Physiological performance measures and tolerance limits of estuarine indicator species in South Florida

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PHYSIOLOGICAL PERFORMANCE MEASURES AND TOLERANCE LIMITS OF

ESTUARINE INDICATOR SPECIES IN SOUTH FLORIDA

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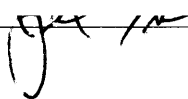BIOLOGY

by

Pamela Marie Bachman

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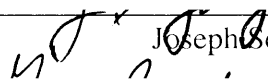
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This dissertation, written by Pamela Marie Bachman, and entitled Physiological Performance Measures and Tolerance Limits of Estuarine Indicator Species in South Florida, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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The dissertation of Pamela Marie Bachman is approved.

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ABSTRACT OF THE DISSERTATION

PHYSIOLOGICAL PERFORMANCE MEASURES AND TOLERANCE LIMITS OF

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Professor Evelyn Gaiser, Major Professor

Current water management practices in South Florida have negatively impacted many

species inhabiting Florida Bay. Variable and high salinity has been identified as a key

stressor in these estuaries. The Comprehensive Everglades Restoration Plan (CERP)

includes water redistribution projects that will restore natural freshwater flows to

northeastern Florida Bay. My studies focused on the following central theme and

hypotheses: Biological performance measures (i.e., growth, reproduction, survival),

behavior (i.e., habitat preference and locomotor behavior) and diversity of estuarine fish

will be controlled by changes in salinity and water quality that will occur as a result of

the restoration of freshwater flow to the bay. A series of acute and subchronic

physiological toxicity studies were conducted to determine the effects of salinity changes

on the life stages (embryo/larval, juvenile, adult) and fecundity of four native estuarine

fish (*Cyprinodon variegatus, Floridichthys carpio, Poecilia latipinna, and Gambusia

holbrooki*). Fish were exposed to a range of salinity concentrations (freshwater to

hypersaline) based on salinity profiles in the study areas. Growth (length, weight) and

survival were measured. Salinity trials included both rapid and gradual change events.

Results show negative effects of acute, abrupt salinity changes on fish survival, development and reproductive success as a result of salinity stress. Other studies targeted reproduction and critical embryo-larval/neonate development as key areas for detecting long-term population effects of salinity change in Florida Bay. Adults of *C. variegatus* and *P. latipinna* were also examined for behavioral responses to pulsed salinity changes. These responses include changes in swimming performance, locomotor behavior and zone preference. Finally, an ecological risk assessment was conducted for adverse salinity conditions in northeastern Florida Bay. Using the U.S. EPA's framework, the risk to estuarine fish species diversity was assessed against regional salinity profiles from a 17-year database. Based on the risk assessment, target salinity profiles for these areas are recommended for managers.

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Chapter I

INTRODUCTION

The southern Florida peninsula encompasses approximately 28,000 km^2 and includes a diverse system of interconnected subtropical habitats that function as a myriad of wetlands, uplands and coastal and marine areas. Comprised of 16 counties, this area is under the jurisdiction of the South Florida Water Management District (SFWMD) and the United Stats Department of the Interior (U.S. DOI. It extends from Orlando to the Florida Keys and is dominated by the watersheds of the Kissimmee River southward through Lake Okeechobee and the Everglades into highly productive estuaries and nearshore coastal waters, including Biscayne and Florida Bays.

South Florida has a low topographic gradient, is surrounded by thousands of miles of coastline and receives approximately 101-165 cm of rainfall annually (60% within the wet season), making it vulnerable to flooding. Water quality and quantity issues in South Florida ecosystems have resulted from human changes to the hydrologic system which began in the late 1800s, with dredging that channelized the Caloosahatchee River and connected it to Lake Okeechobee. Population growth began to escalate in the early 1900s and changes in the hydrologic structures of South Florida culminated with U.S. Congressional authorization of the Central and Southern Florida (C & SF) Flood Control Project in 1948, which focused on flood control, drainage and water supply. The C & SF Project consisted of 1400 miles of primary canals and more than 100 water control projects. The C& SF Project, in trying to provide for the water needs of South Florida, led to hydrologic fragmentation of the Greater Everglades and affected the quantity and

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natural timing of freshwater deliveries to and through the surrounding areas. It has thus created land-use changes that have decreased the land available for water storage and recharge. For the next 50 years following implementation of the C&SF project, population growth, urbanization and agricultural practices significantly exacerbated the degradation and loss of natural ecosystems in South Florida.

Less than half of the original wetland acreage that functioned as natural filters and water retention areas (WTAs) was lost or adversely impacted due to development and drainage changes. Presently, surface water discharges from canals into Florida and Biscayne Bays are heavier during wet periods and lighter during dry periods, producing higher salinity fluctuations in the bays and adversely affecting habitats. Impact on groundwater storage capacity is particularly significant since municipal water (~95%) and agricultural water (~5%) supplies in South Florida come from aquifers.

Water quality was also degraded in some areas by phosphorus, pesticides and metals from agriculture and urban runoff (Science Subgroup, 1996). Evidence that changes in surface, groundwater and water quality have adversely affected plant and animal species and habitats includes:

*wading bird populations have declined (~90%)

*69 species are listed on the federal endangered and threatened list by U.S. Fish and Wildlife Service

*spread of invasive, non-indigenous, exotic plant and animal species

*declines in commercial and recreational fisheries in Biscayne and Florida Bays

*decrease in the number of Everglades tree islands

In the last 30 years, the Florida legislature and U.S. Congress produced legislation to manage growth and protect the natural environment of South Florida, including the Everglades, through state, federal and tribal programs. This began in 1972 with Florida's Water Development Act to establish water policy and the Land Conservation Act that authorized the issuance of bonds to purchase land. In 1996, U.S. Congress, under the Water Resources Development Act, formally established the South Florida Ecosystem Restoration Task Force to coordinate restoration efforts and authorized a review study of the C& SF Project known as the Restudy. The goal of the Restudy was to modify the C& SF project to restore the natural hydropattern and maintain flood control and to improve urban and agricultural water supplies. The U.S. Department of the Interior in 1996 also prepared a report entitled "A Comprehensive Plan for the Restoration of the Everglades" and in 2000, the U.S. Congress authorized the Comprehensive Everglades Restoration Plan (CERP) as part of the Water Resources Development Act which will be implemented at the cost of approximately 8 billion dollars over 40 years. The goal of the CERP is to modify the C& SF project to restore and preserve the hydrology of the pre-drainage ecosystem to a more natural state, to protect the quality of the remaining habitats, to promote the return of populations of plants and animals and to foster human development compatible with sustaining a healthy ecosystem while providing for flood protection and water supply. CERP is led by the U.S. Army Corps of Engineers (USACE) and SFWMD and it is the framework for ecosystem restoration activities in South Florida since it focuses on the quantity, quality, timing and deliveries of freshwater supplying the system.

Much of the South Florida restoration effort originally concentrated on water quantity versus water quality (Scott et al., 2002). However, it is evident from chemical monitoring programs in South Florida that organic and inorganic contaminants are present at concentrations in surface waters and sediment that may present potential adverse effects and risks to organisms. For example, since the mid-1980s, the SFWMD has been monitoring pesticides in South Florida surface waters and sediment, and several compounds including DDT, DDE, DDD, ametryn, atrazine, dicofol, diquat and endosulfan sulfate were frequently detected (Miles and Pfeuffer, 1997). Detectable residues of endosulfan were consistently present in surface water of one canal location (C-111), adjacent to Everglades National Park and occasionally exceeded Florida Department of Environmental Protection (FDEP) and U.S. Environmental Protection Agency (U.S.EPA) water quality criteria. Surface water sampling by the National Oceanic and Atmospheric Administration (NOAA) confirmed these findings (Scott et al., 1994). NOAA also conducted monitoring studies from 1993-1997 and indicated the presence of endosulfan, atrazine, chlorpyrifos and chlorothalonil in surface waters of canals adjacent to agricultural areas that drain into the C-111 and in northeast Florida Bay (Scott et al., 2002). Waters from canals contained detectable concentrations of endosulfan that sporadically exceeded the U.S. EPA freshwater quality criterion. Florida Bay waters also occasionally exceeded the U.S.EPA saltwater quality criterion for endosulfan. In addition, results of sediment toxicity tests indicated that sediment samples from a freshwater canal (C-111) and northeastern Florida Bay were toxic to copepods (*Amphiascus tenuiremis*) and juvenile clams (*Mercenaria mercenaria*), as a result of chronic exposures. The U.S. EPA in 1995 also detected endosulfan, other organochlorine

insecticides, PCBs and PAHs at several sediment sites on the C-111 system, and parts of northeast Florida Bay (Goodman et al. 1999). NOAA conducted a study from 1998 to 2000 and found endosulfan, chlorpyrifos, metolachlor and atrazine concentrations were highest in canal sites in the Everglades panhandle, south of the C-111, but concentrations decreased toward northeast Florida Bay (Fulton et al., 2004). Concentrations of endosulfan exceeded U.S.EPA freshwater and saltwater criterion. NOAA also found that grass shrimp (*Palaemonetes intermedius*) had significantly reduced levels of the enzyme acetylcholinesterase when exposed to canal waters (Military and North Canals) that empty into Biscayne Bay (Key et al., 2003). A large-scale sediment quality program conducted by NOAA in Biscayne Bay (North to South Biscayne Bay) from 1995-1996 found that: high molecular weight PAHs exceeded guideline concentrations more than low molecular weight PAHs, PCBs exceeded guideline concentrations but spatial scales of contamination were small, and copper, lead, mercury and zinc most frequently exceeded guideline concentrations and concentrations of cadmium, copper, lead and zinc were high relative to background levels (Long et al., 2002). Furthermore, the highest chemical concentrations were found in the Miami River which empties into North Biscayne Bay. In the most recent monitoring study by the U.S. Department of Agriculture atrazine, endosulfan, chlorpyrifos, metolachlor, and chlorothalonil were the most frequently detected pesticides in water in South Florida canals and Biscayne Bay (Harman-Fetcho et al., 2005).

The U.S. General Accounting Office (U.S.GAO) prepared a report for the U.S. Department of the Interior, on gaps that exist in scientific information on the restoration of South Florida and indicated the need for studies on "more system-wide work on

screening for contaminants" and the "risks faced by organisms living in areas with contaminants" and, "if not addressed soon, will hinder the success of restoration" (U.S.GAO, 2003).

As highlighted above, several toxicological and ecological risk assessment studies have been conducted over recent years to assess the effects of anthropogenic contamination in South Florida. However, patterns of naturally occurring abiotic factors such as salinity, when disrupted, can also be viewed as a contaminant or stressor to the native biota. Freshwater inflows into coastal areas and the resultant changing salinities are a major issue in the CERP and can be examined from an environmental toxicology viewpoint.

Water management practices for the Everglades have resulted in changes in salinity patterns that exceed the normal variability of the Florida Bay system, particularly in northeast Florida Bay (Marshall et al., 2009). These management practices (as briefly discussed above) have resulted in altered hydropatterns, reduced hydroperiods, as well as a general increase in salinities in the Bay (Marshall et al., 2009). The changes in salinity in the Bay have been linked to declines native forage fish species and the higher trophic levels that depend upon them (Lorenz and Serafy, 2006). Current salinity patterns in the bay can range from hyper- to hypo-saline and vary spatially and temporally (Nuttle et al., 2000). Figure 1.1 (Hittle and Patino, 2000) gives an example on the daily salinity variability within a two-year period for two areas of northeastern Florida Bay.

Hence, salinity in Florida Bay, due to increased in both the variability and average value, can be views in the context of a toxicological stressor. Herein follows an overview of salinity as a stressor.

Estuaries and salinity

Estuaries are dynamic systems characterized by rapidly changing physio-chemical environments. The energetic demands on organisms are great, and many species are excluded from these habitats as a result (Day Jr. et al. 1989).

Vernberg and Vernberg (1976) described the physiological and behavioral effects of estuarine environments on animals. They postulated that when exposed to a stressful environment such as an estuary, there are three primary responses from the organism: 1) avoidance of the stressor; 2) adaptation or adjustment to the stressor, and hence survival; and 3) death. Across the environmental gradient of an abiotic factor, an organism will encounter a range that is conducive to life (Zone of Compatibility) as well as conditions which are detrimental (Zones of Resistance) (Vernberg and Vernberg, 1976). This dynamic is illustrated in Figure 1.2.

The most difficult stressor for estuarine fish is salinity and the physiological need to osmoregulate (Day Jr. et al, 1989). In order to maintain internal osmotic concentrations, estuarine fish have physiological and behavioral adaptations to minimize osmotic stress. These adaptations include, but are not limited to: scales/skin that are relatively impermeable; a mucous coating; control of drinking rate and/or excretion of water in urine; and avoidance of adverse conditions (Day Jr., et al 1989).

Though distribution of estuarine organisms is controlled by no single factor, salinity plays a major deterministic role. Other factors that influence distribution are temperature, oxygen, substrate and food availability (Vernberg and Vernberg, 1972).

Salinity as a stressor

A stressor is any condition or situation that causes a biological system to mobilize its resources and increase its energy expenditure (Lugo, 1978). Stress is the response of the system to the stressor via increase in energy expenditure. Energy is "stored" as either glycogen or lipids with the fish. It is documented that lipid constituents within various organs (eye, gill, liver, digestive tract and gonads) and muscles are influenced by environmental salinity changes (Daikoku et al., 1982). As changes in survival, growth, and development may occur via an energetic route, salinity thus becomes is an important limiting and modifying factor in the physiology of estuarine fish.

Salinity and osmotic pressure of fluids are interrelated, since reduced salinity typically results in reduced osmotic pressure. Disruption of the normal pattern of hyper/hypoosmoregulation by salinity changes may affect tolerances to metals and organic contaminants. Thermal tolerance may also be modified by changes in salinity. For marine organisms, the upper lethal temperature decreases as salinity decreases (Kinne, 1964). Larval fish move into estuaries where they mature as post-larval juveniles to adults before they migrate to marine environments to spawn. Daily and seasonal fluctuations in salinity and temperature are characteristic of estuaries and although post-larval fish are tolerant to a wide range of water quality exposure regimes during these developmental stages, their survival, growth and development can be adversely affected by rapid fluctuations caused by altered flow patterns.

Survival

Some species can slowly acclimate to high salinity and some intertidal organisms can and do live in low salinity waters. But often these intertidal species cannot reproduce there and their repopulation depends on invasion of larvae or adult migratory populations from higher salinity waters. For example, the amphipod *Corophium volutator* was found in salinities as low as 2 ppt, but breeding occurred in salinities greater than 7.5 ppt (McLusky, 1968). Temperature and salinity can act jointly affect the survival of estuarine organisms. Survival time of the polychaete worm, *Clymenella torquata* at 37° C is markedly less at low salinities (Kenny, 1969). However, at low temperatures, the worms can tolerate low salinities for prolonged periods of time (Kenny, 1969). Though tolerant of a wide range of salinities (0-164 ppt) when gradually acclimated, *Cyprinodon variegatus* (Sheepshead minnow) has shown a high degree of mortality when exposed to rapid changes in salinity (Serafy, 1997; Nordlie, 2006). Hence, the rate of salinity change that an organism experiences is a likely factor in its ability to tolerate variable salinity environments (Nordlie, 2006).

Reproduction and Development

There is also evidence that salinity tolerances of progeny may depend upon the salinity to which the parent stock is adapted. Offspring of low salinity adapted parent stocks tolerate transfer to freshwater better than offspring from parent stocks grown at high salinities (Kinne, 1953). Salinity differentially influences various stages of the reproductive process. Fertilization, for example may occur over a wider range of salinities than subsequent larval growth and development. Fertilization, in *Mytilus edulis* occurred in salinities from 15-40 ppt, but successful development to the trochophore

stage occurred only in salinities of 30-40 ppt (Bayne, 1965). In addition, larval development of some estuarine intertidal crabs is limited to the lower salinities of estuarine waters. For example, the wharf crab, *Sesarma cinereum*, has four zoeal stages and a megalops stage before it undergoes development to the crab stage, and it is the fourth zoeal stage which is most sensitive. Maximum survival and molting of this stage to megalops occurs in salinities of 26.7 ppt, and if higher salinities exist the crabs may die (Costlow et al., 1960). In the lingcod (*Ophiodon elongatus*), salinity and temperature have been shown to jointly influence egg hatchability and development and the incidence of deformities (Cook et al., 2005). In the flagfish, *Jordanella floridae*, increases in temperature were shown to increase egg development, while egg hatchability increased with increasing salinity (St. Mary et al., 2004). Gestation time and growth rate of neonates of the livebearer, *Poecelia latipinna*, were shown to be affected by changes in salinity (Kumaraguru et al., 2005). Trexler and Travis (1990) showed that low salinity negatively affected the size and maturation of female *P. latipinna*, fed at a constant food level, when compared to females raised at higher salinities.

In addition to differences among species, previous studies have demonstrated that salinity tolerance is size- and age-dependent for penaeid shrimp (Alvarez et al. 2004). Size is critical in determining postlarval survival to salinity stress (Alvarez et al., 2004). Tsuzuki and Cavalli (2000) found that developmental stage also influenced tolerance to salinity fluctuations. Their studies concluded that larger and older postlarvae tend to be better able to cope with salinity changes likely due to their more developed osmoregulatory capacity. In teleosts, prelarvae are able to osmoregulate via the

integument and shift to use of the gills during the larval stage, hence their ability to osmoregulate also increases with age (Varsamos et al., 2005).

Metabolism

Since changing salinity is a characteristic of estuaries, estuarine animals exhibit a wide diversity of metabolic responses to variable salinities (Kinne, 1967). Laboratory studies with aquatic organisms have shown that standard metabolic rates and osmoregulatory capabilities are affected by salinity (Plaut 1999, Nordlie 1985). Wuenschel et al. (2005) reported that O_2 consumption in juvenile gray snapper was higher at higher salinities. The authors further extrapolated that higher metabolic costs, at higher salinities, could affect overall juvenile production. Haney et al. (1999) showed that even the widely salinity tolerant sheepshead minnow (*Cyprinodon variegatus*) exhibited depressions in its resting metabolic rate (RMR) at freshwater and hypersaline (>40 ppt) conditions resulting in overall lower metabolism and reduced energy expenditure. Fluctuating salinity as well as less-than-optimal salinity levels have been reported to have direct effects on the metabolic response, distribution, growth and survival in penaeid shrimp (Browder et al. 1999, Ramirez-Rodriguez et al. 2003, Tsuzuki et al. 2003, Allan et al. 2006); even so, the physiological responses of shrimp to fluctuating salinities are not fully understood.

Growth

Increases in temperature resulted in increased linear and specific growth rate as well as overall growth efficiency in juvenile gray snapper, *Lutjanus griseus* (Wuenschel et al., 2004) However, an increase in salinity was found to negatively affect the growth efficiency of this same species (Wuenschel et al., 2004). Hypersaline events in Florida

have also been linked to growth reductions in juvenile American crocodiles (*Crocodylus acutus)* (Richards et al., 2004). Juvenile cobias (*Rachycentron canadum*) have shown negative growth effects in relation to lowered salinity (Denson et al., 2003). Hurtado et al. (2006) found significant differences in growth for the penaeid *Litopenaeus vannamei* at high and low osmotic pressure.

Body Fluid Homeostasis

The absence of mortality does not necessarily mean the absence of adverse effects. Fluctuating salinity has been shown to cause a disruption in the osmotic balance in juvenile shrimp (Chen et al. 1996). These salinity fluctuations result in compensation by the organism in an attempt to readjust the osmotic balance, which causes a considerable expenditure of energy possibly leading to growth and reproductive effects (Chen et al. 1996). Plasma osmotic concentration in teleost fish has been shown to increase with increases in salinity (Jordan et al., 1993). Sulikowski and Maginniss (2001) also documented salinity induced changes in the blood fluid homeostasis of stingrays. Hyposaline conditions were shown to reduce both plasma osmolality and hematocrit count in juvenile cobia (Denson et al., 2003). Erythrocyte count in fish has also been shown to be influenced by changes in salinity (Nespolo and Rosemann, 2002). As erythrocytes are linked with O_2 transport efficiency, changes in their volume can thus influence overall metabolic performance of the organism (Nespolo and Rosemann, 2002).

Endocrinology

The hormone prolactin (PRL) has been linked to freshwater adaptation in euryhaline fish, by preventing the uptake of water and increasing ion retention (Manzon, 2002). Prolactin is a versatile hormone also linked to growth, reproduction, behavior,

metabolism and immunoregulation in vertebrates (Manzon, 2002). PRL levels have been shown to be greatly influenced by salinity in estuarine fish, with levels increasing with freshwater adaptation(Manzon, 2002).

Behavior

The behavior of aquatic organisms can be affected by low concentrations of a chemical stressor that at higher concentrations can influence physiology and biochemistry (Rand, 1984). Hence, an organism's behavior may prove to be a more sensitive indicator of stress. Both food intake and food selection were shown to be influenced by salinity in the European sea bass (*Dicentrarchus labrax*) (Rubio et al., 2005). The authors found that changes in salinity altered the preference for proteins and carbohydrates in sea bass subjected to salinity changes.

Distribution

Salinity also limits the distribution of estuarine animals. The number of species present or species diversity in an estuary decreases with a decrease in salinity (Wells, 1961) but it should be noted that other abiotic factors (e.g., temperature, oxygen, contaminants) may also vary with salinity changes. Salinity fluctuations have been shown to be the key drivers in influencing fish abundance and distribution in estuaries (Whitfield, 1996; Paperno et al., 2004). A decrease in the growth of plants and diversity of animal communities has been noted in Biscayne Bay as a result of canal-driven freshwater inflow (Brook, 1982; Serafy et al., 1997; Lorenz et al., 1997). There is a general pattern of low densities of juveniles (Berkeley and Campos, 1984) that may reflect poor growth and/or survival of early life stages at low salinities (<20 ppt).

South Florida Regional Considerations

Along with animal distribution, salinity can also be a limiting factor in the distribution of plants. Marsh plants were shown to respond to salinity gradients in several Florida tidal rivers, though it should be noted that salinity was not the sole contributing factor to distribution and abundance (Clewell et al. 2002). Other authors (Clewell et al., 1999; Mattson 2002, and Alexander and Dunton 2006) have linked salinity and changes in the salinity regime of a given area as a driver of plant communities.

Chesnes (2002) studied the effects of pulsed salinity changes (14-28 ppt) on several species of Florida seagrasses. The response to salinity stress was species specific, with turtle grass (*Thalassia testudinum*) showing the most sensitivity and widgeongrass (*Ruppia maritima*) showing the least. The author concluded that in areas subject to fluctuating salinity like northern Florida Bay, salinity is a primary driver for the abundance and distribution of submerged macrophytes. Additionally, La Peyre and Rowe (2003) found that growth of widgeongrass was better in constant salinity conditions vs. pulsed conditions. Both pulsed and gradual salinity changes (to hypersaline conditions) were examined Koch and Durako (2005) and Koch et al.(2007) in multiple species of seagrass. In both papers it was shown that while rapid changes in salinity produced negative effects, gradual changes which allowed a period of osmotic adjustment resulted in acclimation and survival.

The American crocodile (*Crocodylus acutus*), a resident of Florida Bay, is also sensitive to salinity. Mazzotti and Dunson (1989) showed that juvenile *C. acutus* lacked a functioning salt secreting gland and cannot survive in salinities above 20 ppt for extended periods without drinking freshwater (rainwater). Earlier work by Dunson

(1970) examined the effects of marine conditions (35 ppt) on crocodile hatchlings and concluded that such exposures caused a loss of body mass (1 % per day) and were not conducive to survival. However, a population of crocodiles in South Florida exposed to fluctuating salinitity regimes was shown to be able to survive higher salinities because of periodic exposure to lower salinities. Ingestion of fresh or hyposaline water during these low salinity periods allowed for sufficient osmoregulation by the crocodiles (Mazzotti et. al. 1986).

Rapid changes in salinity and effects on mortality were studied by Serafy et al (1997) for 10 species of estuarine fish native to South Florida. In these studies fish, acclimated to 32 ppt, were directly transferred to freshwater and returned to 32 ppt within a two hour timeframe representing real-time salinity changes that occur in Biscayne Bay due to water management practices (e.g. opening of a flood gate). Results showed that the degree of mortality was species specific, with certain resident species experiencing 100% mortality (e.g. *Cyprinodon variegatus*) while others were unaffected (e.g. *Lutjanus griseus*).

Because of their dynamic nature, estuaries are subject to daily changes in both salinity and temperature corresponding to the timing and quantity of freshwater and saltwater delivery. However, the *quality* of the freshwater delivery also becomes an important consideration in areas with upstream contamination (Eddy, 2005). An additional consideration in bay health is the presence and availability of contaminants. Salinity and/or temperature induced stress changes in organismal physiology may also alter the availability of a contaminant by effecting changes at the site of uptake or in it's metabolic activities.

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Components of the Comprehensive Everglades Restoration Plan (CERP) especially storage, seepage control, redirection of canal flows to overland water and wastewater reuse plants will be used to restore more natural freshwater flows to Florida Bay. However, marine and estuarine communities may be affected by abrupt salinity changes, long-range salinity shifts and a decline in water quality from contaminants resulting from land use changes, human activities and water reuse.

Toxicity of metals is inversely related to salinity in that decreases in salinity produce increases in sensitivity of fish (Voyer et al., 1979; 1982). In addition, organic contaminants are more soluble and bioavailable in fresh waters and are less soluble in salt waters (Hamelink et al., 1994). Studies have demonstrated the presence of contaminants (metals and organics like pesticides, PCBs, PAHs) in the waters and sediments of Florida Bay and adjoining canals, rivers, and streams (Fulton et al., 2004). According to Pait et al., (1992) Dade County ranks second in the nation for hazards associated with pesticide applications in coastal areas. Long et al. (2000) and Carriger et al. (2006) have also shown sediment toxicity in peripheral canals and tributaries of the bay. Other than these studies little toxicological work has been performed for the bay and its freshwater sources. Furthermore, depressions in the pH of saltwater that result from its dilution with freshwater in estuaries may increase either salinity-induced stress and/or the bioavailability of heavy metals and other organic contaminants in the bay (Hamelink et al., 1994). Ammonia toxicity in estuaries has also been associated with lowered pH as a result of freshwater influx (Eddy 2005). Data on the interaction of salinity and/or temperature and contaminants is limited (Hall Jr. and Anderson, 1995).

Central Theme of Dissertation

Timing, volume, delivery and quality of freshwater to Florida Bay can affect the structure and functional aspects of the diverse fish communities in the bay. Therefore, the biological performance measures of estuarine fish will be controlled by changes in salinity, temperature and water quality that will occur as a result of the restoration of freshwater flow to the bay.

With respect to the CERP, freshwater redistribution that ensures a salinity gradient that will support mangrove and estuarine communities is required to restore and maintain these critical South Florida ecosystems (Davis et al., 2005). Data gaps on the effects of water redistribution on bay communities (or lack of redistribution) must be addressed to maximize the restoration of Florida Bay and associated natural systems in this watershed. As altered salinity patterns in the Bay have been linked to declines in the abundance of both dermersal fish and the higher trophic level species that depend upon them, examination of the effects of salinity changes on native fish species can help resource managers understand the potential risks and reduce uncertainties concerning biological impacts of the CERP.

This dissertation will focus on the following two central hypotheses.

Hypothesis 1: Salinity is a significant limiting/modifying factor for optimum growth, development, survival and distribution of indigenous fish.

Hypothesis 2: Changes (decreases and increases) either abrupt or long-term in salinity of saltwater will exert adverse effects on growth, development, survival and distribution of acclimated indigenous fish.

I proposed to test these hypotheses to fill data gaps that must be addressed to maximize restoration of Florida Bay and associated natural systems in this watershed. This will allow us to understand potential risks and reducing uncertainties concerning biological impacts of proposed CERP activities.

I addressed these hypotheses through the use and quantification of performance measures (e.g., survival, growth, development, behavior) in indigenous fish species as the measurement endpoints to assess responses to the stressor (salinity). Each chapter was written with the goal of providing information and tools that can be used by regulatory agencies for management of South Florida systems in regards to the CERP.

Chapter II explores the biological effects of salinity change on four native, estuarine fish species. The endpoints selected were determined by the U.S. EPA as regulatory endpoints perceived by Congress to be of sufficient societal or economic importance to be protected by legislation. Chapter III moves beyond the physiological effects and focuses on the behavioral changes associated with variable salinity, particularly attraction and avoidance. For Chapter IV, I conducted a probabilistic ecological risk assessment to estimate the risk of real-time salinity profiles to the presence and diversity of estuarine fish. This risk assessment was conducted for four embayments of northeastern Florida Bay that are of critical importance as feeding grounds for higher trophic level species. The approach and endpoints are presently being used in assessing potential ecological risks of chemicals under TSCA and FIFRA by the U.S.EPA.

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Daily Salinity at Joe Bay Creeks and Trout Creek. 1999-2001
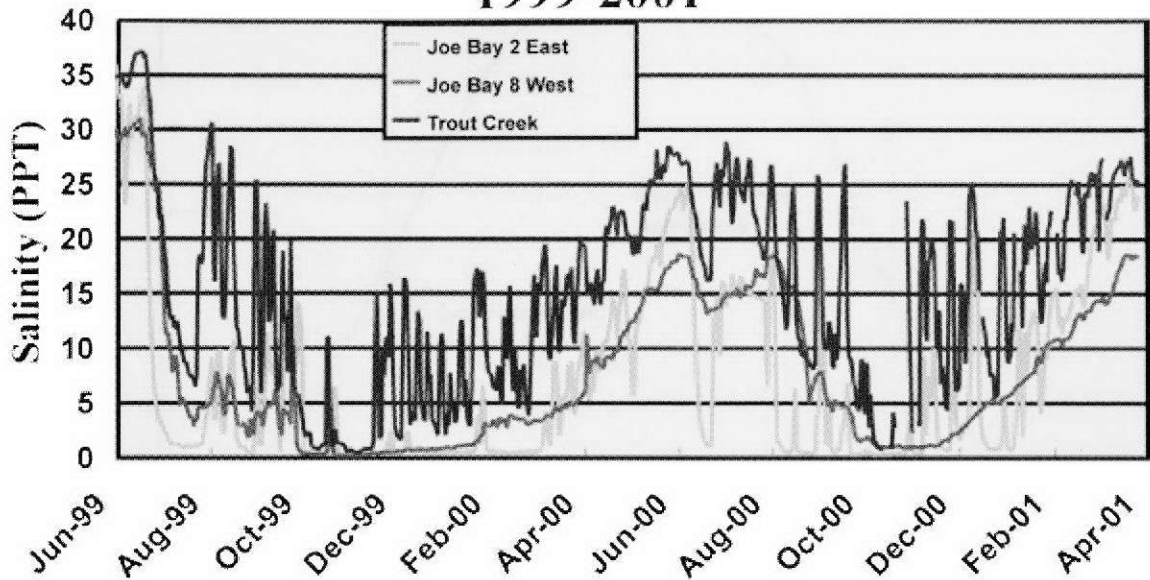
Figure 1.1. Example of spatial and temporal salinity variability in two areas of northeastern Florida Bay, Joe Bay and Trout Creek from 1999-2001. (Data and figure from Hittle and Patino, 2000)
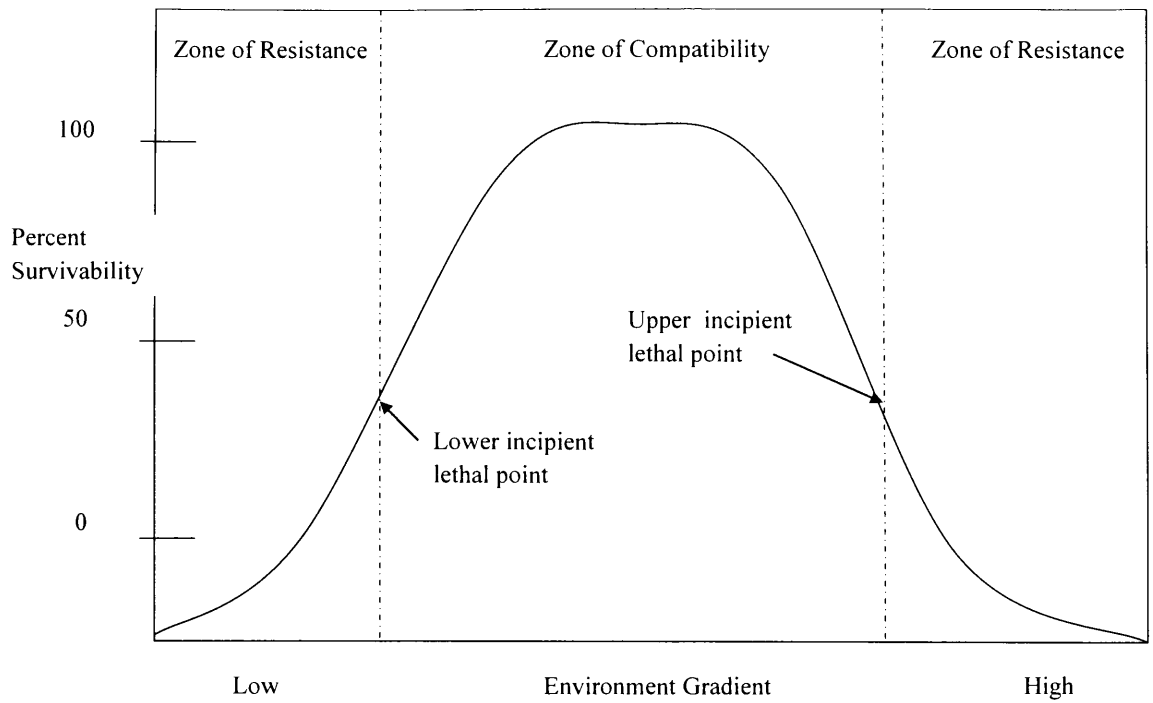
Figure 1.2. Zones of Resistance and Compatibility (after Vernberg and Vernberg, 1976).

Chapter II

EFFECTS OF SALINITY ON NATIVE ESTUARINE FISH SPECIES IN SOUTH
FLORIDA: TOXICOLOGY STUDIES

Abstract

Variable and high salinities have been identified as key stressors in Florida Bay. The

Comprehensive Everglades Restoration Plan (CERP) includes water redistribution

projects that are intended to restore natural freshwater flows to northeastern Florida Bay.

The present salinity regimes in the area, which span from hypo- to hypersaline, will be

altered as a result of these actions. My research examined biological performance

measures (i.e., growth and survival) of estuarine fish under varying salinity regimes that

will occur as a result of the restoration of freshwater flow to the bay. A series of acute

and subchronic studies were conducted to determine the effects of salinity changes on

various life stages (embryo/larval, juvenile, adult) of four native estuarine fish

(*Cyprinodon variegatus, Floridichthys carpio, Poecilia latipinna,* and *Gambusia*

holbrooki). Fish were exposed to a range of salinity concentrations (freshwater to

hypersaline) based on current salinity profiles in the study areas. Growth (length, weight),

and survival were measured. Salinity exposures included both rapid and gradual change

events. Results show adverse effects of acute, abrupt salinity changes on fish survival

and development because of salinity stress.

Introduction

Estuaries and coastal ecosystems experience a range of stresses, both natural and anthropogenic. Two of the most important factors affecting the overall functionality of an estuary are the timing and quality of the freshwater inflow, which subsequently affect the salinity regimes of the area (Montagna et al., 2002). Salinity is considered to be one of the primary environmental factors affecting the growth and survival of aquatic organisms because it can influence both physiological and ecological processes (Chen et al. 1996, Tsuzuki et al. 2003, Varsamos et al., 2005, Nordlie, 2006).

Florida Bay (referred to as the Bay) is a large, shallow (average depth 6-9 feet), subtropical estuary on the southern coast of Florida that, along with the Gulf of Mexico, receives water from the Everglades system (Lodge, 2005). The Bay (and its natural tributaries) is within the boundaries of Everglades National Park (Figure 2.1).

Salinity, circulation and water quality in the Bay depend primarily on freshwater inflow, wind-driven circulation and ocean exchange. Florida Bay provides critical habitat for the American crocodile and West Indian manatee and also supports a diverse natural assemblage of seagrass, hardbottom, and coastal mangrove communities despite hydrological alterations from urban development and the Central and South Florida (C&SF) Flood Control project. The Bay also acts as a nursery for the larvae and juveniles of many critical species, including piscivorous fish and numerous wading bird species (Lorenz and Serafy, 2006).

Analysis of monitoring station data from areas of northeastern Florida Bay shows variable and hypo- to hypersaline events on both short (daily) and long (yearly)

temporal scales (DBHYRDO Database, SFWMD). Salinities in these areas can range from freshwater conditions to over 40 ppt (DBHYDRO Database, SFWMD).

A key component of the Bay is the large population of demersal fish that support Higher Trophic Levels (HTL) species. Declines in the abundance of HTL species (such as Roseate Spoonbill) that rely upon these forage fish have been linked to food stress (Ley et. al, 1994, Lorenz & Serafy, 2006). There is evidence to suggest that the altered salinity patterns in the Bay are, in part, responsible for the decline in overall abundance of forage fish (Lorenz and Serafy, 2006).

Timing, volume, delivery and quality of freshwater to Florida Bay can affect the structure and functional aspects of the diverse fish communities in the bay. Therefore, the biological performance measures (survival, growth, development) of estuarine fish will be controlled by changes in salinity, temperature and water quality that will occur as a result of the restoration of freshwater flow to the Bay.

Field studies in northeastern Florida Bay have reported changes in demersal fish populations and abundance due to changes in salinity patterns (Lorenz and Serafy, 2006). Adults and juveniles of Bay fish species have been shown to exhibit different tolerances to salinity changes, with juveniles being less sensitive than adults (Christensen et al., 1997). Within a fish species, the geographic location and history of salinity exposure can influence the local population's tolerance for salinity change (Holt and Holt, 2003).

Currently, there is little laboratory research available that examines the optimal salinities or tolerance ranges for key forage fish species native to Florida Bay, especially on early life stages. (Nordlie, 2006). The following studies were conducted to determine

if salinity is a significant limiting/modifying factor for optimum growth, development and survival of native fish.

Materials and Methods

Organisms and Water Quality

Four species of native estuarine fish collected from northeastern Florida Bay were used in these studies. They are as follows: Sailfin Molly *(Poecilia latipinna)*; Gold-Spotted Killifish (*Floridichthys carpio*); Sheepshead Minnow (*Cyprinodon variegatus*); and Eastern Mosquitofish (*Gambusia holbrooki*). The species chosen comprise the bulk of demersal fish biomass found in these areas (Lorenz and Serafy, 2006). These species represent a broad range of salinity tolerances and life history strategies. Two species (*Cyprinodon variegatus and Floridichthys carpio*) are egg-layers and the other two species (*Poecilia latipinna and Gambusia holbrooki)* are live-bearers.

To establish cultures, fish were collected from Little Blackwater Sound, in northeastern Florida Bay using a ¼" mesh seine net. Capture and transport techniques were developed to minimize pain and distress to fish and handling was minimized. Fish were transported in aerated water from the collection site water in a cooler. The maximum time from capture to arrival at the laboratory did not exceed three hours for all sampling trips. Sampling trips were conducted to supply individuals to establish in-house breeding cultures at the Florida International University Ecotoxicology & Risk Assessment Laboratory.

Upon arrival at the laboratory, fish were acclimated to culture water and temperature conditions (15 ppt and 22° C, respectively). Fish were separated by species and visually inspected for parasites and outward signs of infection. Two types of

prophylactic treatments, a paracide (formalin) and an antibiotic (nitrofurazone) were administered to all wild-caught fish entering the laboratory to prevent contamination of clean stocks. For ecto-parasite treatment, fish were dipped for 30 minutes in a 250 mg/L formalin solution with aeration and then placed back in clean water. Twenty-four hours after parasite treatment, a dose of 20 mg nitrofurazone per liter of tank water was administered for a period of 3 days as an antibiotic measure. Hospital tanks for the fish had no filtration (to prevent loss of antibiotic) and were held with constant aeration, temperature and light regime for the duration of the prophylactic treatment. A 100% water renewal and fresh batch of antibiotic were prepared for each 24 hours of treatment.

All fish were maintained on a recirculating water system at the appropriate salinity. Each recirculating system consisted of a particle (sand) filter, biological filter and UV sterilizer. Tanks ranged in size from 10 gallon glass aquaria to 300 gallon fiberglass tanks. Each tank was provided continous aeration and controlled temperature (22°C). Indoor tanks had a controlled lighting regime of 16 hours daylight to 8 hours darkness.

All fish were cultured at the optimum conditions for health and welfare, and to minimize distress. Water quality was monitored regularly, with temperature and salinity monitored daily while dissolved oxygen (D.O.) and pH were measured on a weekly basis. The loading volume of fish per tank was based on organism and tank size, and structured in such a way as to not cause undue stress to fish.

Fish used in the studies were euthanized post-test using a concentrated solution of MS-222. Returning fish to the wild, or back into the laboratory broodstock culture was not a viable option. These fish may have been compromised by their exposure to the

substances we were testing, and we did not wish to further compromise wild or breeding stocks by their re-introduction.

Our laboratory has developed Standard Operating Procedures for the culturing of the above mentioned animals. These procedures are based upon the following souces: Guidelines for Use of Fishes in Research (American Fisheries Society, 2004); U.S. EPA Good Laboratory Practice (GLP) Standards 160.90: Animal and other test system care; and U.S. EPA methods for fish culture as described in document EPA/600/4-90/027F August 1993. Fish collection, husbandry and testing were conducted in accordance with FIU IACUC Protocols 04-029 and 04-030.

Offspring (F_1) were produced through both natural and artificial means from the field-collected parental generation (F_0). Artificial reproduction of sheepshead minnows was conducted through use of Human Chorionic Gonadotropin (HCG) under the procedure outlined in U.S. EPA document 1005.0 (in EPA-821-R-02-014, 2002).

Laboratory Resources

All experimentation was carried out at Florida International University's Southeast Environmental Research Center Ecotoxicology Laboratory located on Biscayne Bay in North Miami, Florida, a NELAC certified facility.

The laboratory's location and computer-interface systems provided the capability to conduct toxicity exposures in natural full-strength seawater as well as freshwater. The laboratory contains a salinity control system to automatically produce a range of salinities for estuarine studies based upon study needs.

The source of full-strength seawater (30 ppt) was a saltwater aquifer supplied by Biscayne Bay. Dilution of the 30 ppt source water for the laboratory was controlled by the automated salinity system. Hypersaline water (>30 ppt – 60 ppt) was formulated from the laboratory source saltwater combined with Instant Ocean Sea Salts (Aquarium Systems, Inc.).

Study Design

In salinity toxicity studies, water hardness and alkalinity of freshwater were measured and ranged from 60 to 100 mg/l (as $CaCO_3$) and from 54 to 83 mg/l, respectively. Salinity, conductivity, dissolved oxygen and temperature were monitored daily, while pH and total ammonia (mg/l) were monitored weekly.

For all studies, pH ranged from 7.20 to 8.68 and total ammonia never exceeded 0.7 mg/l. A temperature of 25 °C (+/- 1 °C) and a photoperiod (16L: 8D) were maintained throughout the duration of the studies. The study areas were curtained off from the main laboratory to minimize any disturbance. Salinities were maintained at +/- 1 ppt of the target value, and dissolved oxygen was maintained at greater that 80% saturation in all treatments. In long-term studies, fish were fed daily with Aquatox (Zeigler Bros. Inc.) flake food until satiation. Young fish (neonates and fry up to 2 weeks old) were fed less than 24-hour old brine shrimp nauplii (*Artemia* spp., NABS, LLC). Uneaten food was siphoned from tanks daily.

Static renewal and flow-though water exposures were used for the toxicity studies to simulate salinity regime changes in Florida Bay. Static renewal was used to mimic abrupt salinity changes. Flow-through systems were used to conduct short-term (acute)

and long-term (chronic) exposures with gradual salinity change to determine effects on survival, growth, and reproduction of fish. The flow-through system produces five different salinity concentrations and is controlled by Windows software.

Acute Toxicity of Salinity Changes to Adult Estuarine Fish Under Static Conditions

Each of the four fish species were exposed to abrupt salinity changes to evaluate the direct effects of salinity on survival. Adults of each species were acclimated under culture conditions for 14 days to a salinity of 30 ppt and exposed to an abrupt salinity change, via direct transfer, resulting in final salinities of 15, 8, 4, 2 and 0 ppt respectively. Control fish were maintained at 30 ppt. Observations were made for 10 days. Each salinity treatment contained two replicates with 10 fish per replicate in 10L glass aquaria with aeration. The study was conducted under static-renewal conditions with 50% water exchange daily.

Acute Toxicity of Salinity Changes to Adult Estuarine Fish Under Flow-Through Conditions

Fish were exposed to gradual salinity changes and evaluated for effects on survival for 96 hours. To simulate a more natural and less-abrupt change in salinity, a flow-through diluter system was used. The study was initiated with all tanks at the control salinity (30 ppt or 15 ppt) and employed the automated flow-through diluter to gradually change salinity to the target concentration over a 24 hour period. Two scenarios were conducted with baseline initial salinities from near-marine (30 ppt) and estuarine (15 ppt) conditions.

Adults of each species were first acclimated for 14 days to a salinity of 30 or 15 ppt (control scenario I & II, respectively) and exposed to a gradual change in salinity resulting in final salinities of 30, 15, 8, 4, 2 and 0 ppt. Control groups for each scenario were kept at their respective acclimated salinity, either 30 or 15 ppt. Each salinity treatment contained two replicates with 10 fish per replicate. Exposure tanks were 18-liter glass aquaria provided with aeration.

Fish Early Life Stage (ELS) Toxicity Study

Fish ELS studies were conducted to determine the effects of salinity change on survival and growth of *C. variegatus* embryos/larvae and *P. latipinna* neonates. Procedures followed U.S. EPA toxicity methodology for fish ELS studies (U.S. EPA, 2002). Exposure of *C. variegatus* was initiated within 24 hr after fertilization and exposure of *P. latipinna* was initiated with < 24hr old neonates that were all initially maintained in water with a salinity of 15ppt. Embryos (*C. variegates)* or neonates (*P. latipinna)* were distributed, five at a time, by stratified random assignment to each labeled chamber using a serological pipette until each chamber contained 40 embryos or neonates. There were 40 embryos or neonates per replicate with two replicates per salinity treatment concentration and six salinity concentrations (30, 15, 8, 4, 2 and 0 ppt) held under flow through conditions for 60 days after test initiation. The controls for both species were the treatment groups that were maintained consistently at a salinity of 15 ppt. Dead eggs and fry were removed daily and counted. When hatch was complete the number of live and dead organisms was recorded. Percentage hatch was calculated as the number of live, normal fry in each chamber/40 embryos. Fry were fed 24 hr old brine shrimp nauplii, two times per day. After 14 d, young fish were switched to a diet of

Aquatox flake food. Fish were fed to satiation on both diets, and excess food was siphoned daily from tanks.

Daily observations were recorded for both species on fry mortality, behavior and appearance. At the end of the 60d exposure periods, the fry from each chamber were anaesthetized with MS-222 and percentage survival, mean total length and wet weight were determined. Fry were measured individually to the nearest mm to calculate a mean and standard deviation for total length and fry were weighed to the nearest mg to calculate average wet weight.

Acute Studies- Hypersalinity

Studies were conducted with two different genetic strains of *C. variegatus* to identify response differences between native and commercially produced stock. Embryos of a non-Florida Bay population were purchased from a commercial supplier (Aquatic Biosystems, Ft. Collins, CO). As this stock was cultured at 25 ppt, the control salinity for this study was set at 25 ppt instead of 15 ppt.

Cyprinodon variegatus embryos (<24 hrs) were exposed to salinities ranging from 0-60 ppt in 9 d studies to determine the response of acute exposure. The study duration, and age of the organisms were chosen to target life stages likely to be affected by salinity change. These studies were conducted following U.S. EPA methods (U.S. EPA, 2002) and contained the following salinity treatments 60, 50, 40, 30, 15, 8, 4, 2 and 0 ppt with four replicates per treatment and 15 organisms per replicate. Studies were conducted under static-renewal conditions in 600 ml beakers. The control salinity for these studies

was either 15 ppt or 25 ppt, the salinity at which the parent stock were cultured and held in the laboratory.

Daily observations were recorded on hatching, behavior and appearance. When hatching was complete, the number of live and dead fry was recorded from each chamber, and percentage hatch was recorded. At the end of the 9d exposure period, the fry from each chamber were anaesthetized with MS-222 and percentage survival, mean total length and wet weight were determined. Fry were measured individually to the nearest mm to calculate a mean and standard deviation for total length and fry were weighed to the nearest mg to calculate average wet weight.

Reproduction Studies

The breeding success of the sheepshead minnow was examined as a factor of salinity stress. Breeding groups of *C. variegatus* (1 male, 4 females) acclimated to 15 ppt, were direct transferred to flow-through aquaria (18 L) at the following salinities: 30, 15 (Control), 8, 4, 2 and 0 ppt. Each salinity treatment consisted of two replicates, and the study was conducted for 2 weeks.

The sheepshead minnow is a broadcast spawner with negatively buoyant eggs. To prevent predation on the eggs, the fish were placed in floating breeding tanks 27.94 cm x 15.24 cm x 15.24 cm (Aquatic Ecosystems, Inc. Apopka, FL.). The breeding tanks were constructed of clear plastic, and slotted on the sides and bottom to allow the eggs to fall through and water to circulate. Plastic trays with nitex mesh bottoms were placed on the bottom of each aquaria to facilitate in egg recovery. Due to aggressive behavior, males

and females were separated by a slotted, plastic divider. The divider was removed from 8:00 am til 12:00 pm each day to allow for mating.

Eggs were collected the morning following each mating prior to 8:00 am. Eggs were siphoned from trays and either further examined for hatching success or transferred to Protocol Safe-Fix II fixative (Fisher Scientific, Atlanta, GA) for dry weight analysis.

For the hatching studies, fertilized eggs (< 24 hrs. old) from each salinity treatment were transferred to 600 ml beakers with aerated water of the corresponding salinity. Each salinity treatment consisted of 4 replicates, with 10 eggs per replicate. Embryos were allowed to mature, hatch and grow-out for 7-days post-hatch. Mean dry weights (mg) were determined for each larvae following the grow-out period.

Eggs not used in the hatching study were preserved using a fixative, counted and weighed (dry weight in mg) to determine the mean number of eggs per salinity treatment and mean egg weight.

Statistical Analysis

Statistical analysis followed the guidelines outlined by the U.S. EPA (U.S. EPA, 2002). Figures 2.2 and 2.3 outline the statistical approach used in these analyses. Data for length, weight, survival and hatchability were tested for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's tests, respectively. The estimation of the effects endpoints for normally distributed and homogenous data were made using a parametric ANOVA followed by Dunnett's test. When data were not normally distributed or homogenous, chronic data were analyzed using the nonparametric Steel's Many-one

Rank test or the Wilcoxon Rank Sum test as appropriate. All statistical analyses were conducted using SPSS for Windows, version 14.0.

Results

Acute Toxicity of Salinity Changes to Adult Estuarine Fish Under Static Conditions

Mortality occurred within 24 hours in the rapid transfer of fish from the control salinity (30 ppt) to the lowest salinity treatments (0 ppt, FW) for all species studied (Figure 2.4). When transferred from 30 ppt to 0 ppt there was 60% mortality in *C. variegatus*; 40% in *P. latipinna*; 10% in *G. holbrooki* and 100% in *F. carpio*. Of the three species tested, *G. holbrooki* has the greatest preference for freshwater conditions. *Floridichthys carpio* is the least tolerant of freshwater conditions (Figure 2.5). Survival for *C. variegatus, P. latipinna,* and *F. carpio* was significantly different from the control in the 0 ppt treatment at $p < 0.05$.

Acute Toxicity of Salinity Changes to Adult Estuarine Fish Under Flow-Through Conditions

No mortality or behavioral changes were observed in any of the gradual salinity change studies for *C. variegatus, P. latipinna,* and *G. holbrooki,* regardless of the acclimation salinity (15 ppt or 30 ppt). However, the gradual change for both acclimation scenarios resulted in mortality for *F. carpio* in the 0 ppt treatment. In Scenario I (marine acclimation- 30 ppt), 70% mortality occurred in the 0 ppt treatment by 96 hours. In Scenario II (estuarine acclimation-15 ppt), 50% mortality was observed in the 0 ppt treatment by 96 hours. The latter study was extended for 8d, at which 100% mortality

occurred for the 0 ppt treatment. For both Scenarios, survival of *F. carpio* at 0 ppt was significantly different from the controls at $p < 0.05$.

Fish Early Life Stage (ELS) Toxicity Study

Results for the 60-day *C. variegatus* study are presented in Table 2.1. Hatching success of *C. variegatus* eggs was significantly decreased ($p < 0.05$) in the hyposaline treatments of 8, 2, and 0 ppt. Fry survival in the 0 and 2 ppt treatments at 9 days was significantly less than the control (15 ppt). Both the 28-day and 60-day survival of the larvae was significantly decreased in the 0, 2 and 30 ppt treatments.

Table 2.2 illustrates the results of the early life stage study with *P. latipinna*. A significant decrease ($p < 0.05$) in survival on days 7, 28 and 60 occurred in the 0 ppt treatment. All other salinity treatments showed no significant differences from the control (15 ppt).

Hyper Salinity Acute Studies

Results of the 9-day acute early life stage tests with *C. variegatus* varied with the origin of the organisms (Table 2.3). The Florida Bay stock showed no significant differences in hatching, survival, or growth in any of the treatments when compared to the 15 ppt control ($p < 0.05$).   In the non-Florida Bay Stock, no significant differences occurred in hatching success or 9-day survival in any of the salinity treatments; however, growth was significantly reduced. Larvae in the freshwater treatments (FW), 2 ppt, and 60 ppt had significantly smaller mean length than the control (25 ppt). The mean weight in the freshwater treatment and the 60 ppt treatment was also significantly lower than the control.

Reproduction Studies

Significant effects on egg production, egg weight and hatched larvae weight were observed in the reproduction studies at $p < 0.05$.

In the embryo-larval hatching study, no significant differences in survival or hatching success in any of the salinity treatments were observed. However, the 7-day old larvae weighed significantly less than the control larvae in the 4 ppt and 8 ppt treatments. It should be noted here that while a small amount of eggs was produced in the FW treatment, none were fertilized and that treatment was not used in this study. Results of this study are presented in Figures 2.6, 2.7 and Tables 2.4, 2.5).

Fish fecundity (as mean number of eggs per 2-week period) was affected by salinity in the both the highest (30 ppt) and lowest (FW) treatment (Figure 2.8). Both treatments resulted in fewer number of eggs than the control salinity of 15 ppt. The mean dry weight (mg) of the eggs was also significantly different ($p < 0.05$) than the control in the 30 ppt, 4 ppt, and 2 ppt treatments. In all three of these treatments, the mean egg weight was significantly greater than the control (Figure 2.9).

Discussion

Parts of the Comprehensive Everglades Restoration Plan (CERP) propose to modify the South Dade Conveyance System (SDCS) and redirect freshwater back into the main slough entering Florida Bay (Lorenz and Serafy, 2006). Restoration related changes in the timing and delivery of freshwater can have direct and indirect effects on the aquatic biota in the area (Ault et al. 1999).

Salinity has been shown to differentially influence the growth and development of many species of fish (Beouf and Payan, 2001). Effects on egg fertilization, yolk sac absorption, early development and growth have all been attributed to salinity as a causative factor (Beouf and Payan, 2001). The energetic costs of osmoregulation in teleost fishes have been estimated ranging from 10 to > 50% of the total energy budget of the organism (Beouf and Payan, 2001). There is a trend that the energetic costs of osmoregulation increase with the degree of change away from iso-osmotic conditions (Morgan and Iwama, 1991).

My research showed that acute, abrupt salinity changes had adverse effects on fish survival, growth and hatching success as a result of salinity stress. All species, to a varying degree and dependant upon life stage, exhibited sensitivity to extreme changes to hypo-and hypersaline conditions. In comparison to adults, the larvae and neonates of *C. variegatus* and *P. latipinna* were more tolerant to rapid upward and downward shifts in salinity. This finding supports field data for these two species in that they both spawn in shallow, nearshore areas of variable temperature and salinity and their offspring are subject to such conditions during growout. (Nordlie, 2000). Nordlie (2000) also reported that the reproduction for these species is maximized in the summer months, which coincides with the wet season in South Florida. As such, northeastern Florida Bay is subject to fluctuating and sometimes rapid inputs of freshwater. Therefore, the ability of young fish to withstand these changes becomes paramount. Unlike larvae and neonates, the adults of these species have a much greater ability to move out of areas that are experiencing a dramatic salinity shift. Thus, salinity could become a driver affecting the distribution of various life stages.

The temporal nature of the salinity change was shown to have a pronounced effect and thus must also be taken into consideration when implementing the CERP. Although tolerant of a wide range of salinities (0 to >125 ppt), when gradually acclimated, *C. variegatus* has shown a high degree of mortality when exposed to rapid changes in salinity (Serafy, 1997; Nordlie, 2006). Hence, the rate of salinity change that a fish experiences is a likely factor in their ability to tolerate variable salinity environments (Nordlie, 2006). As stated above, adult *C. variegatus* in the current study showed a higher overall sensitivity (as mortality) to salinity change than young fish. However, this only occurred during rapid change (direct transfer) events. Rapid change events can occur in northeastern Florida Bay in association with storm events and anthropogenic activities such as the opening of sluice gates. The more gradual change (over 24 hrs) scenarios examined in this study showed that all species, except for *F. carpio*, could adequately adapt to the change. There is little life history information available on *F. carpio*, and previous work has suggested the use of *C. variegatus* as a surrogate due to these data gaps (Faunce and Serafy, 2002). This study showed that *F. carpio* has a much narrower salinity tolerance than *C. variegatus* and may serve as a good indicator for establishing a low salinity value for areas of Florida Bay.

For *C. variegatus* populations in Florida Bay, variable salinity conditions are an ever-present challenge. Both phenotypic plasticity and genetic selection within the species allows for adaptation to the erratic environmental conditions in the area. With the exception of full freshwater, *C. variegatus* breeding groups were able to successfully re produce in all salinities tested. Though some significant differences were found in fecundity and egg size over different salinities, the fact that successful fertilization, larval

development, and hatching occurred indicates the plasticity of this species. In comparison to the C. *variegatus* embryos obtained from a commercial supplier, the Florida Bay stock exhibited a high degree of tolerance for salinity change. This response indicates that the local populations of C. *variegatus* have a genetically selected tolerance to wide scale salinity changes that is not found in non-Florida Bay populations. Holt and Holt (2003) showed that Spotted Seatrout eggs and larvae responses to salinity change were linked to a heritable adaptation to their local salinity regimes. It therefore becomes essential to prioritize the study of the responses of native and local organisms when making management decisions.

With respect to the CERP, maintenance of a salinity gradient supportive of mangrove and estuarine communities is required to restore critical South Florida ecosystems (Davis et al., 2005). Data gaps on the effects of water redistribution (or lack of redistribution) on bay communities must be addressed to maximize the restoration of Florida Bay and associated natural systems in this watershed, and to understand potential risks and reduce uncertainties concerning biological impacts of proposed CERP activities. Based on field sampling data, Lorenz (1999) postulated that the history the water management practices in south Florida has lowered both the abundance and biomass of the demersal fish community in mangrove habitat of northeastern Florida Bay. The toxicological salinity studies conducted herein indicate that rapid and prolonged changes in salinity will indeed affect the survival, growth and reproduction of key resident species of demersal fish in Florida Bay. The implications to higher trophic levels that depend upon these fish as a prey base is clear, and both anecdotal and scientific evidence exists to link the decline in

prey base as a factor in the declines in such predatory species as wading birds, alligators, crocodiles, and piscivorous fishes (Lorenz, 1999).

Future studies are required to target reproduction and critical embryo-larval development as key areas for detecting long-term population effects of salinity change on fish populations in Florida Bay. Osmotic, metabolic and behavioral effects associated with salinity changes must also be evaluated. Further work on hypersaline effects is also necessary for a broader understanding of the full salinity regime of Florida Bay.

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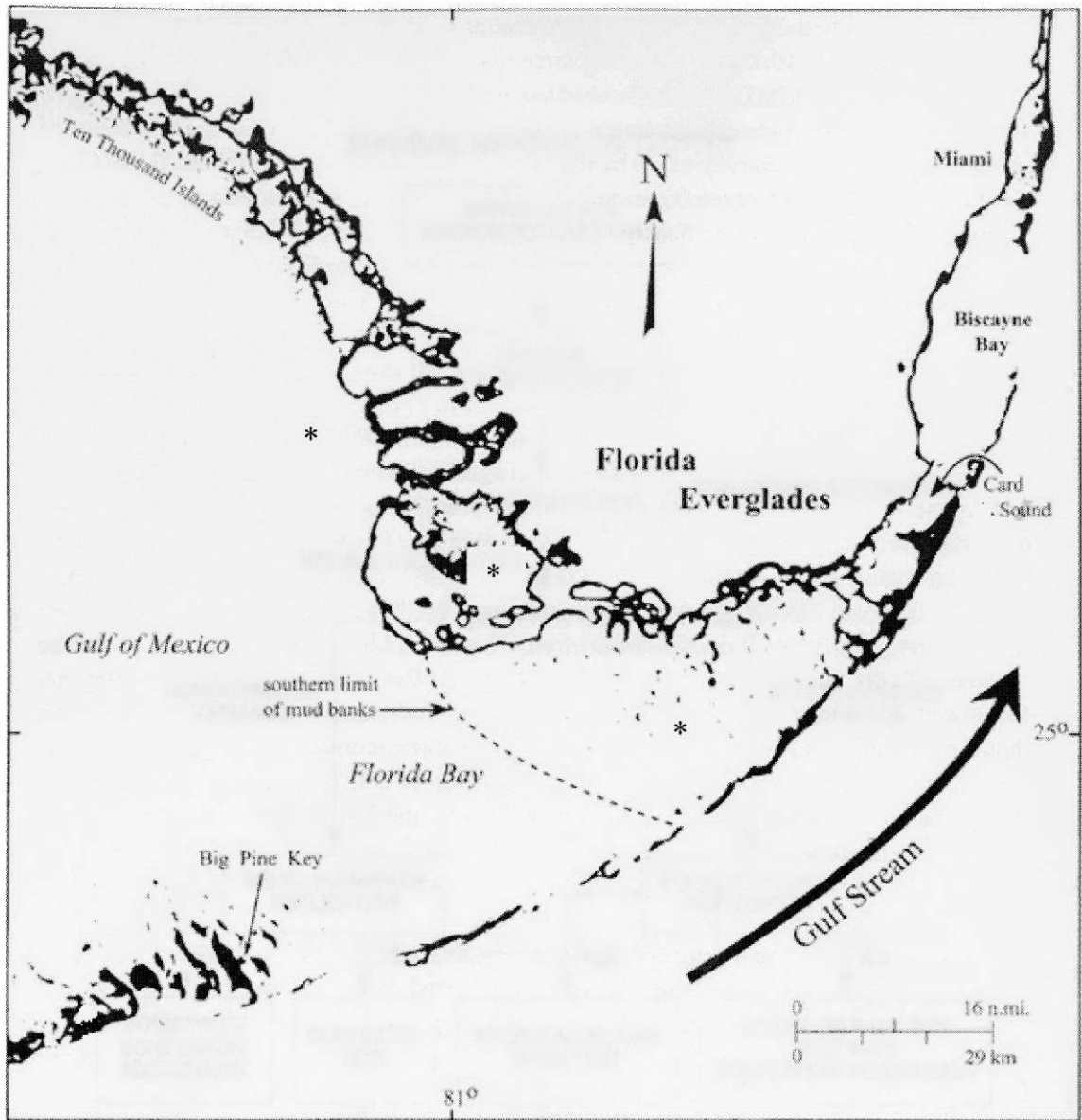
Figure 2.1 South Florida and Florida Bay (from USGS).
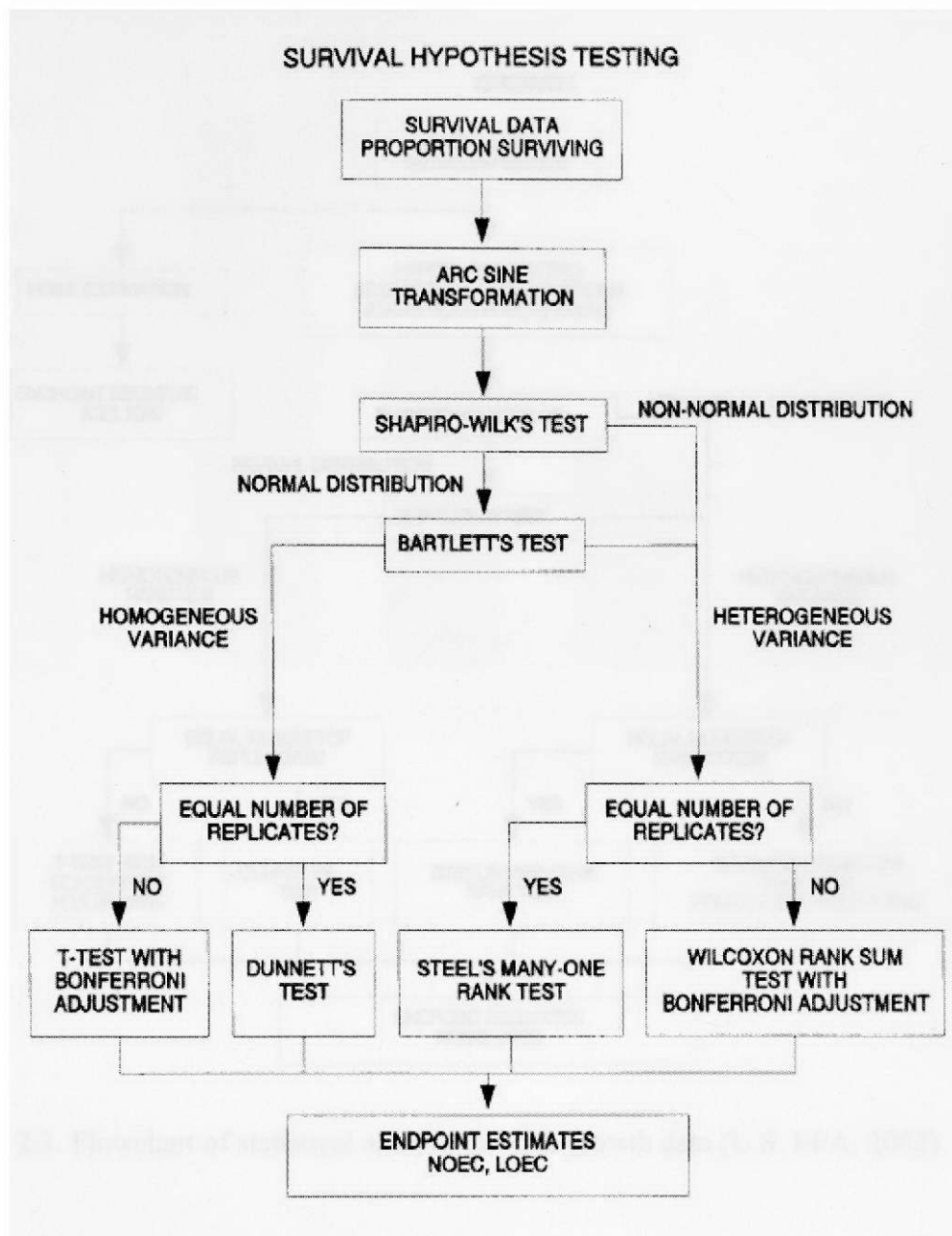
Figure 2.2. Flowchart for statistical analysis of fish survival data (U.S. EPA, 2002).
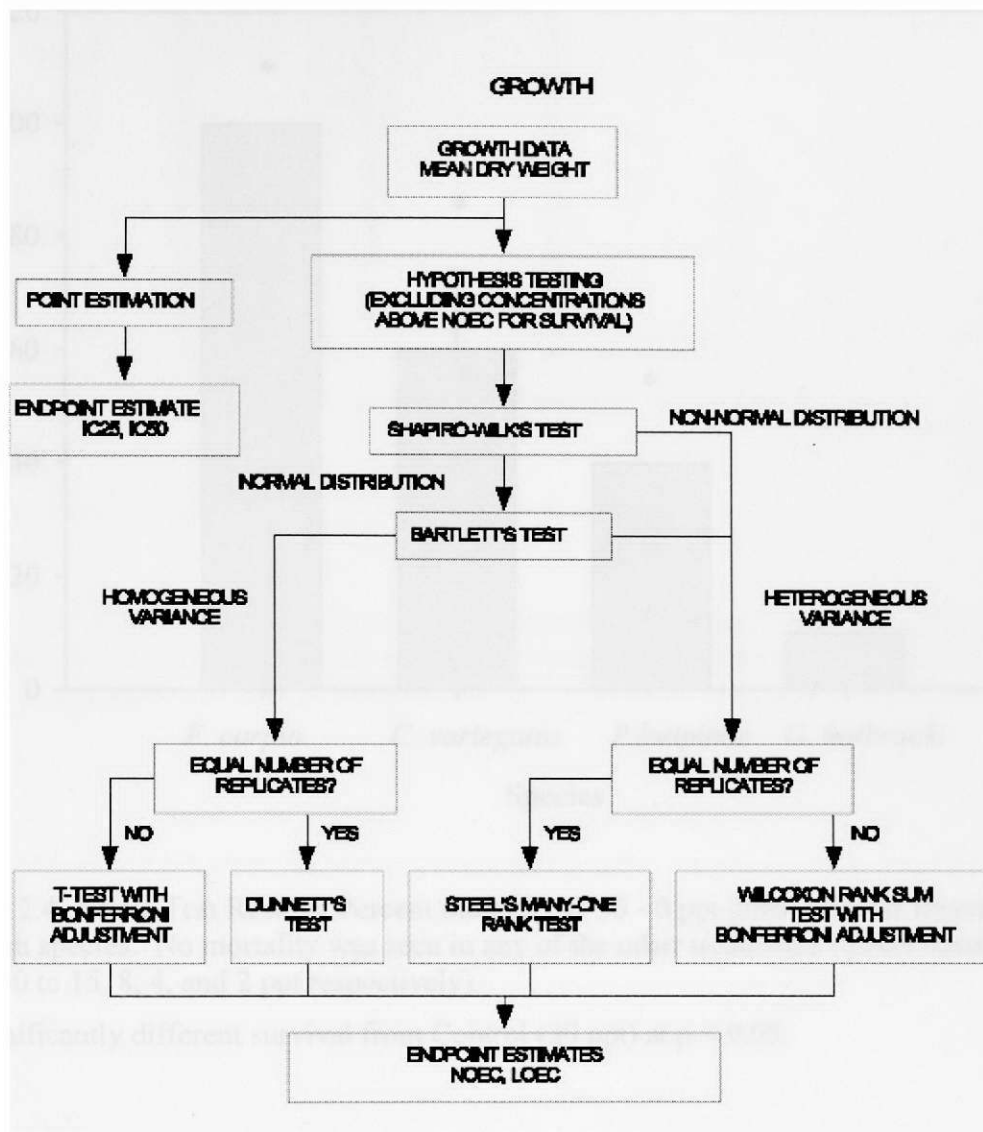
Figure 2.3. Flowchart of statistical analysis for fish growth data (U.S. EPA, 2002).
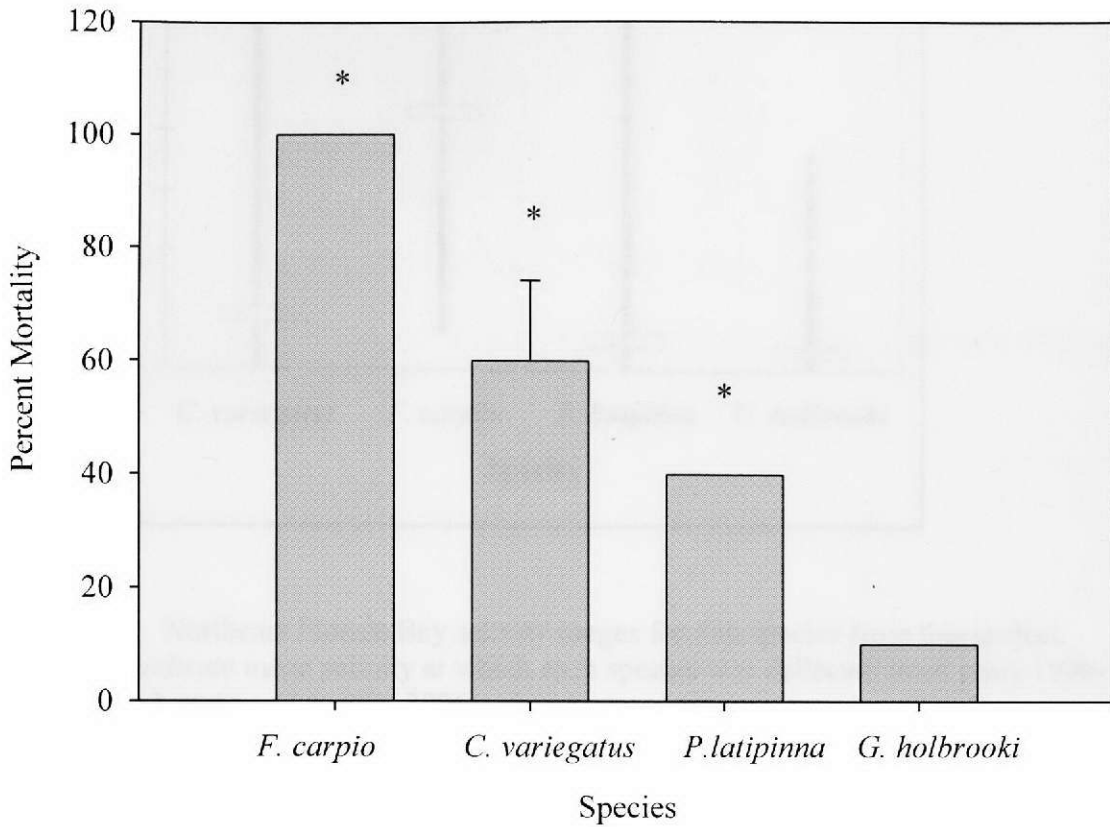
Figure 2.4. Static Test Results: Percent mortality at 30 - 0 ppt direct transfer treatment for each species. No mortality was seen in any of the other treatments (direct transfer from 30 to 15, 8, 4, and 2 ppt respectively).

 * Significantly different survival from Control (30 ppt) at p < 0.05.
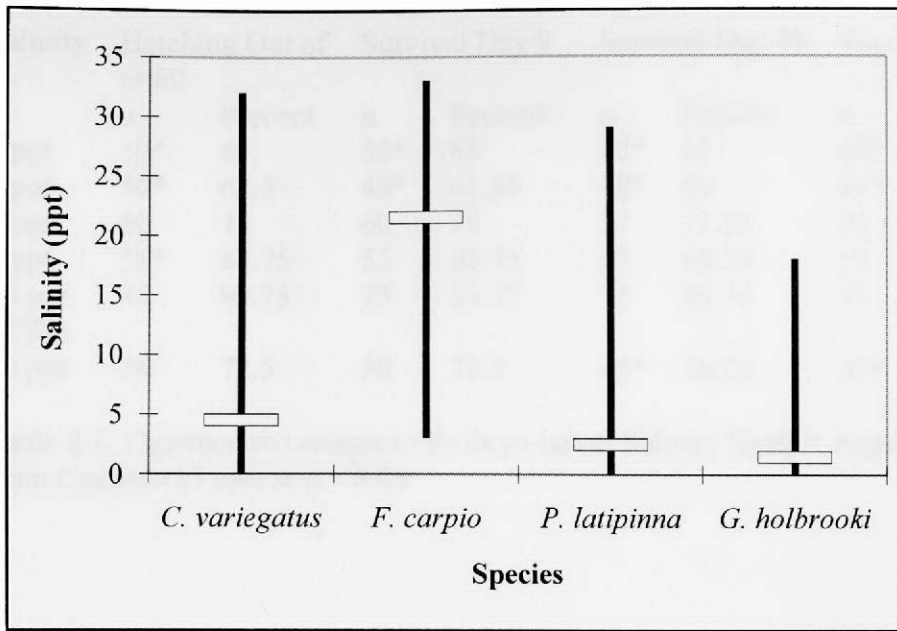
Figure 2.5. Northeast Florida Bay salinity ranges for fish species from this project. Crossbars indicate mean salinity at which each species was collected from years 1990-2006 (from Lorenz and Serafy, 2006).

Salinity	Hatching Out of n=80		Survival Day 9		Survival Day 28		Survival Day 60	
	n	Percent	n	Percent	n	Percent	n	Percent
0 ppt	52*	65	52*	65	52*	65	49*	61.25
2 ppt	50*	62.5	49*	61.25	48*	60	44*	55
4 ppt	60	75	60	75	57	71.25	53	66.25
8 ppt	55*	68.75	55	68.75	53	66.25	53	66.25
15 ppt (CTL)	75	93.75	75	93.75	75	93.75	73	91.25
30 ppt	58	72.5	58	72.5	45*	56.25	43*	53.75

Table 2.1. *Cyprinodon variegatus* Embryo-larval Salinity Study.* Significantly different from Control (15 ppt) at $p < 0.05$

Salinity	Survival Day 7		Survival Day 28		Survival Day 60	
	n= 80	Percent	n	Percent	n	Percent
0 ppt	66*	82.5	66*	82.5	62*	77.
2 ppt	79	98.75	79	98.75	76	95
4 ppt	77	96.25	77	96.25	77	96.25
8 ppt	80	100	80	100	79	98.75
15 ppt (CTL)	80	100	80	100	80	100
30 ppt	80	100	80	100	80	100

Table 2.2. *Poecilia latipinna* Chronic Neonate-fry Salinity Study. *Significantly different from Control (15 ppt) at $p < 0.05$

Salinity	Hatching Out of n=60		Survival Day 9		Growth (Avg.)	
	n	Percent	n	Percent	Weight (mg)	Length (mm)
0 ppt	59	98.3	59	98.3	0.51	4.72
2 ppt	60	100	60	100	0.47	4.91
4 ppt	60	100	59	98.3	0.35	4.38
8 ppt	60	100	60	100	0.30	4.43
15 ppt (CTL)	59	98.3	59	98.3	0.56	5.28
20 ppt	60	100	60	100	0.71	5.73
25 ppt	59	98.3	56	93.3	0.64	5.10
30 ppt	60	100	60	100	0.64	5.01
40 ppt	60	100	60	100	0.56	4.98
50 ppt	56	93.3	51	85	0.57	4.82
60 ppt	53	88.3	53	88.3	0.40	4.09

a) *Cyprinodon variegatus* 9-Day Embryo-larval Study: Native Stock
No treatments were significantly different from Control (15 ppt) at $p < 0.05$

Salinity	Hatching Out of n=60		Survival Day 9		Growth (Avg.)	
	n	Percent	n	Percent	Weight (mg)	Length (mm)
0 ppt	59	98.3	59	98.3	0.58*	5.63*
2 ppt	60	100	60	100	1.14	7.34*
4 ppt	60	100	60	100	1.23	8.41
8 ppt	59	98.3	58	96.7	1.10	8.27
15 ppt	60	100	58	96.7	1.23	8.42
20 ppt	60	100	60	100	1.24	8.36
25 ppt (CTL)	59	98.3	58	96.7	1.07	7.96
30 ppt	60	100	60	100	1.18	8.12
40 ppt	52	83.3	52	86.6	1.05	7.81
50 ppt	56	93.3	50	83.3	0.93	7.46
60 ppt	52	86.7	45	75	0.75*	6.96*

b) *Cyprinodon variegatus* 9-Day Embryo-larval Study: Non-native Stock.
  *Significantly different from Control (25 ppt) at $p < 0.05$.

Table 2.3. Results of Embryo-larval studies with native (a) and non-native stock (b).
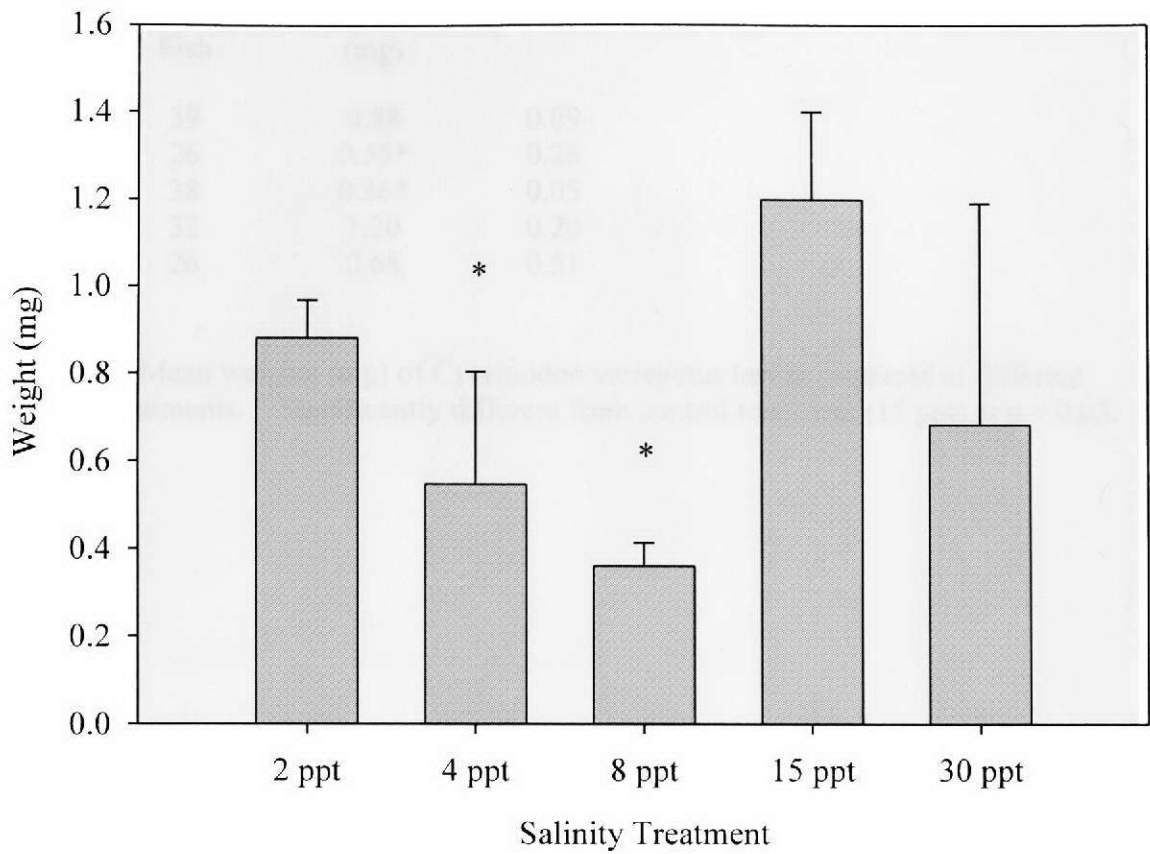
Figure 2.6. Mean weights (mg) of *Cyprinodon variegatus* larvae produced in different salinity treatments. * Significantly different from control treatment (15 ppt) at p < 0.05. (4ppt, p = 0.0141; 8 ppt, p = 0.0021)

Salinity	Number Fish	Mean Weight/TRT (mg)	St.Dev
2 ppt	39	0.88	0.09
4 ppt	26	0.55*	0.26
8 ppt	38	0.36*	0.05
15 ppt	32	1.20	0.20
30 ppt	26	0.68	0.51

Table 2.4. Mean weights (mg) of Cyprinodon variegatus larvae produced at different salinity treatments. * Significantly different from control treatment (15 ppt) at $p < 0.05$.
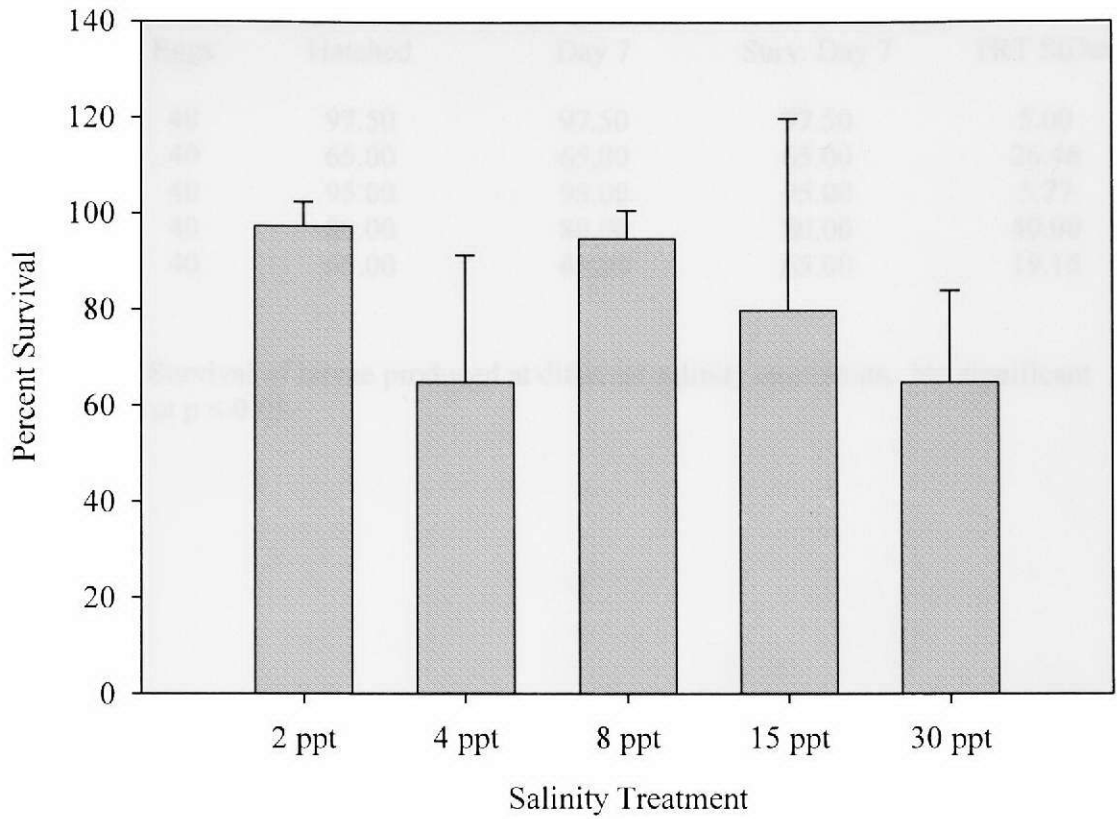
Figure 2.7. Survival of larvae produced at different salinity treatments. No significant differences at p < 0.05.

Salinity	Number Eggs	Percent Hatched	Percent Surv. Day 7	TRT Mean Surv. Day 7	TRT StDev
2 ppt	40	97.50	97.50	97.50	5.00
4 ppt	40	65.00	65.00	65.00	26.46
8 ppt	40	95.00	95.00	95.00	5.77
15 ppt	40	80.00	80.00	80.00	40.00
30 ppt	40	65.00	65.00	65.00	19.15

Table 2.5. Survival of larvae produced at different salinity treatments. No significant differences at $p < 0.05$.
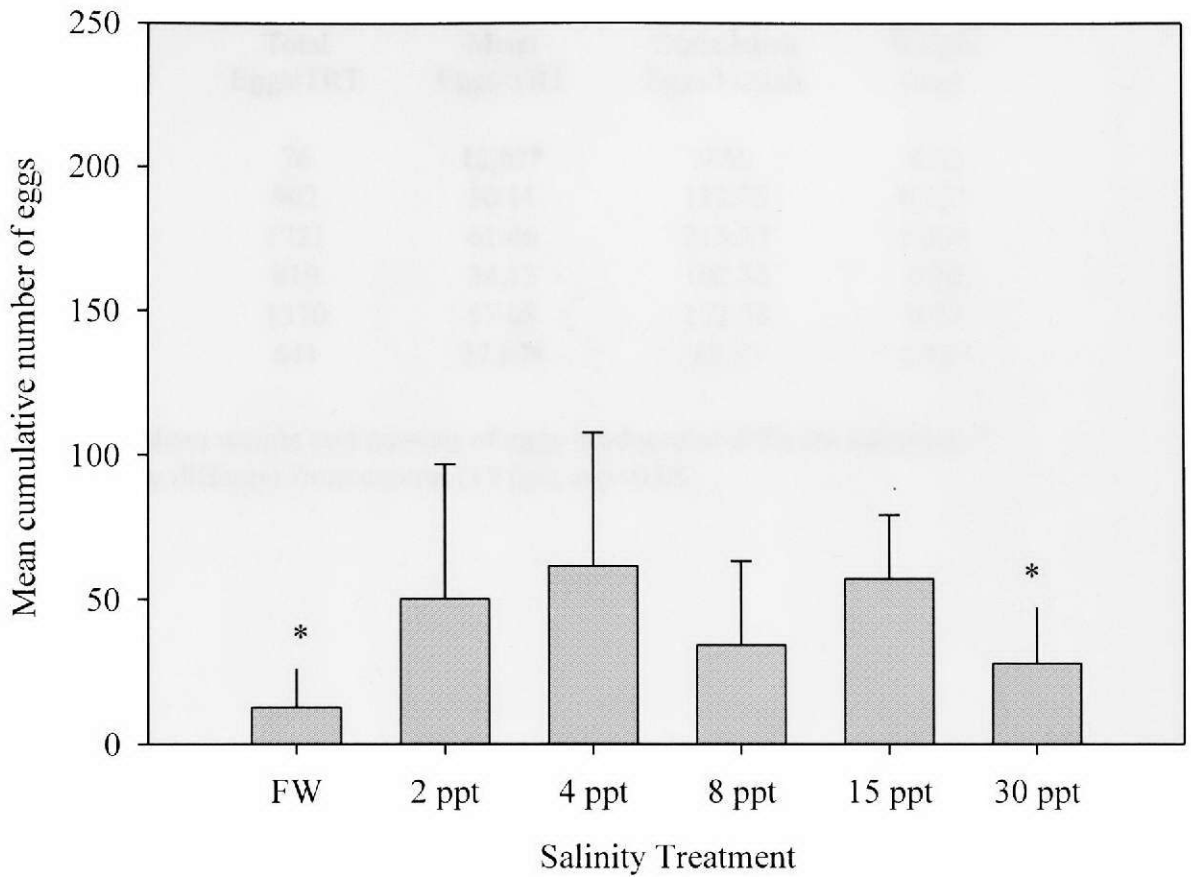
Figure 2.8. Mean cumulative number of eggs produced at different salinity treatments. * Significantly different from control (15 ppt) at p<0.05. (FW, p = 0.0263; 30 ppt, p = 0.0210).

Salinity	Total Eggs/TRT	Mean Eggs/TRT	Mean Cumulative Eggs/Female	Mean Egg Weight (mg)
FW	76	12.67*	9.50	0.22
2 ppt	902	50.11	112.75	0.33*
4 ppt	1721	61.46	215.13	0.33*
8 ppt	819	34.13	102.38	0.28
15 ppt	1370	57.08	171.25	0.27
30 ppt	641	27.87*	80.13	0.33*

Table 2.6. Mean weight and number of eggs produced at different salinities. * Significantly different from control (15 ppt) at $p < 0.05$.
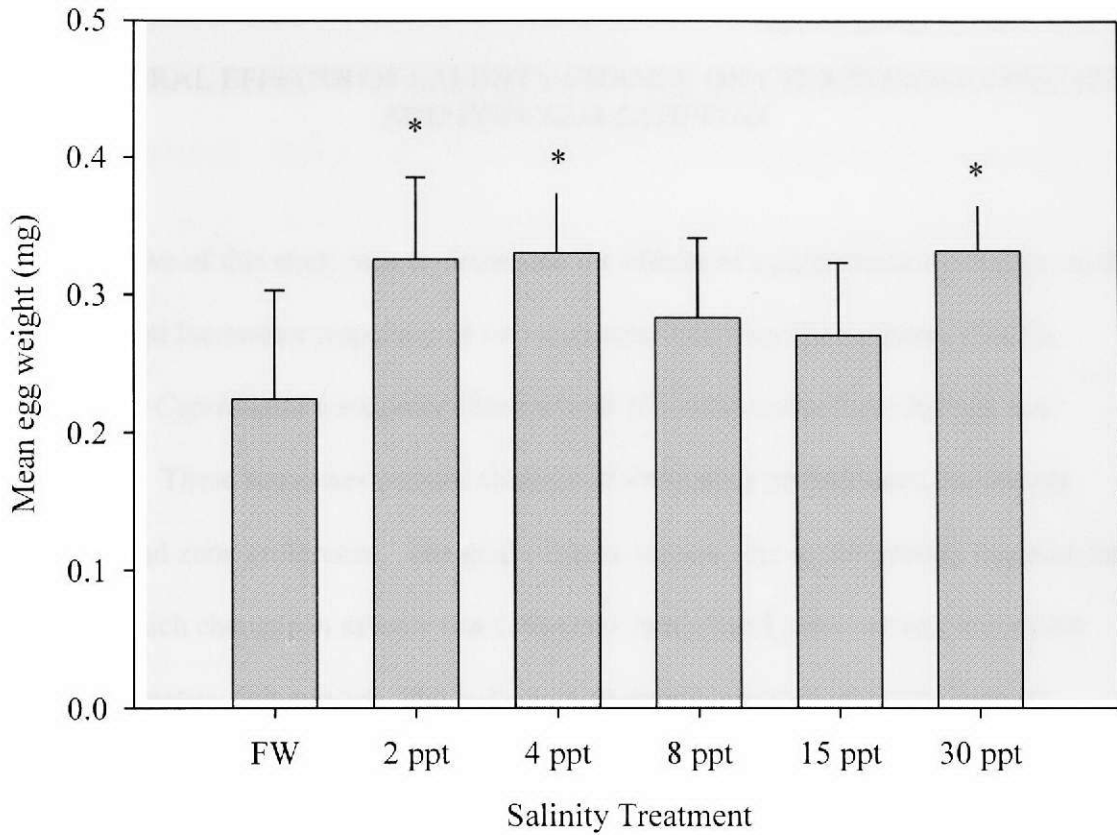
Figure 2.9. Mean egg weight (mg) produced at different salinity treatments. *
Significantly different from control (15 ppt) at $p < 0.05$.

BEHAVIORAL EFFECTS OF SALINITY CHANGE ON *CYPRINODONVARIEGATUS* AND *POECELIA LATIPINNA*

Abstract

The objective of this study was to determine the effects of a pulsed salinity change on the behavior and locomotor responses of two estuarine fish: *Poecilia latipinna* (Sailfin Molly) and *Cyprinodon variegatus* (Sheepshead Minnow) under flow-through test conditions. These responses include changes in swimming performance, locomotor behavior and zone preference. The goal of these studies is to quantitatively examine the ways in which changes in salinity can effects the established behavior and locomotor patterns of native fish species. The endpoints analyzed included velocity; distance moved; distance to a point; distance to a zone border; presence and time in zone; heading; turn angle; angular velocity; and meandering. Data were analyzed using the Before-After Control-Impact (BACI) model. Statistically significant differences in locomotor pattern endpoints ($p < 0.05$, two-tailed test) were found for *P. latipinna*. The endpoints of mean meander, mean angular velocity, mean heading and mean turn angle showed generally higher values in the impact fish after the point source of freshwater was introduced. For the distance and time parameters, no statistical differences were found. For *C. variegatus*, no statistical differences between the control and impact fish were found between the before and after periods for any endpoint.

Introduction

Salinity is one of the strongest drivers for the abundance and distribution of aquatic organisms. Understanding the role that salinity plays in both physiological and behavioral responses is the focus of this dissertation and is of applied importance to management activities in northeastern Florida Bay. The goal of these studies is to quantitatively examine the ways in which changes in salinity can affect the established behavior and locomotor patterns of native fish species.

Laboratory bioassays, though relevant, are not and should not be the sole source information used to make relevant environmental management decisions. Behavioral endpoints are considered to be of great value for elucidating the sub-lethal effects of stressors (Olla et al 1980). Verplanck (1957) defined behavior as the "complex of observable, recordable, or measureable activities of a living animal." Many behavioral responses are the result of either biological, physical, or chemical stimuli and are considered adaptive in origin (Rand, 1985). When confronted with an adverse environmental condition, this adaptive behavior allows the animal to move to or maintain a favorable environment thereby eliminating the negative effects upon itself. However, behavioral changes manifested as a result of anthropogenic changes to the environment are considered non-adaptive (Olla et al 1980). Often, though not initially, departures from behavioral norms can have negative consequences on the individual organism or the population as a whole.

When confronted with an environmental perturbation, the initial response of an organism is a change in behavior, such as avoidance of a negative stimulus (Slobodki,

1968).   If such a behavioral response removes or mitigates the adverse effects of the stimuli, then the cost(s) to the organism is reduced or eliminated entirely. Olla et al. (1980) proposed that in order for an organism to successfully respond to a stimuli, three criteria must be met: (1) the organism must be able to sense the stimuli; (2) the organism must recognize the stimulus as deleterious; and (3) the organism must be able to respond appropriately. The response pathways of an animal exposed to an environmental disturbance are outlined in Figure 3.1.

Behavioral toxicity (Marcucella and Abramson, 1978; Rand 1985) describes a situation where a stressor (chemical or otherwise) precipitates a behavioral change in an organism that exceeds the normal variability for that organism. In an ecological context, those stressors that affect an organism's ability to survive, tolerate, or adapt to the environment are the most significant.   Chemical stressors can alter both the sensory perception and motor activity of an organism. In fish, the sensory modalities include chemoreception, phototaxis and rheotaxis while the motor activities include locomotor patterns and swimming performance. Natural adaptive responses such as feeding behavior, courtship and mating rituals, preference/avoidance of certain environmental conditions, and territoriality can ultimately be affected by the introduction of a stressor to the aquatic environment. Hence, altered behavior in any of the above circumstances could not only reduce the fitness and/or survival of the individual, but could also have long ranging implications on the population level (Rand 1985).

The locomotor movement of an aquatic organism may be characterized as directed or undirected. Directed movement is in response to an orientative cue such as light, food odor, pheromones or an abiotic gradient (temperature, salinity). Conversely,

undirected movement is random and not related to a directional stimulus (circadian and seasonal rhythms). Under natural conditions, directed movements lead to the distribution of organisms within a favorable environment or gradient and allow the organism to exist within conditions conducive to normal physiological performance. The adaptive response is also critical for the localization of food and prey and the identification of conspecifics, mates and nesting/spawning sites.

When exposed to a toxic substance in the environment, a fish may respond in several ways: avoidance, whereby the fish orients away from the substance; attraction, whereby the fish approaches the substance; or the fish may ignore the substance altogether. Though initially beneficial, avoidance of toxic substances (especially in sub-lethal concentrations) can also cause chronic problems as the fish may be forced into sub-optimal conditions for long-term survival, feeding or reproduction.

Laboratory behavioral studies with salinity have been conducted for many taxa in relation to examining the effects of salinity on organism distribution. Some recent work by Jury et al. (1994) examined the behavioral effects of salinity change on American lobsters (*Homarus americanus*). Their studies were designed to establish (1) whether lobsters detected salinity changes that were on the same magnitude as occurred in estuaries, and (2) what locomotor responses (if any) the lobsters exhibited to such change. The lobsters were found to not only detect reductions in salinity, but also avoid them. An interesting outcome of the studies was the finding that there exists a gender difference in the behavioral response of the lobster to salinity changes. Female lobsters found lower salinities more aversive than males, leading the authors to conclude that gender-driven behavioral responses to salinity were, in part, responsible for the

distribution and movements of lobsters in estuaries. Male lobsters have been show to dominate in the lower salinity estuarine habitats.

Much work has been done examining the effects of salinity change on the movements and distribution of crustacean larvae (Forward Jr., 1989). Behavioral responses to salinity gradients have been shown to dictate the location of zoeal larvae within the water column. However, there is less work that examines the rate and/or degree of salinity change required to elicit a behavioral response. Forward Jr. (1989) conducted behavioral studies on the larvae of two species of crab *(Rhithropanopeus harrisii* and *Neopanope sayi)* to determine the lowest rate and absolute amount of salinity change required to evoke a behavioral response. The determined rates were then compared to salinity gradients encountered within an estuarine environment. Sensitivities to salinity changes were found to be species specific and correlated to the different estuarine zones (upper vs. lower) that were the crab's habitats. Both species responded, in different degrees, to increases in salinity, however neither species exhibited a behavioral response to decreases in salinity. As increases in salinity usually occur lower in the water column, a behavioral response that directs the organisms away from higher salinity conditions ensures that the larvae stay off the bottom and away from benthic predators.

In northeastern Florida Bay, many of the small embayments and mangrove fringe areas experience a wide range of salinities with both pulsed and gradual changes from hypo- to hypersaline conditions. Fish in these areas are subjected to both freshwater inputs from rain and canal discharges as well as wind-driven intrusion of more saline water from the Bay. In this sense, water of a different salinity can be viewed as the

introduction of a chemical agent into these areas. With the exception of extreme highs and lows, the average salinities in the embayments of northeastern Florida Bay fall within the tolerance ranges for many of the demersal fish species native to the area. However, upper and lower physiological tolerance limits are not necessarily the conditions for optimal performance. The exposure to sub-optimal salinities may be one of the most prevalent threats to fish in these areas. The behavioral changes that result from these exposures may not lead directly to death but may nonetheless cause major ecological disturbances. The lack of a forage fish base in this area, as a probable result of salinity stress, has been identified as a key driver in the success of higher trophic level species (Lorenz, 1999).

Due to the inherent variability and broad range of an organism's natural response to stimuli, behavioral toxicology studies require strict control and reliable, repeatable and quantifiable measures.

Materials and Methods

The objective of this study was to determine the effects of a pulsed salinity change on the behavior and locomotor pattern of two estuarine fish: *Poecilia latipinna* (Sailfin Molly) and *Cyprinodon variegatus* (Sheepshead Minnow) under flow-through test conditions. These species are common residents of northeastern Florida Bay and are representative of forage species important to higher trophic levels.

A behavioral analysis system with flow through tanks was used for this study (Figure 3.2). The behavior tank was constructed of 5/8" clear Plexiglas and measured 48" x 21" x 8" (Figures 3.3 and 3.4). The outside of the tank, excluding the bottom was painted with white Paint for Plastic (Rustoleum Corp., Vernon Hills, IL) to reduce glare

and reflection. The bottom of the tank was left clear as to let in light from below. The Plexiglas tanks were placed in recirculating water bath for temperature control if needed. The center of the bottom of the recirculating water bath was removed and replaced with ¾" clear Plexiglas and sealed creating a 'light box'. The water bath and behavior tanks were mounted on a wooden frame and elevated to waist height. Two 4-foot fluorescent light fixtures (40 watts) were mounted beneath the light box to project light upwards. A high contrast black and white camera (Panasonic CCTV Camera WV-BP330) was mounted on the ceiling above the tanks to record behavior. The entire system was enclosed behind floor-to-ceiling blackout curtains to prevent the fish from being startled during a recording. Extraneous noise in the lab was kept to a minimum during all recordings.

Water of different salinities was pumped into the system via magnetic drive pumps and not recirculated. A ½" PVC bulkhead fitting was mounted at one end of the tank to provide drainage. Nitex mesh was siliconed over the drain hole to prevent animals from escaping. The dilution water was pumped from an aerated 55-gallon holding tank via a 450 gpm magnetic drive pump and delivered into the behavior tank via two plastic manifolds, mounted horizontally one above the other. (Figure 3.4). Each manifold had 8 valves which delivered water through a 1/8" nylon tube. The upper manifold projected water into the head of the tank whilst the lower manifold delivered water to the midfield of the tank. To prevent fish from accessing the delivery tubes, a 'false bottom" (1/4" white opaque Plexiglas) was installed to cover the tubing. The 'false bottom' was gently sloped (2" to 1") to aid in water flow. The delivery manifolds were separated from the filmed portion of the tank by a 5/8" thick Plexiglas divider painted

white. Holes were drilled in the divider for each delivery tube. The end of each delivery tube was capped with a male luer locking nut (Cole-Parmer No. 30504-10) to direct flow and prevent the tube from sliding out of position. The flow rate of the delivery tubes was controlled by individual valves on the manifolds. The flow rate of 10-12 gpm was established to maintain a salinity gradient while not interfering with normal fish movement.

The test substance was delivered as a point source roughly 3/4 of the way down from the apex the behavior tank. Water was delivered to the point source via a 350 gpm magnetic drive pump submerged in an aerated 55-gallon holding tank. The point source delivery system was constructed ¾" Nalgene tubing with a nylon elbow that was siliconed to the behavior tank wall and angled towards the bulkhead drain. A tubing clamp was used to control the point source at a flow rate of 10-12 gpm.

The dilution water was a stable salinity saltwater (~30 ppt, ~45.0 mS conductivity) pumped from a sub-surface well in North Miami, Florida. The test substance was freshwater (0.2 ppt, ~300 μS conductivity) from the North Miami, Florida, municipal water supply. Both waters were vigorously aerated, passed through activated carbon, filtered (10-micrometers), UV Sterilized and then re-aerated before being used in these studies. The freshwater had a hardness of 50 to 90 milligrams per liter (mg/L) as calcium carbonate ($CaCO_3$). The initial pH of the dilution water was between 7.0 - 8.0.

Fish were collected from the wild in Little Blackwater Sound, Florida. Animals were treated with Nitrofurazon (20 mg/l) on arrival in laboratory and kept in isolation of for two weeks before becoming part of the laboratory culture population. Animals were cultured under flowthrough conditions outdoors at 28° C (+/- 2° C) and 30 ppt salinity.

Adult fish of uniform size were used in these studies; the longest fish was no more than twice the standard length of the shortest fish. Wet weights and standard lengths of the fish were measured and recorded at the end of the study (Table 3.1).

Fourteen days prior to test initiation, the study fish were brought inside to flow-through holding tanks of stable salinity and temperature (30 ppt, 22° C). Fish were gradually acclimated to within 2 degrees Celsius (C) of the test temperature in laboratory culture water of 30 ppt.

To initiate studies, individual fish were introduced into the behavioral analysis system tanks in a stable salinity (30 ppt) with an established laminar flow of water. The curtains around the tank were closed after introduction, and all subsequent test activities (recording, water control, etc.) were conducted remotely. Laboratory noise was kept to a minimum during recording sessions. Fish were allowed to acclimate to the tank for 5 minutes unrecorded. After recordings were started, fish were allowed 7.5 minutes of swim time before the point source was turned on. For control runs, the point source delivered the same salinity water as in the entire tank creating a 'no-change' scenario. For experimental (impact) trials, the point source introduced freshwater (0.2 ppt). The flow rates and design created a 'zone' of alternate salinity at one end of the tank to challenge fish (Figure 3.5). The fish were recorded an additional 7.5 minutes with the point source supplying water, for a total recorded time of 15 minutes for each behavioral trial.

Each study consisted of 20 fish: 10 fish for the control group and 10 fish for the impact group. All 20 fish were first exposed to the flow-through tank system with no salinity change to establish a behavioral baseline. After the baseline exposure, fish were exposed to the system a second time for the point-source trials. During this second

exposure, 10 randomly selected fish were exposed to the 'no-change' scenario and served as the control group. The remaining 10 fish were exposed to a 'salinity-change' scenario and served as the impact group. A single fish occupied each behavior tank during each recording session.

The flow-through tank (s) was calibrated before each study for consistent flow rate and salinity concentrations during point source delivery (Figure 3.5). Salinity concentrations were recorded 3 times from each of 10 locations in the flow-through tank. The average salinity value for each point was recorded. Salinity values did not deviate between studies by more than 1 ppt. The average salinity for the 'no-change' scenarios was 30.3 ppt throughout the entire arena. In the 'salinity-change' scenario, the salinity in the point source vector ranged from 4.5 – 14.6 ppt, while the rest of the behavior arena was maintained at salinities above 25 ppt (Figure 3.5). Aeration was provided to the source water tanks to maintain dissolved oxygen concentrations greater than 80 percent of saturation.

Water temperature was ambient laboratory temperature, maintained at $22 \pm 1^\circ$ C. A 16-hour light and 8-hour dark photoperiod was maintained for fish while in holding for this study.

To reduce stress in transfer into the behavior arena, fish were netted and transferred underwater to a glass bowl while in the holding tank. The bowl was then transferred to the behavior arena and the fish was allowed to swim out. Fish were handled minimally throughout the holding and study periods.

Between each trial, the system was drained and refilled with the main source water (30 ppt). Salinity, conductivity, temperature and dissolved oxygen were verified

before each trial. A second, marked aquarium was established at the same environmental conditions as the initial holding tank to receive post-trial fish and eliminate the chance of the same fish being recorded more than once. Post-trial fish were transferred into the second tank via the same method as described above.

Conductivity, salinity, temperature, ammonia concentration, pH, total alkalinity, and total hardness of the saltwater and freshwater were measured at the start of the study.

Observations of behavior were recorded via a ceiling mounted camera and analyzed using Noldus Ethovison 3.0 software. With this system, the user defines the individual endpoints to use and assigns a sampling interval for recording each endpoint value. Due to the short length of these recordings (15 minutes), a one second sampling interval was chosen to retrieve as many data points as possible. Descriptive statistics (mean, standard deviation, standard error, etc.) were calculated for each endpoint at each time interval.

The endpoints analyzed included swim velocity; distance moved; distance to a point; distance to a zone border; presence and time in zone; heading; turn angle; angular velocity; meandering; and distance between objects (tank wall or point source delivery).

The endpoints used in these studies are described below and grouped categorically.

Distance and time:

This category comprises parameters which provide information about the position in space of an object relative to a user-defined zone or point.

In zone: This parameter specifies if a fish is within a defined zone and is employed to analyze the usage of space by the fish. For these studies, In Zone was used to determine how much time the fish spent in the treated area.

Distance to zone border: The shortest distance between the fish and the border of a zone of interest at a given point in time. In these studies, the zone border was defined as the walls of the tank. A common fish behavior is 'walling', whereby the fish swims along the walls of the tank, usually in one direction.

Distance to point: The distance between the fish and a fixed point of interest (e.g. point source location) and is an indicator of spatial orientation.

In the case of these studies, for analysis of swimming behavior of the fish in a vector (plume) of alternate salinity, the vector itself was defined as a zone, while the point source (of alternate salinity) was regarded as a point. The endpoint was used to measure the fish's proximity towards the point source at any moment in time.

Distance moved: The distance 'traveled' by the center of gravity of the fish between two points in time. The distance moved parameter is used in the calculation of several other endpoints such as velocity and heading. Both the mean distance moved per recorded time interval (1 second) and the total distance moved during pre- and post- point source addition was analyzed.

Velocity: The distance moved by the fish per given unit of time.

Locomotor activities:

These endpoints describe the geometrical shape of the path traveled by the fish in the exposure system.

Heading: The direction of movement of the fish relative to a user-defined reference line. The heading has a range from 0 to 360 degrees. The endpoint was used to determine the direction of movement of the fish relative to the point source and point source vector of alternate salinity.

Turn angle: The change in direction of the fish's movement between two consecutive samples. For these studies, turn angle was calculated as with a signed value (positive or negative) to distinguish right and left-handed turns. A clockwise turn (right) was assigned a positive value, a counterclockwise turn (left) was assigned a negative value. Hence the relative turn angle ranged from -180° to 180°. The analysis of turn angles can aid in determining stereotypical movements of the fish.

Angular velocity: The speed of change in direction of movement of the fish per unit of time. Again, the angular velocity was determined relative to direction and assigned a positive or negative value for clockwise (positive) or counterclockwise (negative) movement. This endpoint is used to detect any abnormalities of movement (e.g. reaction to toxicant).

Meander: The change in the direction of movement of the fish relative to the distance moved (amount of turning per unit distance). For theses studies, meander was calculated relative to direction as described above with a range from -180°/cm to 180°/cm.

Statistical Analysis

Data were analyzed using the Before-After Control-Impact (BACI) model and JMP version 8.0.1 statistical software (SAS Institute, Inc., Cary, NC). The BACI model is used to assess whether an impact has occurred as a result of a stressor on a test system, and if an impact has occurred, determine which components/endpoints are affected and to

what degree (Smith, 2002). In this model, data from two sites (control site and impact site) were paired and compared before and after an activity occurred.

Often used in the assessment of environmental impacts (Smith, 2002; Stewart-Oaten, 2003; Trexler et al., 2005) before and after models fall into three main permutations. The simplest approach is using the Before-After (BA) model described by Green in 1979 (Smith, 2002). In this case, data collected from before an activity is compared to data collected after an activity using a two-sample test. Differences found between the before and after data are thus attributable to the activity and are viewed as an effect. One criticism of this design is that no control exists with which to compare the collected data. A second approach, which includes a control, is known as the Before-After Control-Impact (BACI) model (Smith, 2002). A variant of the BA, the BACI design compares data collected from before and after an activity at a control site against an impact site. The third approach (BACIP), adds the element of pairing data points and calculating the differences between them to the BACI design. Data are collected based on pairs of observations and N pairs of samples are taken before the effect and after the effect at both sites. Stewart-Oaten (2003) referred to a 'tracking relationship' being established between the control (site) and impact (site) that would reflect potential changes as evidence of an effect. The differences between the pairs can then be compared for the period before the effect and after using a two sample test. If there is no impact, the differences in the before period should be similar to the differences in the after period. A t-test can be used to compare the differences for both assumed equal and unequal variances. Figure 3.6 is a graphical representation of the different before-after design approaches taken from Smith, 2002.

For these studies, the 10 fish that had the same salinity water delivered by the point source serve as the control data set, whereas the 10 fish that had the point source deliver different salinity water serve as the impact data set. The time prior (0-7.5 minutes) to the point source being turned on is the before period whilst the time following the point source addition (7.5-15 minutes) is the after period. The data points are the mean value of each end point for the individual fish for both before and after periods. Using the endpoint of velocity as an example, the mean velocities of fishes in the control are compared to the mean velocities fishes in the impact for both before the point source addition and after it. If the differences between the means of the control versus the impact are dissimilar in the before and after periods, then a behavioral impact is indicated for that endpoint.

Results

Statistically significant differences in locomotor pattern endpoints ($p < 0.05$, two-tailed test) were found for *P. latipinna* (Table 3.2). The endpoints of mean meander, mean angular velocity, mean heading and mean turn angle showed generally higher values in the impact fish after the point source of freshwater was introduced (Figure 3.7). Such pattern changes are indicative of hyperlocomotion, a common reaction by a fish to a sublethal stressor as shown in numerous chemical behavior studies (McKim et al, 1987). For the distance and time parameters, no statistical differences were found.

For *C. variegatus*, no statistical differences between the control and impact fish were found between the before and after periods for any endpoint (Table 3.3)

Discussion

The behavioral effects of toxicants have been recorded in many groups of aquatic taxa (Little and Finger, 1990; Bernot et al., 2005) and provide critical information on sublethal effects that can alter feeding and anti-predator behaviors. Behavioral effects are often observed prior to physiological effects and can foreshadow more severe downstream effects (Bernot, 2005). In an ecological context, impairment of such behaviors as locomotion, predator avoidance and foraging/feeding can ultimately lead to reduced fitness for the organism. Changes in locomotor behavior, especially, can make aquatic organisms more or less susceptible to predation. By linking biochemical factors with physiological effects, behavioral endpoints can provide insight into both the individual and potential community level effects of a contaminant (Kane, et al., 2004). Juvenile recruitment can be affected by behavioral changes and thus result in both population and community changes over time (Bridges, 1997).

Both *P. latipinna* and *C. variegatus* are found in the shallows of northeastern Florida Bay and occupy similar salinity-preference niches. The mean salinity that *P. latipinna* has been collected in is approximately 4 ppt while that for *C. variegatus* is approximately 5 ppt (Lorenz and Serafy, 2006). However, *C. variegatus* has a greater range of salinity tolerance than *P. latipinna*, especially in its tolerance for higher salinities. Haney and Nordlie (1997) showed that the metabolic rate of gradually acclimated *C. variegatus* was only affected at salinities above 40 ppt. *C. variegatus* are egg layers, whilst *P. latipinna* are livebearers. Both species have similar feeding habits and are omnivorous, being known to feed on algae, detritus and small invertebrates (Hassan-Williams et al., 2007).

The lack of an effect of salinity change on responses of *C. variegatus* suggests that, at least on the short-term scale of the salinity change used in these studies (under 15 minutes), this species will tolerate osmoregulatory changes. Longer exposure to salinity changes may result in different behavioral patterns, as previous work on this dissertation (see Chapter 2), showed *C. variegatus* to be physiologically sensitive (growth and survival effects) to salinity changes. In the toxicological studies, the fish had no escape from the changed salinity and thus were forced into a situation to either compensate or not. In Florida Bay, unless the fish are physically trapped in an area due to a barrier, it is likely that fish will move to avoid osmotic stress. It should be noted here that for physiological studies involving a gradual change in salinity, there was no difference in survival for adults of the two species used in these behavioral studies. Hence, given time to acclimate to the change, one would expect behavioral changes and/or avoidance of an area not to occur.

Alternately, lack of a measureable behavioral effect on *C. variegatus* may be the result of the concentration of the salinity gradient used in these studies. Due to the design of the exposure system, a shallow concentration gradient was produced. In studies with copper and the goldfish, *Carassius auratus,* Kleerekoper et al., (1972) found that the magnitude of the concentration gradient (shallow verses steep) affected the attraction/avoidance response of the fish. Shallow gradients of copper (with much higher concentrations) attracted fish whilst steeper, less concentrated gradients repelled fish (Kleerekoper et al., 1972). The salinity gradient presented in these studies may not have been dramatic enough to elicit a response in *C. variegatus*.

It is interesting that *P. latipinna* exhibited an increase in locomotor activity when challenged with a drop in salinity in these behavioral studies. Due to the short time frame of each behavioral trial (15 minutes), it is likely that the activity changes observed here are the result of a 'first response' after sensing a change in the environment. Flashy and quick movements in a fish are often associated with panic and/or stress. Saline stress has been shown to increase serotonin levels in fish, which in turn regulates cortisol levels that play a role in salinity adaptation (Kahn and Deschaux, 1997). In this case, the recorded change in behavior of *P. latipinna* could be evidence of a more sensitive osmoregulatory response as compared to *C. variegatus*. As mentioned above, *P. latipinna* has a narrower salinity tolerance range than that of *C. variegatus*. Perhaps this refined response, and potentially quicker physiological adaptation, contributed to the greater success of *P. latipinna* in the previously mentioned toxicological studies. Early detection and reaction to salinity changes in *P. latipinna* may also aid in mitigating osmoregulatory stress by avoiding area of changing salinity.

In terms of Florida Bay, understanding the behavioral and physiological responses of native fish to salinity change can help elucidate the potential impacts to demersal fish community structure from both gradual and rapid/pulsed change events. As highlighted in the physiological toxicity studies, *C. variegatus* and *P. latipinna* showed marked differences in response to acute and abrupt salinity change based on age and life stage. *P. latipinna* showed greater physiological robustness to downwards shifts in salinity, as well as more refined detection of such changes in behavioral studies when compared to *C. variegatus*. Hence, *P. latipinna* may be more likely to inhabit areas of northeastern

Florida Bay and inland habitats that are subject to rapid downwards shifts in salinity than *C. variegatus* would.

Behavioral toxicity studies can be used to complement and add a level of realism to both acute and chronic toxicity studies as well as the regulatory actions associated with such studies. The subtle, sub-lethal effects highlighted in behavioral studies are often precluded by the design of the toxicity study (Atchison et al, 1987). Regulatory agencies in the United States and abroad base water quality criteria wholly or in part on the results of standard toxicity tests with endpoints such as growth, survival, and reproduction in aquatic organisms. Combining behavioral with toxicity studies can present a broader view of an organism's response to toxicant allowing for better management and protection of aquatic life.

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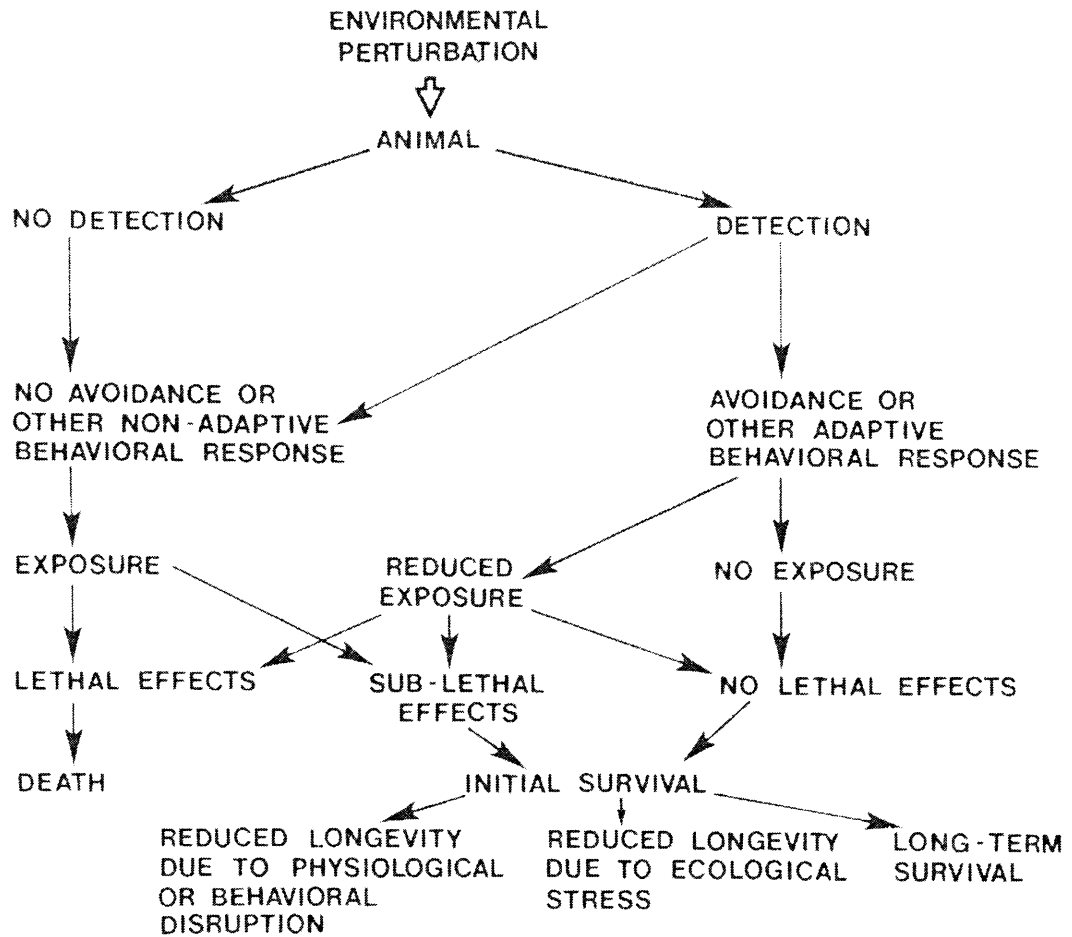
Figure 3.1: Flow chart of behavioral responses to environmental perturbation and possible consequences. From Olla et al. 1980.

Computer Interface

Video camera

Exposure Tank

Water Bath

Light
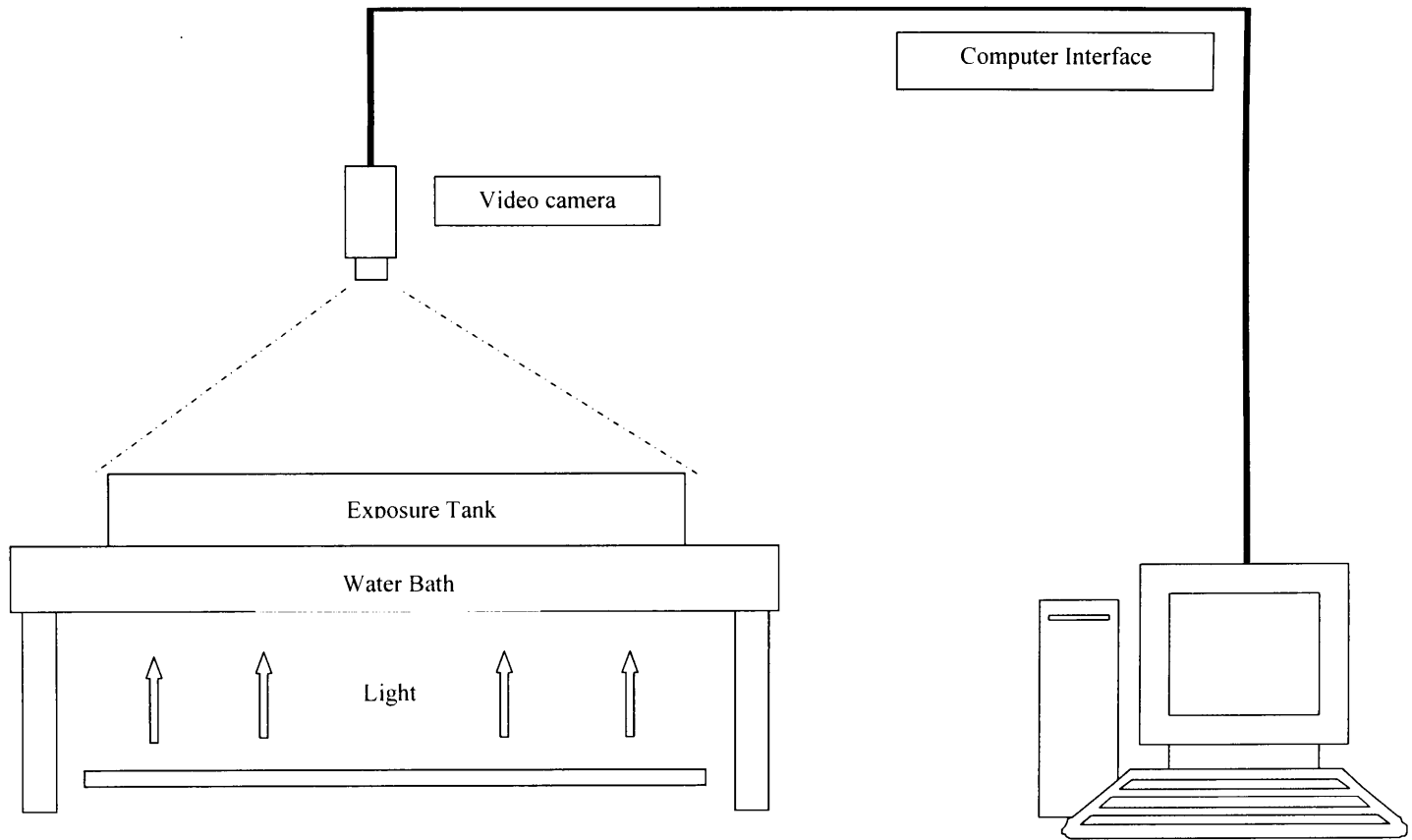
Figure 3.2: Behavioral analysis system
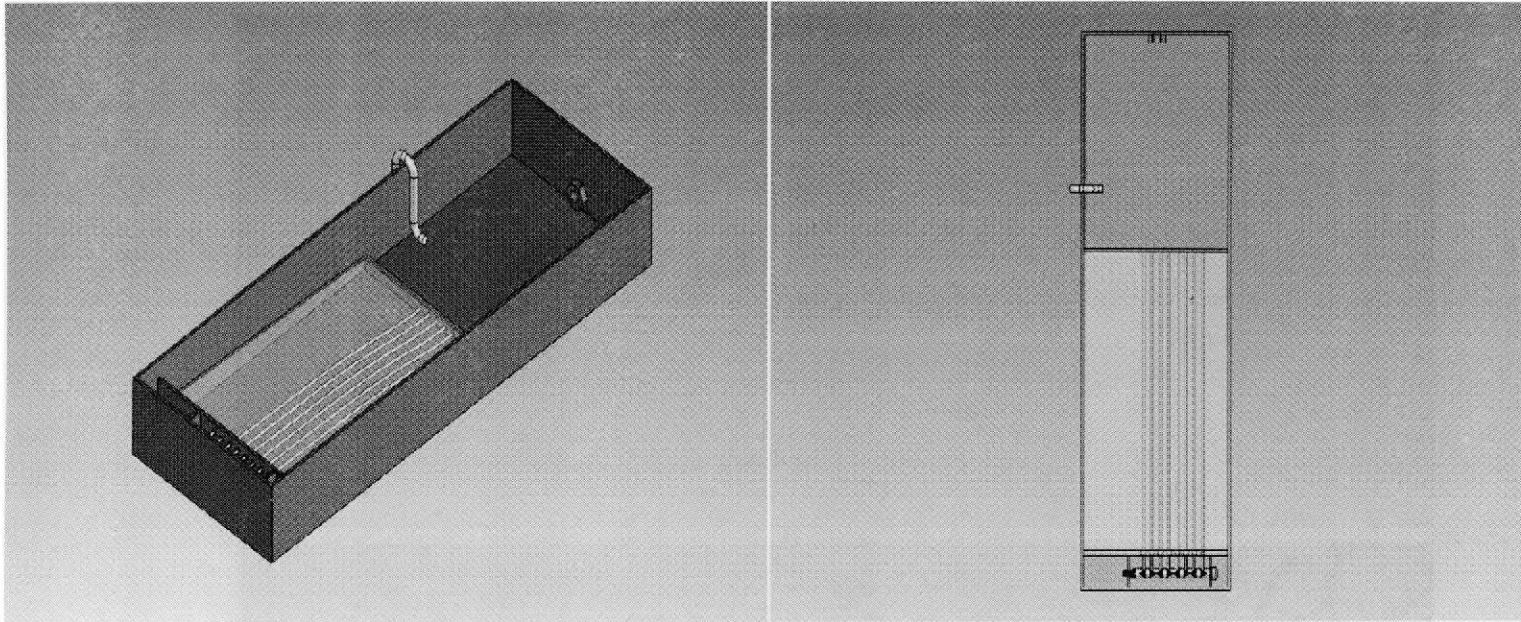
Figure 3.3: (L) Three-dimensional view of behavior tank with water delivery tubes exposed. (R) Top view of with water deliver tubes and manifold exposed.
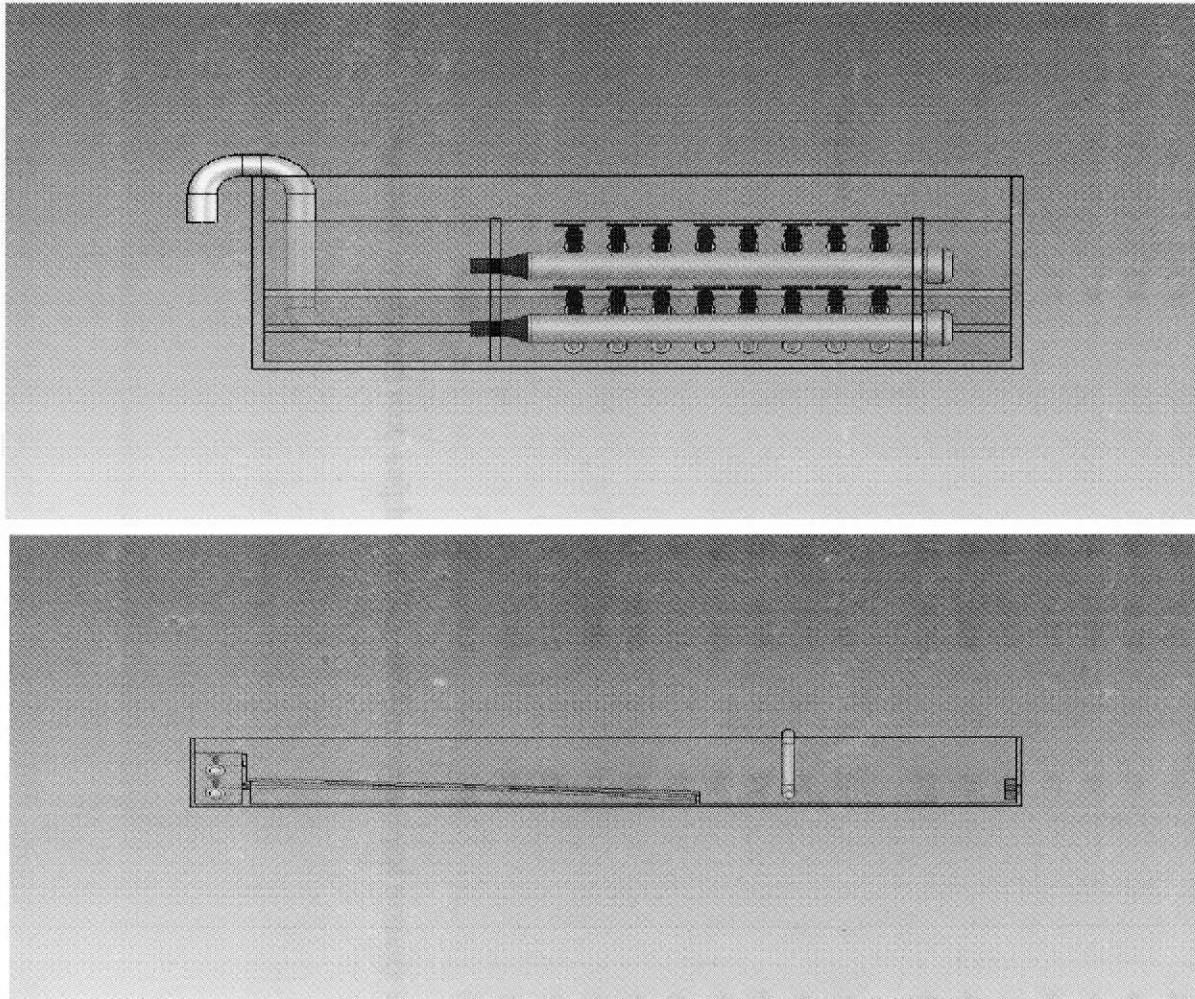
Figure 3.4: Lateral views of delivery manifold (top) and flow through tank with sloping false bottom (below).

Species	Control fish	Standard length (cm)	Wet weight (g)	Impact fish	Standard length (cm)	Wet weight (g)
Cyprinodon variegatus	C1	4.5	0.78	I1	4.4	0.8
	C2	4.3	0.74	I2	4.7	0.81
	C3	4.3	0.75	I3	4.6	0.77
	C4	4.9	0.82	I4	4.8	0.81
	C5	4.4	0.78	I5	4.2	0.72
	C6	5.1	1.06	I6	4.1	0.7
	C7	5.2	1.07	I7	4.2	0.72
	C8	4.9	0.89	I8	4.2	0.75
	C9	4.8	0.88	I9	4.8	0.86
	C10	4.1	0.71	I10	5.0	0.98
Poecilia latipinna	C1	5.3	0.91	I1	5.3	0.92
	C2	5.9	1.23	I2	5.8	1.11
	C3	5.5	0.96	I3	6.1	1.37
	C4	5.8	1.09	I4	5.3	0.92
	C5	5.8	1.13	I5	5.9	1.2
	C6	5.3	0.93	I6	5.9	1.22
	C7	5.4	0.96	I7	5.6	1.01
	C8	5.3	0.9	I8	5.7	1.03
	C9	6.2	1.45	I9	5.7	1.09
	C10	5.2	0.9	I10	5.6	1.01

Table 3.1 Standard length and wet weights of fish used in behavioral toxicity studies.
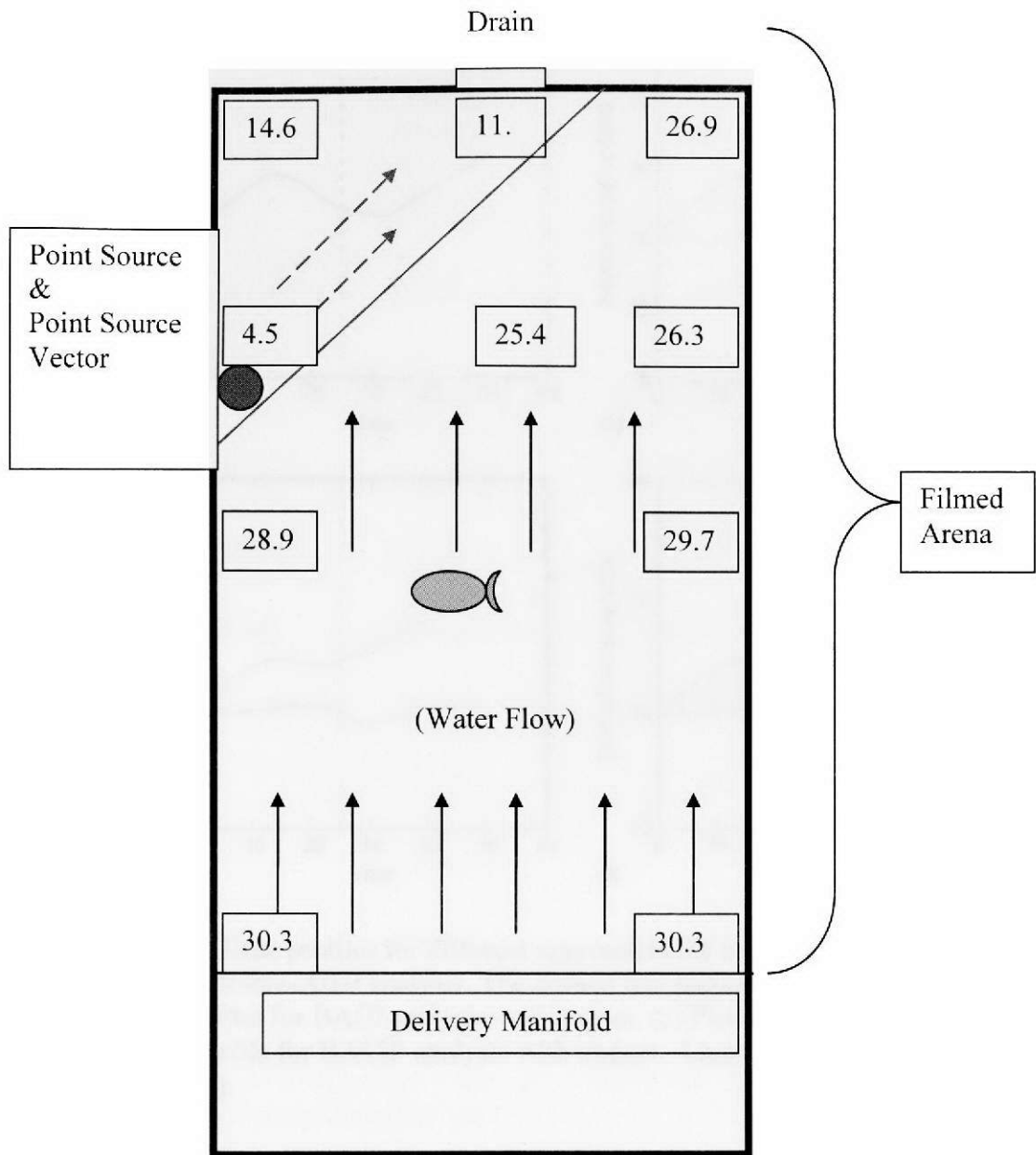
Figure 3.5: Location of salinity change vector in behavior tank. Numbers indicate average salinities (ppt) in each location during the addition of the freshwater point source.
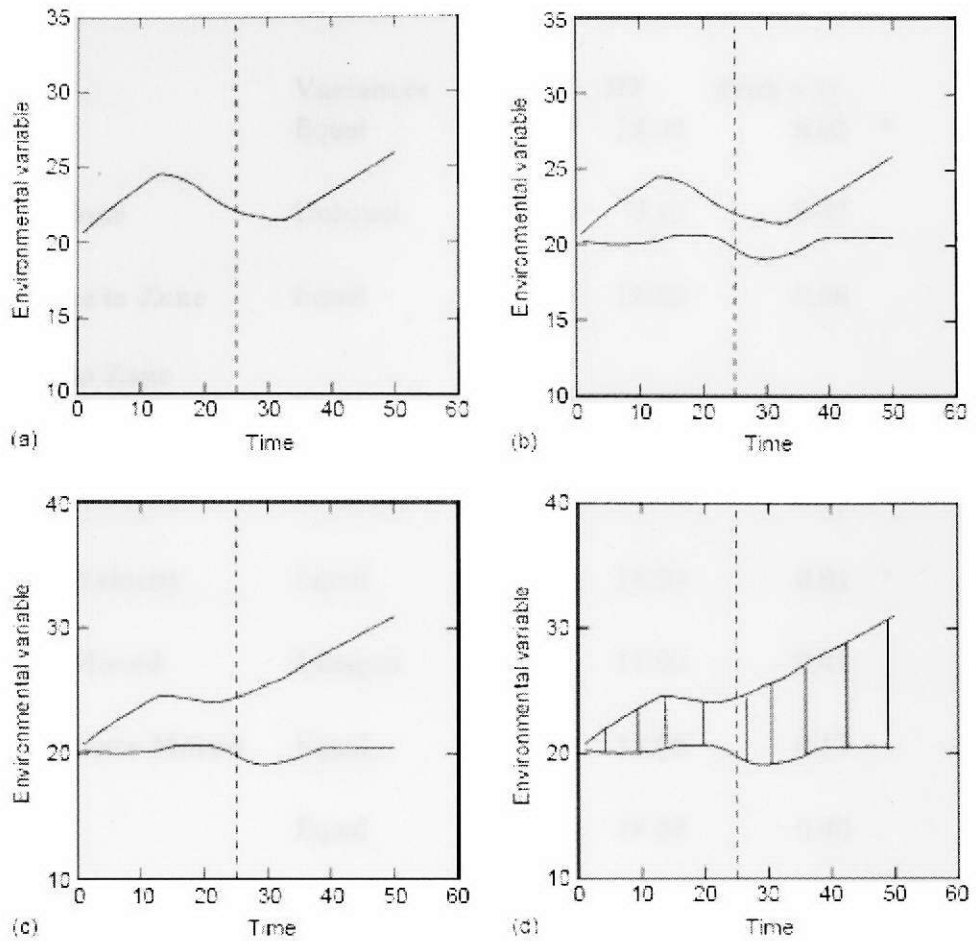
Figure 3.6. Data profiles for different approaches for impact assessment. (a) Data profile pattern for Before-After analysis. The dashed line indicates the time of activity/event. (b) Plot for BACI analysis – no impact. (c) Plot of BACI analysis – with impact. (d) Data profile for BACIP analysis with impact. Lines indicate pairs of samples. (From Smith, 2002).

Endpoint	Variances	t Ratio	DF	Prob > |t|	
Mean Meander	Equal	-2.62	18.00	**0.02**	*
Mean Time in Zone	Unequal	-2.04	9.12	0.07	
Mean Total Time in Zone	Equal	-1.85	18.00	0.08	
Mean Distance to Zone Border	Equal	1.42	18.00	0.17	
Mean Distance to Point	Unequal	-1.75	9.45	0.11	
Mean Angular Velocity	Equal	-2.99	18.00	**0.01**	*
Mean Distance Moved	Unequal	0.86	11.92	0.41	
Mean Total Distance Moved	Equal	1.43	18.00	0.17	
Mean Velocity	Equal	0.86	18.00	0.40	
Mean Heading	Unequal	-2.59	11.54	**0.02**	*
Mean Turn Angle	Equal	-2.18	18.00	**0.04**	*

Table 3. 2. Summary of BACI analysis data for *Poecilia latipinna*. Significant differences ($p < 0.05$) are indicated by an *.

Endpoint	Variances	t Ratio	DF	Prob > |t|
Mean Meander	Equal	-0.412	18	0.66
Mean Time in Zone	Equal	-0.48472	18	0.63
Mean Total Time in Zone	Equal	-1.22417	18	0.24
Mean Distance to Zone Border	Equal	-0.00824	18	0.99
Mean Distance to Point	Equal	-1.14983	18	0.27
Mean Angular Velocity	Equal	-0.43624	18	0.67
Mean Distance Moved	Equal	-0.52819	18	0.60
Mean Total Distance Moved	Equal	-1.12414	18	0.28
Mean Velocity	Equal	-0.058	18	0.95
Mean Heading	Equal	-0.42135	18	0.68
Mean Turn Angle	Equal	-0.74959	18	0.46

Table 3. 3. Summary of BACI analysis data for *Cyprinodon variegatus*. No significant differences were found
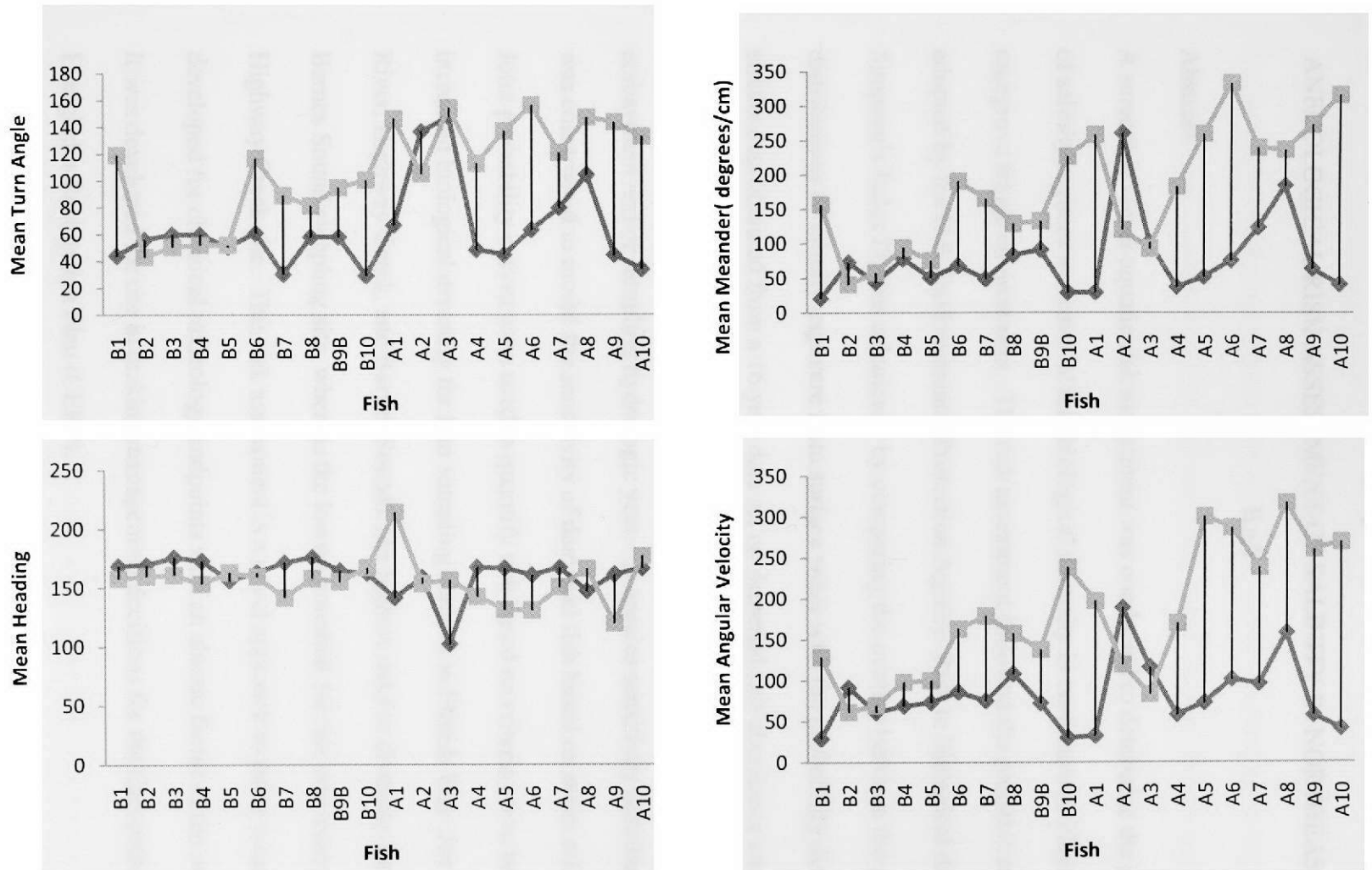
Figure 3.7. Statistically significant locomotor endpoints for *P. latipinna* (p<0.05). The diamond shape indicates the Control data, whereas the square marker indicates Impact data. Vertical lines connect paired data points for BACI analysis

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Chapter IV

ANECOLOGICAL RISK ASSESSMENT OF SALINITY IN NORTHEAST FLORIDA BAY

Abstract

A screening –level aquatic risk assessment was conducted to determine the potential risk of salinity regimes to demersal fish biological diversity in northeastern Florida Bay's mangrove fringe environment. The risk assessment followed the probabilistic framework adopted by the U.S. Environmental Protection Agency. Risk to biological diversity (as Simpson's Index D) was calculated by comparing the overlap between the probability distributions of salinity exposure from surface water with the probability distribution of effects data obtained from a 16 year data set on demersal fish abundance and distribution. Salinity concentrations in surface waters of northeast Florida Bay were summarized by embayment and/or sampling hydrologic year. A species sensitivity distribution (SSD) was constructed to model the sensitivity of demersal fish based on mean salinity affinity. Joint probability curves were used to quantify risk based on criteria set to be protective of increased biological diversity for four sampling stations in Florida Bay (Joe Bay, Taylor River, Highway Creek, and Barnes Sound).The highest risks to diversity were at the Barnes Sound sampling site, whereas the lowest potential for risk was associated with the Highway Creek site. This risk assessment is a novel approach to using a methodology developed for chemical toxicology endpoints with an abiotic factor of the environment. It was developed for use in making management decisions for the Comprehensive Everglades Restoration Plan (CERP).

Introduction

Biological changes in the South Florida hydroscape have been linked to changes in the complex hydrologic patterns of the natural system resulting from water management projects to control floods and water distribution (Science Subgroup, 1996). Therefore, the basic premise behind all restoration activities identified by the Interagency Restoration Task Force for south Florida is that hydrologic restoration is a prerequisite to achieve ecosystem restoration and a sustainable south Florida ecosystem. The Comprehensive Everglades Restoration Plan (CERP) was thus formulated to reconstruct some key features of the natural hydrologic system by restoring the hydrology of pre-drainage conditions through re-establishment of historical flows through the Everglades to the estuaries.

In Florida Bay, the inflow of freshwater was affected by the South Dade Conveyance System (SDCS), a system of canals, pumps, and other water control structures. This changed the spatial and temporal distribution of salinity. As a result of reduced freshwater flow and salinity changes, there have been reductions in the abundance of pink shrimp, piscivorous fish (snook, redfish), alligator, American crocodiles, and wading birds (McIvor et al., 1994). Many of these groups rely on small demersal fish in Florida Bay as a food source. Therefore, changes in the population of small demersal fish in Florida Bay have been suggested as the cause of declines in these important taxa. The distribution, abundances, and presence or absence of small demersal fish communities are strongly influenced by salinity changes. Salinity changes will thus be a stressor and a limiting factor which may directly affect small demersal fish, and indirectly affect the biota in Florida Bay which depends on these fish.

Salinity patterns in Florida Bay have been examined in several studies in order to evaluate their potential effects on aquatic communities (McIvor et al., 1994; Lorenz and Serafy 2006). Additionally, demersal fish communities have been investigated by scientists because they play a key role in estuaries and are good indicators of changes in physico-chemical conditions of water (Bachman and Rand, 2008).

The use of monitoring data on salinity patterns and biological responses of representative demersal fish communities have not been extensively investigated by scientists, regulators, and natural resource managers in an ecological risk context. Furthermore, scientists, regulators, and natural resource managers have relatively few tools or procedures to assist them in predicting the risks of salinity changes on native demersal fish communities. This section focuses on the use of an ecological risk assessment (ERA) approach as a decision support tool to assess the risk of salinity changes to aquatic biota in northeast Florida Bay. It will discuss the general approach and methods and an example will be presented of an ERA to evaluate risk as a result of salinity changes in Florida Bay. The salinity ERA approach is intended to assess potential risks of salinity changes in surface water and not to determine the actual causes of declines in the populations of native invertebrates, fish or plants that may be prevalent in Florida Bay. Assessment of the potential impact of salinity on aquatic communities acknowledges that other chemical and non-chemical factors (e.g., temperature, contaminants, development,) may be direct, indirect, or interacting stressors which can alter habitats and affect populations. The latter factors are not being considered here.

ERAs in aquatic ecosystems have traditionally focused on risks of single and/or multiple contaminant stressors (e.g., pesticides, metals) to individual organisms or

populations of key trophic groups (e.g., fish, zooplankton, phytoplankton, macroinvertebrates, vascular plants). Ecological risk assessment is a process that evaluates the likelihood that adverse ecological effects have occurred, may occur, or are occurring as a result of exposure to one or more stressors (U.S. EPA, 1992; 1998). As stated in the most recent U.S. EPA guidelines for ERAs, it is used to systematically evaluate and organize data, information, assumptions, and uncertainties in order to help understand and predict the relationships between stressors and ecological effects in a way that is useful for environmental decision making. An assessment may involve chemical, physical, or biological stressors, and a single and multiple stressors may be considered (U.S. EPA, 1998). In the present context, "adverse ecological effects" are understood to be anthropogenically-influenced salinity changes considered undesirable because they could alter valued structural or functional characteristics of ecosystems.

An ERA is a three-phase scientific process (Figure 4.1) which follows the U.S. EPA framework (U.S. EPA, 1992; 1998): problem formulation, risk analyses, and risk characterization. Problem formulation defines the problem and plan for analyzing and characterizing the risk. Data on stressor characteristics, ecosystem(s) at risk, potential ecological effects, and ecosystem and receptor(s) characteristics are described in this phase. From these data, assessment (i.e., the environmental value we are trying to protect) and measurement (i.e., tools used to measure effects on assessment endpoints) and a conceptual model are developed to prepare the analysis plan. (i.e., where risk hypotheses are evaluated). The conceptual model at the completion of problem formulation uses information on the ecosystems at risk, stressor characteristics, biological effects, and relationships between endpoints to define exposure and effects scenarios.

The objective of the conceptual model is to formulate hypotheses to determine how the stressor may affect the exposed ecosystem(s).

Phase II of the ERA is risk analysis, and it characterizes two major components of risk: exposure and effects. Characterizing exposure includes: results of measurements of exposure to indicate distributions and amount (intensity) at points of potential contact with receptor(s), and spatial and temporal distributions of exposure to the stressor. Characterizing effects includes: results of measurements showing the responses of endpoints to variations in exposure, response analysis (quantitative analysis of data), and defining the relationship between magnitude and duration of exposure and the endpoint effects.

Risk characterization is the final phase where the results of the analysis phase are synthesized to estimate and describe risks. It uses the results of the analysis of exposure (occurrence and magnitude) and the severity of effects resulting from such exposures to develop risk estimates for the ecological entities listed as assessment endpoints. The final step in risk characterization is describing and interpreting results of risk characterization for communication to the risk manager.

In the evolution of the ERA process for contaminants, the first approach was the calculation of a hazard quotient (HQ) which is the quotient of an estimate of the measured (or predicted) exposure concentration (i.e., in water, sediment) divided by the threshold or safe-level determined from a concentration-response relationship. Screening benchmarks such as U.S. EPA water and sediment quality criteria for contaminants have been typically used as thresholds. Hazard quotients are therefore point estimates used to protect species. The simple HQ approach is classically used to rebut presumptions of a

potential for adverse effects and it is typically used with contaminants in the early stage of an ecological risk assessment. If the quotient exceeds 1, an adverse effect (i.e., hazard) is expected to occur and cannot be rebutted. The HQ approach is conservative for contaminants and very protective. Since the HQ approach is based on point estimates it cannot be used to establish risk, which implies likelihood and is defined by probabilities. Risk is a product of likelihood of exposure and consequence (or magnitude of the effect). Risk is therefore expressed as probability of harm to a biological component of the environment. In the approach described in this section, risk to aquatic biota as a result of salinity change will be developed. For salinity, there are no screening benchmarks and because of the potential variability of salinity in a system a probabilistic approach will be more applicable.

Over the last twenty years ecological risk assessment has used probabilistic risk assessment (PRA) methods (Suter, 2007). PRA allows the inclusion of estimates of uncertainty as well as the stochastic properties of exposures and biological responses. This approach includes recognition that there are no situations where there is no risk of effect and there are few, if any, situations of complete certainty that an effect will occur. There is, in fact, a continuum of potential exposure and effect scenarios. This enables a natural resource manager to make decisions on the degree of overlap between the exposure and effects functions that are acceptable. For example, a natural resource decision manager can determine that a certain percentage of the species in a specific aquatic system must be protected. Based on the results of a PRA, a natural resource manager could predict the probability that this could be achieved. The manager may suggest that the concentration of contaminants (or salinity change) associated with this

level of protection be exceeded only x% of the time. For contaminants, like pesticides, application rates and patterns can be adjusted to obtain a high degree of certainty that the desired level of protection will be obtained.

The CERP includes extensive water management operational changes, structural changes to improve how and when water is delivered to the Everglades and removal of canals and levees that prevent natural overland sheet flow. Monitoring data are presently being generated to determine ecosystem conditions, identify cause-effect relationships and problems, and to evaluate restoration activities. The CERP monitoring and assessment plan (MA) identifies and describes key performance measures as indicators of the natural and man-made systems that are characteristic of a healthy system that will be used to assess restoration success.

Under the Southern Estuary Module of RECOVER (Restoration, Coordination and Verification) one hypothesis is that the return (or restoration) of natural salinity regimes along the shoreline as a result of CERP will increase diversity and density of fish assemblages along the mangrove shorelines of Florida Bay and Biscayne Bay (RECOVER, 2008). RECOVER is a support program within the CERP designed to assess, evaluate and improve the Plan during its implementation. The use of a PRA approach will be a valuable tool to assist scientists and natural resource managers with the performance measures used by RECOVER under CERP to determine how salinity changes affect the health of fish assemblages in the Florida Bay ecosystem.

General Probabilistic Risk Assessment (PRA) Approach and Methods

The specific risk characterization methods for conducting probabilistic ERAs have been extensively used by the U.S.EPA and discussed in detail with contaminants

(ECOFRAM 1999; Giddings et al. 2000; Giesy et al. 1999; SETAC 1994; Solomon et al. 1996; Hall et al., 1998, 1999, 2000; Schuler and Rand, 2008). PRA can incorporate principles of population dynamics, community resiliency and recovery, and functional redundancy in ecosystems. This approach acknowledges that occasionally exceedence of an adverse exposure condition (e.g., high toxic contaminant concentration) to a sensitive species may be accepted if the effected species can recover, is not critical to the function of the system under investigation and is not important as an endangered or threatened species or one of economic importance.

PRAs have had limited use for evaluating other non-contaminant stressors (Hart et al. 2003) although Bayesian probability methods have recently received considerable attention for a number of ecological issues including salinity changes (Sadoddin et al. 2005; Hickey et al. 2008). Bayesian networks can predict oxygen demand on the basis of carbon input (Borsuk et al. 2001a), nutrient reduction strategies for estuaries (Borsuk et al. 2001b), and levels of cyanobacteria (Stow et al. 1997).

Using probabilistic risk assessment in Risk Characterization-Phase III of the U.S.EPA ERA characterizes risk by comparing environmental exposure concentrations (either actual or modeled for stressor(s)) with a distribution of species sensitivity response (or toxicity) data (ECOFRAM 1999). The overlap of the distributions is proportional to the degree (or magnitude) of risk. A major advantage of this approach is that it uses all species response data and when combined with the exposure distributions from a site allows a quantitative estimation of risk to aquatic organisms. The response data (or measurement endpoints) are typically from laboratory studies. For contaminants, the response data used in the distributions are survival, growth and reproduction of

species tested singly in the laboratory and the assessment endpoint (environmental value to be protected) usually includes community and ecosystem structural and functional attributes in the field. Although the PRA approach is a valuable tool for a natural resource manager the data needed may not always be available therefore it will also serve to identify important data gaps.

To link measurement and assessment endpoints, species sensitivity distributions (SSDs) are used which were developed to estimate water quality standards that would protect some percentage of species in the environment. SSD curves are exposure-response models that use a large laboratory data set of species responses (or test endpoint values) with emphasis on using local or site-specific data. Therefore, a percentile (or proportion) of the distribution of responses for different species can be used to represent a concentration that may affect that percentage of the exposed community. For example, the SSD may be used to estimate a level of effect associated with a given level of exposure or to estimate the level of exposure corresponding to a level of effect (Suter, 2007).

It is assumed that the laboratory data used in the SSD can also be used to protect single species in the field and that an appropriate level of single-species protection will also protect populations, communities and ecosystems although many species to be exposed have not been tested. A major requirement of the SSD method is that enough species have to be tested to define the SSD and that they are representative of the receiving community. The U.S.EPA uses eight species from eight different families in developing SSDs for water quality criteria (Stephan et al. 1985) but in the Netherlands they use as few as four species in an SSD (Aldenberg and Slob 1993). When few data are

available for developing SSDs significant uncertainty is added to the extrapolation. Variance due to laboratory test conditions and test protocols are another source of uncertainty for interpretation of SSDs. In contrast, the salinity approach described below uses data of responses from the field for developing the SSD. This not only eliminates some of the uncertainty in the SSD but also provides a more accurate indication of risk since the distribution of salinity responses are being compared to actual environmental salinity exposure distributions at a field site.

All response data for SSDs are assumed to fit a log-logistic distribution and responses are plotted against rank-assigned percentiles but other functions may be used (ECOFRAM 1999; de Zwart 2002). Any statistical software used for regression analysis can be used to fit the distribution and calculate percentiles. Although the assumptions of the SSD method have been criticized (Forbes and Forbes 1993; Smith and Cairns 1993) recent investigators showed that SSD curves resulting from laboratory data were similar to those from semi-field mesocosm data which supports the SSD method for predicting safe concentrations (Hose and Brink 2004; Schroer et al.2004; Van den Brink et al. 2002).

The last step in the PRA may also consist of applying use of joint probability curves (JPCs, or exceedence profiles). JPCs characterize "the relationship between magnitude of effect and the probability of occurrence for that effect" (ECOFRAM 1999, Solomon et al. 2000). Both exposure and response distributions are converted to straight line transformations of probability functions using probit transformation. JPCs are used to determine the probability of exceeding a concentration associated with a particular response (or effect). A point on the JPC for a sampling site thus represents the probability

(%) of the surface water exposure concentrations (e.g., of a contaminant or salinity) exceeding the response endpoint for x% of the species. The curve represents the foundation of PRA because it establishes the proportion of species that will be affected and the frequency or percentage of time for such effects based on the available exposure observations (Solomon et al. 2000). This approach provides a means of comparing relative potential risk at sites when the response and exposure information are adequate.

Figures 4.2 and 4.3 provide an example of the PRA approach using the joint probability method. In Figure 4.2, the exposure concentrations and responses (effects) are characterized by regression lines and are used to generate a joint probability curve (JPC) of the exceedence data (Figure 4.4). In Figure 4.2, the concentration value at which 10% of the species responses will be exceeded is approximately 60 µg/L. Around 95% of the exposures (e.g., water concentrations of a contaminant or salinity) would be equal too or less than this value. In addition, 5% of the time this value (or exceedence value for exposure) will be exceeded. The lower the concentration value, the higher the protection because a smaller number of species would be affected. However, there is a higher probability of exceeding this value. For example, in Figure 4.3 the probability of exposures exceeding the responses for 10% of the species responses is roughly 50%. Figure 4.4 illustrates the decision making interpretation of a JPC, with risk decreasing as the curve approaches the axes.

Another approach to quantifying risk is to calculate the area under the curve (AUC) from the joint probability functions. The AUC is mathematically equivalent to the mean risk, and can be used to compare and rank risk curves (Giddings et al. 2005). The AUC can be expressed as a percentage estimating risk likelihood.

There are four major sources of uncertainty in an ecological risk assessment: stochasticity (natural variation), lack of information (i.e., data gaps), human and analytical error, and flawed model assumptions (Suter, 1993). The nature of uncertainties in the findings is discussed in light of the exposure and response analysis and risk characterization.

An Ecological Risk Assessment of Salinity in Northeast Florida Bay using the U.S.EPA Framework and PRA Approach

The present paper proposes a modified framework for an environmental risk assessment of salinity in northeast Florida Bay. I have used the U.S. EPA paradigm for risk assessment as the conceptual and procedural framework for this analysis. The EPA framework was designed for use with chemical and metal toxicants. Effects data are usually generated in the laboratory from a series of acute and chronic toxicity tests on various organisms representing single or multiple taxa.

With many contaminants, the determination of negative effects and risk is straightforward and characterized by a measureable endpoint such as an LC50 (lethal concentration whereby 50% mortality observed). The LC50 is but one endpoint amongst several acute, chronic and sublethal endpoints generated by toxicity studies and used in risk assessments. Exposure values that exceed the LC50 or other endpoint are considered conditions of risk whereas those exposure values that fall below the endpoint are considered to pose less risk. The percent and distribution of the exceedences are then used to calculate the risk in a given scenario.

For estuarine fish, salinity presents a two-tailed challenge for risk assessment. Exposure values that exceed as well as fall below the salinity tolerance range must be

considered. In this case, it is not adequate to only consider the exceedences. Hence,

modifications were made to the U.S. EPA risk framework for the analysis of salinity risk.

The following terminology was used in this risk assessment and may prove

helpful to the reader.

Diversity as Simpson's Index (D) -- A robust measure of biological diversity where $0 < D < 1$. As the value of D increases, diversity decreases (Magurran, 2004).

Salinity guild – The numerical designation of salinity designed for this ERA and based upon the categories used in the Venice System for salinity classification (1959).   The range is 1-5 (Table 4.1).

Desired Conditions/Criteria – The salinity conditions related to higher diversity as defined by $D < 0.5$.

Salinity affinity – The mean salinity at which a particular species was collected from 1990-2006 from the National Audubon Society data set for northeast Florida Bay.

Hydrologic (Hydro) year (HY) – The 365 or 366 day period starting June 1 and ending May 31, coordinated with the wet-dry cycle in this subtropical landscape.

Problem Formulation

Study Area and Ecosystem at Potential Risk

Florida Bay is a large, shallow (average depth 6-9 feet), subtropical estuary on the

southern coast of Florida that, along with the Gulf of Mexico, receives water from the

Everglades system (Lodge, 2005). The Bay (and its natural tributaries) is within the

boundaries of Everglades National Park (Figure 4.5). In the past century, the ecology,

hydrology and water quality of the Florida Bay ecosystem has been altered by

anthropogenic activities (Science Subgroup, 1996). Increased levels of salinity have been

detected in northeast Florida Bay surface water (Davis and Ogden, 1994) as a result of

changes in freshwater flow patterns in the area due to anthropogenic manipulation of the

Everglades system.

Although salinity has been identified as a driver for organism distribution and abundance, a quantitative aquatic risk assessment has not been conducted for salinity in south Florida aquatic ecosystems. Recent chemical ERAs have been performed for fresh and saltwater environments in south Florida that determined risk from levels of pesticides and other contaminants in surface waters, sediments and biota (Carriger and Rand, 2008a & 2008b; Schuler et al., 2008; Schuler and Rand, 2008). Ecological risk assessments have been conducted for salinity (Hart et al., 2003) as a result of saltwater intrusion in Australian terrestrial systems, but for estuarine systems like northeast Florida Bay, the potential ecological risk is largely unquantified.

This risk assessment will build upon, and contribute to, the existing body of work from many researchers on the patterns, effects and implications of salinity change in Florida Bay in the context of the CERP. Of particular importance to this risk assessment is the data set collected the National Audubon Society, Tavernier Science Center (Lorenz and Serafy, 2006 and additional unpublished data provided by the authors). This data set is comprised of demersal fish monitoring data and salinity from multiple locations in northeast Florida Bay sampled seasonally over a 16 year timeframe. Results from this monitoring study indicate that the reduction of freshwater flow into the Bay has reduced the demersal fish population and that restoration of freshwater inflows may aid in the rebound of these populations (Lorenz and Serafy, 2006).

Assessment and Measurement Endpoints

The U.S.EPA (1998) provides three criteria for the selection of assessment endpoints: (1) ecological relevance, (2) susceptibility to the potential stressor (i.e., salinity change), and (3) relevance to management goals. In this analysis, diversity and

abundance (Simpson's index as D) of small demersal fish assemblages in Florida Bay are the environmental values worthy of protection and are therefore the assessment endpoints. Small demersal fish are an important food base in the diet of piscivorous fish, alligators, crocodiles and wading birds (McIvor et al., 1994; Lorenz and Serafy, 2006). In addition, this assessment endpoint is consistent with the performance measures, restoration expectations and targets under the CERP Southern Estuaries Module (RECOVER, 2008).

Conceptual model

The primary objective was to conduct an ecological risk assessment to quantify the likelihood and extent that adverse effects (as a decrease in biological diversity of demersal fish) will occur from exposure to various salinity regimes using the U.S. Environmental Protection Agency (U.S. EPA) Ecological Risk Assessment Framework. Within this context, the objectives were to determine: (1) where and when are the effects likely to be greatest; and (2) which salinity communities are at greatest risk?

Risk Analysis

Exposure Assessment

The exposure assessment was designed to examine the co-occurrence of the stressor salinity in surface waters of Florida Bay with the ecological responses (or effects) discussed below. For exposure, data on daily salinity concentrations (in ppt) in Florida Bay were obtained by using actual measured concentrations (AMCs) in surface waters from monitoring studies from 1990 to 2006. The data were collected by the National Audubon Society, Tavernier Science Center, and provided by Everglades National Park.

For construction of the SSD, data from up to 21 sampling sites over the 16 year monitoring program were used. Exposure data were obtained from AMCs of salinity on the day of and at the site of fish collection.

Four sites (Figure 4.6) were chosen as germane to this risk assessment: Joe Bay (JB); Taylor River (TR); Highway Creek (HC); and Barnes Sound (BS). These sites, located in northeast Florida Bay, are habitat for a variety of demersal fish, have experienced high fluctuations in salinity, and are critical feeding grounds for higher trophic level species. They were selected, amongst other similar sites, due to their use in current monitoring studies with the National Audubon Society and Everglades National Park to allow for alignment of this risk assessment with ongoing CERP projects (Lorenz, 1999; Bartell et al., 2004). For each of the above mentioned sampling sites, six hydro year (HY) periods at 3-year increments were analyzed: 1990-1991, 1993-1994, 1996-1997, 1999-2000, 2002-2003, and 2005-2006. For the JPCs, daily AMC salinity values for each hydro year and sampling site were ranked from smallest to largest. Due the interannual variability within these measurements, the ranked salinity values were assigned a numerical salinity guild (1-5) based upon the Venice Classification (1959) system for salinity as shown in Table 4.1.

Probability distributions of exposure data for the salinity guilds at the different sites were prepared based on the AMCs at each site to evaluate spatial and temporal trends.

Salinity guild values were ranked to develop cumulative distributions (Giddings et al. 2000). Guilds were ranked from smallest to highest and assigned a centile ranking (j) at each site using the equation:

$$j \times 100/(n+1)$$

where j is the rank number assigned to the individual data point and n is the total number of sample observations at each site. When at least four guild values were available at each site, a log-logistic distribution for salinity exposure data was developed at the site (Hall Jr. et al. 2000). Linear regression was performed using the algorithms found in PRAT-1 software (Solomon et al., 2000).

Response (Effects) Assessment

For the purpose of the SSD, the term 'mean salinity affinity' is used to describe the salinity at which a particular species was most often collected over the course of 16 years of monitoring. The affinity of a given species is used as the response as predicated upon by the driver of environmental exposure. The field data were collected by the National Audubon Society, Tavernier Science Center and provided by Everglades National Park. The dataset was comprised of the species identification and abundance of fish collected at specific dates for each location from the years 1990 – 2006 for the same sites as described above (see Appendix A for individual species data). To calculate ranks for all species salinity affinity response data were ordered by concentration according to the same equation in exposure assessment. The calculated ranks were fit to a log-logistic distribution and the resulting SSD was plotted graphically using Microsoft Excel 2007. For a distribution to be considered for analysis, at least four suitable species endpoints (mean salinity affinity responses) had to be available (Aldenberg and Slob 1993).

For generation of the JPCs, the response used was biological diversity as expressed as Simpson's Index D. To calculate D, the abundance and species of fish collected during each HY was tabulated. Each HY contained 6-8 monthly sampling

events. Simpson's D was calculated for each sampling event within each HY for the sites and HYs listed above using the following equation:

$$D = \Sigma \, (n_i \, (n_i - 1))/(N(N\text{-}1)$$

where n_i is the number of individuals in the ith species and N is the total number of individuals (Magurran, 2004). Calculations were performed using Microsoft Excel 2007 with software developed by Danoff-Burg and Chen (2005). Fish data were screened to only include fish with a total length no greater than 6.5 cm. This value was selected to screen for the size of fish that comprise up to 90% of the demersal fish community found in the study area (Lorenz and Serafy, 2006).

Traditionally, laboratory endpoints (e.g. LC50, EC50, NOEC) with measureable units (e.g. mg/L) are used as the response data for generating a JPC. The LC50 is used to compare exposed organisms with unexposed organisms to establish criteria for deleterious effects. Inherent in this endpoint is the assumption that any concentration above this value will have greater effects (as % mortality) towards the group of organisms tested. I employed the LC50 as the conceptual and procedural basis for using a measure of diversity as an effects endpoint.

As mentioned above, D is a value between 0 and 1. As D increases towards 1, diversity decreases. For this risk assessment I established a value of D less than or equal to 0.50 as the desired criteria for biological diversity. Conceptually this is akin to the LC50 derived from toxicity studies. D-values greater than 0.5 were categorized as above the desired criteria (as would an LC75) and were not used in this risk assessment. To interface with the exposure data and place D in the context of salinity, the D-values were paired with the salinity guild corresponding to the average salinity for the 60 days

antecedent to the sampling event for which they were calculated (Data presented in Appendix B). Lorenz (1999) found that a determining factor in fish abundance and distribution in the study area was the mean salinity for the 60 days prior to sampling. The resulting guild values were ranked and treated the same way as the exposure data to create probability distributions.

Risk Characterization

The potential risk of salinity changes at sites in Florida Bay was examined by integrating the probability distributions of the salinity guild exposure concentrations with the effects distribution of diversity (as salinity guild) for each site and hydro year. From the resulting distributions, the degree of overlap between the salinity guild exposure distributions from surface water field data and the salinity guilds that were correlated to increased diversity were determined.

For each JPC generated, the AUC was calculated to yield a measure of risk. The trapezoid rule was used for the AUC calculation in this risk assessment. In this method, the curve was divided into a series of trapezoids based on each data point. The individual area of each trapezoid (A) was calculated using the formula:

$$A = (Y_n + Y_{n-1})/2 * (X_n - X_{n-1})$$

Where Y_n is the Y-column value for the JPC at the nth cell and X_n is the X-column value at the nth cell (Liengme, 2002). The sum of the trapezoids is the AUC and is expressed as percent risk.

Uncertainties

Effects

Sources of uncertainty in the exposure analysis are the spatial and temporal limits inherent in the database used for this project and the assumption that the salinities recorded are representative of consistent trends. The entire database spans 16 years and up to 21 sites which include many small embayments with individual hydropatterns. However, for consistency with other on-going projects, this risk assessment employed a subset of sampling sites and hydro years.

Exposure

The potential effects of single action or joint action of salinity with other biotic and/ or abiotic factors such as temperature, dissolved oxygen, water depth, food availability or predation on determining fish diversity, distribution and abundance was not considered in the effects analysis. The goal of this ERA was to examine salinity as a single driver for biological diversity since salinity regimes in the area can be controlled (to a degree) through water management activities whereas other biotic/abiotic factors are less easily regulated.

Results

Species Sensitivity Distribution

The SSD was constructed for 42 demersal fish species collected over a 16 year period in northeast Florida Bay (Figure 4.7). The SSD represents the mean salinity affinity for each species sampled, and species are organized according to salinity category. Though estuarine fish have a wide range of salinity tolerance, the mean salinity affinity is reflective of the most common 'preference' for each species. Based on the

SSD, the 90th centile is 13.4 ppt. Hence, 90% of the species collected are commonly found at this salinity or below.   The 10th centile is 0.209 ppt. Below this salinity less than 10% of the sampled fish populations are commonly found.   Summary tables of salinity conditions for each sampling location are provided in Tables 4.2 – 4.5. The percent of salinity values between 5-15 ppt and between 0.5 – 13.4 ppt are provided for consideration in the context of the Florida Bay/Florida Keys Feasibility Study (FBFKFS) salinity targets, which will be discussed in later in this paper.

Probability distributions

The JPCs constructed for each sampling site are presented in Figures 4.8-4.11. The y-axis for each curve marks the percent of time that the desired criteria are not met either because salinities exceed or fall below the desired range. The x-axis represents the percent of desired criteria that is met. The percentiles used to for the risk assessment are developed by the resource manager and/ or regulatory agency as acceptable for the management goals. In this case, I have assigned a conservative value of 80% for the protection of the desired conditions (i.e. increased diversity). The JPC can than be used to determine what percent of the HY was protective of 80% of the desired conditions. Figure 4.8 is the JPC for Joe Bay. For the hydro year 1993-1994, 80 % of the desired criteria (x-axis) corresponds to a point on the curve that indicates that roughly 10% of the year the desired conditions were not me. Hence, 1993-1994 can be considered a year of low risk as compared to other years by meeting desired conditions slightly over 90% of the time.

Tables 4.6 –4.9 summarize the information in the JPCs. I assigned a value of > 80% of the HY as a target for these tables. The percent time of the HY that is protective

of 80% of the desired condition is listed in the central column of each table. Hydro years that were protective of desired conditions greater than 80% of the time are highlighted (read as 80% protective of higher diversity for $\geq$ 80% of the HY).

An additional way to interpret the JPC is through calculation of the area under the curve. The AUCs, presented as 'percent mean risk', are provided in the right-most column of each table. Note that all AUCs under 50% correspond to the same HYs and locations as those that are 80% protective of diversity greater than 80% of the hydro year.

Discussion and Recommendations

Environmental risk assessment is an analytical tool employed by regulatory agencies to support the decision-making process for environmental stewardship. As such, the ERA can be used to address protection goals and identify those scenarios that likely contribute to 'risk' to a given system.

Declines in seagrass beds, coral communities, and wading bird and fish populations in Florida Bay and the Everglades have been linked to contaminant exposure and changes in the hydrological patterns of the natural system as a result of water management projects to control floods and water distribution (Science Subgroup, 1996). Changes in plant and animal assemblages resulting from habitat alteration/ destruction and salinity changes have also been observed (Davis and Ogden, 1994). Consequently, alterations in the hydrological system are thought to be the main cause of declines of fish and wildlife populations due to habitat changes. The Comprehensive Everglades Restoration Plan (CERP) was formulated as part of the Water Resources Development Act (1996) with the goal of restoring hydrologic conditions that support biodiversity,

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ensure the quality of the remaining habitats and foster sustainable development for the region.

Though a large body of work exists on the effects of salinity on fish species, much of these data are for nonnative species. Especially in regards to salinity, upper and lower tolerances derived in a laboratory (or seen in the field) often exceed those that are biologically beneficial to the organism (Haney et al., 1999; Kefford et al., 2004). It is also known that salinity tolerance can be an inheritable trait and site-specific to a given population of fish (Holt and Holt, 2003; Bachman and Rand, 2008). One goal of this aquatic risk assessment was to focus on south Florida species and tailor the PRA to the CERP and, in particular, northeast Florida Bay. As such, the ENP/Audubon field data provided the 'real-time' response of native and local species to the salinity regimes in this area based on multiple years of data. This factor alone makes this ERA unique as traditionally laboratory derived effects data are used in generating risk assessments.

Currently, the Florida Bay/Florida Keys Feasibility Study (FBFKFS) outlines target salinity ranges for areas of northeast Florida Bay as part of the CERP (Rudnick, 2005). These targets were chosen based on paleoecological data for the area and 'best professional judgment (Rudnick, 2005). The Joe Bay (JB), Barnes Sound (BS) and Highway Creek (HC) sites are all within the boundaries of a zone that has a restoration target salinity range of 5-15 ppt (Figure 4.5). The Taylor River (TR) site is more inland than the other three sites and as such is not included in the FBFKFS salinity zones.

This probabilistic ecological risk assessment focused on data from four sampling sites for 6 hydro years spanning a 16 year period. Several conclusions on risk were reached. The highest potential risk to biological diversity associated with salinity was

seen in Barnes Sound. The mean salinities for this site were all greater than 16.58 (range 16.58 to 20.82 ppt) and BS had the least amount of hydro years that were protective of biological diversity based upon the criteria selected (80% protection for $\geq$ 80% of the HY). The salinity guild categories that corresponded to the low risk years were categories 4 and 5 (18.00 - 30.00 + ppt). The lowest potential risk of salinity to demersal fish diversity was at the Highway Creek sampling site, with only 1 out of 6 years not meeting the protection criteria selected. Mean salinities ranged from 1.96 to 17.15 ppt for this site and low risk values ranged from 30.8 to 45.24%. For HC, four of the five years had salinity guilds values in categories 2 to 3 (range 1.00 – 17.99 ppt) that met the desired criteria. The fifth year had a guild value in category 4 (18.00 -29.99 ppt). The Joe Bay and Taylor River sites both showed low risk 3 out of 6 hydro years. For Joe Bay, the mean salinities ranged from 1.81 to 28.51 ppt and low risk values ranged from 22.43 to 46.89 %. Salinities that met requirements for the 80% of the desired criteria fell into guild categories 2 & 3 for Joe Bay. The Taylor River site had mean salinities ranging from 2.35 to 24.77 ppt. and low risk values of 45.47 to 48.86 %. The corresponding salinity guilds for the low risk years all fell within guild category 2 (1.00 -4.99 ppt).

The majority of low risk years for Joe Bay, Highway Creek and Taylor River coincided with HYs that included significant portions of the year with low salinities indicating that freshwater and oligohaline conditions may be favorable to increased diversity. For all three sites, the majority of low risk years also corresponded to a greater percentage of the HY between 0.0 and 13.4 ppt (the range for the 90th centile determined from the SSD). However, in Joe Bay, those years that had a higher percentage of the HY at salinities < 0.5 ppt corresponded to the years of highest risk. While freshwater

conditions appear to be related to increased diversity, extended freshwater conditions can have adverse effects at this site. Conversely, the Taylor River site (which is more inland and receives freshwater from the Taylor Slough) showed lowest risk for the majority of years that had the highest percentage of < 0.5 ppt conditions. The highest risk (at TR) was seen in the majority of HYs that had a higher frequency of salinities > 13.4 ppt, indicting that very low salinity/freshwater is a driver of diversity at this site. At the Barnes Sound site, the higher salinity years showed less risk, and were related to those years which had a higher percentage of the HY > 13.4 ppt (the SSD 90th centile).

Based on the SSD, a salinity value of 5.00 ppt is equal to the 75th centile. Therefore, targeting a range of 5-15 ppt for these areas (as suggested by the FBFKFS) could likely create adverse conditions for 75% of the fish species commonly found in the area. The 16-year sampling data indicates that these areas are dominated by freshwater and oligohaline species. If one of the management goals for the area is to increase diversity and density of species along the coastal areas of northeast Florida Bay, than tools such as this SSD, which takes into account field recorded responses to environmental conditions, should be used along with other performance measure of ecosystem health and historical data in the decision making process.

In the context of the FBFKFS, this risk assessment approach has shown that the proposed salinity targets may not meet the requirements for increased biological diversity in the sampling sites analyzed. Three of the four sites (JB, HC, and TR) exhibited a higher frequency of meeting the requirements for increased diversity within a salinity range that encompassed freshwater, oligohaline and mesohaline salinities. Based on the data generated in this ERA, the proposed salinity target of 5-15 ppt for these areas is too

narrow and may exclude those species that have more affinity for freshwater and oligohaline conditions. The duration of freshwater conditions also contributed to the risk for Joe Bay, thus consideration should be given to length of the freshwater conditions in these areas that are proximal to the Bay rather than inland like the TR site. Barnes Sound had overall higher salinities, with the years of lowest risk at BS corresponding to salinities greater than the 90th centile in the SSD. In the case of BS, management decisions need to be made as to whether target salinities will favor the freshwater, oligohaline and mesohaline as indicted by the SSDs 90th centile, or different salinity categories such as meso- and polyhaline. The data analyzed for this risk assessment indicate that increased diversity can be achieved with higher salinities in this area.

In the case of management decisions for northeast Bay, the SSD and ERA are tools that can be used to establish salinity criteria that are protective of a majority of the demersal fish population. Using field responses in lieu of laboratory data in the ERA can assure greater certainty in risk prediction when the primary measure of exposure is from actual salinity monitoring in Florida Bay and not model predicted values.

This is a novel approach to risk assessment using a natural abiotic component of an ecological system that has become altered due to anthropogenic activities, and as such can be viewed similar to a contaminant. Using the U.S. EPA framework, I have shown that a risk assessment using salinity is feasible and can be used as a supplemental tool to environmental regulators/agencies in the decision making process for the CERP.

Environmental risk assessment plays an important role in the regulatory decision making process. However, risk assessments should be viewed as but one part of a larger process that factors in scientific predictions alongside economic, political, and social

concerns (Johnson et al., 2007). The valuation placed upon ecosystems or their

functional components, the amount of risk that is deemed 'acceptable', and the feasibly

both economically & technologically of mitigating the risk all play a role in driving the

decision making of resource managers and regulators.

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Ecological Risk Assessment

PROBLEM FORMULATION

Discussion Between the Risk Assessor and Risk Manager (Planning)

Data Acquisition; Verification and Monitoring

ANALYSIS

Characterization of Exposure | Characterization of Ecological Effects

RISK CHARACTERIZATION

Discussion Between the Risk Assessor and Risk Manager (Results)

Risk Management
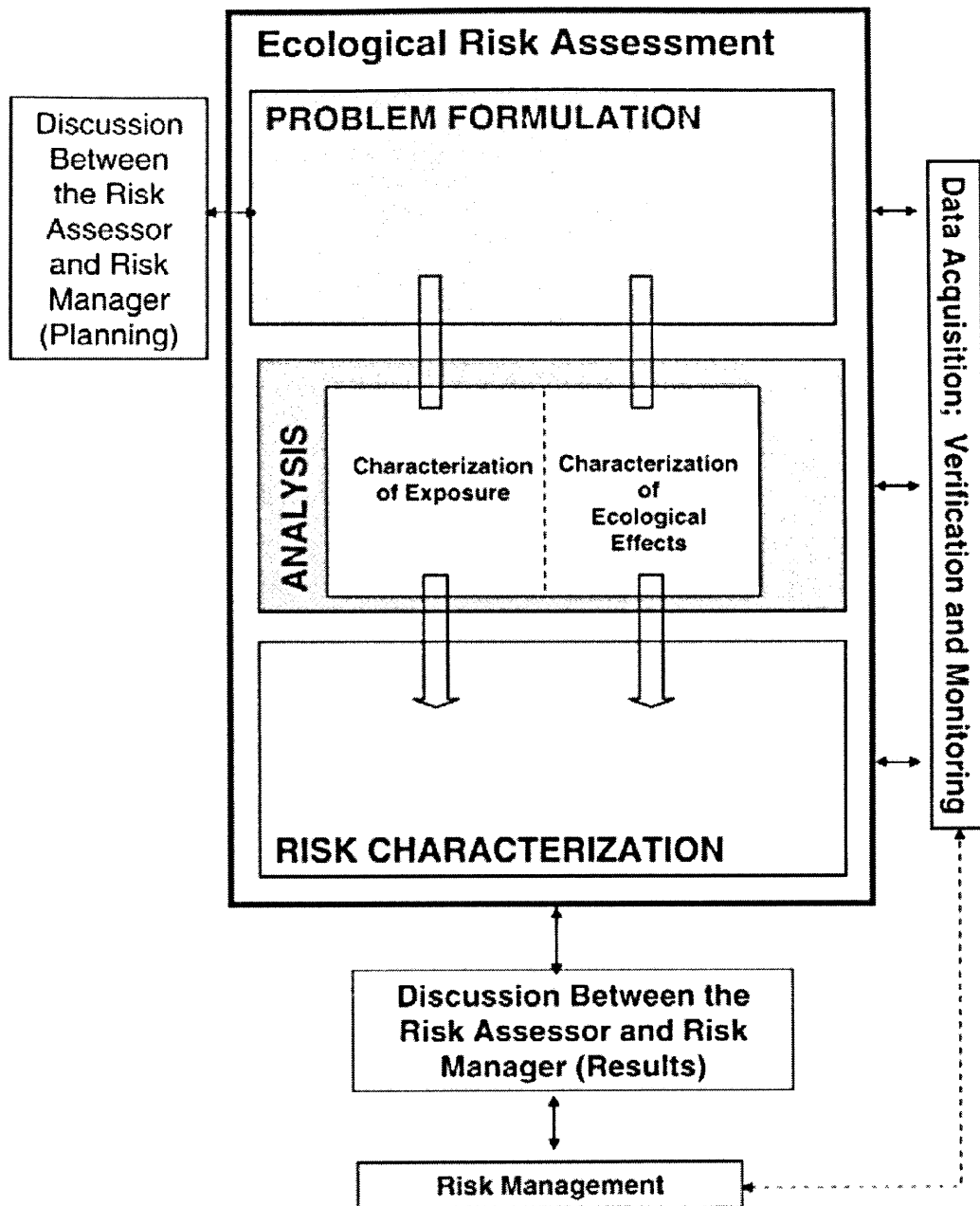
Figure 4.1. The U.S. EPA's (1992) frame work for Ecological Risk Assessment (ERA).
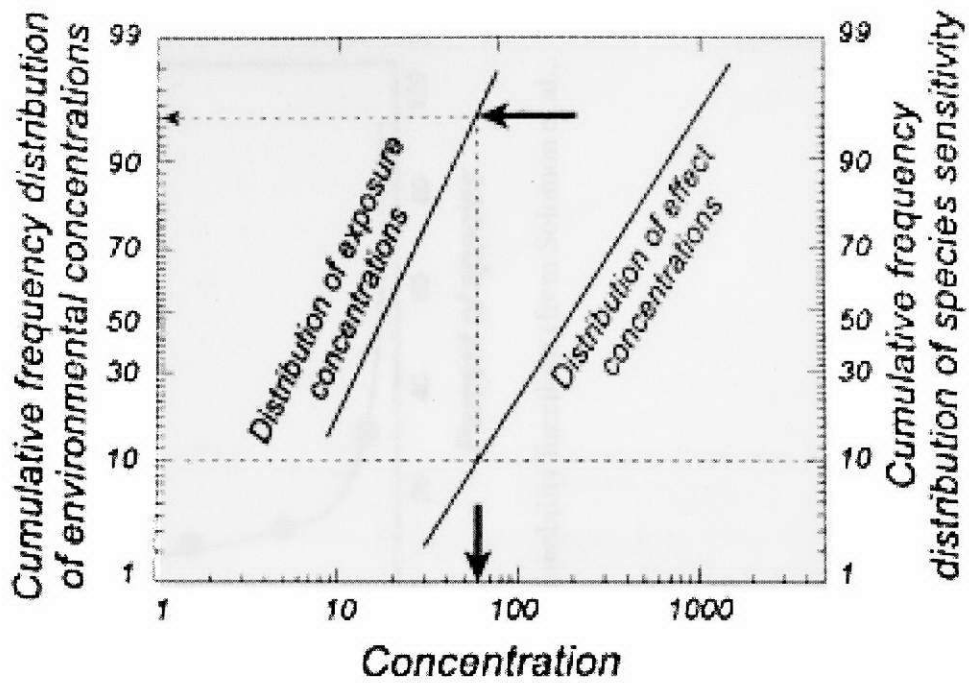
Figure 4.2. Linearized probability distributions of exposure and response (effect) data (from Solomon et al., 2000).
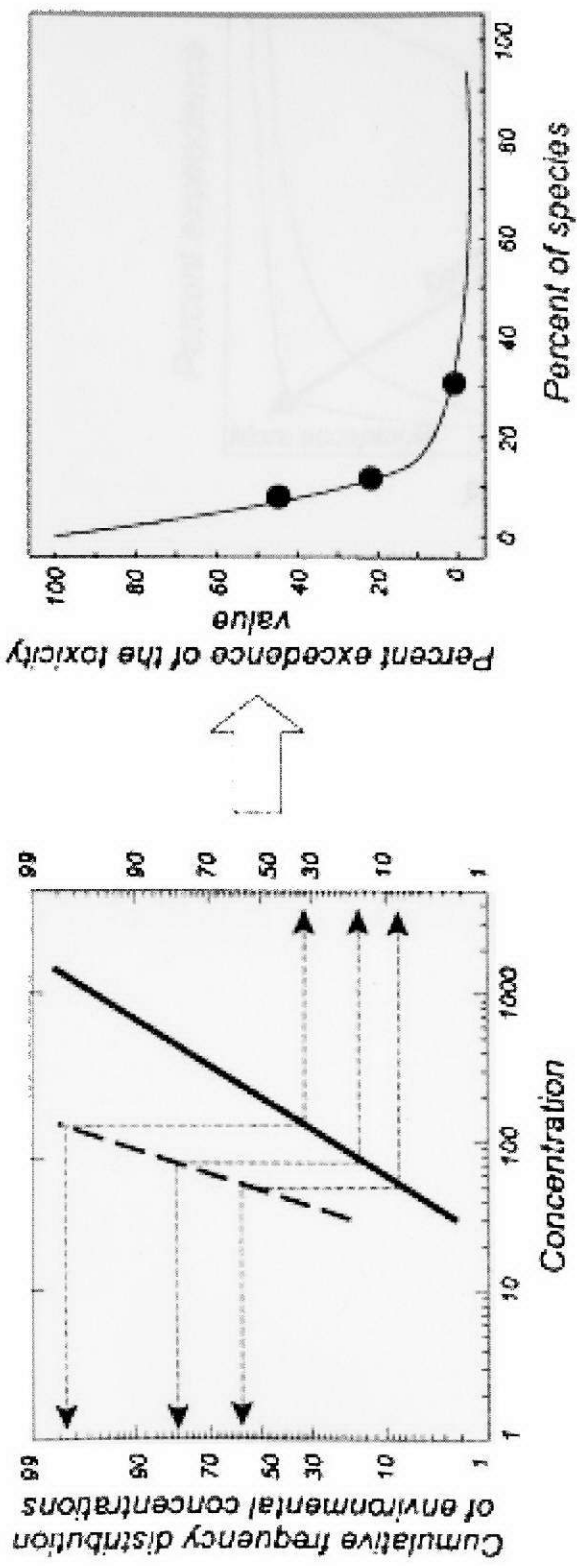
Figure 4.3. Derivation of joint probability curve (JPC) from exposure and response probability functions (from Solomon et al., 2000).

129
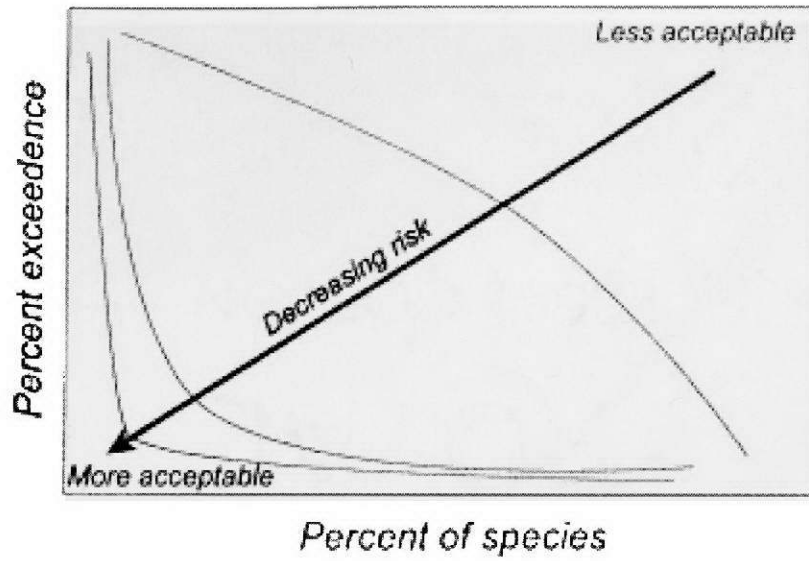
Figure 4.4. Interpretation of JPC in decision making (from Solomon et al., 2000).

Florida Bay Florida Keys Feasibility Study
Annual Salinity Performance Measure Zones

Zone 14a: 10 - 20
Zone 13: 5 - 15
Zone 1: 5 - 15
Zone 1a: 15 - 25
Zone 13a: 10 - 30
Zone 15: 0 - 5
Zone 5: 15 - 35
Zone 16: Base
Zone 3: 25 - 30
Zone 2: 15 - 30
Zone 14: 15 - 30
Zone 4: 30 - 35
Zone 8: Base
Zone 6: 25 - 35
Zone 7: Base
Zone 12: No Change Base
Zone 9: Base
Zone 7: Base
Zone 9: Base
Zone 11: No Change Base
Zone 10: No Change Base
Zone 11: No Change Base

Monitoring Stations
• ENP
• USGS

N
W   E
S

Miles
0   3   6   12

ZONEID	TARGET
1	5 - 15
1a	15 - 25
2	15 - 30
3	25 - 30
4	30 - 35
5	15 - 35
6	25 - 35
7	Base
8	Base
9	Base
10	No Change Base
11	No Change Base
12	No Change Base
13	5 - 15
13a	10 - 30
14	15 - 30
14a	10 - 20
15	0 - 5
16	Base

Figure 4.5. Florida Bay map illustrating embayments and target salinity zones from the Florida bay/Florida Keys Feasibility Study (Rudnick, 2005).
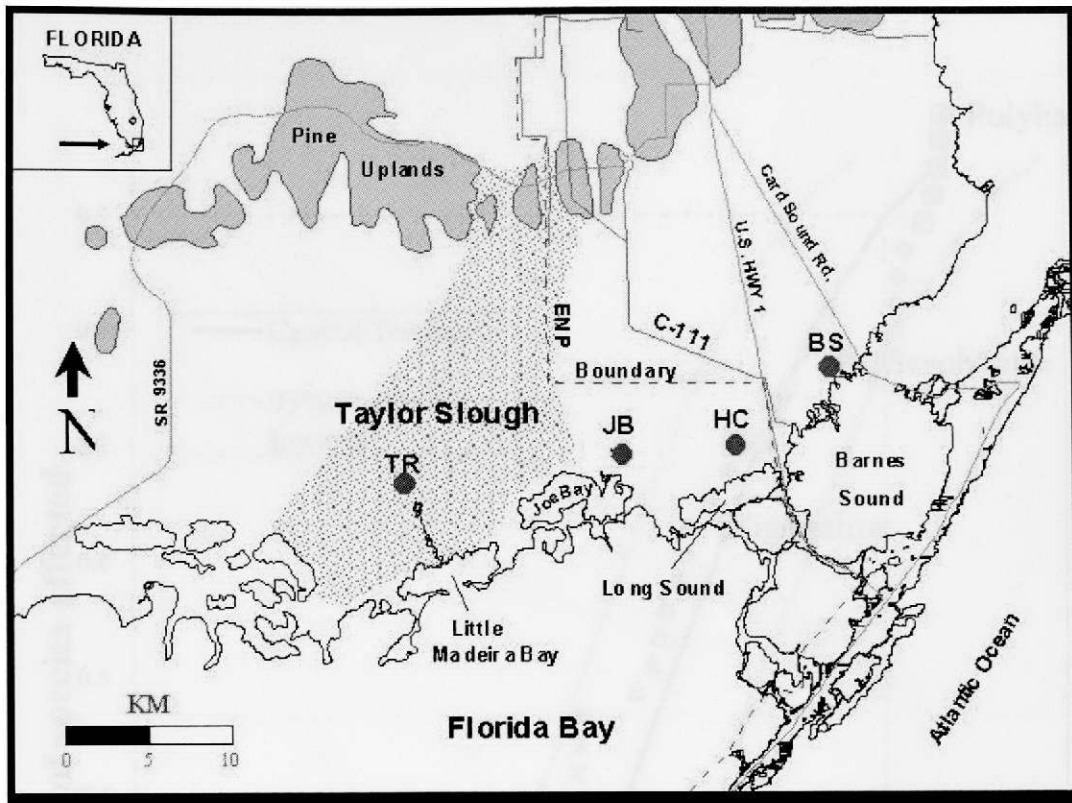
Figure 4.6 Audubon Sampling sites. JB: Joe Bay; HC: Highway Creek; BS: Barnes Sound; TR: Taylor River. (from Bartell et al. 2004.)
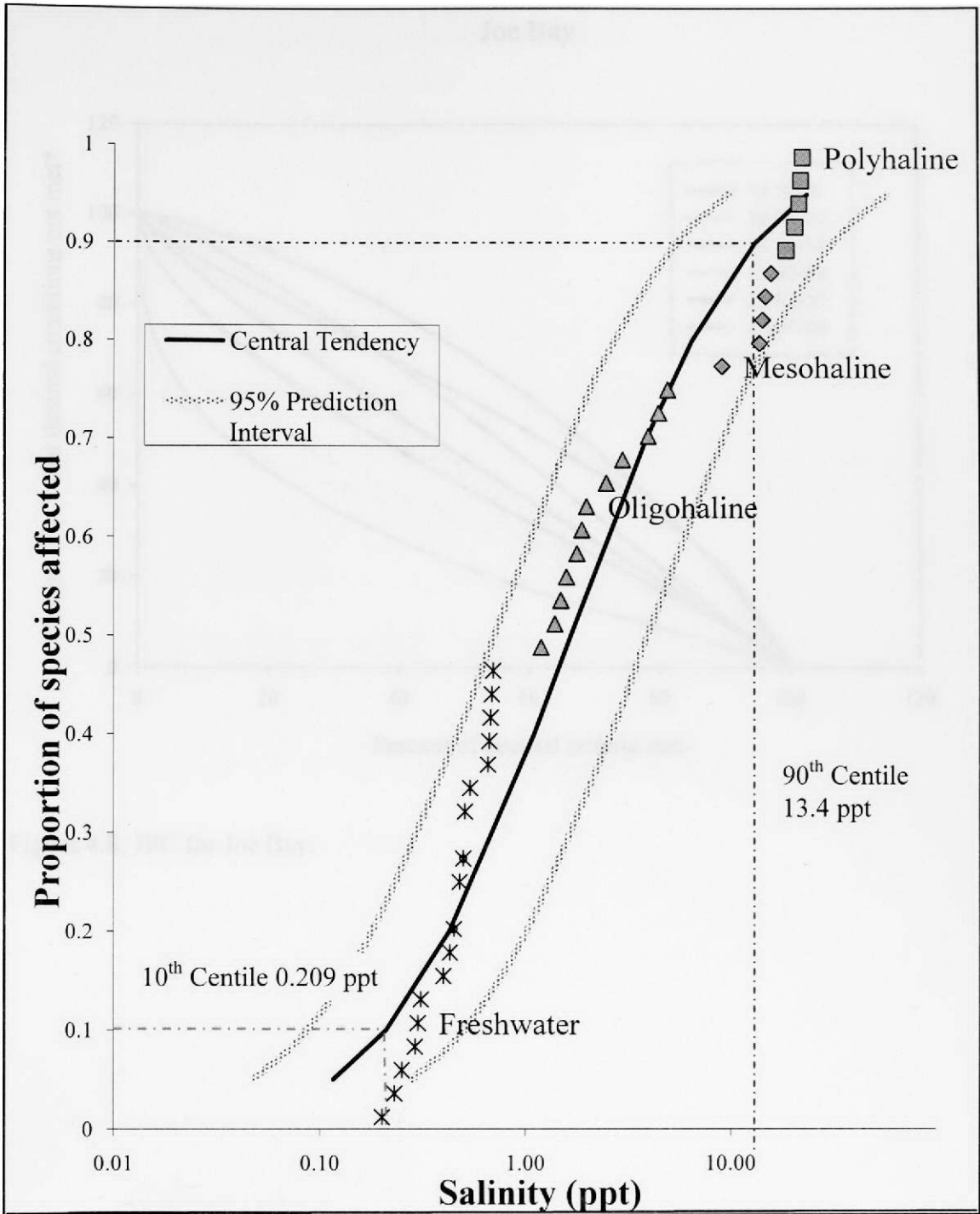
Figure 4.7. Species Sensitivity Distribution (SSD) for mean salinity affinity for demersal fishes in northeast Florida Bay.

Joe Bay
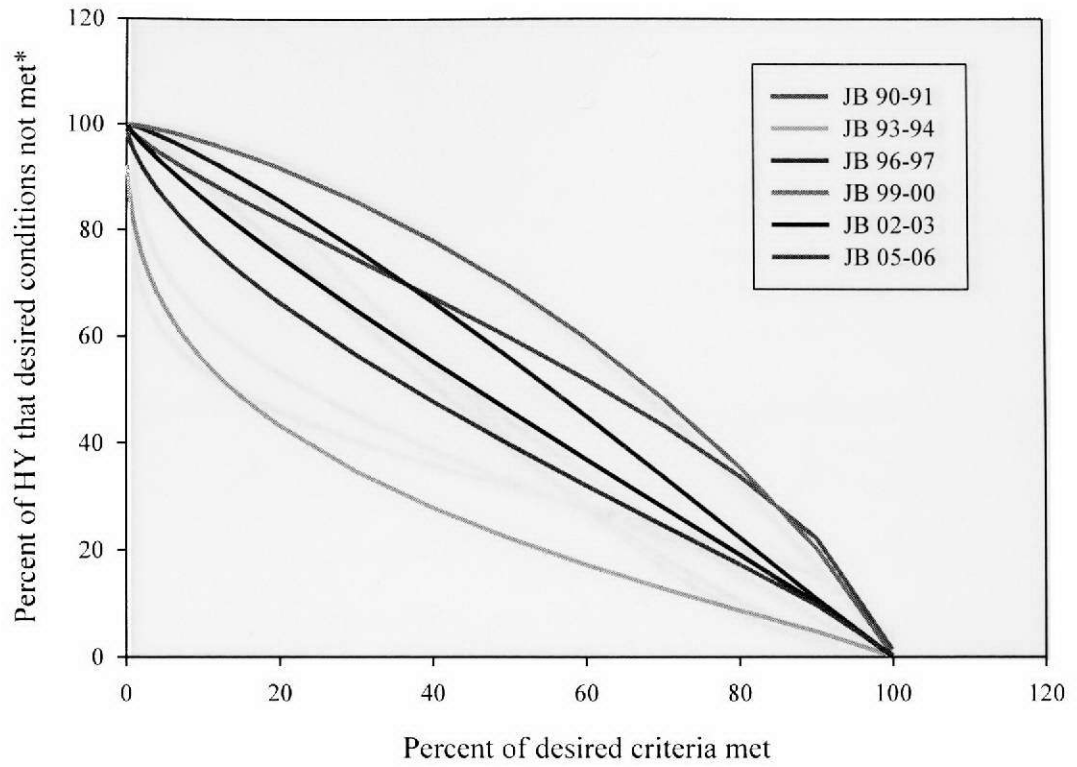
Figure 4.8. JPC for Joe Bay.

Highway Creek
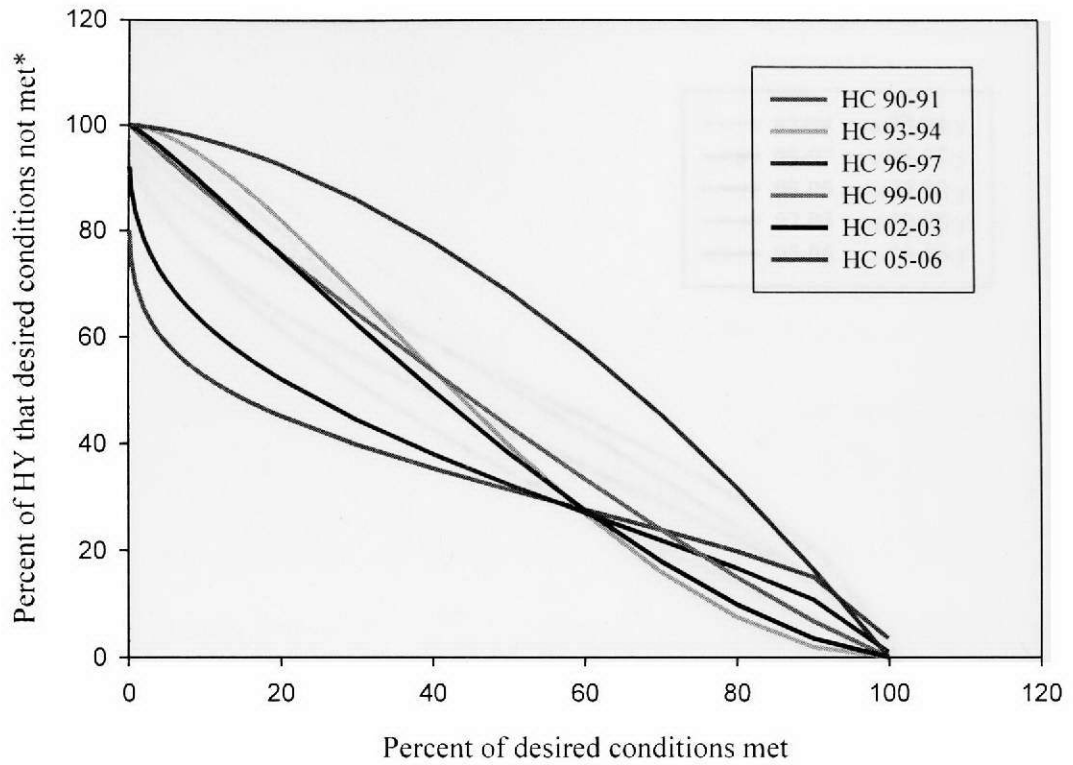
Figure 4.9. JPC for Highway Creek.

Barnes Sound
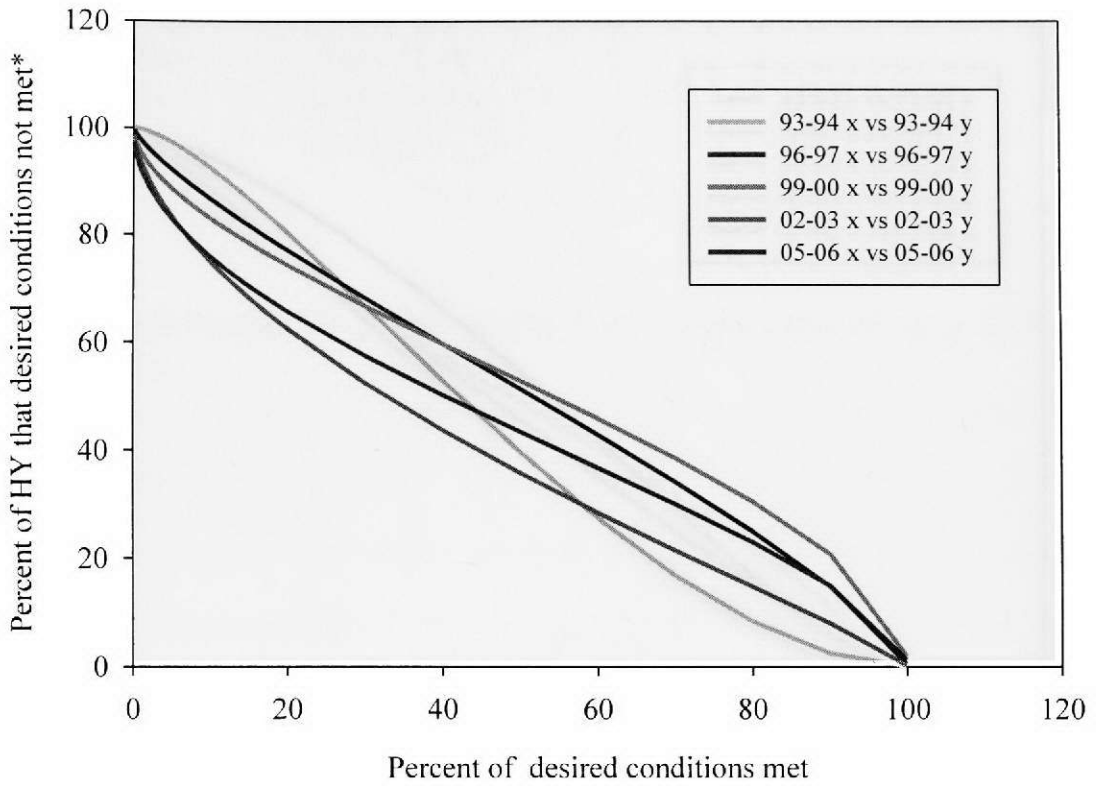
Figure 4.10. JPC for Barnes Sound.

Taylor River
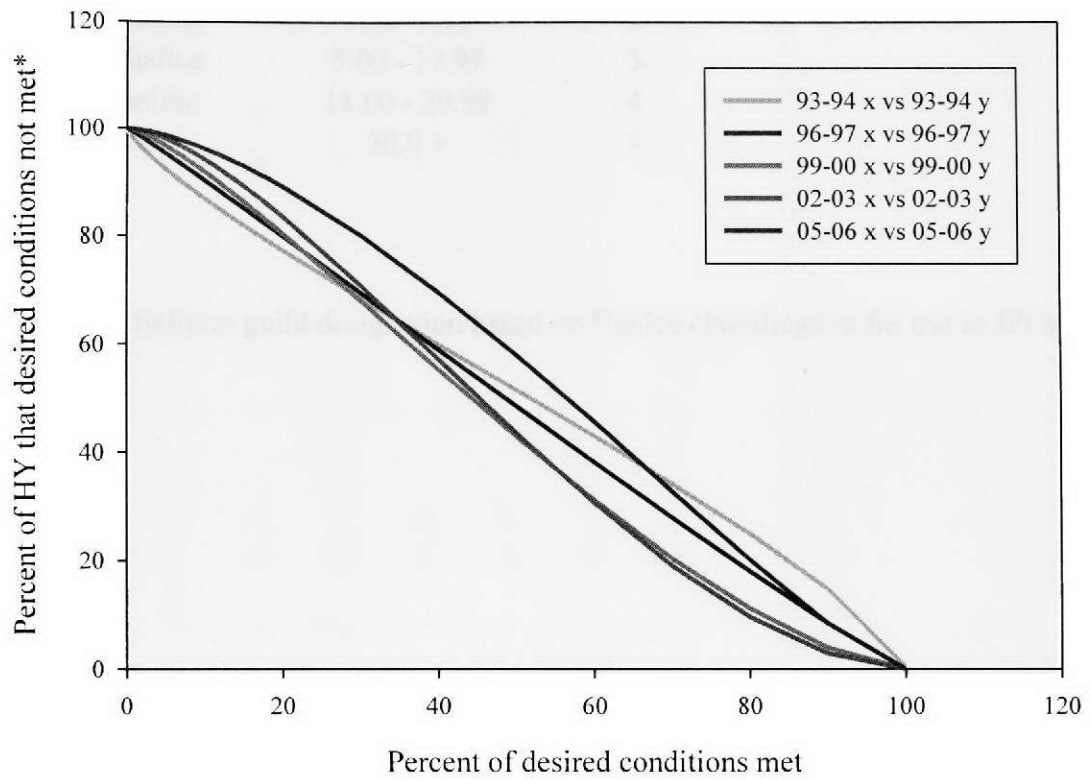
Figure 4.11. JPC for Taylor River.

Venice Classification	Salinity range (ppt)	Salinity Guild
Freshwater	0.00 - 0.99	1
Oligohaline	1.00- 4.99	2
Mesohaline	5.00 - 17.99	3
Polyhaline	18.00 - 29.99	4
Marine	30.0 +	5

Table 4.1. Salinity guild designation based on Venice classification for use in JPCs.

JOE BAY	Maximum Salinity (ppt)	Minimum Salinity (ppt)	Mean Salinity (ppt)	Percent of HY Salinity bet. 0-13.4 ppt	Percent of HY Salinity bet. 0.5-13.4 ppt	Percent of HY Salinity below 0.5 ppt	Percent of HY Salinity above 13.4 ppt	Percent of HY Salinity bet. 5-15 ppt
1990-1991	56.62	1.18	28.51	12.63	12.33	3.61	84.06	11.20
1993-1994	25.26	0.22	1.81	99.28	86.13	13.15	0.72	3.70
1996-1997	32.43	0.24	6.40	90.68	66.81	23.87	9.32	10.72
1999-2000	36.99	0.26	4.87	91.02	60.77	30.25	8.98	11.13
2002-2003	29.02	0.31	5.92	86.29	68.47	17.82	13.71	10.13
2005-2006	45.52	0.25	9.43	83.03	72.60	10.43	16.97	13.98

TABLE 4.2. Salinity profile for Joe Bay. Highlights indicate hydro years in which comparatively lower risk was calculated based upon protection for increased diversity (80% protection for D< 0.5) and risk percentage less than 50%.

	Maximum Salinity ppt	Minimum Salinity ppt	Mean Salinity ppt	Percent of HY Salinity bet. 0-13.4 pp	Percent of HY Salinity bet. 0.5-13.4 ppt	Percent of HY Salinity below 0.5 ppt	Percent of HY Salinity above 13.4 ppt	Percent of HY Salinity bet. 5-15 ppt
HIGHWAY CREEK								
1990-1991	57.78	0.19	17.15	53.78	46.27	7.51	46.22	24.86
1993-1994	7.42	0.00	1.96	100.00	77.33	22.67	0.00	6.87
1996-1997	24.71	0.16	5.76	86.02	66.56	19.46	13.98	16.62
1999-2000	26.02	0.11	5.14	90.31	72.88	17.75	9.37	16.94
2002-2003	31.37	0.23	6.40	85.53	73.02	12.51	14.47	18.65
2005-2006	46.35	0.10	10.34	79.59	64.47	15.12	20.41	19.61

TABLE 4.3. Salinity profile for Highway Creek. Highlights indicate hydro years in which comparatively lower risk was calculated based upon protection for increased diversity (80% protection for D< 0.5) and risk percentage less than 50%.

	Maximum Salinity ppt	Minimum Salinity ppt	Mean Salinity ppt	Percent of HY Salinity bet. 0-13.4 pp	Percent of HY Salinity bet. 0.5-13.4 ppt	Percent of HY Salinity below 0.5 ppt	Percent of HY Salinity above 13.4 ppt	Percent of HY Salinity bet. 5-15 ppt
BARNES SOUND								
1990-1991			Data not available					
1993-1994	29.58	5.02	19.26	13.65	13.60	0.29	86.11	18.65
1996-1997	38.11	0.17	18.50	34.95	34.85	0.11	65.04	37.00
1999-2000	37.69	0.00	16.58	46.84	46.44	0.67	52.89	40.45
2002-2003	42.26	0.86	20.82	33.31	33.31	0.00	66.69	26.61
2005-2006	42.60	0.78	19.95	36.96	36.96	0.00	63.04	27.32

TABLE 4.4. Salinity profile for Barnes Sound. Highlights indicate hydro years in which comparatively lower risk was calculated based upon protection for increased diversity (80% protection for D< 0.5) and risk percentage less than 50%.

	Maximum Salinity ppt	Minimum Salinity ppt	Mean Salinity ppt	Percent of HY Salinity bet. 0-13.4 pp	Percent of HY Salinity bet. 0.5-13.4 ppt	Percent of HY Salinity below 0.5 ppt	Percent of HY Salinity above 13.4 ppt	Percent of HY Salinity bet. 5-15 ppt
TAYLOR RIVER								
1990-1991	63.81	1.32	24.77	39.50	39.50	0.00	60.46	20.70
1993-1994	29.55	0.08	2.35	99.00	45.89	53.13	0.98	2.50
1996-1997	24.43	0.15	4.83	94.10	41.50	52.60	5.90	6.10
1999-2000	26.37	0.14	3.45	95.00	41.30	53.67	5.03	6.10
2002-2003	26.64	0.23	3.24	94.60	49.90	44.66	5.44	5.40
2005-2006	50.14	0.27	8.88	88.30	69.30	19.00	11.70	12.08

TABLE 4.5. Salinity profile for Taylor River. Highlights indicate hydro years in which comparatively lower risk was calculated based upon protection for increased diversity (80% protection for D< 0.5) and risk percentage less than 50%.

Site	Hydro Year	Salinity Guild at which 80% of Desired Criteria Met	Percent of HY salinities that fall within protective range*	Percent of HY salinities that exceed or fall below protective range*	AUC (%Risk)
Joe Bay	1990-1991	4.63	66.2	33.8	56.67
	1993-1994	**2.79**	**91.36**	**8.64**	**22.43**
	1996-1997	2.91	77.66	22.34	53.93
	1999-2000	2	64.47	35.53	63.34
	2002-2003	**2.99**	**80.92**	**19.08**	**46.89**
	2005-2006	**3.77**	**82.75**	**17.25**	**41.68**

*Protective range inclusive of 80% of salinities that meet desired criteria

TABLE 4.6. Risk profile for Joe Bay. Highlights indicate hydro years in which greater than 80% of the salinities were protective of increased diversity (D < 0.5). Note that the percent risks in these years (as AUC) are less than 50% indicating comparatively lower risk.

		Salinity Guild at which 80% of Desired Criteria Met	Percent of HY salinities that fall within protective range*	Percent of HY salinities that exceed or fall below protective range*	AUC (% Risk)
Site	Hydro Year				
Highway	**1990-1991**	**4.62**	**80.12**	**19.88**	**30.80**
Creek	**1993-1994**	**2.85**	**92.51**	**7.49**	**44.01**
	1996-1997	**3.13**	**83.34**	**16.66**	**34.11**
	1999-2000	**3.16**	**85.03**	**14.97**	**45.24**
	2002-2003	**3.99**	**90.13**	**9.87**	**42.32**
	2005-2006	2.92	68.22	31.78	62.28

* Protective range inclusive of 80% of salinities that meet desired criteria

TABLE 4.7. Risk profile for Highway Creek. Highlights indicate hydro years in which greater than 80% of the salinities were protective of increased diversity (D < 0.5). Note that the percent risks in these years (as AUC) are less than 50% indicating comparatively lower risk.

Site	Hydro Year	Salinity Guild at which 80% of Desired Criteria Met	Percent of HY salinities that fall within protective range*	Percent of HY salinities that exceed or fall below protective range*	AUC (% Risk)
Barnes Sound	1990-1991	N/A	N/A	N/A	N/A
	1993-1994	**4.61**	**91.66**	**8.34**	**43.69**
	1996-1997	4.19	75.03	24.97	50.54
	1999-2000	3.94	69.51	30.49	51.00
	2002-2003	**5.13**	**85.25**	**14.75**	**38.62**
	2005-2006	4.58	77	23	43.66

* Protective range inclusive of 80% of salinities that meet desired criteria

TABLE 4.8. Risk profile for Barnes Sound. Highlights indicate hydro years in which greater than 80% of the salinities were protective of increased diversity (D < 0.5). Note that the percent risks in these years (as AUC) are less than 50% indicating comparatively lower risk.

Site	Hydro Year	Salinity Guild at which 80% of Desired Criteria Met	Percent of HY salinities that fall within protective range*	Percent of HY salinities that exceed or fall below protective range*	AUC (% Risk)
Taylor River	1990-1991	N/A	N/A	N/A	N/A
	1993-1994	1.77	75.05	24.95	50.67
	1996-1997	**2.69**	**82.03**	**17.97**	**48.86**
	1999-2000	**2.87**	**88.84**	**11.16**	**45.47**
	2002-2003	**2.99**	**90.43**	**9.57**	**46.13**
	2005-2006	3.36	79.87	20.13	54.96

* Protective range inclusive of 80% of salinities that meet desired criteria

TABLE 4.9. Risk profile for Taylor River. Highlights indicate hydro years in which greater than 80% of the salinities were protective of increased diversity (D < 0.5). Note that the percent risks in these years (as AUC) are less than 50% indicating comparatively lower risk.

Appendix A: Species used in Species Sensitivity Distribution (from Lorenz and Serafy, 2006).

Species	Salinity Affinity (ppt)	Venice Classification System
Erimyzon sucetta	0.5	
Lepomis gulosus	0.5	
Lepomis microlophus	0.5	
Elassoma evergladei	0.5	
Notropis spp.	0.5	
Ameiurus sp.	0.5	
Micropterus salmoides	0.5	
Lucania goodei	0.5	
Fundulus chrysotus	0.5	
Fundulus seminolis	0.5	Freshwater -- 0.2-0.99 ppt
Cichlasoma bimaculatum	0.5	
Lepomis marginatus	0.5	
Enneacanthus gloriosus	0.5	
Jordanella floridae	0.5	
Heterandria formosa	0.5	
Clarias batrachus	0.5	
Lepomis macrochirus	0.5	
Noturus gyrinus	0.5	
Lepomis punctatus	0.5	
Tilapia mariae	0.5	
Giosoma bosc	1	
Lophogobius cyprinoides	1	
Gobiosoma robustum	1	
Cichlasoma urophthalmus	1	
Lepomis spp.	1	
Belonesox belizanus	1	Oligohaline -- 1-4.99 ppt
Gambusia holbrooki	2	
Adinia xenica	2	
Fundulus confluentus	3	
Poecilia latipinna	4	
Lucania parva	4	
Cyprinodon variegatus	5	
Menidia spp	9	Mesohaline -- 5-17.99 ppt

Trinectes maculatus	14	
Microgobius gulosus	14	
Fundulus grandis	15	
Strongylura notata	16	
Opsanus beta	19	
Floridichthys carpio	21	
Eucinostomus spp.	22	Polyhaline -- 18-30 ppt
Syngnathus scovelli	22	
Fundulus similis	23	

Appendix B. Diversity Calculations by HY and Sampling Site.

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Joe Bay	1990-1991	11/1/1990	0.34	5.54	3
		11/29/1990	0.26	10.68	3
		12/21/1990	0.39	12.13	3
		1/21/1991	0.28	18.45	4
		2/26/1991	0.39	26.08	4
		3/21/1991	0.29	30.13	5
	1993-1994	6/21/1993	0.49	12.14	3
		9/30/1993	0.34	1.94	2
		12/2/1993	0.41	1.79	2
		1/25/1994	0.25	2.36	2
		3/14/1994	0.20	1.52	2
		4/20/1994	0.15	1.51	2
	1996-1997	6/25/1996	0.28	12.74	3
		8/27/1996	0.20	0.66	1
		10/28/1996	0.26	0.35	1
		11/28/1996	0.16	0.74	1
		1/9/1997	0.19	1.58	2

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Joe Bay	1996-1997	2/24/1997	0.31	1.58	2
		3/22/1997	0.17	3.56	2
		4/21/1997	0.22	11.36	3
	1999-2000	7/22/1999	0.53	21.43	4
		9/18/1999	0.56	0.51	1
		11/23/1999	N/A	0.51	1
		12/15/1999	0.42	0.76	1
		1/30/2000	0.30	0.9	1
		2/24/2000	0.19	0.89	1
		3/23/2000	0.20	1.25	2
	2002-2003	6/18/2002	0.52	25.14	4
		10/2/2002	0.43	0.43	1
		11/16/2002	0.18	1.87	2
		12/19/2002	0.41	2.81	2
		1/16/2003	0.19	1.91	2
		2/18/2003	0.22	4.2	2
		3/21/2003	0.53	13.02	3
		4/30/2003	0.37	14.35	3
	2005-2006	6/1/2005	0.44	38.73	5

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Joe Bay	2005-2006	10/18/2005	0.49	1.37	2
		11/13/2005	0.20	1.25	2
		12/20/2005	0.25	1.76	2
		1/10/2006	0.23	1.21	2
		2/7/2006	0.16	3.2	2
		3/7/2006	0.13	6.81	3
		4/4/2006	0.23	15.42	3

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Highway Creek	1990-1991	12/18/90	0.42	6.13	3
		2/28/1991	0.27	19.95	4
		3/28/1991	0.37	22.29	4
		4/29/1991	0.17	29.88	4
	1993-1994	6/8/1993	0.37	12.26	3
		10/5/1993	0.26	0.61	1
		11/8/1993	0.23	0.4	1
		1/26/1994	0.41	3.45	2
		3/22/1994	0.23	3.23	2
		4/21/1994	0.34	2.86	2
	1996-1997	7/3/1996	0.31	7.43	3
		9/6/1996	0.48	1.14	2
		10/22/1996	0.60	0.39	1
		10/22/1996	0.42	1.11	2
		1/16/1997	0.39	3.66	2
		2/27/1997	0.26	3.51	2
		3/26/1997	0.30	6.48	3
		4/24/1997	0.28	13.74	3
	1999-2000	7/15/1999	0.42	15	3

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Highway Creek	1999-2000	9/30/1999	0.51	0.59	1
		11/16/1999	0.33	0.56	1
		12/12/1999	0.33	1.38	2
		1/20/2000	0.36	1.74	2
		2/21/2000	0.22	2.49	2
		3/20/2000	0.29	4.81	2
		04/20/00	0.46	8.4	3
	2002-2003	6/11/2002	0.32	29.18	4
		11/4/2002	0.30	1.51	2
		12/12/2002	0.27	3.5	2
		1/9/2003	0.61	3.09	2
		2/11/2003	0.75	4.44	2
		3/12/2003	0.29	10.57	3
		4/8/2003	0.25	14.51	3
	2005-2006	6/1/2005	0.55	36.48	5
		10/18/2005	0.27	0.64	1
		11/15/2005	0.23	0.7	1
		12/16/2005	0.26	1.2	2
		1/20/2006	0.22	1.93	2

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Highway Creek	2005-2006	2/18/2006	0.42	5.79	3
		3/7/2006	0.58	9.12	3
		4/3/2006	0.64	16.61	3

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Barnes Sound	1990-1991	NO DATA			
	1993-1994	6/11/1993	0.32	24.86	4
		10/7/1993	0.58	21.29	4
		11/23/1993	0.38	21.76	4
		2/4/1994	0.27	21.69	4
		3/18/1994	0.19	20.01	4
		4/26/1994	0.24	16.68	3
	1996-1997	7/11/1996	0.21	17.99	3
		8/31/1996	0.41	9.96	3
		10/31/1996	0.29	11.14	3
		11/30/1996	0.37	10.23	3
		1/24/1997	0.18	18.2	4
		3/3/1997	0.21	19.77	4
		4/1/1997	0.22	24.65	4
		4/30/1997	0.27	29.24	4
	1999-2000	7/29/1999	0.29	23.2	4
		10/7/1999	0.31	17.32	3
		11/6/1999	0.29	9.21	3
		12/8/1999	0.59	5.23	3
		1/13/2000	0.21	5.86	3
		2/15/2000	0.20	8.49	3

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Barnes Sound	1999-2000	3/13/2000	0.13	12.89	3
		4/15/2000	0.37	20.16	4
Barnes Sound	2002-2003	6/6/2002	0.37	44.35	5
		9/17/2002	0.31	9.93	3
		11/1/2002	0.32	16.01	3
		12/4/2002	0.23	20.33	4
		2/6/2003	0.33	24.8	4
		3/6/2003	0.31	32.72	5
		4/3/2003	0.36	33.05	5
	2005-2006	6/7/2005	0.30	36.74	5
		10/11/2005	0.31	9.32	3
		11/8/2005	0.25	9.67	3
		12/2/2005	0.25	11.11	3
		1/10/2006	0.16	18.62	4
		2/3/2006	0.37	24.06	4
		3/3/2006	0.39	25.9	4
		4/6/2006	0.30	29.85	4

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Taylor River	1990-1991	NO DATA			
	1993-1994	6/16/1993	0.50	1	2
		9/28/1993	0.32	0.4	1
		12/1/1993	0.45	0.3	1
		1/21/1994	0.38	0.4	1
		3/9/1994	0.41	0.4	1
		4/19/1994	0.44	0.5	1
	1996-1997	6/30/1996	0.22	11.1	3
		8/22/1996	0.00	0.3	1
		10/10/1996	0.26	0.3	1
		11/10/1996	0.22	0.2	1
		1/6/1997	0.18	0.3	1
		2/20/1997	0.26	0.5	1
		3/19/1997	0.39	1.4	2
		4/17/1997	0.48	8	3
	1999-2000	7/7/1999	0.20	18.4	4
		9/9/1999	0.27	2.3	2
		11/9/1999	0.30	2.8	2
		12/22/1999	0.20	0.2	1
		2/3/2000	0.24	0.26	1

Site	HY	Sampling Date	D	60d Salinity Avg.(ppt)	Salinity Guild
Taylor River	1999-2000	2/29/2000	0.18	0.27	1
		3/16/2000	0.27	0.29	1
	2002-2003	6/27/2002	0.30	20.51	4
		10/8/2002	0.26	0.33	1
		11/21/2002	0.20	0.44	1
		12/22/2002	0.20	0.45	1
		1/22/2003	0.25	0.35	1
		2/12/2003	0.16	0.36	1
		3/21/2003	0.23	4.12	2
		4/25/2003	0.30	7.79	3
	2005-2006	6/8/2005	0.29	43.53	5
		10/11/2005	0.28	1.57	2
		11/8/2005	0.22	0.86	1
		12/3/2005	0.37	0.94	1
		1/13/2006	0.19	0.62	1
		2/9/2006	0.15	0.93	1
		3/3/2006	0.32	1.11	2
		4/11/2006	0.26	6.79	3

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