regards to issues such as feeding later in the production cycle.

An economic analysis to determine the net returns of the directly stocked group and of the nursery stocked group was not done. The data, however, showed a wide difference in final standing crop and survival, much better for the nursery stocked group. Profitability will be determined by the farmer's individual management system and how he utilizes increased survival and yield in a management plan in his farm that allows him to use his resources in a way that maximizes profits.

The vaccination part of the study showed no major benefits of utilizing the AQUACAC ESC vaccine. Benefit of vaccination at older age was only observed in survival between the non-vaccinated group and the 32-d vaccinated group. However, the results of the vaccination component of the study were confounded by the mixed infections of ESC and columnaris disease. Weeds and birds also had a huge impact on the total number of dead fish that were documented during the disease outbreak.

In conclusion, the use of a primary nursery phase by itself can increase final standing crop and survival. Further studies are recommended to properly continue to evaluate the potential of the vaccine in earthen pond conditions.

LITERATURE CITED

Alsagoff, S. A. 1981. An evaluation of management techniques for channel catfish fry in rearing ponds. M.S. Thesis. Auburn University, Auburn, Alabama, USA.

Arias, C., T. Welker, C. Shoemaker, J. Abernathy, and P. Klesius. 2003. Genetic characterization of the fish pathogens Edwardsiella ictaluri and Flavobacterium columnare. Proceedings of the 6th International Meeting on Microbial Epidemiological Markers. Les Diablerets, Switzerland, 27-30 August 2003.

Bailey, L. M., J. C. Jones, and J. R. Morrison. 1996. Early rearing of channel catfish fry in above ground raceways and its impact on fry survival on farm fingerling production. Pages 46-49 in Ebodaghe, D., editor. Proceedings of the National Small Farm Conference. Nashville, Tennessee, USA, 10-13 September 1996. The Plant and Animal Production, Protection and Processing Division of USDA-Cooperative State Research, Education, and Extension Service, Washington DC, USA.

Bernardez, J. G. 1995. Evaluation of an in-pond raceway system and its economic feasibility for fish production. M.S. Thesis. Auburn University, Auburn, Alabama, USA.

Bosworth, B. G., W. R. Wolters, D. J. Wise, and M. H. Li. 1998. Growth, feed conversion, fillet proximate composition and resistance to *Edwardsiella ictaluri* of channel catfish, *Ictalurus punctatus* (Rafinesque), blue catfish, *Ictalurus furcatus* (Lesueur), and their reciprocal F1 hybrids fed 25% and 45% protein diets. Aquaculture Research 29:251-257.

Brown, E. E. and J. B. Gratzek. 1980. Fish farming handbook. The AVI Publishing Company, Incorporated, Westpoint, Connecticut.

Bryan, R. D. and K. O. Allen. 1969. Pond culture of channel catfish fingerlings. Progressive Fish-Culturist 31:38-41.

Bush, R. A. 1981. Enteric redmouth disease (*Yersinia ruckeri*). In: Antigens of Fish Pathogens: Development and Production for Vaccines and Serodiagnostics. Pages 201-226 *in* D. P. Anderson, M. Dorson, and P. Dubourget, editors. Collection Foundation Marcel Merieux, Lyon.

Carpenter, D. B. 2001. In-pond raceway production of channel catfish fingerlings. M.S. Thesis. Auburn University, Auburn, Alabama.

Conrath, T. B. 1972. Handbook of microtiter procedures. Dynatech Corporation, Cambridge, Massachusetts, USA.

Dunham, R. A., C. Hyde, M. Masser, J. A. Plumb, R. O. Smitherman, R. Perez, and A. C. Ramboux. 1994. Comparison of culture traits of channel catfish, *I. furcatus,* and blue catfish *I. furcatus.* Journal of Applied Aquaculture 3(4):211-222.

Dupree, H. K., O. L. Green, and K. E. Sneed. 1970. The growth and survival of Fingerling channel catfish fed complete and incomplete feeds in ponds and troughs. Progressive Fish-Culturist 32(2):85-92.

Francis-Floyd, R., M. H. Beleau, P. R. Waterstrat, and P. R. Bowser. 1987. Effect of water temperature on the clinical outcome of infection with *Edwardsiella ictaluri* in channel catfish. Journal of the American Veterinary Medical Association 191:1413-1416.

Gaikowski, M. P., C. W. Jeffrey, R. G. Endris, and W. H. Gingerich. 2003. Safety of Aquaflor (florfenicol, 50% type A medicated article), administered in feed to channel catfish, *Ictalurus punctatus.* Toxicologic Pathology 31:689-697.

Hatch, U., R. Dunham, H. Hebicha, and J. Jensen. 1987. Economic analysis of channel catfish egg, fry, fingerling, and food fish production in Alabama. Circular 291, Alabama Agricultural Experiment Station, Auburn University, Auburn, Alabama, USA.

Hawke, J. P. 1979. A bacterium associated with disease of pond cultured channel catfish, *Ictalurus punctatus.* Journal of the Fisheries Research Board of Canada 36:1508-1512.

Hawke, J. P., A. C. McWhorter, A. G. Steigerwalt, and D. J. Brenner. 1981. *Edwardsiella ictaluri* sp. Nov., the causative agent of enteric septicemia of catfish. International Journal of Systemic Bacteriology 31:396-400.

Hawke, J. P., R. M. Durborow, R. L. Thune, and A. C. Camus. 1998. Enteric septicemia of catfish. Publication no. 477, Southern Regional Aquaculture Center, Stoneville, Mississippi, USA.

Hebicha, H. A. 1984. Economic analysis of channel catfish fingerling production in Alabama. M.S. Thesis. Auburn University, Auburn, Alabama, USA.

Klesius, P. 1992. Immune system of channel catfish: an overture on immunity to *Edwardsiella ictaluri.* Annual Review of Fish Diseases. Pages 325-338. Pergamon Press, Oxford, United Kingdom.

Klesius, P. H. and C. A. Shoemaker. 1997. Heterologous isolates challenge of channel catfish, *Ictalurus punctatus,* immune to *Edwardsiella ictaluri.* Aquaculture 157:147-155.

Klesius, P. H. and C. A. Shoemaker. 1999. Development and use of modified live *Edwardsiella ictaluri* vaccine against Enteric septicemia of catfish. Pages 523-537 *in* R. D. Schultz, editor. Veterinary vaccines and diagnostics. Advances in Veterinary Science and comparative Medicine, volume 41. Academic Press, San Diego, California, USA.

Lawrence, M. L., K. C. Cooper, and R. L. Thune. 1997. Attenuation, persistence, and vaccine potential of an *Edwardsiella ictaluri purA* mutant. Infection and Immunity 65(11):4642-4651.

Lim, C. and P. H. Klesius. 2003. Influence of feed deprivation on hematology, macrophage chemotaxis, and resistance to *Edwardsiella ictaluri* challenge of channel catfish. Journal of Aquatic Animal Health 15:13-20.

Lock, J. T. 1973. Tank culture, problems and solutions. Proceedings of the Commercial Fish Farming Conference, Texas A&M University pp. 24-28

MacMillan, J. R. 1985. Infectious diseases. Pages 405-496 *in* C. S. Tucker, editor. Channel Catfish Culture. Elsevier, New York, New York, USA.

McGinty, A. S. 1980. Survival, growth, and variation in growth of channel catfish fry and fingerlings. Ph.D. Dissertation. Auburn University, Auburn, Alabama, USA.

Meyer, F. P., K. E. Sneed, and P. T. Eschmeyer, eds. 1973. Second report to the fish farmers: the status of warmwater fish farming and progress in fish farming research. Bureau of Sport Fisheries. Wildlife Resources Publication 113, USDA, Washington D. C. 123 pp.

Morrison, J. R., W. L. Deavours, J. C., and M. A. Tabb. 1995. Early rearing of channel catfish in fry in floating raceways and subsequent survival in ponds. The Progressive Fish-Culturist 57:292-296.

Newton, J. C., L. C. Wolfe, J. M. Grizzle, and J. A. Plumb. 1989. The pathology of experimental enteric septicemia in channel catfish, *Ictalurus punctatus* (Rafinesque), following immersion-exposure to *Edwardsiella ictaluri.* Journal of Fish Diseases 12:335-347.

Petrie-Hanson, L. and A. J. Ainsworth. 1999. Humoral immune responses of channel catfish (*Ictalurus punctatus*) fry and fingerlings exposed to *Edwardsiella ictaluri.* Fish and Shellfish Immunology 9:579-589.

Piper, R. G., I. B. McElwain, L. E. Orne, J. P. McCraren, L. G. Fowler, and J. R, Leonard. 1982. Fish hatchery management. U. S. Department of the Interior, U.S. Fish and Wildlife Service, Washington, D.C.

Plumb, J. A. 1988. Vaccination against *Edwardsiella ictaluri.* Pages 152-161 *in* T. Ellis, editor. Fish Vaccination. Academic Press, London, England.

Plumb, J. A. and S. Vinitnantharat. 1989. Biochemical, biophysical, and serological homogeneity of *Edwardsiella ictaluri.* Journal of Aquatic Animal Health 1:51-56.

Plumb, J. A. and S. Vinitnantharat. 1993. Vaccination of channel catfish, *Ictalurus punctatus* (Rafinesque) by immersion and oral booster against *Edwardsiella ictaluri.* Journal of Fish Diseases 16:65-71.

Plumb, J. A., S. Vinitnantharat, V. Abe. and R. P. Phelps. 1994. Density-dependent effect on oral vaccination of channel catfish against *Edwardsiella ictaluri.* Aquaculture 122: 91-96.

Rodgers, L. J. 1994. Spatial distribution of channel catfish fry in earthen nursery ponds. M.S. Thesis. Auburn University, Auburn, Alabama, USA.

Shoemaker, C. A., P. H. Klesius, and J. M. Bricker. 1999. Efficacy of a modified live *Edwardsiella ictaluri* vaccine in channel catfish as young as seven days post hatch. Aquaculture 176:189-193.

Shoemaker, C. A., P. H. Klesius, and J. J. Evans. 2002. In ovo methods for utilizing the modified live *Edwardsiella ictaluri* vaccine against enteric septicemia in channel catfish. Aquaculture 203:221-227.

Shotts, E. B., V. S. Blazer, and W. D. Waltman. 1985. Pathogenesis of experimental *Edwardsiella ictaluri* infections in channel catfish (*Ictalurus punctatus*). Canadian Journal of Fisheries and Aquatic Sciences 43:36-42.

Silva de Gomez, A. 1990. Influence of shelter and feeding practices on channel catfish fingerling production. M.S. Thesis. Auburn University, Auburn, Alabama, USA.

Smitherman, R. O., R. A. Dunham, and D. Tave. 1983. Review of catfish breeding research 1969-81 at Auburn University. Aquaculture 33:197-205.

Snow, J. R. 1962. A comparison of rearing methods for channel catfish fingerlings. Progressive Fish-Culturist 24:112-118.

Steinbach, D. W. 1977. An intensive culture system for channel catfish fingerlings. Proceedings of the Annual Conference of South Eastern Fish and Wildlife Agencies 31:484-492.

Stickney, R. R., T. Murai, and G. O. Gibbons. 1972. Rearing channel catfish fingerlings under intensive culture conditions. The Progressive Fish-Culturist 34(2):100-102.

Stock, I. and B. Wiedmann. 2001. Natural antibiotic susceptibilities of *Edwardsiella tarda, E. ictaluri,* and *E. hoshinae.* Antimicrobial Agents and Chemotherapy 45(8):2245-2255.

Tamassia, S. T. J. 1993. Techniques for enumeration of channel catfish sac fry and the impact of initial confinement of fry on growth and survival in primary nursery ponds. M.S. Thesis. Auburn University, Auburn, Alabama, USA.

Thune, R. L., J. P. Hawke, and M. C. Johnson. 1994. Studies on vaccination of channel catfish, *Ictalurus punctatus,* against *Edwardsiella ictaluri.* Journal of Applied Aquaculture. 3(1/2):11-23.

Thune, R. L., L. A. Collins, and M. P. Pena. 1997. A comparison of immersion, immersion/oral combination and injection methods for the vaccination of channel catfish, *Ictalurus punctatus* against *Edwardsiella ictaluri.* Journal of the World Aquaculture Society 28:193-201.

Tucker, C. S. and E. H. Robinson. 1990. Channel catfish farming handbook. Van Nostrand Reinhold, New York, New York, USA.

USDA (United States Department of Agriculture). 1997. Part 1: reference of 1996 U.S. catfish health and production practices. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health, National Animal Health Monitoring System, Forth Collins, Colorado, USA.

USDA (United States Department of Agriculture). 2005. Catfish production. National Agricultural Statistics Service, United States Department of Agriculture, Washington, D.C., USA.

Wagner, B. A., D. J. Wise, L. H. Khoo, and J. S. Terhune. 2002. The epidemiology of bacterial diseases in food-size channel catfish. Journal of Aquatic Animal Health 14:263-272.

Wilson, R. P. and W. E. Poe. 1989. Palatability of diets containing sulfadimethoxine, ormetoprim, and romet-30 to channel catfish. Progressive Fish-Culturist 51:226-228.

Wise, D. J. and M. R. Johnson. 1998. Effect of feeding frequency and romet-medicated feed on survival, antibody response, and weight gain of fingerling channel catfish *Ictalurus punctatus* after natural exposure to *Edwardsiella ictaluri.* Journal of the World Aquaculture Society 29(2):169-175.

Wise, D. J. and J. S. Terhune. 2001. The relationship between vaccine dose and efficacy in channel catfish *Ictalurus punctatus* vaccinated as fry with live attenuated strain of *Edwardsiella ictaluri* (RE-33). Journal of the World Aquaculture Society 32(2):177-183.

Wise, D. J., P. H. Klesius, C. A. Shoemaker, and W. R. Wolters. 2000. Vaccination of mixed and full-sib families of channel catfish with a live attenuated *Edwardsiella ictaluri* isolate (RE-33). Journal of the World Aquaculture Society 31(2):206-212.

Wolfinger, R. D., R. D. Tobias, and J. Sall. 1991. Mixed models: A future direction. Proceedings of the Sixteenth Annual SAS Users Group Conference, SAS Institute Inc., Cary, N C 1380-1388.

Wolters, W. R., D. J. Wise, and P. H. Klesius. 1996. Survival and antibody response of channel catfish, blue catfish and hybrids of female channel catfish and male blue catfish following exposure to *Edwardsiella ictaluri.* Journal of Aquatic Animal Health, in press.

Yant, D. R., R. O. Smitherman, and O. L. Green. (1975). Production of hybrid (blue x channel) catfish and channel catfish in ponds. Proceedings of the Annual Conference of Southeastern Association of Game and Fish Commissioners 29:82-86.