2010-01-01

Optimizing Transport of Live Juvenile Cobia (Rachycentron canadum): Effects of Salinity and Shipping Biomass

John Dommerich Stieglitz
University of Miami, jstieglitz@rsmas.miami.edu

Recommended Citation

Stieglitz, John Dommerich, "Optimizing Transport of Live Juvenile Cobia (Rachycentron canadum): Effects of Salinity and Shipping Biomass" (2010). *Open Access Theses*. 21.
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UNIVERSITY OF MIAMI

OPTIMIZING TRANSPORT OF LIVE JUVENILE COBIA (*RACHYCENTRON CANADUM*): EFFECTS OF SALINITY AND SHIPPING BIOMASS

By

John D. Stieglitz

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

May 2010

UNIVERSITY OF MIAMI

A thesis submitted in partial fulfillment of
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OPTIMIZING TRANSPORT OF LIVE JUVENILE COBIA (*RACHYCENTRON CANADUM*): EFFECTS OF SALINITY AND SHIPPING BIOMASS

John D. Stieglitz

Approved:

Daniel D. Benetti, Ph.D. Professor of Marine Affairs & Policy	Terri A. Scandura, Ph.D. Dean of the Graduate School
Joseph E. Serafy, Ph.D. Research Professor of Marine Biology & Fisheries	Kenneth Broad, Ph.D. Professor of Marine Affairs & Policy

STIEGLITZ, JOHN D. (M.S., Marine Affairs and Policy)
Optimizing Transport of Live Juvenile Cobia (May 2010)
(*Rachycentron canadum*):
Effects of Salinity and Shipping Biomass

Abstract of a thesis at the University of Miami.

Thesis supervised by Professors Daniel D. Benetti and Joseph E. Serafy.
No. of pages in text. (42)

Live juvenile cobia (*Rachycentron canadum*) transport methods were examined to determine opportunities for increasing packing density in closed containers for temporal durations up to 24 hours. Juvenile cobia (27 to 46 days-post-hatch (dph)) were tested for salinity tolerance following abrupt transfer from 35 ppt salinity water to salinities ranging from 0 ppt to 55 ppt. Results indicate a wide range of tolerance, with 100% survival at 24 hours post-transfer in salinities between 11 ppt and 45 ppt. Salinity preference was also tested to determine a possible correlation between acclimation salinity and salinity preference using an experimental horizontal salinity gradient with juvenile cobia (87 dph) over a period of 24 hours. Results of the salinity preference trials showed that salinity preference was directly related to acclimation salinity. Using two different salinities within the range tested in the tolerance trials (12 ppt and 32 ppt), a 24 hour simulated shipping trial was conducted comparing final survival between the two salinities at each of four packing densities (5 kg/m^3, 10 kg/m^3, 15 kg/m^3, and 20 kg/m^3). Results indicated a significant relationship between salinity and stocking density on survival of juvenile cobia following a 24 hour simulated shipment. At packing densities above 10 kg/m^3,

survival was significantly higher in the low salinity (12 ppt) treatments as compared to survival rates in the higher salinity (32 ppt) treatments. To help aquaculture professionals make accurate and economical decisions regarding the shipment of live juvenile cobia in closed containers, a bioeconomic model was constructed using survival data at different packing densities (1 kg/m^3 to 20 kg/m^3) and salinities (12 ppt and 32 ppt) obtained in the experimental trials combined with shipping cost and fingerling price data. The resulting model enables cobia fingerling producers to optimize their shipping methods and protocols, allowing for reductions in labor and material costs.

To my family, without your love and support this would not have been possible
…and to Robbie, my newborn son.

ACKNOWLEDGEMENTS

I would like to thank my committee members, Dr. Daniel Benetti, Dr. Joe Serafy, and Dr. Kenny Broad for their guidance throughout my graduate education. Also, before I was ever a graduate student at the University of Miami, Dr. Jerry Lorenz at Audubon of Florida's Tavernier Science Center and Dr. Joe Serafy at NOAA were both instrumental in shaping my research interests and goals, for which I am thankful.

At RSMAS, I am very grateful to Maria Estevanez who selflessly gave her time, advice, and support over the years. Dr. Juan Agar also provided valuable advice as I worked towards completion of this project, and Dr. Joe Serafy provided invaluable assistance with experimental design and statistical analysis. Of course, none of this would have been possible without the tireless support of Dr. Daniel Benetti, whose passion for aquaculture and fostering of my research interests made this project a reality. I simply could not have asked for a better advisor and mentor, and I continue to value his guidance and support, as a student, friend, and colleague.

I am also very thankful for the guidance and analytical assistance of Dr. Gavin Partridge of the Challenger Institute of Technology in Fremantle, Australia, and for the technical assistance provided by Brian Teare and Xaymara Serrano. Finally, I am grateful to my colleagues, Aaron Welch and Ron Hoenig for their help with this project, and all of the University of Miami Experimental Hatchery staff and volunteers: Bruno Sardenberg, Sasa Miralao, Patrick Brown, Dan Farkas, Bryant Bookhamer, and Carlos Fuentevilla for their assistance. Funding for this project was provided by NOAA. Last, but certainly not least, I thank my family for their unfailing support and love throughout this journey, especially my wife, Amanda.

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CHAPTER 1: INTRODUCTION

BACKGROUND

Aquaculture has rapidly become the fastest growing sector of the food production industry, with a compounded growth rate of 9.2% per year since 1970, compared with only 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production systems (Gatlin et al., 2007). The industry is increasingly turning towards the culture of high-value carnivorous marine finfish species. Accompanying the growth of the aquaculture industry is an increase in the transportation of live fish over extended periods of time (>8 hours). In many cases the grow-out sites for farmed fish (ponds, cages, net pens, etc.) are distant from the hatchery or nursery location that supplies the juvenile fish. This necessitates the transportation of mass quantities of fish by land, sea, and air freight (Harmon, 2009). Shipping costs can be substantial, as a result of the aquatic media within which fish must be shipped (Guo et al., 1995; Lim et al., 2003; Paterson et al., 2003). Therefore, it is in the best interest of buyers and sellers to transport fish in ways that minimize shipping costs while maximizing fish survival (Norris et al., 1960; Lim et al., 2003; Harmon, 2009). Traditionally, freshwater and marine fish have been transported in both open and closed systems (Amend et al., 1982; Berka, 1986), using techniques to minimize stress and increase survival of the fish before, during, and after the transportation period (Carmichael et al., 1984; Weirich and Tomasso, 1991; Weirich et al., 1992; Gomes et al., 2003; Harmon, 2009). There is a plethora of scientific literature devoted to fish physiology and the effects of alterations in water quality, temperature, salinity, pH, ammonia, and the use of anesthetics for fish. However, with the ever-increasing variety of species being cultured for both the ornamental and food fish markets, there is no "standard" shipping methodology that applies to all species (Emata, 2000). Nearly all aspects of fish transportation are aimed at reducing the metabolic costs of the fish while supplying the necessary elements for survival in a confined space (Durve, 1975; Weirich et al., 1992; Guo et al., 1995; Gomes et al., 2003; Paterson et al., 2003; Colburn et al., 2008; Harmon, 2009). Fish farmers also need to be conscious of "batch variability" when it comes time to transport fish, as variations in genetic makeup, feeding regime, culture conditions, or size distribution can all have marked impacts on the overall success of live fish transport. The difference between shipping success and failure typically comes down to the small variations between shipping methods and the physiological tolerance levels of the species being transported (Pennell, 1991; Weirich et al., 1992; Chow et al., 1994; Paterson et al., 2003; Pavlidis et al., 2003; Harmon, 2009).

PROBLEM ADDRESSED

Cobia (*Rachycentron canadum*), a large migratory pelagic teleost (Shaffer and Nakamura, 1989), has been identified as one of the most promising species for open ocean marine finfish aquaculture due to their high growth rates, excellent palatability, and resilience in cage culture (Benetti et al., 2006; Faulk and Holt, 2006; Colburn et al., 2008). However, the larviculture of the species remains an industry bottleneck (Benetti et al., 2008a), with many offshore aquaculture operators failing to achieve economically viable results in this portion of commercial aquaculture operations. Juvenile cobia have been raised successfully at the University of Miami Experimental Hatchery (UMEH) in commercial quantities (i.e. sufficient to stock numerous net pen operations) (Benetti et al., 2007; 2008a; 2008b). Various quantities of these fish have been successfully shipped to aquaculture operators throughout the Americas using protocols developed at UMEH, whereby fish are shipped at an average biomass of 5 – 7.5 kg/m^3 in 1000-L transport boxes (SpaceKraft®;www.spacekraft.com) using 300 L of 26 - 35 ppt UV filtered seawater at 20-22°C with virtually no mortality over 24-36 hours (*personal observations*).

However, as the aquaculture industry and the market for live ornamental and food-fish has grown, advancements in fish packaging and shipping methodologies have progressed to allow for shipments of greater density (high biomass relative to water volume) over extended periods of time (>8 hours) (Lim et al., 2003). The shipping biomass levels reported for selected species of live tropical marine fish greatly exceed those utilized by UMEH for shipments of juvenile cobia. A review of literature regarding simulated shipments of juvenile cobia in closed systems suggests that 25 kg/m^3 is the highest biomass that has been tested. However, Colburn et al. (2008) suggest that there were significant mortalities at this biomass and they did not recommend exceeding 20 kg/m^3 for shipping live juvenile cobia. The Colburn et al. (2008) methodology fails to determine optimal shipping parameters for juvenile cobia, such as optimal salinity and biomass. The present study focused on water salinity and shipping biomass to discover optimal levels for juvenile cobia shipment for durations up to 24 hours, while utilizing UMEH tested and manufacturer recommended dosages of water chemistry buffer additives (McFarland and Norris, 1958; 1961).

Following the experimental trials used to determine optimal salinity and biomass packing levels for juvenile cobia, the optimized parameters and shipping cost data were incorporated into a bioeconomic model. This model allowed for the input of key variables, such as average fish weight (g), salinity of transport water (either 32 ppt or 12 ppt), market price per fish, and all shipping costs (air freight, boxes, bags, etc.). Through analysis of the model results and graphical output, the bioeconomic model allows UMEH staff to make case-specific shipping decisions to allow for the optimal use of resources and a reduction in transport costs to the buyer and seller.

PREVIOUS STUDIES

While previous peer-reviewed studies focused on the transport of live juvenile cobia have measured parameters examined in this study, to date no study has determined the optimal shipping parameters for this species. The most recent publication (Colburn et al., 2008) demonstrated success at shipping biomass levels up to 20 kg/m^3, though mortality increased significantly during trials using higher biomass (25 kg/m^3) over extended periods of time (24 hours). The University of Miami Experimental Hatchery (UMEH) has also had considerable success shipping 1-5 g (25 – 50 days-post-hatch (dph)) juvenile cobia at biomass levels up to 7.5 kg/m^3 (*personal observations*), using methods adapted from Benetti et al. (2007), but it is anticipated that biomass levels could be increased significantly by adjusting water quality parameters to further minimize stress and metabolic costs for the fish.

Traditionally, the aquaculture industry has utilized methods to reduce the metabolic costs for fish during shipment that include increasing the salinity for shipping freshwater fish and decreasing the salinity for marine fish. Such practices are applied to decrease the osmoregulatory cost to the fish. Alterations of salinity have also proven to affect fish growth in relation to salinity (Bouef and Payan, 2001), with higher growth rates exhibited at salinities closer to isosmotic levels. However, while there have been studies conducted on salinity tolerances of juvenile cobia (Denson et al., 2003; Atwood et al., 2004; Resley et al., 2006; Faulk and Holt, 2006; Burkey et al., 2007), none of the previous studies compare effects of significantly reduced salinity (<20 ppt) on simulated shipping survivability. Mortality curves are commonly constructed for euryhaline species, whereby salinity tolerance levels are correlated with changes in the osmotic concentration of the blood and/or survivability of the fish (Blaber, 1973; Whitfield and Blaber, 1976; Martin, 1988). This study utilized methods similar to those used in the aforementioned studies to conduct the salinity tolerance trials with juvenile cobia.

Additionally, salinity preference is often neglected when examining the salinity tolerance of a euryhaline species. While cobia may be able to tolerate a particular range of salinities following abrupt transfer, the salinity preference of this species following abrupt transfer to a salinity gradient has not been tested. Many operators engaged in live fish transport believe that fish must be acclimated to a particular salinity prior to transport in order to reduce osmotic stress and ensure survival. Through the use of two different groups of fish acclimated to two different salinities (12 ppt and 34 ppt), it was possible to test for any differences in salinity preferences amongst acclimation groups. These types of analyses have never been completed for the juvenile stage of this species, and the results allowed for improved shipping conditions for juvenile cobia.

While numerous aspects of juvenile pelagic fish stress physiology have been tested (Morgan et al., 1996; Idrisi et al., 2003; Atwood et al., 2004; Burkey et al., 2007; Feeley et al., 2007), there is little scientific documentation of simulated shipping trials

Publication	Species	Size Range	Salinity (ppt)	Temperature (°C)	Biomass Level (kg/m3)	pH	Ammonia /Nitrite	Blood Osmolality	Buffers	Duration of Treatment	Shipping Specific
Colburn et al. (2008)	Cobia (*Rachycentron canadum*)	1.5 - 3.0 g (60 dph)	26	19, 21, 25	5, 10, 15, 20, 25	X			Trizma (8.3 and 7.4) and Amquel	24 hours	X
Rodrigues et al. (2007)	Cobia (*Rachycentron canadum*)	0.82 - 1.85 g	22	26		X	X			96 hours	
Benetti et al. (2007)	Cobia (*Rachycentron canadum*)	1.0 – 5.0 g	32	14 - 24	0.7 - 7.12	X	X		Trizma (8.3 and 7.4) and Amquel	12 - 20 hours	X
Burkey et al. (2007)	Cobia (*Rachycentron canadum*)	9.0 – 43.0 g	2 to 14	24 - 26				X		Variable for each trial	
Resley et al. (2006)	Cobia (*Rachycentron canadum*)	6.0 – 6.7 g	5, 15, 30	27		X	X	X		8 weeks	
Sun et al. (2006)	Cobia (*Rachycentron canadum*)	22.0 g	31.2 - 33.4	23, 27, 31, 35							
Atwood et al. (2004)	Cobia (*Rachycentron canadum*)	1.4 - 41.6 g	2 to 20	22.6 - 27.3		X	X			Variable for each parameter tested	
Denson et al. (2003)	Cobia (*Rachycentron canadum*)	8.5 g (120 dph)	5, 15, 30	23.9		X	X	X		10 weeks	

Table 1.1. Previous Studies: published accounts of juvenile cobia (>1 g) shipping and/or physiological tolerance trials.

using high densities of pelagic teleost fish. The few studies that have been published on cobia (Table 1.1) focus primarily on shipping and/or physiological tolerances of this species at varying juvenile life stages (Denson et al., 2003; Atwood et al., 2004; Resley et al., 2006; Sun et al., 2006; Benetti et al., 2007; Burkey et al., 2007; Rodrigues et al., 2007; Colburn et al., 2008), though none of the studies objectively evaluate the variables examined in this study. All fish transport studies reviewed incorporate methodologies to reduce the metabolic rate and stress of the fish, yet this study differed from previous research in that experimental trials focused on the specific variables of salinity and biomass (while using buffer additives in the shipping water) to develop an optimal shipping environment for juvenile cobia. Though shipping biomass is dependent on numerous other factors (water quality, salinity, temperature, pH, additives, etc.), the results of the salinity tolerance trials were incorporated into the simulated shipping trials in an effort to evaluate the survivability of juvenile cobia at different shipping biomass levels in relation to packing-water salinity. Relative survivability was used as the metric to evaluate the effectiveness of each experimental trial as this study represents an initial attempt to significantly improve the shipping of live juvenile cobia and it also represents the first documented effort to simulate shipment of cobia under isosmotic salinity conditions for up to 24 hours.

OBJECTIVES

The goal of this research was to examine the effects of shipping variables on the survival of juvenile cobia. While cobia fingerlings are routinely shipped at biomass levels ranging from 4 - 7.5 kg/m^3, the shipping expense remains a significant cost for producers and operators. Recently, scientific literature suggesting successful simulated shipping trials of juvenile cobia at biomass levels up to 20 kg/m^3 was reported (Colburn et al., 2008). Such success represents the potential for significant savings on behalf of the producers and operators, as the cost of supplies and freight would be significantly lower at increased shipping biomass. A three-phase experimental design was used to test the following: the effects of salinity on juvenile cobia survival following abrupt transfer to a wide range of salinities, the behavioral preference of juvenile cobia exposed to a horizontal salinity gradient, and the effects of salinity and biomass levels on survival of juvenile cobia using a simulated box shipping trial.

Following the three-phased experimental plan, the survival results from the simulated box shipping trial were incorporated into a bioeconomic model that was designed to assist determination of optimal shipping biomass and salinity levels for juvenile cobia. All costs related to shipping live juvenile cobia over extended time periods (24 hours) were incorporated into the model, in addition to the costs associated with mortalities. Dynamic economic and biological factors, such as average fish weight (in grams) and the price per fingerling (in USD), were built into the model to allow for these variables to change on a per shipment basis. The objective in using this decision-assistance tool was to allow aquaculturists interested in shipping live juvenile cobia to effectively plan for material and labor needs while reducing their cost of shipping live juvenile cobia.

CHAPTER 2: MATERIALS AND METHODS

SALINITY TOLERANCE TRIALS

Juvenile cobia ranging in age from 27 dph to 46 dph were obtained from the University of Miami Experimental Hatchery (UMEH) and were held in a flow-through 2,200-L fiberglass tank equipped with aeration and supplemental oxygen. Water used at UMEH is pumped from adjacent coastal Atlantic/Biscayne Bay waters into a settling tank, two broken glass media solids filters, a UV filter, and finally through 10µm filter bags prior to use in holding or experimental tanks. Mean water quality parameters in the holding tank prior to starting experimental trials were as follows: salinity, 35 ppt; dissolved oxygen, 7.62 mg/L; temperature, 29.1°C; and pH, 7.94. During the holding timeframe prior to experiments the fish were fed a commercial dry pellet diet (Otohime C-2 and EP-1) to satiation 1-2 times per day.

To test the tolerance of cobia to abrupt changes in salinity, three separate, but nearly identical, experimental trials were conducted. The juvenile cobia in the holding tank were fasted for a period of 24 hours prior to use in the experimental trials to allow for the gut to empty and to reduce ammonia excretion. Using randomly selected juvenile cobia from the holding tank, 180 fish were used in each trial. 20-L buckets (18 total) were used as replicate experimental chambers and each was filled with 15-L of water that was adjusted to the desired salinity. Tap water that was dechlorinated with sodium thiosulfate (2.5g/100L) was used to obtain the low salinity treatments (0, 5, and 11 ppt) and Instant Ocean® synthetic sea salt was added to the 35 ppt seawater to obtain the high salinity treatments (40, 45, 50, and 55 ppt). For each experimental trial, the 3 buckets with 35 ppt salinity water were used as the control group for the experiments, as this water was the same salinity as that of the holding tank, to which the cobia were already acclimated. Continuous aeration was supplied to each bucket using a small air stone and the aeration system present at UMEH. Prior to addition of any fish and at the conclusion of the experiment, the water quality parameters of each bucket were recorded (temperature, dissolved oxygen, pH, and salinity). Juvenile cobia were selected at random from the holding tank and placed into the buckets at a density of 10 fish per bucket. A 95% shade cloth was placed over the tanks for the entire 24 hour duration of each trial. The fish were exposed to a natural photoperiod throughout the trial and behavioral differences between treatments were noted following the initial 1 - 2 hours of the experiment. The salinities (ppt) tested in the trials were as follows: Trial 1 (0, 5, 11, 35, 40, 45); Trial 2 (0, 5, 11, 35, 45, 50); Trial 3 (0, 5, 11, 35, 45, 55), and each salinity was tested in triplicate during each trial. The metric used to determine tolerance was survival following 24 hour immersion in the treatment salinities, expressed as a percentage. The following describes specific differences amongst the trials, as the salinity tolerance experiments were conducted using an adaptive methodology in which results from previous trials were analyzed and salinity parameters were adjusted in subsequent trials accordingly.

Experiment #1

Using the aforementioned experimental design, 18 buckets were divided into 6 treatment groups (3 buckets in each group) according to the water salinity (ppt): 0, 5, 11, 35, 40, and 45. The buckets (3) with 35 ppt water were used as the controls for the experiment, as described previously. 180 juvenile cobia (27 dph; 7.24 cm ± 0.78 cm TL;

1.07 g ± 0.35 g; 2.71 c.f. ± 0.22) were randomly selected from the holding tank and 10 fish were directly placed into each of the 18 experimental chambers. Following the experimental design described above, the fish were kept in the treatment chambers for 24 hours, after which survival was enumerated and water quality parameters for each chamber were recorded.

Experiment #2

This experimental trial was exactly the same as the Experiment #1, except for the treatment salinities and the age of the fish used in the chambers. Again, 180 juvenile cobia (46 dph, 9.32 cm ± 1.34 cm TL; 3.31 g ± 1.54 g; 3.85 c.f. ± 0.23) were randomly selected from the holding tank and 10 fish were directly placed into each of the 18 experimental chambers. The 18 buckets were divided into 6 treatment groups (3 buckets in each group) according to the water salinity (ppt): 0, 5, 11, 35, 45, and 50. The buckets (3) with 35 ppt water were used as the controls for the experiment, as described previously. Following the experimental design described above, the fish were kept in the treatment chambers for 24 hours, after which survival was enumerated and water quality parameters for each chamber were recorded.

Experiment #3

This experimental trial was exactly the same as the Experiment #1 and #2, except for the treatment salinities and the age of the fish used in the chambers. Again, 180 juvenile cobia (33 dph, 6.06 cm ± 0.82 cm TL; 0.66 g ± 0.29 g; 2.78 c.f. ± 0.53) were randomly selected from the holding tank and 10 fish were directly placed into each of the

18 experimental chambers. The 18 buckets were divided into 6 treatment groups (3 buckets in each group) according to the water salinity (ppt): 0, 5, 11, 35, 45, and 55. The buckets (3) with 35 ppt water were used as the controls for the experiment, as described previously. Following the experimental design described above, the fish were kept in the treatment chambers for 24 hours, after which survival was enumerated and water quality parameters for each chamber were recorded.

Data Analysis

To reveal the overall pattern of salinity tolerance for juvenile cobia, the relationship between the independent variable of salinity and the dependent variable of survival was analyzed. Logistic regression was performed on binary survival data with salinity and salinity squared as the factors. The fit of the logistic model was determined from its final model concordance index (C), which ranges from 0.5 (poor) to 0.99 (best).

BEHAVIORAL RESPONSE TO SALINITY GRADIENT

Experimental Aquarium

In this study, the response of cobia to a horizontal salinity gradient was examined using 5 specially-built 55 gallon (208.2 L) aquaria (119.5 cm X 30.5 cm X 39.0 cm)(Figure 2.1). Adapting methods used in previous studies examining salinity preference of aquatic animals (Staaland, 1969; Keiser and Aldrich, 1976; Stephenson and Knight, 1982; Bos and Thiel, 2006), each aquarium used in this experiment was divided into five U-shaped chambers using glass dividers sealed in place with aquarium sealant. Each U-shaped chamber was connected to adjacent chambers by a water column of 3 cm at the top of alternating glass dividers. This connective column of water could be disconnected from the adjacent U-shaped chambers by inserting vinyl dividers on top of the glass dividers. Each of the five U-shaped chambers held approximately 11 gallons (41.6 L) of water at the following mean salinities: 0, 11, 20, 26, and 34. The 5 aquariums (N=10 for each mean salinity) were setup on the floor of a windowless laboratory, allowing for the experimental trials to be run in complete darkness for the 24 hour duration of each trial.

Salinity Gradient

The salinity gradient was created in each aquarium using dechlorinated tap water (chlorine removed using active charcoal filtration and extended aeration) and filtered seawater from coastal Atlantic/Biscayne Bay waters. The vinyl dividers were set in place to disconnect each adjacent U-shaped chamber from one another. Using pre-determined ratios of salt to fresh water, each chamber was filled with 11 gallons (41.6 L) of water to obtain the following mean salinities, listed in horizontal order from low to high: 0, 11, 20, 26, and 34. Following initial scoping trials to determine optimal salinity measurement location, the salinity for each U-shaped chamber was measured in the bottom third of the chamber using a YSI 550 salinity probe. Due to the relatively short duration of each trial, as compared to those conducted by Bos and Thiele (2006), and swimming activity of the juvenile cobia, significant haloclines did not develop and salinity measurements of the gradient remained consistent throughout the scoping and experimental trials. The horizontal direction in which the salinity gradient was established in each aquarium was reversed after the first two trials to allow each salinity-acclimated group of fish (12 ppt and 34 ppt) to start the experimental trials at opposite ends of the room as compared to previous trials.

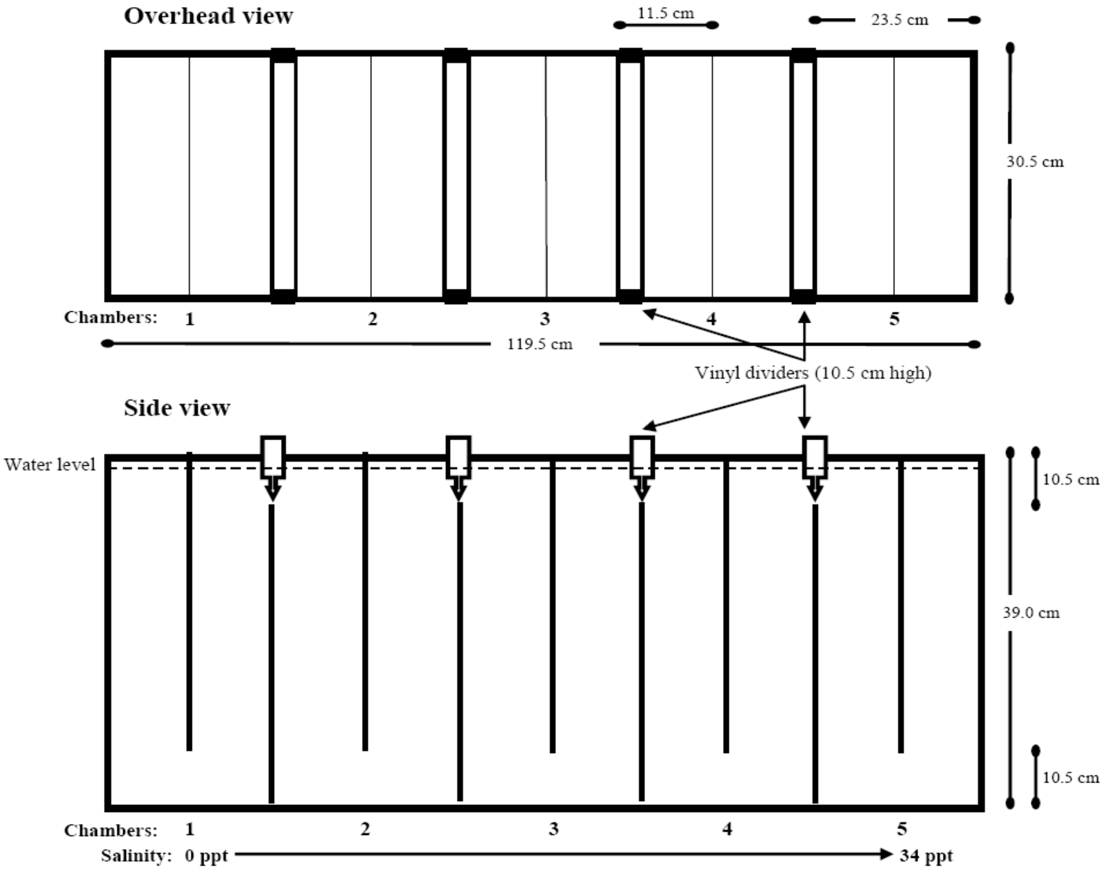

Figure 2.1. Overhead and side view diagrams of the experimental aquaria used in the salinity preference experiments. The five U-shaped chambers in the top view and side view (1 – 5) correspond to the segments used to make the salinity gradients used in this experiment (0 – 34 ppt). The vinyl dividers were put in place during the establishment of the salinity gradient, after which they were removed to allow the fish to swim throughout all the U-shaped chambers in the aquarium.

Introduction of juvenile cobia to the salinity gradient

Juvenile cobia (87 dph; 11.6 cm ± .86 cm TL) were obtained from the University of Miami Experimental Hatchery (UMEH) and were held in two 2,200-L fiberglass tanks equipped with aeration. One of the 2,200-L tanks held fish at an ambient water salinity of 34 ppt under flow-through conditions while the other 2,200-L tank was set at a mean salinity of 12 ppt through the use seawater that was diluted with the addition of dechlorinated tap water. The water in the 12 ppt tank was changed completely every other day and juvenile cobia were allowed to acclimate for an average period of 3 days prior to use in each salinity gradient experimental trial. The juvenile cobia were fed *ad libitum* on Zeigler^{TM} Marine Grower Silver diet once per day. Following establishment of the salinity gradient (see above) one juvenile cobia per aquarium was introduced to the starting chamber (U-segment). The chamber with salinity closest to the acclimation salinity was used as the starting point for the fish. Chamber 2 (mean salinity of 11.17 ppt) was used as the starting point for the fish that had been kept at 12 ppt in the 2,200-L holding tank and chamber 5 (mean salinity of 34.73 ppt) was used as the starting point for the fish that had been kept at 34 ppt in the flow-through 2,200-L tank. The chambers in the aquaria were numbered 1 – 5, with chamber 1 in each aquarium representing the low salinity side of the gradient (mean salinity of 0.37 ppt) and chamber 5 in each aquarium representing the high salinity side (mean salinity of 34.73 ppt). Prior to removal of the vinyl dividers, the juvenile cobia were acclimated to the experimental aquarium for 24 hours, and supplemental aeration was provided in the chamber housing each fish. Following the 24 hour acclimation period the aeration stone was removed and water quality parameters (salinity in ppt, temperature, dissolved oxygen in mg/L and percent saturation) were recorded. Subsequently, the vinyl dividers were removed from each aquarium and cardboard covers were placed on top of the tanks to prevent the fish from jumping. Tanks remained undisturbed for 24 hours in complete darkness to simulate shipping conditions for the juvenile cobia and to eliminate any biases based on visual or photoperiodic cues to the fish.

Data Analysis

The results were analyzed using one-way ANOVAs ($p < 0.05$) to test for variability between treatments and water quality parameters. Any significant differences found in the ANOVA results were analyzed for determination of biological significance. Data were subsequently analyzed using a one-sided median 2-sample test. P values were considered significant when less than 0.05.

SIMULATED SHIPPING TRIALS

In this trial, the effects of decreased water salinity and increased packing biomass on the survival of juvenile cobia following a 24 hour simulated shipment in sealed containers were tested. Utilizing shipping methods described by Benetti et al.(2007), 47 dph juvenile cobia (7.33 cm ± 1.01 cm TL; 1.65 g ± 0.7 g; 3.99 c.f. ± 0.33 c.f.) were fasted for 24 hours prior to use in the experimental trial. Using 12 Styrofoam coolers inside cardboard boxes (39 cm x 71 cm x 43 cm), fish were split into two polyethylene bags (24 bags total) within each box, with each bag containing 12-L of water at the desired salinity (either 32 ppt or 12 ppt). Tap water that was dechlorinated with sodium thiosulfate (2.5 g/100 L) was used to obtain the low salinity treatment (12 ppt) and filtered seawater (solids removed and UV sterilized) was used for the control salinity treatment (32 ppt). In order to maintain sufficient water quality within each bag the following water conditioners were used in equivalent concentrations for each bag: 4 g of

Trizma® Pre-Set crystals FISH grade, Type 8.3-FT, pH 8.3; and 1.2 g of AmQuel® Plus (Kordon LLC). Mean water quality parameters in the bags upon closure were as follows: Temperature 19.28°C ± 0.72°C; Dissolved Oxygen 31.20 mg/L ± 3.67 mg/L; pH 8.32 ± 0.10). The fish were netted out of the holding tank (32 ppt salinity water that was gradually cooled to 19°C) and placed in buckets partially filled with the same tank water which were weighed on a digital scale to the closest 0.01 g. Each bag was filled with the desired shipping biomass level (kg/m^3) and the air space above the water in each bag was filled with pure oxygen prior to tying off each bag and closing the shipping box. The packing biomass levels tested were (in kg/m^3): 5, 10, 15, and 20, with 5 kg/m^3 representing the control treatment, as UMEH has a successful track record shipping juvenile cobia at this biomass using similar packing protocols (*personal observations*). Each box (2 bags) was packed with the desired fish biomass level and water quality parameters were recorded in addition to the time of box closure. To simulate real shipping practices, the boxes were shaken gently every hour during the first two hours and last two hours of the experiment. Fish survival was the metric used to evaluate each treatment. After 24 hours ± 8 minutes, the boxes were opened in the same sequence that they were closed and final water quality parameters were taken from each bag immediately upon opening. Survival was assessed and the length (TL) and weight of a sample of ten surviving fish (when available) were measured, as well as the length (TL) and weight of all mortalities from the experiment.

Data Analysis

Results were analyzed using one-way ANOVAs to test for differences between treatments and water quality parameters. Length-weight frequency histograms were also constructed to examine the size distribution of the population used in the experiment. Any significant differences found in the ANOVA results were analyzed for determination of biological significance. Additionally, two-way ANOVAs were used to determine the effect of stocking density on survival, the effect of salinity on survival, and the interaction of the two variables. All data are presented as mean (± standard deviation) values.

CHAPTER 3: RESULTS

SALINITY TOLERANCE TRIALS

The data from the three salinity tolerance experimental trials revealed that cobia exhibit a wide range of salinity tolerance, with 100% survival after 24 hours in salinities ranging from 11 ppt to 45 ppt and various levels of mortality at salinities below and above this range. Logistic regression results indicated that salinity was a significant explanatory variable for the mortality of juvenile cobia ($C= 0.989$; $P < 0.001$) (Table 3.1). The equation of the final model was as follows:

$$Survival=1/(1+exp(-(b_0+b_1*sal+b_2*salsq))).$$

Where b_0 is the intercept, b_1 is the coefficient for "sal", b_2 is the coefficient for "salsq", "sal" is the salinity of the treatment water, and "salsq" is the salinity squared.

Experiment 1

The mean starting and ending dissolved oxygen level was 6.44 mg/L (±0.13 mg/L) and 6.28 mg/L (±0.07), respectively, among all buckets used in the experiment, with a mean change in dissolved oxygen over the 24 hours of -0.16 mg/L (±0.12 mg/L). Water temperatures at the start and end were 28.23°C (± 0.60°C) and 29.03°C (± 0.32°C), respectively, with a mean change of 0.8°C (±0.5°C) over the 24 hour duration of the experiment. The pH of the water averaged 8.32 (± 0.33) at the start of the experiment and dropped to a mean pH among buckets of 8.13 (± 0.06) by the end of the experiment, with a mean negative change in pH over 24 hours of 0.19 (± 0.29). After 24 hours, there was complete mortality (0% survival) in the 0 ppt salinity treatment, 53% mean survival in the 5 ppt treatment, and 100% survival in all remaining treatments (11 ppt, 35 ppt, 40 ppt, 45 ppt) (Figure 3.1).

Experiment 2

The mean starting and ending dissolved oxygen level was 5.99 mg/L (±0.12 mg/L) and 5.80 mg/L (±0.10), respectively, among all buckets used in the experiment, with a mean change in dissolved oxygen over the 24 hours of -0.19 mg/L (±0.14 mg/L). Water temperatures at the start and end were 32.31°C (± 0.57°C) and 32.19°C (± 0.38°C), respectively, with a mean negative change of 0.12°C (±0.65°C) over the 24 hour duration of the experiment. The pH of the water averaged 8.35 (± 0.26) at the start of the experiment and dropped to a mean pH among buckets of 8.10 (± 0.08) by the end of the experiment, with a mean negative change in pH over 24 hours of 0.25 (± 0.23). After 24 hours, there was complete mortality (0% survival) in the 0 ppt salinity treatment, 93% mean survival in the 5 ppt treatment, 100% survival in the 11 ppt, 35 ppt (control), and 45 ppt treatments, and 97% survival in the 50 ppt treatment (Figure 3.1).

Experiment 3

The mean starting and ending dissolved oxygen level was 7.13 mg/L (±0.12 mg/L) and 6.98 mg/L (±0.07), respectively, among all buckets used in the experiment, with a mean change in dissolved oxygen over the 24 hours of -0.16 mg/L (±0.15 mg/L). Water temperatures at the start and end were 25.11°C (± 0.32°C) and 24.99°C (± 0.10°C), respectively, with a mean negative change of 0.12°C (± 0.27°C) over the 24 hour duration of the experiment. The pH of the water averaged 8.20 (± 0.12) at the start of the experiment and dropped to a mean pH among buckets of 8.13 (± 0.08) by the end of the experiment, with a mean negative change in pH over 24 hours of 0.07 (± 0.08). After 24 hours, there was complete mortality (0% survival) in the 0 ppt salinity treatment, 100% survival in the 5 ppt, 11 ppt, 35 ppt (control), and 45 ppt treatments, and 43% survival in the 55 ppt treatment (Figure 3.1).

Parameter	**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**	**Model Fit C**
Intercept	1	-4.7866	1.1171	18.3587	<.0001	0.989
salinity	1	1.592	0.256	38.6765	<.0001	
Salinity squared	1	-0.0275	0.00431	40.6098	<.0001	

Table 3.1. Salinity tolerance trials. Survival of juvenile cobia after 24 hours following abrupt transfer to hypo- and hyper-osmotic conditions.

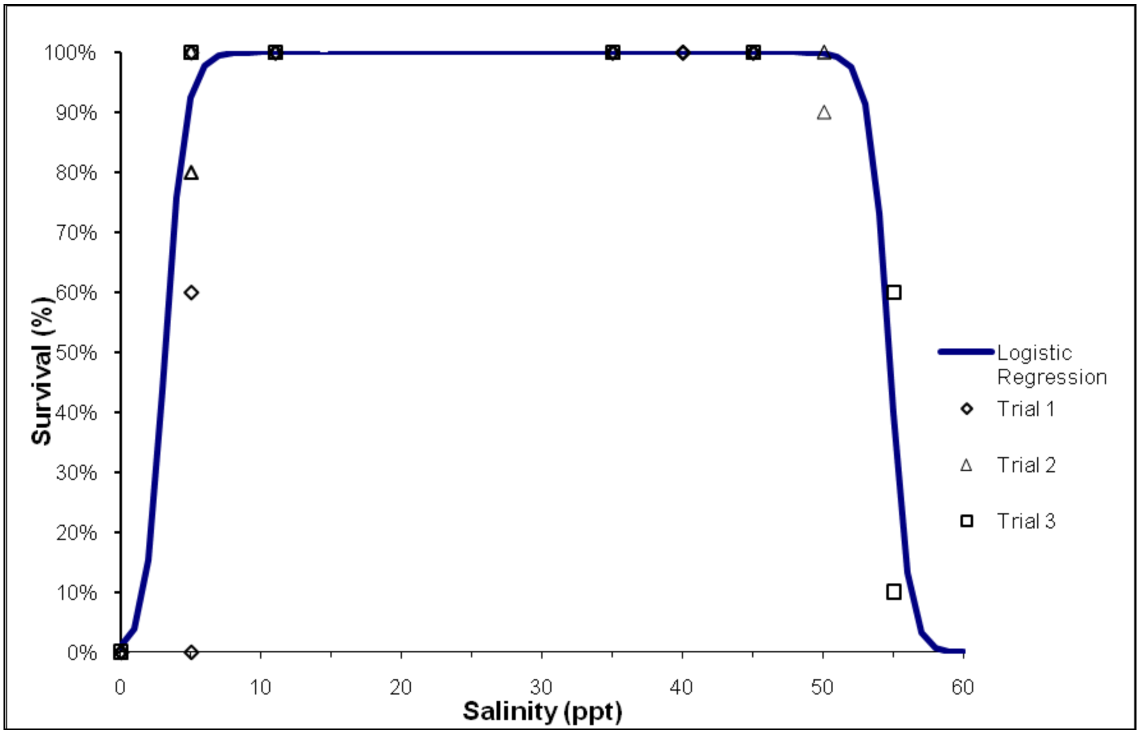

Figure 3.1. Salinity tolerance trials. Juvenile cobia survival after 24 hours following abrupt transfer to hypo- and hyper-osmotic conditions.

BEHAVIORAL RESPONSE TO SALINITY GRADIENT

The medians of the two treatments (34 ppt and 12 ppt) were significantly different, as determined using a one-sided median 2-sample test (P=0.04). This indicates that salinity preference is directly related to acclimation salinity. Fish that were acclimated to a lower salinity chose a lower median salinity than fish that had been acclimated to a higher salinity (Figure 3.2).

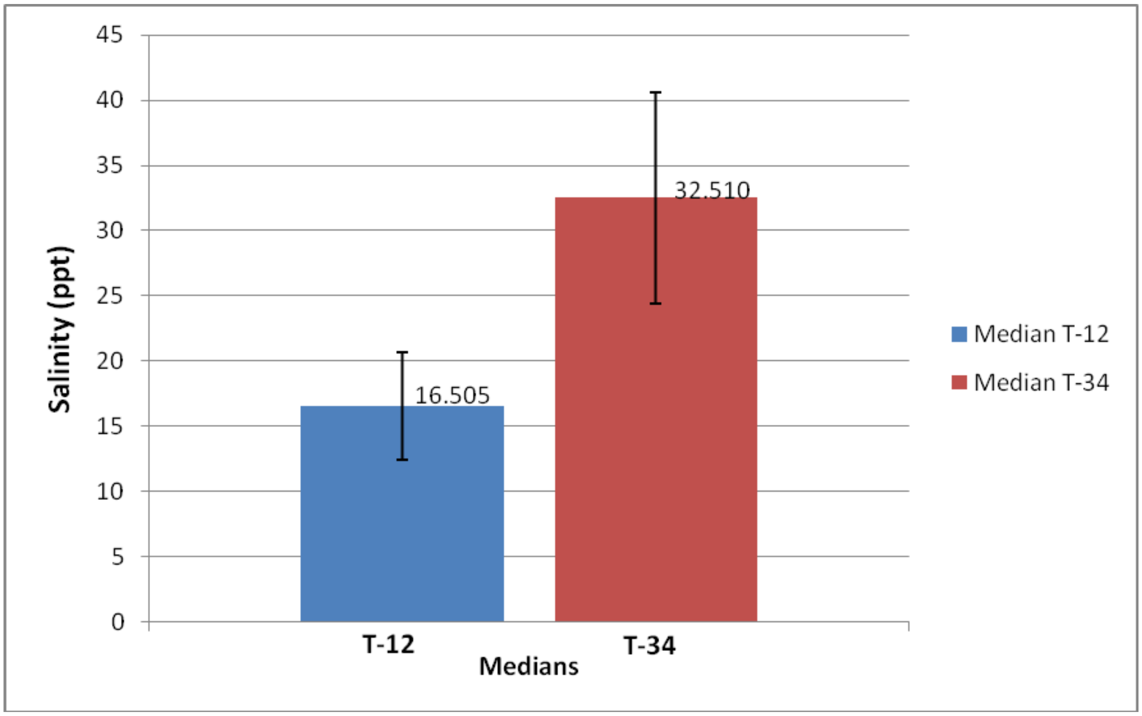

Figure 3.2. Histogram of salinity preference data. The two medians were found to be significantly different (P=0.04).

SIMULATED SHIPPING TRIALS

At the start of the 24 hour simulated shipping trial, the D.O. levels (31.20 ± 3.67 mg/L) and temperatures (19.28 ± 0.72°C) were not significantly different among treatments (as determined by ANOVA). The starting pH of the 32 ppt treatment bags (8.24 ± 0.06) was significantly lower than the starting pH of the 12 ppt treatment bags

(8.39 ± 0.08), yet there was no significant difference in pH values between treatments at the conclusion of the trial (6.54 ± 0.43) or in the mean pH decrease amongst treatments over the course of the 24 hour experiment (1.78 ± 0.39). There was no mortality in the control shipping biomass levels of 5 kg/m^3. Mortality increased with increasing packing biomass regardless of treatment (Figure 3.3). However, mortality was lower in the shipping bags that used the 12 ppt treatment than the bags with the 32 ppt treatment. ANOVA results indicated a significant interaction between salinity and stocking biomass (P=0.005). Specifically, significant differences between treatment groups can be seen in Figure 3.3 and treatments with different letters indicate those which are significantly different (Figure 3.3).

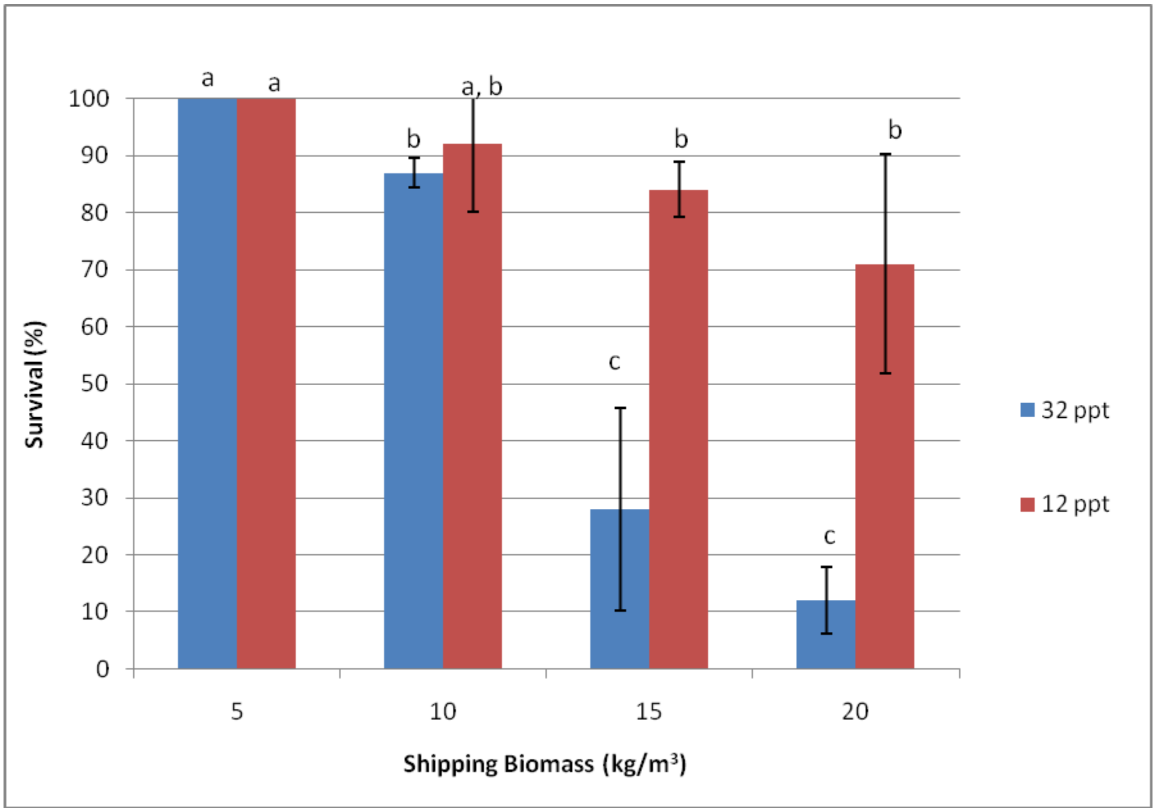

Figure 3.3. Juvenile cobia survival following a 24 hour simulated shipment. Treatments with different letters are significantly different.

CHAPTER 4: DISCUSSION

SALINITY TOLERANCE TRIALS

The results indicate that juvenile cobia have a wide salinity tolerance, with 100% survival after 24 hours following abrupt transfer from full-strength seawater (35 ppt) to salinities ranging from 11 ppt to 45 ppt. This range of salinity tolerance is consistent with experiments in which cobia were gradually acclimated to lower salinities (Denson et al, 2003; Atwood et al., 2004; Burkey et al., 2007) and in some cases reared for extended periods at low salinities (Denson et al., 2003; Resley et al., 2006). However, previous research has not addressed the ability of juvenile cobia to survive abrupt transfers to hypo- and hyper-osmotic conditions and survive for 24 hours, though Faulk and Holt (2006) reported on the survival of cobia larvae (3 – 9 dph) following abrupt salinity change. It is unknown whether mortality would have ensued in salinities at either end of the survival curve (from 11 ppt to 45 ppt) (Figure 3.1) over the coming days and weeks had the experimental trial spanned a time period greater than 24 hours. However, given that this research was focused on optimizing shipping procedures, it is likely that 24 hours would be sufficient to reach even the most distant growout sites, and salinity tolerance studies of longer duration would not be necessary for the purposes of shipping optimization.

The full physiological effect of the abrupt salinity transfer may not have been apparent in these trials since survival was used at the sole performance metric. While the fish survived abrupt transfer to hypo- and hyper-osmotic treatments, it is unclear whether the hypo- and hyper-osmotic treatments caused stress or increased metabolic rate in the fish. In theory, fish held at isosmotic conditions should have reduced energetic costs

(Weirich and Tomasso, 1991), which would be beneficial for live fish transport. Additionally, in some species the stress of a non-anesthesia assisted capture from a holding tank has been shown to induce temporary osmoregulatory dysfunction (Weirich and Tomasso, 1991; Weirich et al., 1992; Lim et al., 2003), and in some cases the stress of capture is more detrimental to the fish's health than the transport itself (Harmon, 2009). If such osmoregulatory disruptions were occurring in juvenile cobia during transfer to experimental and shipping chambers, the isosmotic conditions would likely aid in the fish's ability to survive in its new surroundings. To positively ascertain the metabolic implications of the abrupt salinity transfer, future research may incorporate the use of respirometry to elucidate the physiological effects of salinity tolerance over a 24 hour time period following abrupt transfer. Additionally, the use of anesthetics in the acclimation tank prior to abrupt transfer may help to alleviate the stress of capture and relocation to the experimental chambers, and this technique is used during full-scale shipments of juvenile cobia at UMEH.

BEHAVIORAL RESPONSE TO SALINITY GRADIENT

Results from the salinity preference experiments using the horizontal salinity gradient indicate that acclimation salinity significantly impacts salinity preference. While salinity preference experiments using gradients have been performed on other species (Staaland, 1969; Keiser and Aldrich, 1976; Stephenson and Knight, 1982; Bos and Thiel, 2006), there are no reports of such experiments being conducted with cobia at any life stages. The relation of acclimation salinity to preferred salinity was determined in this experiment, yet it is not clear whether there are any specific physiological benefits to acclimating juvenile cobia to lower salinities prior to shipment in isosmotic water conditions. Serrano et al. (2010) discussed the effect “salinity history” might have in salinity preference experiments, and this may help explain the lower median salinity chosen by cobia that had been acclimated to lower salinity. While this experiment determined that juvenile cobia exhibited differences in salinity preference depending on acclimation salinity, the specific physiological effects of salinity acclimation were not determined, yet could be examined in future research by incorporating the use of respirometry and hematologic examination. Additionally, the salinity preference trials could be repeated using fish at different life stages to determine whether salinity preference changes with age and ontogenetic development, as reported by Bos and Thiel (2006) for flounder, *Pleuronectes flesus*. The ontogenetic development of osmoregulatory abilities in teleost fish varies by species (Varsamos et al., 2005), and performing salinity preference experiments on cobia at different life stages may help track this osmoregulatory development as it changes with age.

SIMULATED SHIPPING TRIALS

The results from the simulated box shipping trials indicated significant salinity and biomass level effects on survival of juvenile cobia following a 24 hour simulated shipment. These findings underscore the importance of considering salinity and biomass when shipping live juvenile cobia for extended time periods. Results from this experiment are consistent with other reports of shipping procedures that utilize salinities at or near isosmotic conditions to reduce stress and increase survival in a variety of marine and freshwater species (Weirich and Tomasso, 1991; Weirich et al., 1992;

Harmon, 2009). Additional evidence supporting the notion that juvenile cobia should be shipped at lowered salinities, at or near isosmotic levels, was the actual appearance of the fish at the conclusion of the trial. Though factors such as appearance and long-term health effects of the simulated live fish transport were not measured quantitatively, the surviving fish packed in 32 ppt salinity water at high densities (> 5 kg/m^3) were in noticeably poorer condition at the conclusion of the trial than fish packed at the same densities in low salinity (isosmotic) water. Continued monitoring of fish health and feeding behavior amongst the trial survivors in the ensuing days following the experimental trial supported this, as there was negligible (<2 %) mortality in fish from the low salinity treatment as compared to roughly 20% - 30% mortality in fish that had survived the 32 ppt treatment in the simulated shipping trial. Future research might focus on the long-term health effects of transporting live fish, as it was evident that in the weeks after the experiment the surviving fish packed in 32 ppt seawater appeared to be in sub-standard condition following the experimental trial.

While this experiment indicated significantly increased survival at high packing biomass levels in low salinity treatments as compared to high salinity treatments, future research should test the low salinity/high biomass methods on full-scale shipping trials using 1000-L SpaceKraft® boxes, as are commonly used for transporting commercial quantities of cobia throughout the Americas (Benetti et al. 2008b). Shipping techniques and methods that are successful under experimental conditions are not always successful when applied to full-scale commercial shipments, as in the case of Colburn et al. (2008), whereby the 20 kg/m^3 shipping biomass levels proven successful in experimental trials were unsuccessful in commercial-scale shipments (*personal communication*). Therefore, full-scale (in a commercial setting) testing of shipping cobia under isosmotic conditions is needed, and dosages of water conditioners (Amquel® and Trizma®) used in the full-scale shipments may need to be altered to accommodate for the increased shipping biomass levels.

CHAPTER 5: BIOECONOMIC MODEL:
A DECISION SUPPORT TOOL

BACKGROUND

Applying the results obtained from the previous experimental trials, a bioeconomic model was constructed to help aquaculture professionals make sound decisions regarding the shipment of live juvenile cobia. This model was based on the methods and protocols used by the staff at UMEH, but simple adjustments to the input variables allow this tool to be used in other facilities as well. Because survival data were obtained specifically for cobia shipped experimentally at two different salinities (12 ppt and 32 ppt), this model should not be used for other species or for juvenile cobia shipped using packing protocols that dramatically differ from the methods described in this and previous sections. Additionally this model should only be used for juvenile cobia weighing 1 – 5 grams that have been fasted for 24 hours prior to packing.

METHODS

The costs (excluding utility costs such as electricity and water) for shipping live juvenile cobia at UMEH were obtained and all materials used in transporting the fish were broken down into a per-fish cost, based on the following assumptions, all of which can be adjusted in the model to account for the inherent variability of each input.

Assumptions:

- A typical larval rearing run at UMEH will result in roughly 80,000 cobia fingerlings (1 – 5 g each).

- Usually about 25% of the 80,000 fish are deemed unsalable due to deformities or poor growth performance. Therefore, it is assumed that each larval rearing run produces 60,000 salable fish.
- The staff required for each larval rearing run are as follows: 1 hatchery manager and 4 technicians.

Based on these assumptions and the costs of labor and materials, one could assume that each cobia fingerling costs UMEH roughly $0.25 to produce (i.e. "cost per fish" = $0.25).

In the same manner that costs were broken down on a per-fish basis, the costs of shipping fish were broken down on a per-box level (Table 5.1), assuming the use of 1000-L SpaceKraft® boxes and standard UMEH shipping materials/protocols (as described in Chapter 1). Depending on variable shipping rates for air-freight and other materials used in the shipping process, the total shipping cost per box may fluctuate. However, this variation was accounted for in the model by linking all inputs in the decision-support spreadsheet. The per-box cost of shipping was assumed to be stable, regardless of the biomass of fish packed inside, as the primary cost of shipping derives from the physical volume that the box occupies on the cargo plane and the weight of the water, with the fish biomass accounting for a small fraction of the final box weight. 300 L of water was used in the 1000-L SpaceKraft® shipping boxes, and once fish biomass was added the amount of water increases by 20 – 40 L for a final total water amount of 320 – 340 L per box.

Shipping Supplies	**Cost per Box ($)**
Amquel	0.30
Trizma	5.80
Pallet	18.90
SpaceKraft Box	105.65
SpaceKraft Shipping	28.00
Truck Rental	20.00
Air Freight	750.00
Total Cost per Box	**928.65**

Table 5.1. Shipping materials and supplies broken down into a per-box cost.

Using the total cost per-box (Table 5.1) input (i.e. Fixed Cost (FC)) in the decision-support spreadsheets (Tables 5.2 and 5.3), along with the variable inputs of "cost per fish" (see above), "average weight of fish (g)", and "Market price per fish", the model was completed. The formula used for calculation of profits at each shipping salinity (12 ppt and 32 ppt) was as follows: ***Profits (π) =***

[(Price per fish)(Total # of fish)]-[(FC)-(Cost per fish)(Total # of fish)]

= TR-TC

Whereby: FC = Fixed Costs, TR = Total Revenue, and TC = Total Costs

Additionally, "IF, THEN" relationships were inserted into the decision-support spreadsheets (Tables 5.2 and 5.3) to provide the number of boxes needed for each packing biomass at each respective salinity. These calculations were determined based on the packing biomass per box that would result in the greatest profit potential (i.e. the lowest overall cost of delivering the live fish). The formula used for the calculation of the "IF, THEN" relationships was as follows:

IF Profits (π) > (2*FC), THEN # of Boxes = 1; IF Profits (π) > (3*FC), THEN # of Boxes = 2; IF Profits (π) > (4*FC), THEN # of Boxes = 3;…

It is customary in nearly all aquaculture operations, research- and commercial-scale, for the buyer to assume responsibility for shipping costs, as is the case at UMEH for all juvenile cobia shipments. Beginning with the total biomass (g) per box (300 L of water), the following linked data columns were created (Table 5.2 and 5.3): “Biomass Level (kg/m^3)”, “Total Biomass (g) in box”, “Total # of fish”, “Survival at 12 ppt salinity (%)”, “Survival at 32 ppt salinity (%)”, “Shipping cost per fish (Live & Dead)”, “Total Variable Cost (TVC)”, “Marginal Cost (MC)”, “Number of fish delivered alive at 12 ppt salinity”, “Total Revenue at 12 ppt salinity (TR-12)”, “Marginal Revenue at 12 ppt salinity (MR-12)”, “Number of fish delivered alive at 32 ppt salinity”, “Total Revenue at 32 ppt salinity (TR-32)”, “Marginal Revenue at 32 ppt salinity (MR-32)”, “Shipping cost per live fish at 12 ppt salinity”, “Shipping cost per live fish at 32 ppt salinity”, “Shipping cost per box (FC)”, “Total Cost (TC)”, “Profits at 12 ppt salinity”, “Number of Boxes needed at 12 ppt”, “Profits at 32 ppt salinity”, and “Number of boxes needed at 32 ppt”.

A sensitivity analysis was run using the model to determine profits at three different costs of production per fingerling ($0, $0.25, and $0.50) and graphs were created illustrating the effects of changes in production costs (Figures 5.1 – 5.3). Additionally, a final scenario was run using the model whereby the cost of production per fingerling was assumed to be the market price per fish ($1.75 in this case) (Table 5.3; Figure 5.4). Due to the heavily subsidized (federal, state, and University supported) fingerling production at UMEH, the cost or value of the fish to each member of the UMEH staff is usually thought of in terms of the market price per fingerling. Because the UMEH facility is not a commercial operation and fish are raised for purposes of

Cost per box (air freight, boxes, supplies, etc.)($)	$ 928.65	Liters per box (pre-fish)	300	Weight of fish (g)	1.5	Market price per fish (not including shipping)	$ 1.75	Cost per fish (Labor and Materials ONLY)	$ 0.25

Biomass level (kg/m^3)	Total biomass (g) in Box (300-L)	Total # of fish	Survival @ 12 ppt (%)	Survival @ 32 ppt (%)	Shipping cost per fish (Live & Dead)	TVC	MC	Number of fish delivered alive (12 ppt)	TR-12	MR-12	Number of fish delivered alive (32 ppt)	TR-32	MR-32	Shipping cost per live fish (12 ppt)	Shipping cost per live fish (32 ppt)	Shipping cost per box (FC)	TC	Profits (12 ppt)	Number of boxes needed (12 ppt)	Profits (32 ppt)	Number of boxes needed (32 ppt)
1.00	300	200	100.00	100.00	$ 4.64	$ 50.00		200	$ 350.00		200	$ 350.00		$ 4.64	$ 4.64	$ 928.65	$ 978.65	$ (628.65)	1	$ (628.65)	1
2.00	600	400	100.00	100.00	$ 2.32	$ 100.00	$ 0.25	400	$ 700.00	$ 1.75	400	$ 700.00	$ 1.75	$ 2.32	$ 2.32	$ 928.65	$1,028.65	$ (328.65)	1	$ (328.65)	1
3.00	900	600	100.00	100.00	$ 1.55	$ 150.00	$ 0.25	600	$1,050.00	$ 1.75	600	$1,050.00	$ 1.75	$ 1.55	$ 1.55	$ 928.65	$1,078.65	$ (28.65)	1	$ (28.65)	1
4.00	1200	800	100.00	100.00	$ 1.16	$ 200.00	$ 0.25	800	$1,400.00	$ 1.75	800	$1,400.00	$ 1.75	$ 1.16	$ 1.16	$ 928.65	$1,128.65	$ 271.35	1	$ 271.35	1
5.00	1500	1000	100.00	100.00	$ 0.93	$ 250.00	$ 0.25	1000	$1,750.00	$ 1.75	1000	$1,750.00	$ 1.75	$ 0.93	$ 0.93	$ 928.65	$1,178.65	$ 571.35	1	$ 571.35	1
6.00	1800	1200	99.25	98.65	$ 0.77	$ 300.00	$ 0.25	1191	$2,084.25	$ 1.67	1184	$2,071.66	$ 1.61	$ 0.78	$ 0.78	$ 928.65	$1,228.65	$ 855.60	1	$ 843.01	1
7.00	2100	1400	97.31	92.17	$ 0.66	$ 350.00	$ 0.25	1362	$2,384.17	$ 1.50	1290	$2,258.15	$ 0.93	$ 0.68	$ 0.72	$ 928.65	$1,278.65	$1,105.52	1	$ 979.50	1
8.00	2400	1600	95.38	85.69	$ 0.58	$ 400.00	$ 0.25	1526	$2,670.54	$ 1.43	1371	$2,399.27	$ 0.71	$ 0.61	$ 0.68	$ 928.65	$1,328.65	$1,341.89	1	$ 1,070.62	1
9.00	2700	1800	93.44	79.21	$ 0.52	$ 450.00	$ 0.25	1682	$2,943.34	$ 1.36	1426	$2,495.03	$ 0.48	$ 0.55	$ 0.65	$ 928.65	$1,378.65	$1,564.69	1	$ 1,116.38	1
10.00	3000	2000	91.50	72.73	$ 0.46	$ 500.00	$ 0.25	1830	$3,202.59	$ 1.30	1455	$2,545.41	$ 0.25	$ 0.51	$ 0.64	$ 928.65	$1,428.65	$1,773.94	1	$ 1,116.76	1
11.00	3300	2200	89.57	66.24	$ 0.42	$ 550.00	$ 0.25	1970	$3,448.28	$ 1.23	1457	$2,550.43	$ 0.03	$ 0.47	$ 0.64	$ 928.65	$1,478.65	$1,969.63	1	$ 1,071.78	1
12.00	3600	2400	87.63	59.76	$ 0.39	$ 600.00	$ 0.25	2103	$3,680.42	$ 1.16	1434	$2,510.08	$ (0.20)	$ 0.44	$ 0.65	$ 928.65	$1,528.65	$2,151.77	1	$ 981.43	1
13.00	3900	2600	85.69	53.28	$ 0.36	$ 650.00	$ 0.25	2228	$3,898.99	$ 1.09	1385	$2,424.36	$ (0.43)	$ 0.42	$ 0.67	$ 928.65	$1,578.65	$2,320.34	1	$ 845.71	1
14.00	4200	2800	83.76	46.80	$ 0.33	$ 700.00	$ 0.25	2345	$4,104.01	$ 1.03	1310	$2,293.27	$ (0.66)	$ 0.40	$ 0.71	$ 928.65	$1,628.65	$2,475.36	1	$ 664.62	1
15.00	4500	3000	81.82	40.32	$ 0.31	$ 750.00	$ 0.25	2455	$4,295.47	$ 0.96	1210	$2,116.81	$ (0.88)	$ 0.38	$ 0.77	$ 928.65	$1,678.65	$2,616.82	1	$ 438.16	1
16.00	4800	3200	79.88	33.84	$ 0.29	$ 800.00	$ 0.25	2556	$4,473.37	$ 0.89	1083	$1,894.99	$ (1.11)	$ 0.36	$ 0.86	$ 928.65	$1,728.65	$2,744.72	1	$ 166.34	1
17.00	5100	3400	77.94	27.36	$ 0.27	$ 850.00	$ 0.25	2650	$4,637.71	$ 0.82	930	$1,627.80	$ (1.34)	$ 0.35	$ 1.00	$ 928.65	$1,778.65	$2,859.06	1	$ (150.85)	1
18.00	5400	3600	76.01	20.88	$ 0.26	$ 900.00	$ 0.25	2736	$4,788.50	$ 0.75	752	$1,315.24	$ (1.56)	$ 0.34	$ 1.24	$ 928.65	$1,828.65	$2,959.85	1	$ (513.41)	1
19.00	5700	3800	74.07	14.40	$ 0.24	$ 950.00	$ 0.25	2815	$4,925.73	$ 0.69	547	$ 957.31	$ (1.79)	$ 0.33	$ 1.70	$ 928.65	$1,878.65	$3,047.08	1	$ (921.34)	1
20.00	6000	4000	72.13	7.91	$ 0.23	$1,000.00	$ 0.25	2885	$5,049.40	$ 0.62	317	$ 554.02	$ (2.02)	$ 0.32	$ 2.93	$ 928.65	$1,928.65	$3,120.75	1	$(1,374.63)	1

Table 5.2. Sample bioeconomic model spreadsheet. Highlighted terms indicate the input variables (note: “Cost per fish” = $0.25).

Cost per box (air freight, boxes, supplies, etc.)($)	$ 928.65	Liters per box (pre-fish)	300	Weight of fish (g)	1.5	Market price per fish (not including shipping)	$ 1.75	Cost per fish (Labor and Materials ONLY)	$ 1.75

Biomass level (kg/m^3)	Total biomass (g) in box (300-L)	Total # of fish	Survival @ 12 ppt (%)	Survival @ 32 ppt (%)	Shipping cost per fish (Live & Dead)	TVC	MC	Number of fish delivered alive (12 ppt)	TR-12	MR-12	Number of fish delivered alive (32 ppt)	TR-32	MR-32	Shipping cost per live fish (12 ppt)	Shipping cost per live fish (32 ppt)	Shipping cost per box (FC)	TC	Profits (12 ppt)	Number of boxes needed (12 ppt)	Profits (32 ppt)	Number of boxes needed (32 ppt)
1.00	300	200	100.00	100.00	$ 4.64	$ 350.00		200	$ 350.00		200	$ 350.00		$ 4.64	$ 4.64	$ 928.65	$1,278.65	$ (928.65)	1	$ (928.65)	1
2.00	600	400	100.00	100.00	$ 2.32	$ 700.00	$ 1.75	400	$ 700.00	$ 1.75	400	$ 700.00	$ 1.75	$ 2.32	$ 2.32	$ 928.65	$1,628.65	$ (928.65)	1	$ (928.65)	1
3.00	900	600	100.00	100.00	$ 1.55	$1,050.00	$ 1.75	600	$1,050.00	$ 1.75	600	$1,050.00	$ 1.75	$ 1.55	$ 1.55	$ 928.65	$1,978.65	$ (928.65)	1	$ (928.65)	1
4.00	1200	800	100.00	100.00	$ 1.16	$1,400.00	$ 1.75	800	$1,400.00	$ 1.75	800	$1,400.00	$ 1.75	$ 1.16	$ 1.16	$ 928.65	$2,328.65	$ (928.65)	1	$ (928.65)	1
5.00	1500	1000	100.00	100.00	$ 0.93	$1,750.00	$ 1.75	1000	$1,750.00	$ 1.75	1000	$1,750.00	$ 1.75	$ 0.93	$ 0.93	$ 928.65	$2,678.65	$ (928.65)	1	$ (928.65)	1
6.00	1800	1200	99.25	98.65	$ 0.77	$2,100.00	$ 1.75	1191	$2,084.25	$ 1.67	1184	$2,071.66	$ 1.61	$ 0.78	$ 0.78	$ 928.65	$3,028.65	$ (944.40)	1	$ (956.99)	1
7.00	2100	1400	97.31	92.17	$ 0.66	$2,450.00	$ 1.75	1362	$2,384.17	$ 1.50	1290	$2,258.15	$ 0.93	$ 0.68	$ 0.72	$ 928.65	$3,378.65	$ (994.48)	1	$(1,120.50)	1
8.00	2400	1600	95.38	85.69	$ 0.58	$2,800.00	$ 1.75	1526	$2,670.54	$ 1.43	1371	$2,399.27	$ 0.71	$ 0.61	$ 0.68	$ 928.65	$3,728.65	$ (1,058.11)	1	$(1,329.38)	1
9.00	2700	1800	93.44	79.21	$ 0.52	$3,150.00	$ 1.75	1682	$2,943.34	$ 1.36	1426	$2,495.03	$ 0.48	$ 0.55	$ 0.65	$ 928.65	$4,078.65	$ (1,135.31)	1	$(1,583.62)	1
10.00	3000	2000	91.50	72.73	$ 0.46	$3,500.00	$ 1.75	1830	$3,202.59	$ 1.30	1455	$2,545.41	$ 0.25	$ 0.51	$ 0.64	$ 928.65	$4,428.65	$ (1,226.06)	1	$(1,883.24)	2
11.00	3300	2200	89.57	66.24	$ 0.42	$3,850.00	$ 1.75	1970	$3,448.28	$ 1.23	1457	$2,550.43	$ 0.03	$ 0.47	$ 0.64	$ 928.65	$4,778.65	$ (1,330.37)	1	$(2,228.22)	2
12.00	3600	2400	87.63	59.76	$ 0.39	$4,200.00	$ 1.75	2103	$3,680.42	$ 1.16	1434	$2,510.08	$ (0.20)	$ 0.44	$ 0.65	$ 928.65	$5,128.65	$ (1,448.23)	1	$(2,618.57)	2
13.00	3900	2600	85.69	53.28	$ 0.36	$4,550.00	$ 1.75	2228	$3,898.99	$ 1.09	1385	$2,424.36	$ (0.43)	$ 0.42	$ 0.67	$ 928.65	$5,478.65	$ (1,579.66)	1	$(3,054.29)	3
14.00	4200	2800	83.76	46.80	$ 0.33	$4,900.00	$ 1.75	2345	$4,104.01	$ 1.03	1310	$2,293.27	$ (0.66)	$ 0.40	$ 0.71	$ 928.65	$5,828.65	$ (1,724.64)	1	$(3,535.38)	3
15.00	4500	3000	81.82	40.32	$ 0.31	$5,250.00	$ 1.75	2455	$4,295.47	$ 0.96	1210	$2,116.81	$ (0.88)	$ 0.38	$ 0.77	$ 928.65	$6,178.65	$ (1,883.18)	2	$(4,061.84)	4
16.00	4800	3200	79.88	33.84	$ 0.29	$5,600.00	$ 1.75	2556	$4,473.37	$ 0.89	1083	$1,894.99	$ (1.11)	$ 0.36	$ 0.86	$ 928.65	$6,528.65	$ (2,055.28)	2	$(4,633.66)	4
17.00	5100	3400	77.94	27.36	$ 0.27	$5,950.00	$ 1.75	2650	$4,637.71	$ 0.82	930	$1,627.80	$ (1.34)	$ 0.35	$ 1.00	$ 928.65	$6,878.65	$ (2,240.94)	2	$(5,250.85)	5
18.00	5400	3600	76.01	20.88	$ 0.26	$6,300.00	$ 1.75	2736	$4,788.50	$ 0.75	752	$1,315.24	$ (1.56)	$ 0.34	$ 1.24	$ 928.65	$7,228.65	$ (2,440.15)	2	$(5,913.41)	6
19.00	5700	3800	74.07	14.40	$ 0.24	$6,650.00	$ 1.75	2815	$4,925.73	$ 0.69	547	$ 957.31	$ (1.79)	$ 0.33	$ 1.70	$ 928.65	$7,578.65	$ (2,652.92)	2	$(6,621.34)	7
20.00	6000	4000	72.13	7.91	$ 0.23	$7,000.00	$ 1.75	2885	$5,049.40	$ 0.62	317	$ 554.02	$ (2.02)	$ 0.32	$ 2.93	$ 928.65	$7,928.65	$ (2,879.25)	3	$(7,374.63)	7

Table 5.3. Sample bioeconomic model spreadsheet. Highlighted terms indicate the input variables (note: UMEH scenario where “Cost per fish” = $1.75).

research, with only the byproducts of this research being sold to buyers, the fish are usually assumed to hold a value that is equivalent to the market price. Through the sensitivity analysis whereby the cost of production per fingerling was equivalent to the market price, the “Number of boxes needed” and “Biomass Level” columns would indicate the maximum biomass level to stock in each box under the unique subsidized fingerling production cost assumptions ($1.75 per fingerling) at UMEH.

RESULTS

The results of the sensitivity analysis indicated that at each production cost level and biomass level, shipping at 12 ppt salinity results in the greatest profits. Additionally, the analysis showed that as production costs rise, profits and the maximum profit packing biomass level (kg/m^3) decrease (Figures 5.1 – 5.4). Using the “IF, THEN” relationships, it was possible to determine the optimal number of boxes to use at each biomass level for each respective packing salinity. In the example model output using a $0.25 cost per fish (Table 5.2), the number of boxes needed for maximum profit on the buyer’s behalf is one box. However, when the model is run using the $1.75 cost per fish (the “UMEH scenario”), the number of boxes needed for maximum profit increases at higher packing densities (Table 5.3).

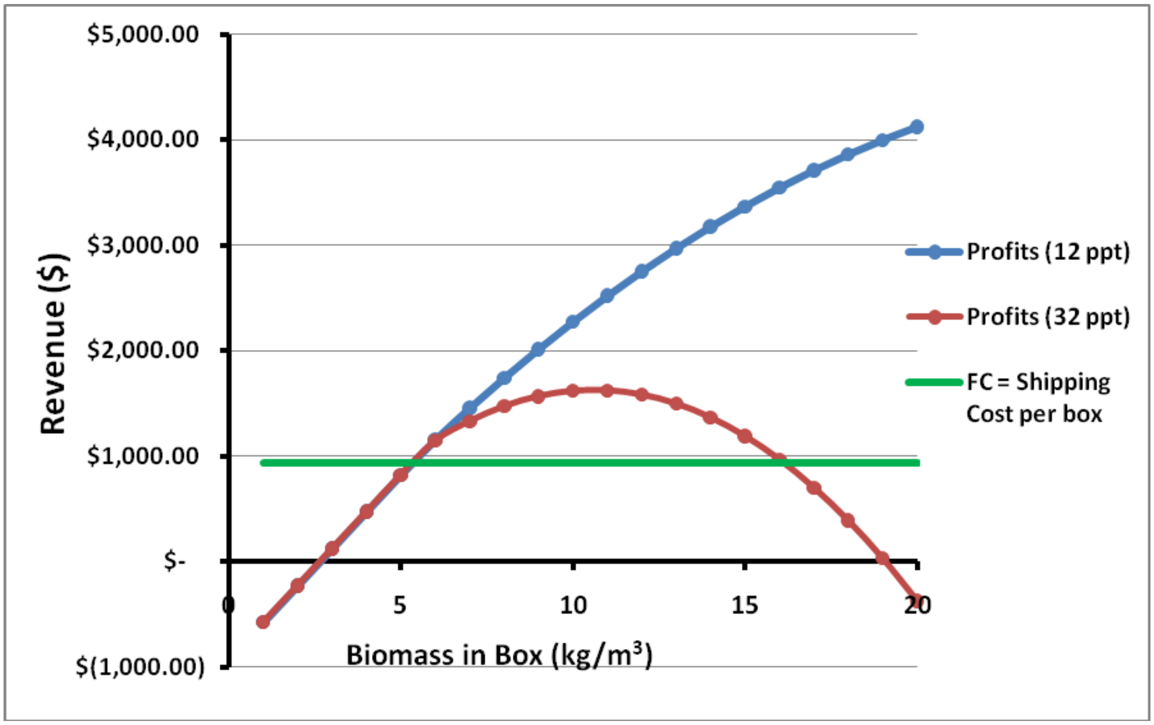

Figure 5.1. Results from sensitivity analysis using a per fingerling production cost of $0.00.

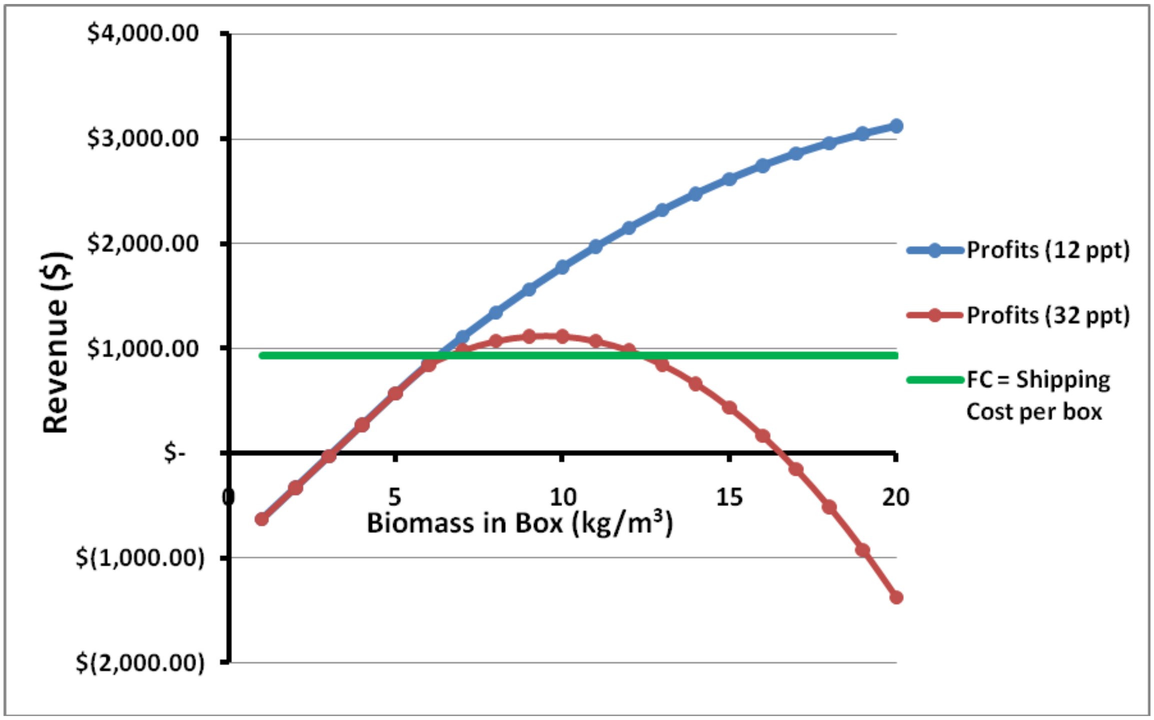

Figure 5.2. Results from sensitivity analysis using a per fingerling production cost of $0.25.

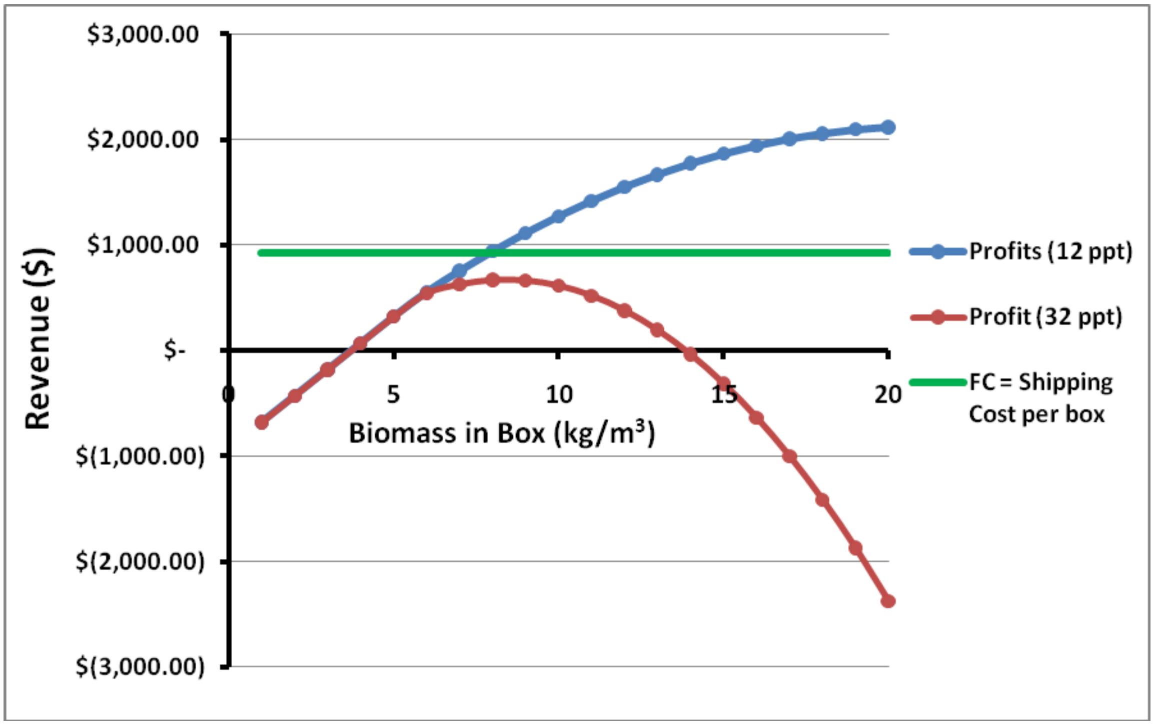

Figure 5.3. Results from sensitivity analysis using a per fingerling production cost of $0.50.

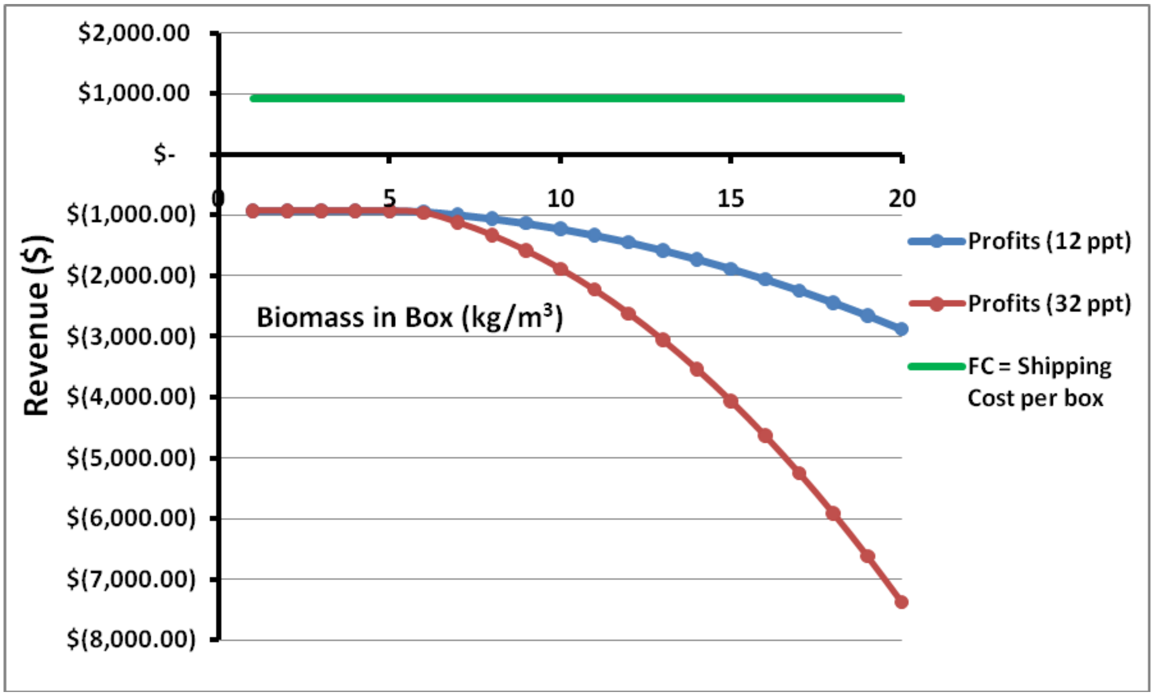

Figure 5.4. Results from sensitivity analysis using a per fingerling production cost of $1.75.

DISCUSSION

Since the buyers typically assume the costs of shipping fingerlings, they would ideally pack each box as densely as possible to minimize their shipping costs. In low cost of production scenarios, the buyers do not care about minor mortality, but rather how many live fish they end up with out of the lowest number of boxes. This is reflected in the “Number of boxes needed” columns for each salinity (Table 5.2 and 5.3), whereby at low costs of production (Table 5.2), the number of boxes needed is one. However, as the cost of production increases (Table 5.3), the opportunity cost of each live fish increases as well, which means the seller (UMEH) would want to pack at lower biomass levels to maximize survival and lose as few fish to dead loss as possible.

In the UMEH scenario, whereby the cost of production is assumed to equal the market price of each fingerling (Table 5.3; Figure 5.4), the number of boxes needed at higher biomass levels increases, as the packing biomass in each box which results in the greatest profit decreases (Table 5.3). This reflects the greater opportunity cost at higher costs of production, and most accurately reflects the way shipping fish at UMEH is viewed. Ideally, UMEH wants to maximize the profits for the buyer through minimization of shipping costs, while not losing too many fish in the process. There is a tendency in aquaculture to either under- or over-pack boxes with fish, with both actions resulting in poor economic results. By using this bioeconomic decision support model, aquaculturists are able to determine the economically optimal packing biomass levels for either 32 ppt or 12 ppt salinity packing water, while reducing labor and material costs of shipping.

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