SWIMMING PERFORMANCE OF JUVENILE FLORIDA POMPANO (*TRACHINOTUS CAROLINUS*) AND ATLANTIC SPADEFISH (*CHAETODIPTERUS FABER*) EXPOSED TO SUBLETHAL CONCENTRATIONS OF ETHYLENE GLYCOL AND METHANOL: INDIVIDUAL AND SYNERGISTIC EFFECTS

A Thesis

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Table of Contents

List of Tables

List of Figures

Abstract

Recently, new technology has pushed petrochemical exploration into increasingly deeper water (>305 m) at increased risks to marine fauna. One risk is from chemical additives used to enhance deepwater production such as ethylene glycol and methanol which are used during the production and treatment to prevent the formation of gas hydrates in deepwater wells and pipelines. Juvenile Florida pompano (*Trachinotus carolinus*) were used in controlled experiments to test the effects of 3.0 % ethylene glycol (EG), 1.07 % methanol (MeOH) and a combination of the two chemicals (EG + MeOH) on swimming performance of individual fish. Swimming performance was evaluated by comparing differences in pre- and post-exposure critical swimming speeds (U_{crit}) for each individual to quantify sublethal effects. The experimental protocol included identical fasting, exposure, acclimation, and swimming experience for each group and required 18 days for the Florida pompano experiment. In juvenile swimming performance tests, single exposure to ethylene glycol and the combination of ethylene glycol and methanol significantly reduced U_{crit} by 13.0 and 42.0 %, respectively. No detectable difference in U_{crit} was found for Florida pompano exposed to methanol or for the controls. Juvenile Atlantic spadefish (*Chaetodipterus faber*) were used to test the single effects of 3.0 % ethylene glycol on swimming performance of individuals. For Atlantic spadefish, there was a 6.9 % reduction in U_{crit} compared to pre-exposure swimming performance and a 17.9 % reduction compared to the controls. The reduced ability of pompano and spadefish to sustain high prolonged and burst performance levels could have affects an individuals ability to avoid predators and feed effectively.

Chapter I.

Overall Introduction

Recently, new technology has pushed petrochemical exploration into increasingly deeper water (>300 m) at increased risks to the marine fauna including the release of contaminants and noise pollution associated with the construction of offshore oil platforms. One group of risks is from chemical additives used to enhance deep-water production such as ethylene glycol and methanol which are used during the production and treatment to prevent the formation of gas hydrates in deep-water wells and pipelines (Anonymous 1996, 2000; Herzhaft and Dalmazzone 2000; Boehm et al. 2001). Ethylene glycol and methanol are often transported long distances through underwater pipelines and by ships to deep-water production platforms increasing the risk of accidental spills. Although the possibility of a large spill is unlikely, the risk remains plausible with increased usage, transport and storage of volumes of up to 300,000 L of ethylene glycol and methanol per platform (Boehm et al. 2001).

A tremendous amount of research has been directed towards understanding the effects of offshore oil development in the northern Gulf of Mexico (Gallaway et al. 1981; Grizzle 1986; Stanley and Wilson 1990; Grossman et al. 1987; Boehm et al. 2001, Hymel et al. 2002). Yet little attention has been paid towards understanding chronic or acute sublethal effects associated with low-level, localized discharges of toxicants, which may reduce an individual fish's ability to avoid predators, feed, reproduce, and resist diseases and parasites. Effects at the level of the organism may, in turn, lead to effects at the population and community levels (Weis et al. 1999). It is, therefore, important to

quantify sublethal effects at the individual level to enhance our understanding of how populations and communities may respond to these impacts.

Ethylene glycol and methanol are toxic to both mammals and fishes to varying degrees. Median lethal concentrations (LC_{50}) of ethylene glycol have been reported for various brackish and freshwater organisms (Bridie et al. 1979; Adbelghani et al. 1989; Pillard 1995; Green and Kocan 1997). However, ethylene glycol LC_{50} values have been reported for only a few freshwater fishes including rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*) (Pillard 1995; Greene and Kocan 1997) and bluegill sunfish (*Lepomis macrochirus*) (Abdelghani et al. 1989). Methanol LC_{50} values have been reported for various freshwater and two saltwater fishes by Bengstsson et al. (1984) for the bleak (*Alburnus alburnus*) and by Portmann and Wilson (1971) for the hooknose (*Agonus cataphractus*). Experiments by Hymel et al. (2002) and Baltz et al. (in prep) found LC_{50} values for ethylene glycol and methanol, respectively, and quantified their sublethal effects through swimming performance on a marine fish, Florida pompano (*Trachinotus carolinus*).

The measurement of swimming performance was first suggested as an important criterion in the determination of sublethal effects of toxicants on fishes by Cairns (1966) and has since been used to determine tolerances of fishes to varying environmental factors and pollutants (Nikl and Farrell 1993; Beaumont et al. 1995; Heath 1995; Kolok et al. 1998; Hymel et al. 2002). Swimming performance is associated with of the ability of fish to feed, escape predation and maintain position in a current and involves the integrated effects of numerous physiological processes (Beamish 1978). Selyle (1950) first defined stress as the sum of all the physiological responses by which an animal tries

to maintain or re-establish a normal metabolism in the face of a physical or chemical force. Stress and resistance to a stressor are energy draining processes (Schreck 1982; Barton and Schreck 1987) and while responding to stress an organism should have less energy available to devote to other life functions (Schreck 1990). Estimation of swimming performance can, therefore, provide an index of stress.

Juvenile Florida pompano and Atlantic spadefish (*Chaetodipterus faber*) were used to evaluate the sublethal effects of ethylene glycol and methanol because they occur in the vicinity of offshore petroleum production platforms and pipelines in the northern Gulf of Mexico. Pompano and spadefish are important commercial and sport fishes but little is known about the life history of both species. Pompano apparently have a protracted spawning season in the northern Gulf of Mexico during the spring and summer (Iverson and Berry 1969; Finucane 1969; Nelson and Murphy 2001) and may spawn year round in the tropical regions of the Gulf of Mexico (Berry and Iverson 1967). Spadefish are believed to spawn from May through September, principally in the northern Gulf of Mexico (Ditty et al. 1993). Both species are believed to spawn offshore and juveniles usually migrate inshore to feed and the young of the year move back offshore when temperatures cool (Finucane 1969; Johnson 1978; Gilbert and Parsons 1986; Hayse 1989; Nelson and Murphy 2001).

The goals of these studies were to extend previous research by testing the separate and combined effects of 3.0 % (volume/volume) ethylene glycol and 1.07 % (v/v) methanol on the swimming performance of individual Florida pompano and to examine sublethal effects of ethylene glycol on Atlantic spadefish. Exposure concentrations were based on LC_{50}, behavior, and recovery experiments by Hymel et al. (2002) and Baltz et

al. (in prep). Previous research on the separate effects of ethylene glycol (Hymel et al. 2000) and methanol (Baltz et al. in prep) found significant reductions in critical swimming speed after exposure. Florida pompano were exposed to combination exposures of both chemicals in a randomized experimental design to test for any additive, synergistic, or antagonistic sublethal effects that ethylene glycol and methanol might have on swimming performance. Atlantic spadefish were exposed to single exposures of ethylene glycol in a randomized experimental design to examine potential sublethal effects on a marine species with a different physiology and ecology.

It is common in studies testing for the sublethal effects of contaminants on fishes to first find an appropriate LC_{50} on the species of interest. Hymel et al. (2002) and Baltz et al. (In prep) found LC_{50} values for ethylene glycol and methanol on juvenile Florida pompano. Due to the lack of sufficient numbers of similarly sized Atlantic spadefish, I did not conduct a LC_{50} range finding experiment.

A secondary goal of both studies was to examine synergistic and individual sublethal impacts by comparing changes in respiration rates of fishes before and after exposure. Water samples from each individual respirometer were taken at the beginning and end of each 10 min velocity increase. Due to technical problems with the oxygen meter no values were reported in this manuscript.

Chapter II.

Swimming Performance of Juvenile Florida Pompano Exposed to Ethylene Glycol and Methanol: Synergistic Effects

Introduction

There are an estimated 4,000 oil and gas platforms in the northern Gulf of Mexico that add an additional 12 km^2 to the 2600 km^2 of natural hard bottom (Stanley and Wilson 1997). As artificial reefs, oil and gas platforms are unique because they extend throughout the water column and are thought to affect benthic, demersal and pelagic fishes (Gallaway et al. 1981; Continental Shelf Associates 1982). Artificial reefs provide additional habitat that attract fish and may increase the environmental carrying capacity and eventually the biomass of reef fishes (Bohnsack 1989). Oil platforms create fishing opportunities, reduce user conflicts, save time and fuel, make locating fish more predictable, and increase public access (Stone 1985; National Academy Press 1988). Nevertheless, oil and gas platforms are also a source of anthropogenic inputs including produced formation water, drilling fluid chemicals, oil-based and water-based drilling muds and cuttings and production additives, some of which are toxic to the fauna that utilizes these structures (Holdway 2002).

Recently, new technology has pushed petrochemical exploration into increasingly deeper water (>300 m) at increased risks to the marine fauna including the release of contaminants and noise pollution associated with the construction and operation of offshore oil platforms. One group of risks is from chemical additives used to enhance deep-water production such as ethylene glycol and methanol which are used during the production and treatment to prevent the formation of gas hydrates in deep-water wells and pipelines (Anonymous 1996, 2000; Herzhaft and Dalmazzone 2000; Boehm et al.

2001). Ethylene glycol and methanol are often transported long distances through underwater pipelines and by ships to deep-water production platforms increasing the risk of accidental spills. Although the possibility of a large spill is unlikely, the risk remains plausible with increased usage, transport and storage of volumes of up to 300,000 L of ethylene glycol and methanol per platform (Boehm et al. 2001).

A tremendous amount of research has been directed towards understanding the effects of offshore oil development on the northern Gulf of Mexico (Gallaway et al. 1981; Grizzle 1986; Stanley and Wilson 1990; Grossman et al. 1987; Boehm et al. 2001, Hymel et al. 2002). Yet little attention has been paid to understanding chronic or acute sublethal effects associated with low-level, localized discharges of toxicants, which may reduce an individual fish's ability to avoid predators, feed, reproduce, and resist diseases and parasites. Effects at the level of the organism may, in turn, lead to effects at the population and community levels (Weis et al. 1999). It is, therefore, important to quantify sublethal effects at the individual level to enhance our understanding of how populations and communities may respond to these impacts.

Ethylene glycol and methanol are common chemicals used in industry and by consumers. Ethylene glycol a common ingredient in antifreeze, is used as a deicing agent on bridges, airplanes and airport runways, and can prevent the formation of hydrates in deep-water pipelines (Abdelghani et al. 1990; Herzhaft and Dalmazzone 2000). Methanol or wood alcohol is used as an organic solvent, in synthetic solid fuels and like ethylene glycol can prevent the formation of hydrates in deep-water wells and pipelines (Tephly 1991; Herzhaft and Dalmazzone 2000). Hydrates are solid structures that form when water molecules crystallize around guest molecules in natural gas and other

petroleum products (Herzhaft and Dalmazzone 2000). Hydrate formation is particularly common in under water pipelines due to the cooling effect of sea water on the pipeline at extreme depths (Anonymous 1996). Hydrate formation is a problem not only during the drilling and extraction process, but also with transport in deep-water pipelines and can potentially clog a pipeline permanently (Herzhaft and Dalmazzone 2000). With the intensified activity in deeper water, larger volumes of ethylene glycol and methanol are being transported, stored and used by industry.

Ethylene glycol and methanol are toxic to both mammals and fishes to varying degrees. Median lethal concentrations (LC_{50}) of ethylene glycol have been reported for various brackish and freshwater organisms (Bridie et al. 1979; Adbelghani et al. 1989; Pillard 1995; Green and Kocan 1997). However, ethylene glycol LC_{50} values have been reported for only a few freshwater fishes including the rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*) (Pillard 1995; Greene and Kocan 1997) and bluegill sunfish (*Lepomis macrochirus*) (Abdelghani et al. 1989). Methanol LC_{50} values have been found for various freshwater and two saltwater fishes by Bengstsson et al. (1984) for the bleak (*Alburnus alburnus*) and by Portmann and Wilson (1971) for the hooknose (*Agonus cataphractus*). Experiments by Hymel et al. (2002) and Baltz et al. (in prep) found LC_{50} values for ethylene glycol and methanol, respectively, and quantified their sublethal effects through swimming performance on a marine fish, Florida pompano (*Trachinotus carolinus*). This study extends previous research on Florida pompano by examining the sublethal effects of both chemicals in combination.

The measurement of swimming performance was first suggested as an important criterion in the determination of sublethal effects of toxicants on fishes by Cairns (1966)

and has since been used to determine tolerances of fishes to varying environmental factors and pollutants (Nikl and Farrell 1993; Beaumont et al.1995; Heath 1995; Kolok et al. 1998; Hymel et al. 2002). Swimming performance is associated with the ability of fish to feed, escape predation and maintain position in a current and involves the integrated effects of numerous physiological processes (Beamish 1978). Selyle (1950) first defined stress as the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force. Stress response and resistance to a stressor are energy draining processes (Schreck 1982; Barton and Schreck 1987) and while responding to stress an organism should have less energy available to devote to other life functions (Schreck 1990). Estimation of swimming performance can, therefore, provide an index of stress (Cech 1990).

Three classes of locomotor activity in studies of the swimming performance of fishes are used (Beamish 1978). Sustained swimming is low speed movement that can be maintained for long periods of time without muscular fatigue. Prolonged swimming is a higher level of activity that can be maintained for minutes to hours depending on velocity, but eventually results in muscular fatigue. Burst swimming is extremely intense activity that can only be maintained for seconds (Hartwell and Otto 1991). In this study, critical swimming speed (Brett 1964) was the locomotor activity of interest and was used to quantify the capacity of individual fish for prolonged swimming activity.

Juvenile Florida pompano were used to evaluate the sublethal effects of ethylene glycol and methanol because they occur in the vicinity of offshore petroleum production platforms and pipelines in the northern Gulf of Mexico and they are a good representative of a number of reef associated species (i.e., family Carangidae). Florida pompano range

from North Carolina to Brazil, with young occasionally found as far north as Cape Cod (Finucane 1969). Pompano are highly prized commercial and sport fish and demand one of the highest prices per pound (Gilbert and Parsons 1986). Pompano apparently have a protracted spawning season in the northern Gulf of Mexico during the spring and summer (Iverson and Berry 1969; Finucane 1969; Nelson and Murphy 2001) and may spawn year round in the tropical regions of the Gulf of Mexico (Berry and Iverson 1967). Spawning site conditions are poorly established for pompano and may vary depending on location within its range. Evidence exists in favor of offshore spawning from the appearance of small larvae in plankton tows up to 24 km offshore in Florida waters and the capture of ten ripe females near the the Desoto Canyon 54-60 m below the surface (Finucane 1969; Gilbert and Parsons 1986 Nelson and Murphy 2001).

The goals of this study was to extend previous research by testing the separate and combined effects of 3.0 % (volume/volume) ethylene glycol and 1.07 % (v/v) methanol on the swimming performance of individual Florida pompano. Exposure concentrations were based on LC_{50}, behavior, and recovery experiments by Hymel et al. (2002) and Baltz (in prep) wherein fish exposed to 3.0 % ethylene glycol and 1.07 % methanol concentration displayed lethargic behavior but improved after the recovery period and few mortalities were observed. Previous research on the separate effects of ethylene glycol (Hymel et al. 2002) and methanol (Baltz et al. in prep) found significant reductions in critical swimming performance after exposure. In this study pompano were exposed to combination exposures of both chemicals in a randomized experimental design to better understand any additive, synergistic, or antagonistic sublethal effects. The synergistic effects of ethylene glycol and methanol were more profound than the effects of single

exposures. Average performance was significantly reduced by 14.2 % after exposure to ethylene glycol, 2.7 % after exposure to methanol and 43.3 % after exposure to both chemicals.

Materials and Methods

The purpose of this experiment was to evaluate potential additive, antagonistic or synergistic effects that sublethal concentrations of ethylene glycol and methanol might have on the swimming performance of Florida pompano. The swimming performance of juvenile Florida pompano was used to test the separate and combined effects of 3.0 % ethylene glycol (EG) and 1.07 % methanol (MeOH) by comparing differences in pre- and post-exposure critical swimming speeds for each individual. The swimming performance experiment was repeated once at two different times to increase sample size. Logistical and time constraints prevented us from conducting a single swimming performance trial with a larger sample size without altering an existing protocol used in previous research on ethylene glycol (Hymel et al. 2000) and methanol (Baltz et al. in prep).

Respirometer Design

Three Blazka-type swimming respirometers were used to simultaneously test swimming performance of three individual fish (Blazka et al. 1960). The respirometer design and techniques developed by Hymel et al. (2002) to quantify swimming performance (U_{crit}) are similar to those used in previous swimming experiments (Brett 1964; Parsons 1994; Gregory and Wood 1999; Hymel et al. 2002). The inner respirometer chamber had a diameter of 20.3 cm and the total volume of each respirometer was 104 L. Plastic square grids at both ends of the inner chamber contained the fish during swimming trials and reduced turbulent flow characteristics. A propeller

driven by a variable-speed D.C. motor provided water velocities up to a maximum of 100 cm s^{-1}. Propeller speed was adjusted using a hand-held tachometer to set motor revolutions per minute (RPM) to obtain desired velocities for individual chambers based on system calibrations by Hymel et al. (2002):

Swimming chamber 1: Velocity = 0.062(RPM) – 4.0099; (r^2 = 0.9452, P < 0.001),

Swimming chamber 2: Velocity = 0.0627(RPM) – 1.1029; (r^2 = 0.9715, P < 0.001),

Swimming chamber 3: Velocity = 0.0571(RPM) + 0.0582; (r^2 = 0.9886, P < 0.001).

Collection and Exposure

Juvenile Florida pompano were collected at Port Fourchon Beach, Louisiana, using a 3.05 m (10 ft) beach seine and then transferred to holding facilities at Louisiana Universities Marine Consortium. Pompano were initially quarantined for one month to recover from the stress of capture and treated with 0.2 ppm copper solution (Cutrine-Plus) to eliminate ectoparasites, principally Amylodinium species. They were held for a total of eight weeks in a 5,000 L recirculating seawater system at a salinity of 30 ± 1 practical salinity units (psu). Thoughout the holding and experimental phases of this study fish were fed 0.25 g per individual of 45.0 % protein pellet fish food and water quality was monitored once daily for pH, salinity, and ammonia concentrations.

For each experiment twenty-four fish were marked with a latex elastomer (Northwest Marine Technologies, Inc.) to distinguish individuals and later randomized among treatments into eight holding tanks with three individuals per tank. Individuals were anesthetized with 0.2 g L^{-1} of MS-222 and elastomer injected in the dorsal, caudal, and anal fins. Following the marking process, fish were allowed to recover for two weeks. Twenty-four hours prior to the start of each swimming experiment, marked fish

were randomized into eight groups of three individuals and each group was randomly assigned to one of eight 60 L holding tanks. Two groups were randomly assigned to each treatment (ethylene glycol, methanol, ethylene glycol + methanol, and control). The recirculating holding tank system had a total volume of 550 L and was maintained at a salinity of 30 ± 1 psu. A biofilter and a UV light sterilizer were used to maintain water quality in the system. Each holding tank was aerated and kept at a constant temperature of 25 ± 1 °C using 200 W aquarium heaters. Groups were exposed or sham exposed (fake exposed) in one of three round fiberglass tanks filled with 60 L of 30 ± 1 psu sea water at a temperature of 25 ± 1 °C and aerated with two air stones.

The experimental protocol included identical fasting, exposure, acclimation, and swimming experience for each group and required 18 days. Fish were acclimated for 17 hr in the swimming respirometers following a 24-hr exposure and fasted during the entire 41-hr period of exposure (24 hr) and acclimation (17 hr). Swimming performance for Florida pompano before and after exposure to 3.0 % ethylene glycol concentration, 1.07 % methanol, and the combination of the two chemicals was measured for individual fish (Hymel et al. 2002). Sham exposures were conducted in exactly the same manner as real exposure trials except for the addition of the treatment chemical. During the protocol all groups were sham exposed once, except the two control groups were sham exposed twice. Treatment groups were exposed for 24 hrs before the post-exposure swimming test. Homogenous mixing of treatment chemical was tested by taking water samples were taken from exposure tanks at the beginning and end of each 24-hr exposure period for gas chromotography (GC) analysis. GC analyses and paired t-tests (SAS Institute Version 8) of exposure concentrations showed that there were no significant differences

between 3.0 % ethylene glycol initial and final exposure tank concentrations for EG and combination treatment or between 1.07 % methanol initial and final exposure tank concentrations for the MeOH or combination treatments. After exposure, groups were placed in a bucket of clean sea water and immediately transferred to swimming respirometers that were set up in a constant temperature room at 25 ± 1 °C. During the acclimation, respirometers were connected to a recirculating system that included a biofilter and a UV sterilizer and flow in each respirometer was set at a velocity of 5 cm s^{-1}. All air bubbles were removed and respirometers isolated before the start of the swimming trials. Individual fish were tested and re-tested in the same respirometers during the pre- and post-exposure phases of the swimming trials.

Swimming Performance

Swimming performance was tested by determining differences in pre- and post-exposure critical swimming speed for individual fish. Velocities in the respirometers were increased by 10 cm s^{-1} every 10 min, with no rest period between 10-min swimming intervals. Swimming trials ended when the fish fatigued and was momentarily pinned against the back grid for two to three seconds. Pre- and post-exposure critical swimming speed (U_{crit}) was calculated for each trial using the equation formulated by Brett (1964):

$$U_{crit} = u_i + (t_i / t_{ii} \times u_{ii});$$

where u_i is the highest velocity maintained for the prescribed period, u_{ii} is the velocity increment, t_i is the time that fish swam at the fatigue velocity, and t_{ii} is the prescribed period of swimming. Fish that did not fatigue after swimming for 10 min at the

maximum velocity of 100 cm s^{-1} were assigned a U_{crit} value of 100 cm s^{-1}. Dissolved oxygen in mg L^{-1} was measured in each respirometer at the beginning and ending of each swimming trial with a YSI Model 85 handheld oxygen, conductivity, salinity and temperature meter to verify that the fish were not stressed by low oxygen concentrations.

Plasma Samples

Blood plasma from each fish was analyzed for concentrations of ethylene glycol and methanol using GC analysis. At the end of the post-exposure swimming trials, fish were anesthetized using 0.2 g L^{-1} of MS-222, and Body Mass (BM) to the nearest 0.01 g and Fork Length (FL) to the nearest mm were measured. Fish were sacrificed and blood was extracted and pooled for each treatment and control group. Blood was drawn from both the caudal and gill vessels into heparinized capillary tubes and samples were placed in an IECMicro-MB Centrifuge for 15 min at 12,700 RPM to separate red blood cells from plasma. The plasma samples were frozen at –80 °C until the GC analysis.

Statistical Analysis

A split plot design was used to determine the differences in pre- and post-exposure U_{crit} for the Florida pompano swimming performance tests. U_{crit} values were standardized from cm s^{-1} to in body lengths per second (BL s^{-1}) to account for variation in individual size. Replicate experiments were conducted in August, 2001 and September, 2001 to increase total sample size to N = 42. Experiment replicates (1 or 2), exposure tank number (1, 2 or 3) and fish were used as subplots. The Mixed procedure of SAS (SAS InstituteVersion 8) was used to test the model $Y_{ijk} = \mu + \rho_i + \alpha_j + (\alpha\rho)_{ij} + T(\alpha\rho) + \tau_k + (\alpha\tau)_{jk} + (\rho\tau)_{ik} + F(T\rho\alpha)_{ijk} + \varepsilon_{ijk}$ for Florida pompano swimming performance tests where ρ_i is the random effect of replication (experiment 1 or 2), α_j is the fixed treatment

effect, $(\alpha\rho)_{ij}$ is the random interaction of replication by treatment, $T(\alpha\rho)$ is the random interaction of tank (1, 2 or 3) nested within experiment by treatment, τ_k is the fixed trial effect (pre- or post-exposure), $(\alpha\tau)_{jk}$ is the fixed interaction between treatment and trial, and $F(T\rho\alpha)_{ijk}$ is the random interaction of fish nested within tank by experiment by treatment. The Shapiro-Wilks test ($W = 0.8864$; $P < 0.001$) indicated evidence the residuals were not normally distributed for the Florida pompano swimming performance tests, however, residuals plots exhibited a normal symmetrical distribution with a few outliers. The F-test is fairly robust in considering non-normal distributions and no other transformations were deemed necessary (Glass et al. 1972). In post-exposure testing, differences between least-square means (LSMeans) were calculated for all possible treatment comparisons.

Results

Swimming Performance

In the experiments designed to test for potential additive, antagonistic or synergistic effects of simultaneous sublethal exposures, the combination groups exposed to both methanol and ethylene glycol performed significantly more poorly than single exposure and control groups (Figure 2.1). Five fish in the combination treatment groups and one fish in a control group did not survive the swimming performance trials leaving a total sample size of ($N = 42$).

No detectable difference was observed for the main effect of treatment ($df = 3$, $F = 5.42$, $P < 0.0994$); however, the trial ($df = 1$, $F = 31.95$, $P< 0.0001$) and the treatment by trial interaction ($df = 3$, $F= 10.91$, $P < 0.0001$) were significantly different. The significant interaction ($P < 0.0001$) indicated the pre- and post-exposures by EG, MeOH,

EG + MeOH, and Controls differed widely in effects on swimming performance. The LSMeans from the Mixed procedure showed evidence of a significant difference between pre- and post-exposure swimming performance of pompano exposed to 3.0 % ethylene glycol ($P < 0.0058$) and combination treatment ($P < 0.0001$). There was no evidence that pre- and post-exposure U_{crit} differed for pompano exposed to 1.07 % methanol ($P < 0.5793$) or controls ($P < 0.9440$).

Individuals exposed to 1.07 % methanol exhibited a 2.7 % mean reduction in U_{crit} (Figure 2.2) but the reduction was not detectably different ($P < 0.5793$). Mean (± SE) pre-exposure U_{crit} of fish exposed to methanol was 93.14 ± 7.50 cm s^{-1} (9.50 ± 0.44 BL s^{-1}) and the post- exposure U_{crit} was 90.70 ± 7.50 cm s^{-1} (9.25 ± 0.44 BL s^{-1}). Seven of the 12 pompano exhibited a mean decrease in U_{crit} of 5.17 %, three fish showed a minor increase of 1.29 % in U_{crit}, while the remaining two fish performed at equal levels before and after exposure (Figure 2.3, 2.4).

Individuals exposed to ethylene glycol exhibited a mean 14.2 % decrease in U_{crit} (Figure 2.2). Mean (± SE) pre-exposure U_{crit} of exposed fish (Table 2.2) was 91.98 ± 7.50 cm s^{-1} (9.43 ± 0.41 BL s^{-1}). Mean post-exposure U_{crit} of fish treated with 3.0 % ethylene glycol was 79.72 ± 7.50 cm s^{-1} (8.09 ± 0.41 BL s^{-1}). Ten of the 12 fish exhibited a mean 16.74 % reduction in U_{crit}, while the remaining two fish showed a mean increase in U_{crit} of 6.39 % after exposure (Figure 2.3, 2.4).

Individuals exposed to both ethylene glycol and methanol in combination registered the largest drop in performance of all treatment groups. Florida pompano exposed to 3.0 % ethylene glycol and 1.07 % methanol in combination showed a mean

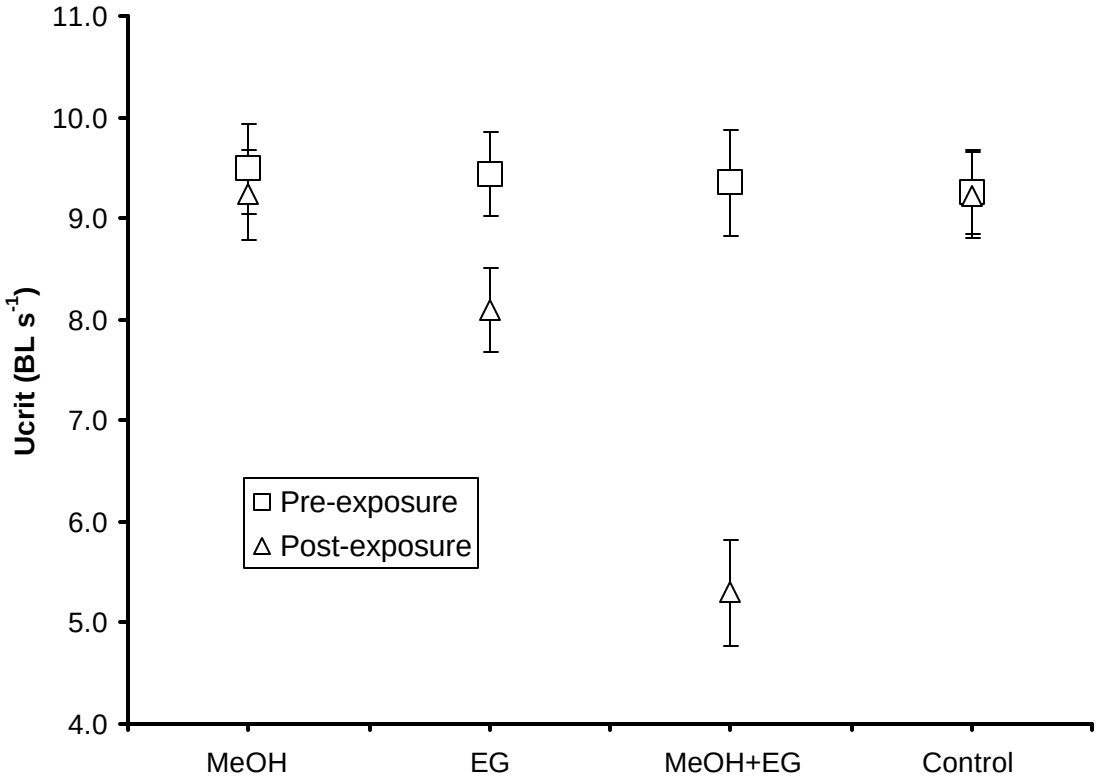

Figure 2.1: Mean (± SE) critical swimming speeds (U_{crit}) of juvenile Florida pompano before and after a 24-hr exposure to 1.07 % methanol (N = 12), 3.0 % ethylene glycol (N = 12), combination exposure (N = 7) and sham controls (N = 11).

decrease in U_{crit} of 43.3 % (Figure 2.2). The mean (± SE) pre-exposure U_{crit} of combination exposed fish (Appendix A) was 90.82 ± 8.06 cm s^{-1} (9.35 ± 0.52 BL s^{-1}). Mean post-exposure U_{crit} for exposed pompano was 53.66 ± 8.06 cm s^{-1} (5.30 ± 0.52 BL s^{-1}). A noticeable amount of individual variation in U_{crit} was observed for groups exposed to the combination of chemicals. Four of the seven fish exhibited a mean decrease of 64.10 % in U_{crit}, while the remaining three fish showed a relatively smaller decrease of 12.72 % after exposure (Figure 2.3, 2.4).

Individuals in control groups performed at relatively equal levels after both sham-exposures. Control groups registered a mean percent decrease of 0.3 % in U_{crit} after sham exposure (Figure 2.2). Mean (± SE) pre-exposure U_{crit} of control fish (Table A.1) was 92.23 ± 7.56 cm s^{-1} (9.26 ± 0.42 BL s^{-1}). Mean post-exposure U_{crit} of control fish was 92.10 ± 7.56 cm s^{-1} (9.23 ± 0.42 BL s^{-1}). Seven of the 11 control fish exhibited a mean percent increase in U_{crit} of 5.06 %, three fish showed a mean percent decrease of 12.38 %, and one fish swam at the same performance level after both sham exposures (Figures 2.3 and 2.4).

Plasma Samples

GC analysis of plasma samples pooled from each group was used to detect traces of ethylene glycol or methanol in each treatment and control group. After 17 hr of acclimation, up to 100 min of experimental swimming trials, and 0.5 hr of post-experimental processing, traces of ethylene glycol were found in fish exposed to the combination (N = 3 groups) and single ethylene glycol treatments (N = 4 groups); however, no trace of methanol was detected in plasma samples from fish exposed to the combination (N = 3 groups) or single methanol (N = 4 groups) treatments. Pooled

plasma collected from juvenile Florida pompano groups (N = 7 groups) exposed to 3.0 % ethylene glycol or combination treatment contained a mean percent (± SE) ethylene glycol concentration of 0.623 ± 0.04 %.

Discussion

The simultaneous exposure to sublethal concentrations of ethylene glycol and methanol significantly reduced the swimming performance of juvenile Florida pompano. The 43.3 % reduction in performance caused by exposure to the combination of ethylene glycol and methanol was more profound then the reductions exhibited by fish exposed to ethylene glycol (a 14.2 % reduction) or methanol (a 2.7 % reduction). According to 24-hr LC_{50} values found by Hymel et al. (2002) and Baltz et al. (in prep), Florida pompano were generally more susceptible to the toxic effects of methanol and ethylene glycol compared to other fish species. A noticeable amount of individual variability in swimming performance was observed within and among all treatment groups. The overall mean reduction in U_{crit} of Florida pompano exposed to 3.0 % ethylene glycol (a 14.2 % reduction) was similar to the 14.0 % reduction observed by Hymel at el. (2002), but for methanol the 2.7 % reduction U_{crit} observed in this experiment was somewhat less than the 5.0 % reduction observed by Baltz et al. (in prep).

Combination Exposure

Pompano exposed to the combination of ethylene glycol and methanol exhibited the most profound reduction in swimming performance. Fish that were exposed to the combination of ethylene glycol and methanol performed two and a half orders of magnitude worse then fish exposed to the single concentration of ethylene glycol.

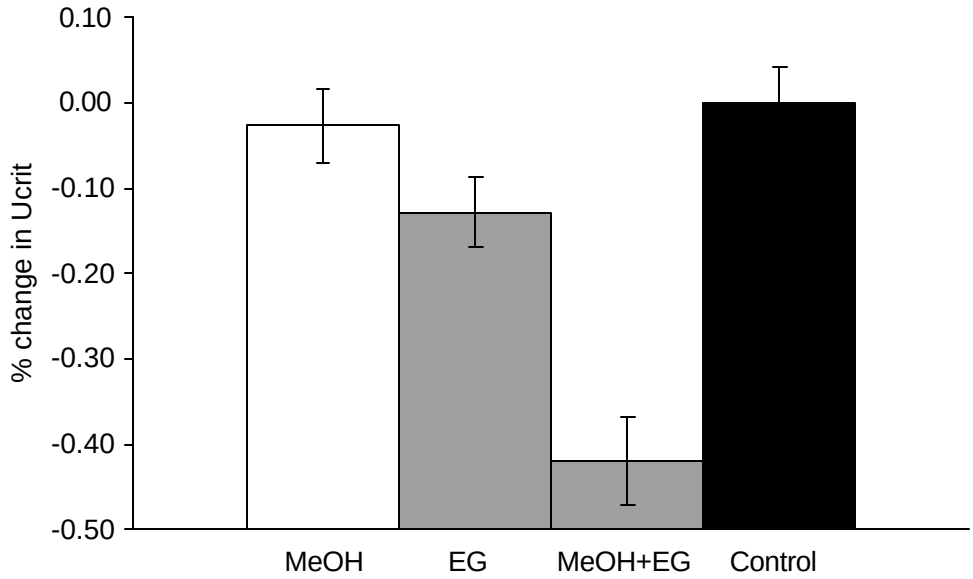

Figure 2.2: Mean percent change (± SE) in critical swimming speed (U_{crit}) of juvenile Florida pompano before and after a 24-hr exposure to 3.0 % ethylene glycol (N = 12), 1.07 % methanol (N = 12), combination exposure (N = 7) and sham controls (N = 11).

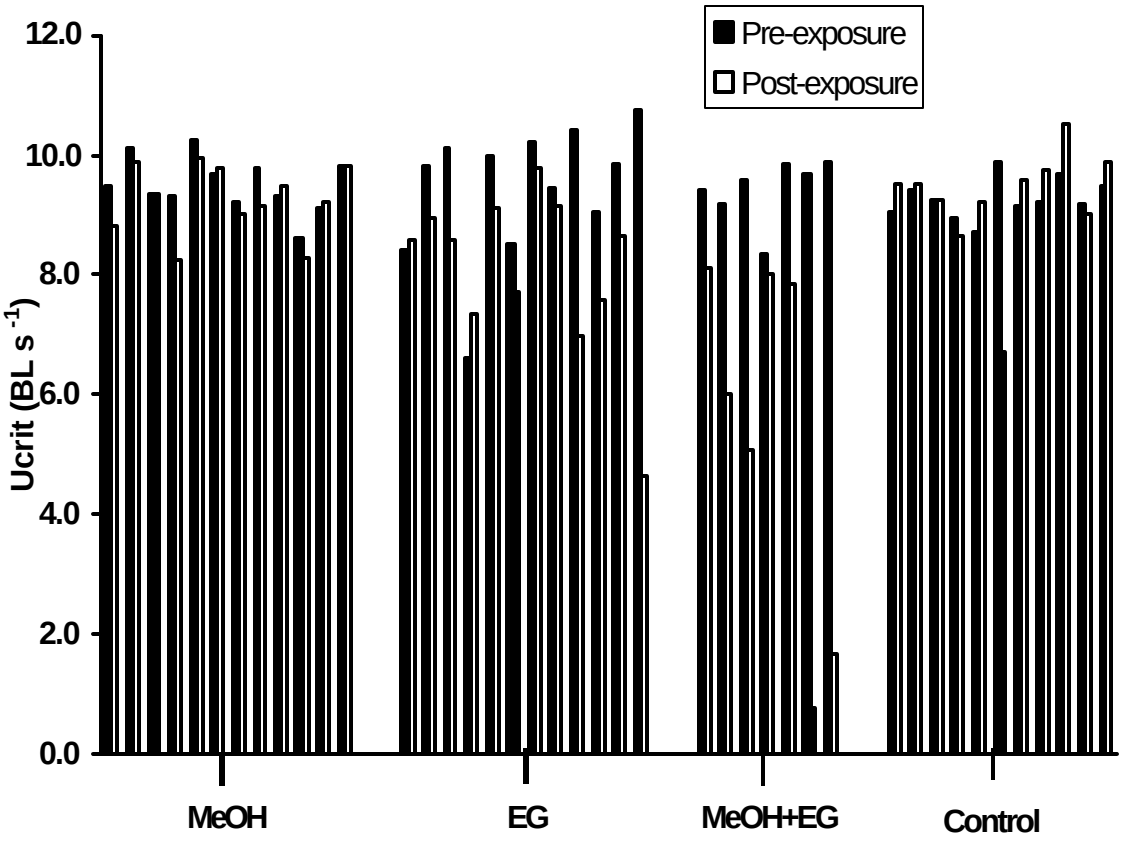

Figure 2.3: Critical swimming speeds (U_{crit}) of Juvenile Florida pompano before and after a 24-hr exposure to 3.0 % ethylene glycol (N = 12), 1.07 % methanol (N = 12), combination exposure (N = 7) and sham controls (N = 11).

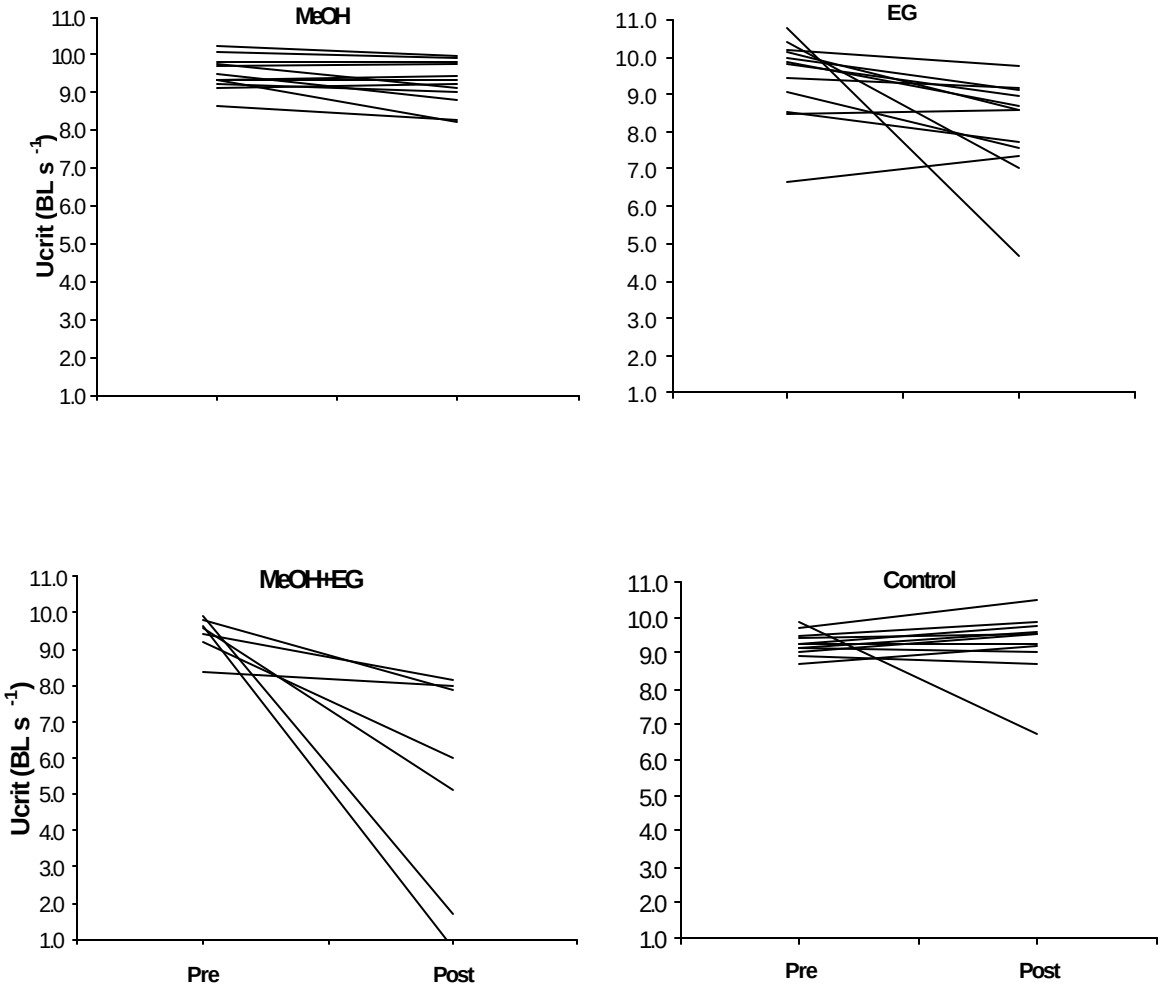

Figure 2.4: Individual variation in the critical swimming speeds (U_{crit}) of juvenile Florida pompano before and after 24-hr exposure to 1.17 % methanol (N=12), 3.0 % ethylene glycol (N = 12), combination exposure (N = 7) and sham controls (N = 11).

Similar joint action or additive interactions occur when toxicants have similar modes of action but act independently resulting in the toxicity of the mixture amounting to the sum of the toxicities of the individual toxicants present (Bliss 1939; Sprague 1970; Marking 1985). Interactive action can be either synergistic (more then additive) or antagonistic (less then additive). These interactions occur when one toxicant alters the toxicity of another toxicant present. The results of this study showed that a synergistic interaction may have occurred after pompano were exposed to the combination of ethylene glycol and methanol. The findings in this study were similar those of Greene and Kocan (1997) who found the toxicity of ethylene glycol increased in freshwater fish when sublethal levels of thiram, a chemical used in an agricultural seed-protectant, was present.

The extensive toxic effects observed in this study may have been the result of interactions between the various components of the formulation, referred to as synergism (Marking 1985). A discharge or spill often involves more then one chemical component and the interaction between two or more chemicals can result in toxicity that is greater than the sum of the toxicities of the individual components (Greene and Kocan 1997). Both ethylene glycol and methanol are competitors for aldehyde dehydrogenase (ALDH) and may inhibit each other from being completely metabolized and increase the toxic effects after exposure (Pietryka et al. 1998). The metabolic pathways of ethylene glycol and methanol are in fact so similar that treatment for ethylene glycol and methanol poisoning is the administration of ethanol, another ALDH competitor (Katarzyna et al. 1998).

Individual Variation

Considerable within group variation was observed for individuals in the swimming performance tests. Florida pompano mean U_{crit} decreased after exposure to ethylene glycol, methanol and combination treatments by 14.2, 2.7, and 43.3 %, respectively. However, there was a considerable amount of individual variation in U_{crit} within treatment and control groups following exposure and sham-exposure (Figure 2.3, 2.4). In previous swimming performance studies, it was common practice to compare U_{crit} means of different treatment and controls groups in a one-way ANOVA design, but this approach fails to address within-group variation. Within-group variability made it difficult to find significant differences between treatment and control group means, which can limit the usefulness of swimming performance tests (Kolok 1998). In this, and related studies (Hymel et al. 2002; Baltz et al. in prep) swimming performance of individual fish before and after exposure was used in a split-plot design with blocks to take advantage of the within-group variability to analyze changes in swimming performance (Kolok 1992).

Previous studies revealed conflicting results concerning the performance variability among individuals after treatment. Kolok (1998) found large within group variation in U_{crit} during swimming performance tests, but this variation was repeatable over replicate experiments. In contrast Kolok and Farrel (1994) found that the reduction in U_{crit} of northern pikeminnow (*Ptychocheilus oregonensis*) after a surgical technique was fairly consistent. The results of this study resembled those of Kolok (1998) in that the individual performance in U_{crit} after exposure to ethylene glycol, methanol, and combination treatment varied between slight increases and traumatic reductions.

The variability in this study implies that individual fish may have lower or higher tolerances to ethylene glycol, methanol or combination treatments. This study used only one concentration for each chemical; therefore, it was not possible to calculate tolerance values for individuals.

Methanol

Reports in the literature are conflicting on the mode of action directly responsible for the toxic effects of methanol. In vivo methanol is quickly metabolized into formaldehyde, which has been suggested as the metabolite that produces the toxic effect (Potts and Johnson 1952, Koivusalo 1970). A more recent study indicates that the second metabolic product, formate, is responsible for the toxicity associated with methanol exposure (Tephly 1991). Formate was found in elevated levels in monkeys and humans who ingested substantial amounts of methanol (McMartin et al.1977, 1980; Sejersted et al.1983). Formate causes the depletion of plasma bicarbonate and the development of metabolic acidosis (Tephly 1991). The buildup of formate and subsequent changes in metabolic equilibrium may reduce a fish's ability to swim for prolonged and burst swimming.

The results of this study showed that exposure to 1.07 % methanol and 17 hr recovery did little to impair the swimming performance of juvenile Florida pompano. Sublethal exposure to methanol had a short-term effect on behavior but little effect on swimming performance after 17 hr of recovery. The lack of detectable level of methanol in the plasma samples suggests that pompano completely metabolize methanol (probably into formaldehyde and formate products) after the ~ 20-hr swimming and recovery

process. In contrast, the mean percentage (± SE) of ethylene glycol still present in pompano plasma samples ~ 20 hours after exposure was 0.623 ± 0.04 % (v/v).

Methanol may be less toxic to certain fish than to some mammals. Although methanol is toxic to humans, other species such as rats (*Rattus rattus*) are less susceptible to methanol poisoning (Telphy 1991). Certain species are able to oxidate formate into carbon dioxide at a faster rate, and thus have a higher tolerance to methanol exposure (Tephly 1991).

Although methanol did not effect performance significantly, previous studies suggest evidence that pompano are more sensitive to the toxic effects of methanol compared to other fish species. Baltz et al. (in prep) found the static 24-hr LC_{50} value for pompano exposed to methanol to be 1.28 %, which is lower than the static 24-hr LC_{50} that Poirier et al. (1986) found for fathead minnows (3.75 %), bluegill (2.41 %) and rainbow trout (2.57 %). Direct comparisons of the LC_{50} values observed by Poirier et al. (1986) and those found by Hymel et al. (2002) are problematic because of the use of fresh and saltwater species. Nevertheless, studies conducted in salt water by Portmann and Wilson (1971) found the LC_{50} for hooknose (*Agonus cataphractus*) ranged from 1.26 to 4.17 % (48- and 96-hr renewal). It is assumed that LC_{50} decreases linearly with time of exposure, therefore, LC_{50} values in studies using different test durations can be grossly compared by using a toxicity index (concentration x time) (French et al. 1996a; Boehm et al. 2001). The 1-hr LC_{50} index of toxicity (Index) value for Florida pompano is 31, compared to 340 for bleak and 400 for hooknose, which indicates that pompano are 11 to 12 times more sensitive to methanol than the other species.

Ethylene Glycol

Toxicity of ethylene glycol varies from species to species and has been shown to be five times more toxic to humans than to poultry (Beasley 1980). Ethylene glycol causes depression of the nervous system similar to ethanol intoxication followed by drowsiness, coma, respiratory failure, convulsions, and renal damage in humans and other primates (Merck 1983). Hymel et al. (2002) found that exposure to as little as 1.0-2.1 % of ethylene glycol caused changes in the behavior of Florida pompano.

The 14.2 % reduction in swimming performance of fish exposed to ethylene glycol may be a result of a buildup of metabolic products similar to that of methanol. Ethylene glycol is oxidized in the liver (Chou and Richardson 1978) and involves several metabolic products, with glycolic acid believed to be responsible for the toxic effects of the chemical. The buildup of metabolic products and increased metabolic cost of removing ethylene glycol from the body may have caused or contributed to the decrease in swimming performance of exposed fish.

Previous studies have shown that pompano are more sensitive to ethylene glycol than freshwater species. Hymel et al. (2002) found a 24-hr LC_{50} value of 5.63 % for pompano statically exposed to ethylene glycol. The 24-hr LC_{50} value for pompano is similar to static 96-hr LC_{50} values found by Green and Kocan (1997) for rainbow trout (6.08 % and 5.65 %) and fathead minnow (6.83 % and 6.95 %). These values cannot be directly compared due to differences in test duration; comparing the 1-hr LC_{50} index values for rainbow trout (Index = 584 and 542) and fathead minnow (Index = 656 and 667) to that of Florida pompano (Index = 135) indicates that pompano are four to five

times more sensitive to ethylene glycol than these species. There are no data for comparisons to other saltwater species.

Modes of Action

In addition to the buildup of metabolic products, the increased metabolic cost of removing ethylene glycol and methanol from the body and gill damage may also reduce swimming performance. Several theories have been proposed on how exposure to a toxicant, such as ethylene glycol or methanol, results in a reduction in swimming performance. A decrease in critical swimming speed has been attributed to impaired transport or exchange of respiratory gases (Sprague 1971; Satchell 1984). Jones (1971) identified several factors that may hinder the swimming performance of fishes: inability to supply adequate oxygen to the gills; inability to supply oxygen to the tissues; inability to remove metabolic products; and inability to provide adequate substrate or to activate enzymatic processes. Wilson and Taylor (1993) found acute levels of copper exposure in freshwater rainbow trout caused a disruption of ion regulation and a progressive hypoxia and plasma acidosis resulting from physical damage to the gills. It was unclear whether substantial gill damage was present in this study as examination of pompano gill lamelle revealed signs of damage and fusion; however, this may have been partially caused by the use of MS-222 before sacrifice.

Spills

This study concentrated on low-level acute exposures to ethylene glycol and methanol individually and in combination for 24 hrs. In the marine environment juvenile fish could be exposed to sublethal concentrations of methanol by a large localized discharge (Boehm et al. 2001). Large offshore storage volumes of methanol in the

northern Gulf of Mexico (up to 380,800 L) pose significant risk of a spill. One such incident was reported on November 19, 1997 where 11,360 L of methanol leaked from an injection line into the Gulf of Mexico (National Response Center 2002 a). Fish would be exposed for a short period of time before the rapid dilution and biodregradation of methanol would occur. Methanol exhibits a relatively fast biodegradation rate and has a half-life of only 6.12 days. Boehm et al. (2001) modeled a possible spill of 380,800 L (100,600 gal) of methanol and found that the predicted concentrations would be two orders of magnitude smaller then the lowest 96-hr LC_{50} (12,539 mg/l or 1.58 %) for the harpacticoid copepod (*Nitroca spinipes)*. However, Florida pompano appear to be more sensitive to the toxic effects of methanol than the species mentioned by Bohem et al. (2001). The 1-hr LC_{50} index values for Florida pompano (Index = 31) would be five times lower than that found for a harpacticoid copepod (Index = 152) (Boehm et al. 2001). Sublethal concentrations of methanol found by Baltz et al. (in prep) for Florida pompano would be six (Index = 26) times lower then the 1-hr LC_{50} index values for the harpacticoid copepod (Index = 152) suggesting that a spill would effect Florida pompano populations and other marine fishes to a greater extent than predicted for other species.

A large localized spill of ethylene glycol poses a similar risk with offshore storage volumes up to 416,400 L per platform (Boehm et al. 2001). Significant spills have been reported in recent years, 13,510 L of ethylene glycol leaked from an underwater pipeline into the Gulf of Mexico on June 29, 1998 (National Response Center 2002 b). Fish would most likely be exposed for only a short period of time due to the rapid dilution and biodegradation of ethylene glycol in the natural environment. The half-life of ethylene glycol in the water column and marine sediments is longer then that of methanol and is

estimated at 32.50 days. Boehm et al. (2001) modeled a possible spill of ethylene glycol (416,400 L) and found the predicted exposure concentrations would be an order of magnitude smaller then the lowest 48-hr LC_{50} (34,400 mg/l or 3.13 %) for the water flea (*Ceriodaphnia dubia)*. However, it appears that Florida pompano are more sensitive to the toxic effects of ethylene glycol. The 1-hr LC_{50} index values for Florida pompano (Index = 135) would be noticeably lower then those found for the water flea (Index = 150) by Boehm et al. (2001). Sublethal concentrations found by Hymel et al. (2002) were only half as large (Index = 72) as the 1-hr LC50 index found for the water flea (Index = 135), suggesting that a spill could effect pompano populations and, perhaps, other marine fishes to a greater extent than previously expected.

Ecological Effects

An episodic spill of ethylene glycol and methanol could have severe ecological implications at individual, community, or population levels. The results of this study showed that sublethal exposure to small concentrations of ethylene glycol and methanol in combination could greatly reduce the swimming performance of individuals. Nevertheless it is difficult to interpret how the effects of exposure at the individual level translate to the population or community. Although post-exposure critical swimming speeds were well above those used in normal sustained swimming, the ability for prolonged or burst speeds would be reduced in the event of exposure, which may result in the reduced ability to capture prey or avoid predators. However, fish populations have been shown to exhibit compensatory responses to stress or mortality; therefore, a spill may not necessarily affect a species at the population level if the exposure is not sustained or widespread (Houde 1989). But a spill during a peak spawning period could

seriously reduce recruitment and add an additional stress to populations already burdened by overfishing, reduced habitat quality and other contaminants. An in-depth model including parameters on food sources, avoidance behavior, dispersion of ethylene glycol and methanol and life histories of marine fishes may be the best way to understand the implications of sublethal exposures at the population level.

Conclusion

The results of this study showed conclusively that both a single exposure to ethylene glycol and combination exposure of ethylene glycol and methanol significantly reduced the swimming performance of juvenile Florida pompano. Although Boehm et al. (2001) found that realistic chemical spills of ethylene glycol or methanol to be well below the lowest LC_{50} values for other species found in the literature, pompano appear to be more sensitive to the two chemicals. The reduced ability of pompano to sustain high prolonged and burst performance levels could have affects an individual's ability to avoid predators and feed effectively. Effects at the level of the organism may, in turn, lead to effects at the population and community levels (Weis et al. 1999). The additive or synergistic affects of exposure to ethylene glycol or methanol and other sublethal contaminants in combination could ultimately effect population and community structure in the vicinity of a spill around a contaminated platform.

Chapter III:

Swimming Performance of Juvenile Atlantic Spadefish Exposed to Sublethal Concentrations of Ethylene Glycol

Introduction

There are an estimated 4,000 oil and gas platforms in the northern Gulf of Mexico that, as artificial reefs, constitute most of the known hard substrate off the Louisiana and Texas coasts (Stanley and Wilson 2000). Oil and gas platforms extend throughout the water column and are thought to affect benthic, demersal and pelagic fishes (Gallaway et al. 1981; Continental Shelf Associates 1982). They attract available recreational fishes and may enhance production of other species (Bohnsack 1989; Grossman et al. 1997). They also create fishing opportunities, reduce user conflicts, save time and fuel, make locating fish more predictable, and increase public access (Stone 1985; National Academy Press 1988). The success of platforms as artificial reefs is indicated by the concentration of fishes and recreational fishing around these structures off the coast of Louisiana (Stanley and Wilson 1990). Unfortunately, oil and gas platforms are also a source of anthropogenic inputs including produced formation water, drilling fluid chemicals, oil-based and water-based drilling muds and cuttings and production additives, some of which are toxic to the fauna that utilizes these structures (Holdway 2002).

As petrochemical exploration moves into increasingly deeper water (>300 m), facilitated by new technology, there are risks to the marine fauna including the release of contaminants (Boehm et al. 2000) and the noise pollution associated with the construction and operation of offshore oil platforms (Richardson 1995). New chemical additives and combinations of additives are used to enhance deep-water production. For

example ethylene glycol is used during the production and treatment to prevent the formation of gas hydrates in deep-water wells and pipelines (Anonymous 1996, 2000; Herzhaft and Dalmazzone 2000; Boehm et al. 2001). It is often transported long distances through underwater pipelines and by ships to deep-water production platforms. Although the possibility of a large spill may be low, the risk remains plausible with increased usage, transport and storage of volumes of up to a 300,000 L of ethylene glycol per platform (Boehm et al. 2001).

Ethylene glycol is a common chemical used by both industry and consumers. It is a common ingredient in antifreeze, is used as a deicing agent on bridges and airport runways, and can prevent the formation of hydrates in deep-water pipelines (Abdelghani et al. 1990; Herzhaft and Dalmazzone 2000). Hydrates are solids that form when water molecules crystallize around "guest" molecules in natural gas and other petroleum products (Herzhaft and Dalmazzone 2000). Hydrate formation is particularly common in underwater pipelines due to the cooling effect of sea water on pipeline contents at extreme depths (Anonymous 1996). Hydrates form during drilling and extraction process, but also during transport in deep-water pipelines and can result in permanent blockage (Herzhaft and Dalmazzone 2000). With intensified activity in deeper water larger volumes of ethylene glycol are being transported, stored and used by the offshore industry. Ethylene glycol is toxic to both mammals and fishes to varying degrees (Bridie et al. 1979; Adbelghani et al. 1989; Pillard 1995; Green and Kocan 1997 and Hymel et al. 2002).

Although research has been directed towards understanding the effects of offshore oil development on the northern Gulf of Mexico (Gallaway et al. 1981; Grizzle 1986;

Stanley and Wilson 1990; Grossman et al. 1987; Boehm et al. 2001, Hymel et al. 2002), little attention has been paid to understanding the chronic or acute sublethal effects associated with low-level, localized discharges of toxicants. Such sublethal effects may reduce an individual fish's ability to avoid predators, feed, reproduce, and resist diseases and parasites, and may, in turn, lead to effects at population and community levels (Weis et al. 1999). Therefore, it is important to quantify effects at the individual level to enhance our understanding of how populations and communities may respond.

Induced changes in swimming performance were first suggested as an important tool for assessing the sublethal effects of toxicants on fishes by Cairns (1966) and have since been used to determine tolerances of fishes to varying environmental factors, stresses and pollutants (Nikl and Farrell 1993; Beaumont et al. 1995, Heath 1995; Kolok et al. 1998; Hymel et al. 2002). Swimming performance is an indicator of the ability of an individual to capture prey, escape predation, migrate and maintain position in a current and involves the integrated effects of numerous physiological processes (Beamish 1978). Selyle (1950) first defined stress as the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force. Stress response and resistance to a stressor are energy draining processes (Schreck 1982; Barton and Schreck 1987) and by responding to stress an organism should have less energy available to devote to other life functions (Schreck 1990). Estimation of swimming ability can, therefore, provide an index of stress caused by exposures to chemical additives that can reduce an individual's scope for activity (Cech 1990).

In this study, critical swimming speed (Brett 1964) was used to quantify the capacity of individual fish for prolonged swimming activity. Prolonged swimming is a higher level of activity that can be maintained for minutes to hours depending on velocity, but eventually results in muscular fatigue (Beamish 1978). Burst swimming is extremely intense activity that can only be maintained for seconds (Hartwell and Otto 1991). Burst swimming would probably be a better ecological index, but is too difficult to assess in laboratory experiments.

Atlantic spadefish (*Chaetodipterus faber*) were selected to evaluate the sublethal effects of ethylene glycol because they often congregate in the vicinity of deep-water pipelines and gas and oil wells. The Atlantic spadefish is the only member of the family Ephippidae in the Atlantic Ocean (Hayse 1989). Spadefish are one of the most common fish found near oil platforms and are sometimes caught by sport fishermen, but little is known about their life-history. Spadefish feed on a variety of hydroids, epifaunal amphipods, and anthozoans. A study on the stomach contents by Hayse (1989) found the cannonball jelly (*Stomolophus meleagris*) to be the dominant organism by all numeric indicators. Spadefish are believed to spawn from May through September, principally in the northern Gulf of Mexico (Ditty et. al 1993). Juveniles migrate inshore during summer and young of the year move into deeper offshore waters once temperatures cool. Sexual maturity is reached after 2 years at a size of 135 mm total length (Johnson 1978).

The goal of this study was to extend previous research on the effects of ethylene glycol on Florida pompano (Hymel et al. 2000) by testing the effects of a 3.0 % (volume/volume) exposure on swimming performance of another marine fish species. Atlantic spadefish were exposed to the same concentration of ethylene glycol in an

experimental design developed by Hymel et al. (2002) wherein fish exposed to 3.0 % ethylene glycol concentration displayed lethargic behavior but improved after the recovery period and few mortalities were observed. Ethylene glycol did reduce the swimming performance of juvenile Atlantic spadefish by an average of 7.3 % after exposure and by 18.4 % compared to the improvement exhibited by the control group.

Materials and Methods

The purpose of this experiment was to evaluate potential effects that sublethal concentrations of ethylene glycol have on the swimming performance of a juvenile Atlantic spadefish. Spadefish were used to test the effects of a 24-hr exposure to 3.0 % ethylene glycol on the swimming performance of individual fish. Swimming performance was evaluated by comparing differences in pre- and post-exposure critical swimming speeds for each individual. Spadefish were exposed to the same concentration of ethylene glycol used in previous experiments by Hymel et al. (2002). The swimming performance experiment was repeated once at two different times to increase sample size. Logistical and time constraints prevented us from conducting a single swimming performance trial with a larger sample size without altering the protocol used in previous research on ethylene glycol (Hymel et al. 2002).

Respirometer Design

Three Blazka-type swimming respirometers were used to simultaneously test swimming performance of three individual fish (Blazka et al. 1960). The respirometer design and techniques developed by Hymel et al (2002) to quantify critical swimming speed (U_{crit}) are similar to those used in previous swimming experiments (Brett 1964; Parsons 1994; Gregory and Wood 1999; Hymel et al. 2002). The inner respirometer

chamber had a diameter of 20.3 cm and the total volume of each respirometer was 104 L. Plastic square grids at both ends of the inner chamber contained the fish during swimming trials and reduced turbulent flow characteristics. A propeller driven by a variable-speed D.C. motor provided water velocities up to a maximum of 100 cm s^{-1}. Propeller speed was adjusted using a hand-held tachometer to set motor revolutions per minute (RPM) to obtain desired velocities for individual chambers based on system calibrations by Hymel et al. (2002):

Swimming chamber 1: Velocity = 0.062(RPM) – 4.0099; (r^2 = 0.9452, P < 0.001),

Swimming chamber 2: Velocity = 0.0627(RPM) – 1.1029; (r^2 = 0.9715, P < 0.001),

Swimming chamber 3: Velocity = 0.0571(RPM) + 0.0582; (r^2 = 0.9886, P < 0.001).

Collection and Exposure

Atlantic spadefish were captured by inshore shrimpers in Terrebone Bay, Louisiana. Fish were maintained in live-wells and then transferred to Louisiana Universities Marine Consortium. Spadefish were held in captivity for one month to recover from the stress of capture and treated with 0.2 ppm copper solution (Cutrine-Plus) to eliminate ectoparasites, principally Amylodinium species. They were held for a total of eight weeks in a 5000 L recirculating seawater system at a salinity of 30 ± 1 practical salinity units (psu). Two weeks before the start of the swimming experiment, 1.3 g of Praziqantel dissolved in 20 ml of 95.0 % ethanol was added to an isolated 1000 L tank containing experimental fish to remove gill flukes**.** Thoughout the holding and experimental phases of this study spadefish were fed 1.5 g per individual of frozen shrimp once daily and water quality was monitored once daily for pH, salinity, and ammonia concentrations.

For each experiment eighteen fish were uniquely marked with latex elastomer (Northwest Marine Technologies, Inc.) to distinguish individuals and later randomized among treatments into eight holding tanks with three individuals per tank. Individuals anesthetized with 0.2 g L^{-1} of MS-222 and elastomer injected in the dorsal, caudal, and anal fins. Following the marking process, spadefish were allowed to recover for two weeks. Twenty-four hours prior to the start of swimming experiment trials, marked fish were randomized into six groups of three individuals and each group was randomly assigned to one of eight 60 L holding tanks. Five groups were randomly assigned as treatment groups and the remaining group was designated as the control group. The recirculating holding system had a total volume of 550 L and was maintained at a salinity of 30 ± 1 psu. A biofilter and a UV light sterilizer were used to maintain water quality in the system. Each holding tank was aerated and kept at a constant temperature of 25 ± 1 °C using 200 W aquarium heaters. Groups were exposed or sham exposed in one of three round fiberglass tanks filled with 60 L of 30 ± 1 psu sea water at a temperature of 25 ± 1 °C and aerated with two air stones.

The experimental protocol included identical fasting, exposure, acclimation, and swimming experiences for each group and required 15 days. Fish were acclimated for 17 hr in the swimming respirometers following the 24-hr exposures, and were fasted during the entire 41-hr period of exposure (24 hr) and acclimation (17 hr). Swimming performance for Atlantic spadefish before and after exposure to 3.0 % ethylene glycol concentration was measured for individual fish (Hymel et al. 2002). Sham exposures (fake exposures) were conducted in exactly the same manner as real exposure trials except for the addition of the treatment chemical. During the protocol all groups were

sham exposed once, except the control group was sham exposed twice. Treatment groups were exposed for 24 hrs before the post-exposure swimming test. Homogenous mixing of treatment chemical was tested by taking water samples from exposure tanks at the beginning and end of each 24-hr exposure period for gas chromotography (GC) analysis. The GC analysis and paired t-test (SAS Institute Version 8) of exposure concentrations revealed no significant difference between 3.0 % ethylene glycol initial and final exposure tank concentrations. After exposure, groups were placed in a bucket of clean sea water and transferred to swimming respirometers that were set up in a constant temperature room at 25 ± 1 °C. During the acclimation, respirometers were connected to a recirculating system that included a biofilter and a UV sterilizer and flow in each respirometer was set at a velocity of 5 cm s^{-1}. All air bubbles were removed and respirometers were isolated before the start of the swimming trials. Individual fish were tested and retested in the same respirometers during the pre- and post-exposure phases of the swimming trials.

Swimming Performance

Swimming performance was tested by determining differences in pre- and post-exposure critical swimming speed for individuals. Velocities in the respirometers were increased by 10 cm s^{-1} every 10 min, with no rest period between 10-min swimming intervals. Swimming trials ended when the fish fatigued and was momentarily pinned against the back grid for two to three seconds. Pre- and post-exposure critical swimming speed (U_{crit}) was calculated for each trial using Brett's (1964) equation:

$$U_{crit} = u_i+(t_i/t_{ii} \text{ x } u_{ii});$$

where u_i is the highest velocity maintained for the prescribed interval, u_{ii} is the velocity increment, t_i is the time that fish swam at the fatigue velocity, and t_{ii} is the prescribed interval of swimming. Fish that did not fatigue after swimming for 10 min at the maximum velocity of 100 cm s^{-1} were assigned a U_{crit} value of 100 cm s^{-1}. Dissolved oxygen in mg L^{-1} was measured in each respirometer at the beginning and ending of each swimming trial with a YSI Model 85 handheld oxygen, conductivity, salinity and temperature meter to verify that the fish were not stressed by low oxygen concentrations.

Plasma Concentration

Blood plasma from each fish was analyzed for concentrations of ethylene glycol using GC analysis. At the end of the post-exposure swimming trials, fish were anesthetized using 0.2 g L^{-1} of MS-222, and Body Mass (BM) to the nearest 0.01 g and Fork Length (FL) to the nearest mm were measured. Fish were sacrificed and blood was extracted and pooled for each treatment and control group. Blood was drawn from both the caudal and gill vessels into heparinized capillary tubes. Blood samples were placed in an IECMicro-MB Centrifuge for 15 min at 12,700 RPM to separate red blood cells from plasma. The plasma from the each group was frozen at –80 °C until GC analysis.

Statistical Analysis

A split plot design was used to determine the differences in pre- and post-exposure U_{crit} for the Atlantic spadefish swimming performance tests. U_{crit} values were standardized from cm s^{-1} to body lengths per second (BL s^{-1}) to account for variation in individual size. Replicate experiments were conducted in September, 2000 and January 2002 to increase the sample size to a total of N = 36 individuals. Experiment replicates

(1 or 2), exposure tank number (1, 2 or 3) and fish were used as subplots. The Mixed procedure of SAS (SAS Institute Version 8) was used to test the model $Y_{ijk} = \mu + \rho_i + \alpha_j + (\alpha\rho)ij + T(\alpha\rho) + \tau_k + (\alpha\tau)_{jk} + (\rho\tau)_{ik} + F(T\rho\alpha)_{ijk} + \varepsilon$ for Atlantic spadefish swimming performance tests where ρ_i is the random effect of replication (experiment 1 or 2), α_j is the fixed treatment effect, $(\alpha\rho)_{ij}$ is the random interaction of replication by treatment, $T(\alpha\rho)$ is the random interaction of tank (1, 2 or 3) nested within experiment by treatment, τ_k is the fixed trial effect (pre- or post-exposure), $(\alpha\tau)_{jk}$ is the fixed interaction between treatment and trial, and $F(T\rho\alpha)_{ijk}$ is the random interaction of fish nested within tank by experiment by treatment. The Shapiro-Wilks test ($W = P < 0.0225$; $W = 0.9484$) indicated evidence the residuals were not normally distributed for the Atlantic spadefish swimming performance tests, however residuals plots exhibited a normal symmetrical distribution with a few outliers. The F-test is fairly robust in considering non-normal distributions and no other transformations were deemed necessary (Glass et al. 1972). In post-exposure testing, differences between least-square means (LSMeans) were calculated for all possible treatment comparisons.

Results

Swimming Performance

The treatment groups exposed to ethylene glycol performed significantly more poorly than control groups (Figure 3.1). Five treatment fish did not survive the swimming performance experiment leaving 25 treatment and 6 control fish for the analysis. The overall F-test ($df = 32$, $F = 2.63$, $P < 0.0050$), the main effects of treatment ($df = 1$, $F = 315.62$, $P < 0.0359$) and trial ($df = 1$, $F = 0.0593$, $P < 0.0893$) and the treatment by trial interaction ($df = 1$, $F = 3.15$, $P < 0.0861$) were all significant at the $\alpha =$

0.10 level. The significant interaction ($P < 0.0861$) indicated the pre- and post-exposures by treatment differed widely in effects on swimming performance. The Least Square Means (LSMeans) from the Mixed procedure showed evidence of a significant difference between post-exposure U_{crit} for treatment groups and post-exposure control groups ($P < 0.0085$). No detectable difference in U_{crit} was observed within pre- and post-exposure groups for treatment groups ($P < 0.0921$), control ($P < 0.2699$), or between the pre-exposure treatment and control groups ($P < 0.7462$).

Sublethal exposure to 3.0 % v/v ethylene glycol reduced the swimming performance of juvenile Atlantic spadefish by 7.3 % compared to their initial performance ($P < 0.0921$) and by 18.4 % compared to the final performance ($P < 0.0085$) of the controls. The mean (± SE) pre-exposure critical swimming speed of treatment fish (Appendix B) was 66.90 ± 5.54 cm s^{-1} (6.59 ± 0.36 BL s^{-1}). Mean post-exposure critical swimming speed of treatment fish was 62.44 ± 5.54 cm s^{-1} (6.14 ± 0.36 BL s^{-1}). Values for pre- and post-exposure U_{crit} of treatment fish may be slightly conservative due to one fish (1 out of 31) that did not fatigue after 10 min at 100 cm s^{-1} and was subsequently assigned a U_{crit} value of 100 cm s^{-1} in both pre- and post-trials.

Individuals in control groups performed at a higher level after their second sham exposure (Figure 3.1), showing a mean 8.9 % increase in U_{crit} in the second trial. The mean pre-exposure critical swimming of the control groups (Table B.2) was 82.85 ± 8.24 cm s^{-1} (6.73 ± 0.56 BL s^{-1}) and the mean post-exposure U_{crit} was 77.25 ± 8.24 cm s^{-1} (7.34 ± 0.56 BL s^{-1}).

Individual variation was observed between pre- and post-exposure swimming performance of Atlantic spadefish (Figures 3.2 and 3.3). Following exposure 10 of the

25 treatment fish showed a mean 7.2 % increase in U_{crit} while the remaining 15 treatment fish exhibited a mean 17.4 % decrease in U_{crit}. Five of the six control fish showed a 19.1 % increase in U_{crit}, while one control fish exhibited a 30.0 % decrease in U_{crit} following sham-exposure.

Plasma Samples

GC analysis of plasma samples pooled for each treatment and control group was used to detect traces of ethylene glycol after 17 hr of acclimation, up to 100 min experimental swim time and ~ 0.5 hr of post-experimental processing. Pooled plasma from juvenile Atlantic spadefish treatment groups (N = 10 groups) contained a mean (± SE) ethylene glycol concentration of 0.891 ± 0.09 %.

Discussion

Sublethal exposure to ethylene glycol significantly reduced the swimming performance of juvenile Atlantic spadefish by 7.3 % compared to their initial performance and by 18.4 % compared to the final performance of the controls. In the event of exposure, the inability of spadefish to swim at prolonged and burst swimming performance levels could have an effect on individuals due to reduced ability to avoid predators and feed effectively. The overall mean reduction of 7.3 % in critical swimming speed of Atlantic spadefish exposed to 3.0 % ethylene glycol was roughly half the 14.2 % reduction observed for Florida pompano.

Ethylene Glycol

The severity of ethylene glycol toxicity varies from species to species and has been shown to be five times more toxic to humans than to poultry (Beasley 1980). In

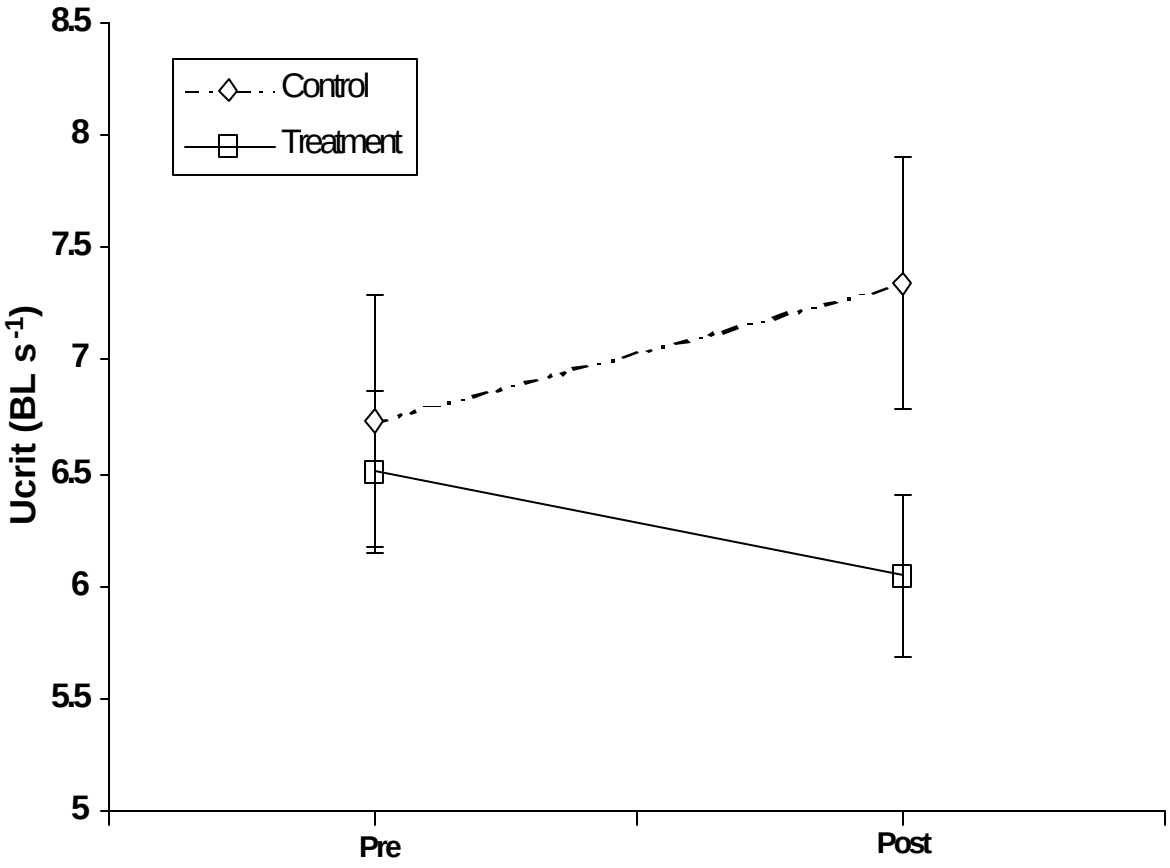

Figure 3.1: Mean (± SE) critical swimming speeds (U_{crit}) of juvenile Atlantic spadefish before and after a 24-hr exposure to 3.0 % ethylene glycol (N = 25) and sham controls (N = 6).

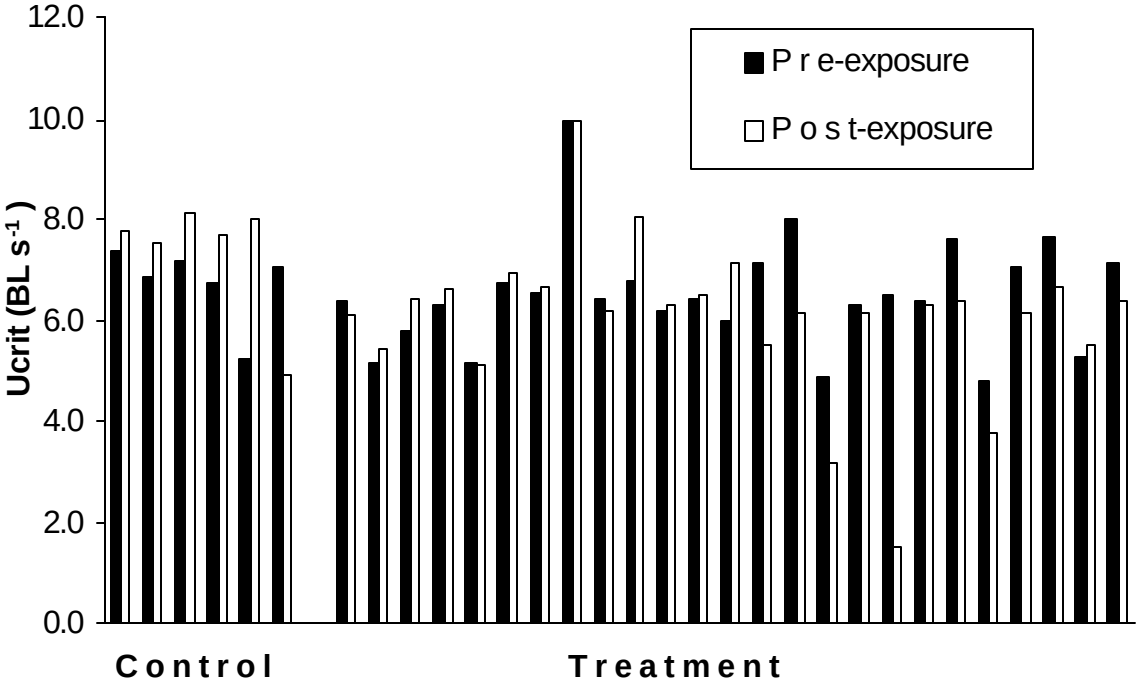

Figure 3.2: Critical swimming speeds (U_{crit}) of juvenile Atlantic spadefish before and after a 24-hr exposure to 3.0 % ethylene glycol (N = 25) and sham controls (N=6).

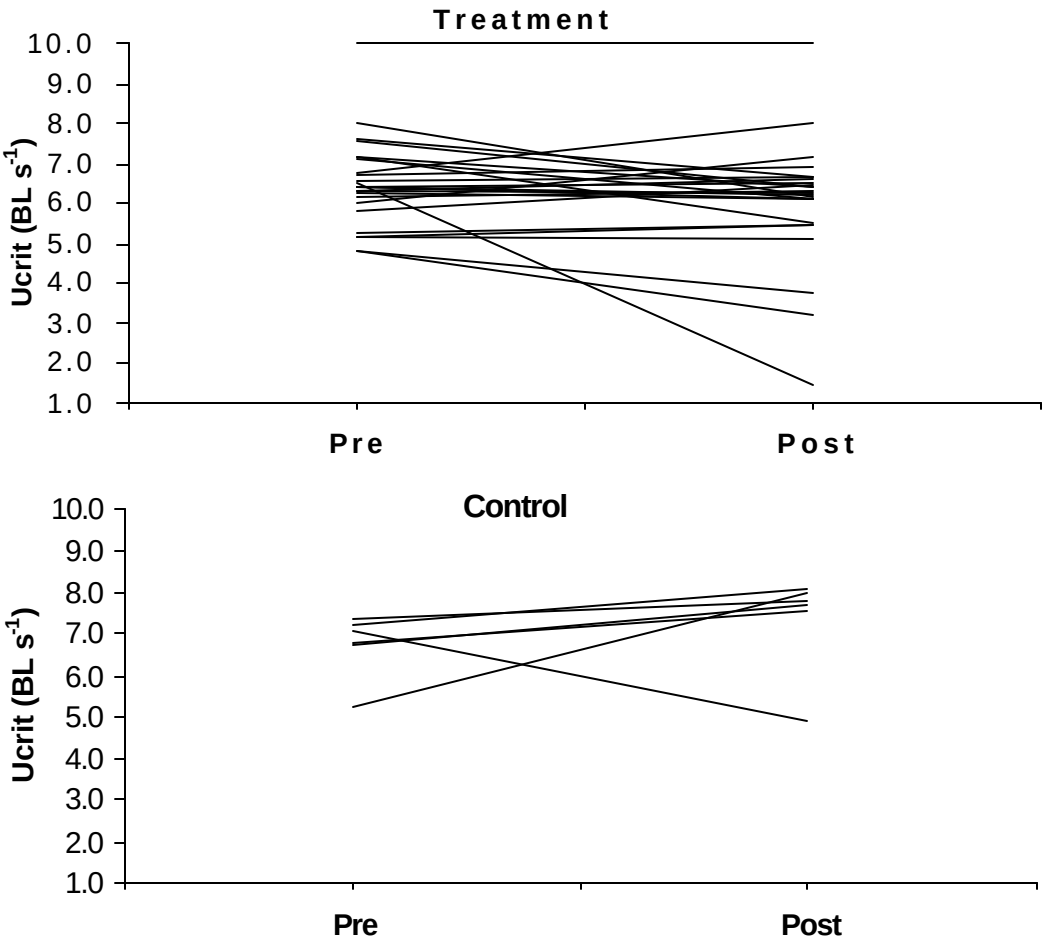

Figure 3.3: Individual variation in the critical swimming speeds (U_{crit}) of juvenile Atlantic spadefish (N = 25) before and after exposure to 3.0 % ethylene glycol and sham controls (N = 6).

primates, ethylene glycol causes depression of the nervous system that is similar to ethanol intoxication followed by drowsiness, coma, respiratory failure, convulsions, and renal damage (Merck 1983). Hymel et al. (2002) found that exposure to as little as 1.0-2.1 % of ethylene glycol causes changes in the swimming behavior of Florida pompano after exposure.

The 7.3 % and 18.4 % reductions in U_{crit} compared to initial performance and post-treatment control means, respectively, may be a result of a buildup of metabolic products. In mammals ethylene glycol is oxidized in the liver (Chou and Richardson 1978) and involves several metabolic products, with glycolic acid believed to be the metabolic product that is responsible for the toxic effects of the chemical. The buildup of metabolic products and increased metabolic cost of removing ethylene glycol from the body may have directly caused or contributed to the decrease in swimming performance of exposed fish.

Modes of Action

In addition to the buildup of metabolic products, the increased metabolic cost of removing ethylene glycol from the body and reduced gas exchange from gill damage may reduce swimming performance. Several theories have been proposed in the literature on how exposure to a toxicant, such as ethylene glycol, results in a reduction in swimming performance. A decrease in critical swimming speed has been attributed to impaired transport or exchange of respiratory gases (Sprague 1971; Satchell 1984). Several factors may hinder the swimming performance of fishes: the inability to supply adequate oxygen to the gills; the inability to supply oxygen to the tissues; the inability to remove metabolic products; and the inability to provide adequate substrate or to activate enzymatic

processes (Jones 1971). Wilson and Taylor (1993) found acute levels of copper exposure in freshwater rainbow trout caused a disruption of ion regulation and progressive hypoxia and plasma acidosis resulting from physical damage to the gills. It was unclear whether gill damage was a result of the ethylene glycol, as examination of spadefish gill lamelle revealed signs of damage and fusion; however, this could have also been caused by the use of MS-222 before sacrifice.

Individual Variation

Considerable within group variation was observed for individuals in the swimming performance tests (Figure 3.2, 3.3). Traditionally in swimming performance studies, it is common practice to compare the mean U_{crit} of different treatment and control groups, in a one-way ANOVA design, but this approach fails to address within-group variation. In the traditional approach, high within-group variation makes it difficult to find significant differences between treatment and control group means, which can limit the usefulness of swimming performance tests (Kolok 1998). In this and related studies (Hymel et al. 2002; Baltz in prep) swimming performance of individual fish before and after exposure was used in a split-plot design with blocks to take advantage of within-group variability to analyze changes in Florida pompano swimming performance (Kolok 1992).

Previous studies revealed conflicting results concerning the performance variability among individuals after treatment. Kolok (1998) found large within group variation in U_{crit} during swimming performance tests, but this variation was repeatable over replicate experiments. In contrast Kolok and Farrel (1994) found that the reduction in U_{crit} of northern pikeminnow (*Ptychocheilus oregonensis*) after a surgical technique

was fairly consistent. The results of this study resembled those by Kolok (1998) where individual performance in U_{crit} after exposure to ethylene glycol varied between slight increases and traumatic reductions. The variability in this study implies that individual fish may have lower or higher tolerances to ethylene glycol. This study used only one concentration of ethylene glycol and it was not possible to calculate tolerance values for individuals.

Spills

A large localized spill of ethylene glycol poses a risk with large offshore storage volumes up to 416,400 L per platform (Boehm et al. 2001). Significant spills have been reported in recent years, 13,510 L of ethylene glycol leaked from an underwater pipeline into the northern Gulf of Mexico on June 29, 1998 (National Response Center 2002 b). Fish would most likely be exposed for only a short period of time due to the rapid dilution and biodegradation of ethylene glycol in the natural environment. The half-life of ethylene glycol in the water column and marine sediments is estimated at 32.50 days. Boehm et al. (2001) modeled a possible spill of ethylene glycol (416,400 L) and found the predicted exposure concentrations would be an order of magnitude smaller than the lowest 48-hr LC_{50} (34,400 mg/L or 3.13 %) for the water flea (*Ceriodaphnia dubia)*. However, marine fish may be more sensitive to the toxic effects of ethylene glycol. If it is assumed that LC_{50} decreases linearly with time of exposure, then LC_{50} values in studies using different test durations can be grossly compared by using a toxicity index (concentration x time) (French et al. 1996a; Boehm et al. 2001). Using the index of toxicity (Index) to estimate 1-hr LC_{50} found by Hymel et al. (2002) for Florida pompano (Index = 135) would be lower than those found by Boehm et al. (2001) for the water flea

(Index = 150). Sublethal concentrations (Index = 72) used in this experiment would be half as large as values found for the water flea suggesting that a spill could affect juvenile Atlantic spadefish to a greater extent than predicted.

Ecological Effects

An episodic spill of ethylene glycol could have ecological implications at individual, community, or population levels. The results of this study showed that sublethal exposure to small concentrations of ethylene glycol can greatly reduce the swimming performance of an individual. Nevertheless, it is difficult to forecast how the effects of exposure at the individual level translate to the population or community level. The reduced ability of an individual to feed and avoid predators could have effects on a population. Although post-exposure critical swimming speeds were well above those used in normal swimming speeds, the ability for prolonged or burst swimming speeds would be reduced in the event of exposure, which may result in the reduced ability to capture prey or avoid predators. However, mortality in fish populations can be compensatory; therefore, a spill may not necessarily affect a species at the population levels if the exposure is not sustained or widespread (Houde 1989). But a spill during peak spawning period could reduce recruitment and add an additional stress to populations already burdened by overfishing, pollution and reduced habitat quality. An in-depth model including parameters on food resources, avoidance behavior, dispersion patterns of ethylene glycol and life histories of marine fishes may be the best way to understand the implications of sublethal exposures at the population level.

Conclusion

The results of this study showed conclusively that a single exposure to ethylene glycol significantly reduced the swimming performance of juvenile Atlantic spadefish. Although Boehm et al. (2001) found that realistic chemical spills of ethylene glycol to be well below the lowest LC_{50} values for other species found in the literature, at least two marine fish, Florida pompano and Atlantic spadefish appear to be more sensitive to the toxic effects of the chemical. The reduced ability of Atlantic spadefish to swim at high prolonged and burst performance levels could have effects on an individual's ability to avoid predators and feed effectively. Effects at the level of the organism may, in turn, lead to effects at population and community levels (Weis et al. 1999). Exposure to ethylene glycol could ultimately affect population and community structure in the vicinity of a spill if recurrent or combined with other population level effects.

Chapter IV.

General Summary

The results of the juvenile Florida pompano study showed that exposure to 1.07 % methanol and 17 hr recovery did little to impair the swimming performance of juvenile Florida pompano. Sublethal exposure to methanol had only a short-term effect on swimming behavior but little effect on measured swimming performance. The lack of detectable levels of methanol in the plasma samples suggest that pompano completely metabolized methanol (probably into formaldehyde and formate products) during the ~ 20-hr swimming and recovery process. In contrast, the mean percentage (± SE) of ethylene glycol still present in pompano plasma samples ~ 20 hours after exposure was 0.623 ± 0.04 % (v/v).

The reduction in swimming performance of juvenile Florida pompano (a 13.0 % reduction) exposed to ethylene glycol was substantially higher then the reduction in performance of Atlantic spadefish (a 6.9 % reduction). The severity of ethylene glycol toxicity varies from species to species and has been shown to be more toxic to humans than poultry (Beasley 1980). Spadefish may be more efficient at buffering against or removing the metabolite responsible (glycolic acid) for the toxic effects of ethylene glycol.

Pompano exposed to the combination of ethylene glycol and methanol exhibited the most profound reduction in swimming performance. Fish that were exposed to the combination of ethylene glycol and methanol performed 250.0 % worse then fish exposed to the single concentration of ethylene glycol. Methanol and ethylene glycol may become more toxic when fish are exposed to a combination of both chemicals.

Similar joint action or additive interactions occur when toxicants have similar modes of action but act independently resulting in the toxicity of the mixture amounting to the sum of the toxicities of the individual toxicants present (Bliss 1939; Sprague 1970; Marking 1985). Interactive action can be either synergistic (more then additive) or antagonistic (less then additive). These interactions occur when one toxicant alters the toxicity of another toxicant present. The results of the Florida pompano study showed that a synergistic interaction may have occurred after juveniles were exposed to the combination of ethylene glycol and methanol. These findings were similar those of Greene and Kocan (1997) who found the toxicity of ethylene glycol increases in freshwater fish when sublethal levels of thiram, a chemical used in an agricultural seed-protectant, is present.

The extensive toxic effects observed might have been the result of interactions between the various components of the formulation, referred to as synergism (Marking 1985). A discharge or spill often involves more then one chemical component and the interaction between two or more chemicals can result in toxicity that is greater than the sum of the toxicities of the individual components (Greene and Kocan 1997). Both ethylene glycol and methanol are competitors for aldehyde dehydrogenase (ALDH) and may inhibit each other from being completely metabolized and increase the toxic effects after exposure (Pietryka et al. 1998). The metabolic pathways of ethylene glycol and methanol are in fact so similar that treatment for ethylene glycol and methanol poisoning is the administration of ethanol, another ALDH competitor (Katarzyna et al. 1998).

Both species exhibited a high amount of individual variation in swimming performance after exposure to ethylene glycol. Kolok (1998) found large within group

variation in U_{crit} during swimming performance tests, but this variation was repeatable over replicate experiments. In contrast Kolok and Farrel (1994) found that the reduction in U_{crit} of northern pikeminnow (*Ptychocheilus oregonensis*) after a surgical technique was fairly consistent. The results of both the Florida pompano and Atlantic spadefish swimming performance studies resembled those of Kolok (1998) in that the individual reduction in U_{crit} after exposure to ethylene glycol, methanol, and combination treatment was substantial but repeatable. The variability in these studies implies that individual fish may have lower or higher tolerances to ethylene glycol, methanol or combination treatments. Both studies used only one concentration for each chemical; therefore, it was not possible to calculate tolerance values for individuals.

Future studies should concentrate on accumulating more data on the sublethal and lethal effects of ethylene glycol and methanol on a variety of species associated with deepwater offshore oil and gas platforms (i.e., encrusting organisms, planktonic species, etc.). This data could aid in developing a working model to better understand predator prey interactions, chemical avoidance, behavior and feeding activity of fishes after exposure. Quantifying the sublethal and lethal effects of ethylene glycol and methanol at all levels of the reef ecosystem would be a good first step in understanding the effects of deepwater oil development on fisheries in the northern Gulf of Mexico.

Due to lack of available fish, finding an appropriate LC_{50} value of ethylene glycol and methanol and testing the potential and additive or synergistic effects of ethylene glycol and methanol on the swimming performance of Atlantic spadefish was not possible. LC_{50} values would allow comparisons of the toxic effects of ethylene glycol and methanol on physiologically different species and is important in understanding the

overall toxicity of a substance. Answering the question of whether the exposure to methanol and the combination of ethylene glycol and methanol would effect a physiologically different species would aid in understanding the overall effect of a localized spill and begin to extend our understanding from the population to community level. Future studies should concentrate on finding LC_{50} values for several marine fish at risk of exposure.

Only one sublethal concentration was used to evaluate the sublethal effects of ethylene glycol, and methanol on Florida pompano and Atlantic spadefish. Although behavior and recovery trials by Hymel (2002) and Baltz (in prep) were used to find sublethal test concentrations; testing a range of sublethal concentrations on swimming performance would help in identifying the effects of a differing exposure ranges.

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Appendix A

Data for Chapter II.

Table A.1: Individual Body Mass (g), Fork Length (mm) and pre-exposure and post-exposure U_{crit} (cm s^{-1}, BL s^{-1}) for the treatments and control groups of Florida pompano.

Florida pompano			Pre-exposure Ucrit		Post-exposure Ucrit	
Treatment	**FL (mm)**	**Body Mass (g)**	**cm s^{-1}**	**BL s^{-1}**	**cm s^{-1}**	**BL s^{-1}**
MeOH	102.0	23.32	96.93	9.50	89.95	8.82
MeOH	98.0	19.29	99.07	10.11	96.97	9.89
MeOH	107.0	25.67	100.0	9.35	100.0	9.35
MeOH	90.0	15.24	83.85	9.32	74.17	8.24
MeOH	92.0	16.11	94.18	10.24	91.62	9.96
MeOH	91.0	15.50	88.13	9.68	89.03	9.78
MeOH	91.0	14.71	83.88	9.22	82.08	9.02
MeOH	93.0	15.66	90.83	9.77	85.02	9.14
MeOH	94.0	17.11	87.40	9.30	89.07	9.48
MeOH	110.0	26.51	94.93	8.63	91.10	8.28
MeOH	108.0	24.70	98.48	9.12	99.41	9.20
MeOH	102.0	22.14	100.0	9.80	100.0	9.80
LSMean	**98.17**	**19.66**	**93.14**	**9.49**	**90.70**	**9.24**
SE	**2.12**	**1.30**	**7.50**	**0.44**	**7.50**	**0.44**
EG	111.0	27.45	93.53	8.43	95.33	8.59
EG	102.0	24.31	100.0	9.80	91.30	8.95
EG	99.0	19.79	100.0	10.10	84.97	8.58
EG	112.0	28.70	74.18	6.62	82.23	7.34
EG	100.0	21.34	100.0	10.0	91.20	9.12
EG	108.0	24.03	91.97	8.52	83.32	7.71
EG	91.0	15.00	92.86	10.20	89.03	9.78
EG	97.0	18.51	91.63	9.45	88.88	9.16
EG	89.0	15.08	92.65	10.41	62.32	7.00
EG	90.0	14.78	81.43	9.05	68.12	7.57
EG	91.0	17.41	89.73	9.86	78.68	8.65
EG	89.0	14.57	95.83	10.77	41.30	4.64
LSMean	**98.25**	**20.08**	**91.98**	**9.43**	**79.72**	**8.09**
SE	**2.48**	**1.46**	**7.50**	**0.41**	**7.50**	**0.41**
MeOH+EG	106.0	26.81	100.0	9.43	86.05	8.12
MeOH+EG	109.0	24.66	100.0	9.17	65.48	6.01
MeOH+EG	87.0	13.32	83.32	9.58	44.22	5.08
MeOH+EG	89.0	14.44	74.26	8.34	71.25	8.01
MeOH+EG	92.0	14.20	90.48	9.83	72.23	7.85
MeOH+EG	89.0	16.50	86.03	9.67	6.916	0.78
MeOH+EG	88.0	14.78	86.95	9.88	14.78	1.68
LSMean	**94.29**	**17.82**	**90.82**	**9.35**	**53.66**	**5.30**
SE	**2.65**	**1.59**	**8.06**	**0.52**	**8.06**	**0.52**
Control	105.0	27.30	94.95	9.04	100.0	9.52
Control	105.0	22.30	99.12	0.94	100.0	9.52
Control	108.0	25.60	100.0	9.26	100.0	9.26
Control	112.0	27.92	100.0	8.93	96.85	8.65
Control	108.0	25.50	94.05	8.71	99.50	9.21
Control	89.0	16.13	88.08	9.90	59.83	6.72
Control	95.0	16.34	87.08	9.17	91.17	9.60
Control	94.0	16.64	86.78	9.23	91.53	9.74
Control	88.0	16.22	85.10	9.67	92.65	10.5
Control	92.0	16.28	84.43	9.18	82.82	9.00
Control	94.0	17.81	89.06	9.48	92.95	9.89
LSMean	**99.09**	**20.73**	**92.22**	**9.26**	**92.10**	**9.23**
SE	**2.48**	**1.44**	**7.56**	**0.42**	**7.56**	**0.42**

Appendix B

Data for Chapter III.

Table B.2: Individual Body Mass (g), Fork Length (mm) and pre-exposure and post-exposure U_{crit} (cm s^{-1}, BL s^{-1}) for treatment and control groups of Atlantic spadefish.

Spadefish			**Pre-exposure U_{crit}**		**Post-exposure U_{crit}**	
Treatment Fish	**FL (mm)**	**BM (g)**	**cm s^{-1}**	**BL s^{-1}**	**cm s^{-1}**	**BL s^{-1}**
1	97	34.8	69.33	7.15	53.40	5.51
2	110	54.3	87.93	7.99	67.75	6.16
3	83	16.1	40.25	4.85	26.63	3.21
4	93	25.9	58.55	6.30	57.08	6.14
5	105	41.5	68.38	6.51	15.82	1.51
6	88	22.0	55.97	6.36	55.38	6.29
7	108	48.9	82.07	7.60	69.17	6.40
8	88	18.6	42.43	4.82	33.00	3.75
9	98	34.7	69.45	7.09	60.12	6.13
10	76	15.5	58.03	7.64	50.73	6.68
11	116	39.0	61.18	5.27	63.80	5.50
12	119	71.3	84.78	7.12	76.05	6.39
13	119	59.9	75.98	6.38	72.75	6.11
14	101	33.3	51.87	5.14	55.05	5.45
15	115	48.6	66.85	5.81	73.90	6.42
16	95	26.8	60.28	6.35	62.75	6.61
17	116	52.4	59.85	5.16	59.13	5.10
18	115	52.6	77.05	6.70	79.98	6.95
19	110	49.0	100.0	9.09	100.0	9.09
20	134	93.5	87.92	6.56	89.02	6.64
21	68	9.8	43.57	6.41	42.17	6.20
22	89	26.8	60.17	6.76	71.47	8.03
23	136	95.9	84.12	6.19	85.92	6.32
24	84	20.0	53.87	6.41	54.47	6.48
25	109	46.1	65.25	5.99	77.92	7.15
Mean	**102.9**	**41.5**	**66.90**	**6.59**	**62.44**	**6.13**
SE	**3.40**	**4.48**	**5.54**	**0.36**	**5.54**	**0.36**
Control Fish	**FL (mm)**	**BM (g)**	**cm s^{-1}**	**BL s^{-1}**	**cm s^{-1}**	**BL s^{-1}**
1	112	58.2	82.43	7.36	87.30	7.79
2	120	61.3	81.85	6.82	90.04	7.53
3	96	31.1	69.22	7.21	77.80	8.10
4	109	42.9	73.33	6.73	83.92	7.70
5	112	39.6	58.58	5.23	89.48	7.99
6	139	105.7	98.08	7.06	68.22	4.91
Mean	**114.7**	**56.5**	**77.25**	**6.73**	**82.85**	**7.34**
SE	**5.82**	**0.89**	**8.24**	**0.55**	**8.24**	**0.55**

Vita

Mark Alan Stead was born March 16, 1978, in New Brunswick, New Jersey. Mark is the son of Donald H. Stead and Karen J. Stead and the brother of Scott W. Stead. He graduated from Slidell High School in May of 1996. Mark attended Louisiana State University where he graduated with a Bachelor of Science degree in wildlife and fisheries with a minor in zoology in May 2000. He enrolled in graduate school and became a graduate assistant under Dr. Donald Baltz in August of 2000 in the Department of Oceanography and Coastal Sciences at L.S.U. Mark will receive the degree of Master of Science in oceanography and coastal sciences in May 2003.