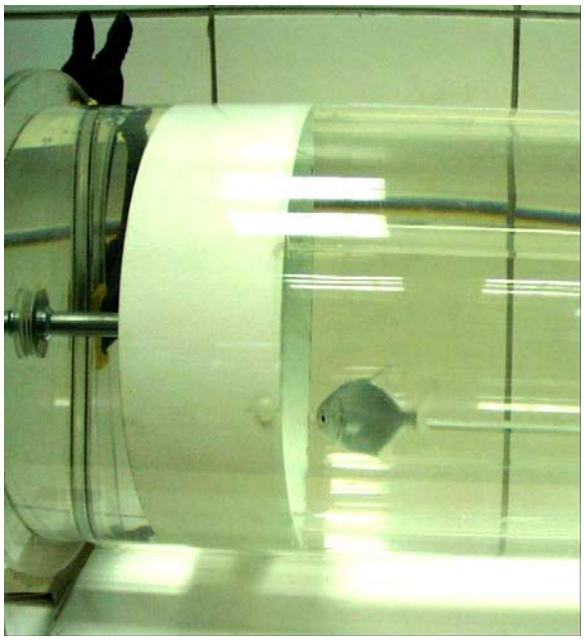
OCS Study
MMS 2005-054

Coastal Marine Institute

Evaluating Sublethal Effects of Exposure to Petroleum Additives on Fishes Associated with Offshore Platforms



U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

Cooperative Agreement
Coastal Marine Institute
Louisiana State University

OCS Study
MMS 2005-054

Coastal Marine Institute

Evaluating Sublethal Effects of Exposure to Petroleum Additives on Fishes Associated with Offshore Platforms

Editors

Donald M. Baltz
Edward J. Chesney

December 2005

Prepared under MMS Contract
1435-01-99- CA-30951-16802
by
Coastal Marine Institute
Louisiana State University
Baton Rouge, Louisiana 70803

Published by

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

Cooperative Agreement
Coastal Marine Institute
Louisiana State University

DISCLAIMER

This report was prepared under contract between the minerals Management Service (MMS) and Coastal marine Institute. This report has been technically reviewed by the MMS, and it has been approved for publication. Approval does not signify that the contents necessarily reflect the view and policies of the MMS, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. However, it is exempt from review and compliance with the MMS editorial standards.

REPORT AVAILABILITY

Extra copies of the report may be obtained from the Public Information Office (Mail Stop 5034) at the following address:

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region
Public Information Office (MS 5034)
1201 Elmwood Park Boulevard
New Orleans, Louisiana 70123-2394

CITATION

Suggested citation:

Baltz, D. M. and E. J. Chesney. 2005. Evaluating Sublethal Effects of Exposure to Petroleum Additives on Fishes Associated with Offshore Platforms. U.S. Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2005-054. 76 pp.

ABOUT THE COVER

The Florida pompano *Trachinotus carolinus* is commonly caught by anglers around petroleum platforms where it is exposed to contaminants associated with the production and transport of oil and gas. The swimming performances of juvenile pompano were tested in swimming tunnels before and after sublethal exposures to ethylene glycol and methanol to evaluate ecological effects on individuals.

ACKNOWLEDGEMENTS

We appreciate the critical comments on earlier manuscripts by Jaye Cable, Kenny Rose, Larry Rouse, and several anonymous reviewers, John Hawke for assistance with husbandry, and many members of the Louisiana Universities Marine Consortium staff for all manner of assistance, especially Russell Martin for fabricating the swimming chambers. Field and laboratory work would not have been possible without the help of Marcus Bradley, Cody Short, Murt Verburg, Ted Switzer, Ken Riley, and Chris Salmonsen and the captain of the R/V *Acadiana*, Craig LeBoeuf. We are also grateful to the Minerals Management Service for funding (contract 1435-01-99-CA-30951).

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 of petroleum to prevent the formation of gas hydrates in deepwater wells and pipelines. Deepwater petroleum production requires that a number of additives, such as ethylene glycol and methanol, be transported and stored offshore in large quantities posing the risk of spills. Although these additives may not be highly toxic per se, it is important to evaluate and understand the risks to marine organisms, especially fishes that are often strongly associated with oil production facilities. Juvenile Florida pompano *Trachinotus carolinus* were used in three separate controlled experiments to test the sublethal 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. Florida pompano 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 protocols included identical fasting, exposure, acclimation, and swimming experience for each group and required up to18 days for the experimental trial. An additional ethylene glycol exposure experiment was conducted on a second species, juvenile Atlantic spadefish *Chaetodipterus faber*. Ethylene glycol toxicity tests on juvenile Florida pompano and subsequent probit analysis identified the LC50 as 5.63 % (volume per volume) at 30 practical salinity units and 25 °C. Behavioral observations of treatment and control groups during 24-h exposures and 15-h recovery trials showed that 2.1 % ethylene glycol was the lowest concentration at which individuals displayed lethargic behavior relative to controls after 24 h. Fish exposed to concentrations of 3.1 % or less became lethargic or distressed relative to controls, but showed signs of recovery after 15 h in clean seawater. The mean (± SE) U_{crit} of juveniles (23.1±4.73 g) was evaluated before and after exposure to a 3.0 % concentration. The mean U_{crit} declined significantly by 13.5 % (P<0.0002) from 95.9 ±2.37 cm/s in pre-exposure trials to 83.0 ±3.45 cm/s in post-exposure trials. Exposure to ethylene glycol did not impact all individuals to the same degree, and smaller fish experienced a greater percent decrease in swim performance (%R) than did larger individuals (%R = - 53.5 + [1.7 x Body Mass], R^2 = 0.3148, df = 13, P<0.0295). We also evaluated the toxicity and sublethal effects of methanol on the swimming performance of juvenile Florida pompano. A 24-h static exposure identified the LC50 of methanol as 1.28% (volume per volume, % v/v) at 30 practical salinity units and 25°C. The mean (± SE) U_{crit} of juveniles (20.5 ± 4.59 g) was evaluated before and after exposure to a 1.07% concentration of methanol and showed that U_{crit} was significantly reduced (P < 0.0002) from 90.1 ± 1.35 cm/s to 84.2 ± 1.36 cm/s in post-exposure trials. Following exposure and a 17-h recovery period in clean seawater, the mean 6.5% decline in performance of the methanol treatment group contrasted sharply with a mean increase of 4.0% in the control group, indicating that conditioning and/or training effects were significantly surpassed by the negative sublethal effects of methanol exposure. In a combination experiment (i.e., Control, EG, MeOH and EG + MeOH) on juvenile Florida pompano swimming performance, single exposures to ethylene glycol and the combination of ethylene glycol and methanol significantly reduced U_{crit} by 13.0 and 42.0%, respectively. In this experiment, no detectable differences in U_{crit} were found for Florida pompano exposed to methanol or for the controls. Juvenile Atlantic spadefish were used to test the single effects of 3.0% ethylene glycol on swimming performance of individuals using the same protocol developed for pompano.

Treatment fish experienced 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 Florida pompano and Atlantic spadefish to sustain high prolonged and burst performance levels could have affected an individual's ability to avoid predators and feed effectively.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1.0 INTRODUCTION

D. M. Baltz[1, 2] and E. J. Chesney[3]

[1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803-7503

[2]Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503

[3]Louisiana Universities Marine Consortium, Chauvin, LA 70344

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). Recently, declining inshore production, rising oil prices and new technology have expanded 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 type of risk is from chemical additives that are used to enhance deep-water production. Oil and gas platforms are a source of anthropogenic inputs including contaminated waters that are separated from crude oil at the extraction point (i.e., 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 reside on and near oil production structures (Holdway 2002).

Additives such as ethylene glycol and methanol are often transported long distances from shore by underwater pipelines up to 96 km in length and by ships to deep-water production platforms where they are stored in volumes of up to 300,000 L per platform (Boehm et al. 2001). These additives are used to prevent the formation of gas hydrates in deep-water wells and pipelines (Anonymous 1996, 2000; Herzhaft and Dalmazzone 2000; Boehm et al. 2001). Although the possibility of a large spill is unlikely, the risk remains plausible with increased usage, transport and storage of large volumes of ethylene glycol and methanol (Boehm et al. 2001). Although we are not aware of any instance were both ethylene glycol and menthanol are used at the same production facility, multiple other chemicals and proprietary mixtures of chemicals are commonly used together in production and transport (Boehm et al. 2001).

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 as a prelude to understanding population and community responses to these impacts.

Ethylene glycol and methanol are toxic to fishes to varying degrees. Median lethal concentrations 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 LC50 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 LC50 values have been reported for various freshwater and two salt-tolerant or saltwater fishes by Bengtsson et al. (1984) for the bleak *Alburnus alburnus* and by Portmann and Wilson (1971) for the hooknose *Agonus cataphractus* .

We used critical swimming speed (Brett 1964) to quantify the capacity of individual fish for prolonged swimming activity. Swimming performance has been used to determine tolerances of fishes to varying environmental stresses and pollutants (Cairns 1966; Sprague 1971; Nikl and Farrell 1993; Beaumont et al.1995; Heath 1995; Kolok et al. 1998; Hymel et al. 2002). 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 a variety of 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 migrate, 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 resisting 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). Thus estimates of swimming performance provide an index of stress.

Juvenile Florida pompano *Trachinotus carolinus* 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. These species were also chosen because they are excellent fish models for many similar species such as amberjack, crevalle jack and other species that reside offshore and are strongly associated with reefs and other structures including pipelines and platforms. Pompano apparently have a protracted spawning season in the northern Gulf of Mexico during the spring and late 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 this study were to understand the separate and combined sublethal effects of ethylene glycol and methanol on the swimming performance of individual fishes with Florida pompano and Atlantic spadefish serving as the fish models. When testing for the sublethal effects of contaminants it is common practice to first find an appropriate LC50 for the species of interest. Hymel et al. (2002) and Baltz et al. (2005) found LC50 values for ethylene glycol and methanol on juvenile Florida pompano. In genral exposure concentrations were based on LC50, behavior, and recovery experiments detailed in Hymel et al. (2002) and Baltz et al. (2005).

Because of the difficulty of collecting numbers of similarly sized Atlantic spadefish, we did not conduct an LC50 range finding experiment for that species.

Chapters 2 through 5 are standalone documents describing four sets of experiments that are or will be published. Chapters 2 and 3 report on the toxicities and sublethal effects on swimming performance of ethylene glycol (Hymel et al. 2000) and methanol (Baltz et al. 2005), respectively, on Florida pompano. Chapter 4 reports on significant reductions in critical swimming speed after Florida pompano were exposed to combinations 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. And Chapter 5 reports on a single exposure of Atlantic spadefish to ethylene glycol in a randomized experimental design to examine potential sublethal effects on a marine species with a different physiology and ecology.

2.0 SWIMMING PERFORMANCE OF JUVENILE FLORIDA POMPANO EXPOSED TO ETHYLENE GLYCOL

M. E. Kay[1], D. M. Baltz[1,2], E. J. Chesney[3], M. A. Tarr[4] and A. S. Kolok[5]

[1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803-7503

[2]Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503

[3]Louisiana Universities Marine Consortium, Chauvin, LA 70344

[4]Department of Chemistry, University of New Orleans, New Orleans, LA 70148

[5]Biology Department, University of Nebraska at Omaha, Omaha, NE 68182

Marine fishes face an array of environmental contaminants that may reduce their ability to perform in ecological situations. The effects of factors producing direct mortality in populations, such as fishing, are often easier to quantify than sublethal impacts. Though not responsible for direct mortality, sublethal impacts may affect scope for activity (Cech 1990), swimming performance (Brett 1964, Wedemeyer et al. 1990), growth, reproduction, and survival of individuals (Little et al. 1990), with consequences for population size and biomass. Quantification of sublethal effects at the individual level is an important step in predicting how individuals and populations may respond ecologically to these impacts.

Marine fisheries are potentially at risk from releases of chemical additives from deepwater petroleum production operations. With the development of new technologies, oil and gas production is now expanding into greater ocean depths (> 305 m), thus posing a new array of environmental risks to marine fishes. Pure chemicals like ethylene glycol and methanol may be transported in small supply lines (i.e., umbilicals) up to 96.5 km in length, stored in large volumes and used at high rates. This expansion has created a need for the ecological evaluation of new practices and chemicals used in petroleum production and transport (Boehm et al. 2001, Minerals Management Service 2002). One such contamination risk comes from the use of ethylene glycol in the production of petroleum products from deepwater wells to prevent the formation of gas hydrates (Anonymous 1996, 2000; Herzhaft and Dalmazzone 2000; Boehm et al. 2001), which can potentially clog pipelines.

The environmental effects of ethylene glycol have been widely studied in relation to its use as an antifreeze, coolant, and deicing agent (Barber 1999). Median lethal concentrations (LC50) have been reported for various freshwater and brackish-water organisms (Abdelghani et al. 1989; Pillard 1995; Greene and Kocan 1997). The toxicity of ethylene glycol to fishes has been assessed on only a few species, including rainbow trout *Oncorhynchus mykiss*, fathead minnow

Pimephales promelas (Pillard 1995; Greene and Kocan 1997), bluegill sunfish *Lepomis macrochirus* (Abdelghani et al. 1989), and goldfish *Carassius auratus* (Bridie et al. 1979).

Sublethal exposure levels can lead to important individual effects measurable by diminished performance (Sprague 1971), which in turn creates an enhanced risk of mortality or an impaired ability to perform normal life functions (Schreck 1990). Sublethal effects may potentially lead to declining numbers in a population (Heath 1995). Changes in critical swimming speed (U_{crit}) have been used to indicate fish performance in many studies assessing sublethal effects of contaminants (Nikl and Farrell 1993; Beaumont et al. 1995; Heath 1995; Kolok et al. 1998). Reduced swimming performance may have ecological significance, such as impairing the ability of larval fishes to avoid predators and catch prey, but has not been demonstrated for larger individuals. Reduced swimming performance and prey capture ability has been demonstrated in fish larvae exposed to sublethal concentrations of methylmercury (Zhou et al. 1996; Samson et al. 2001). Vulnerability to predation is dynamic, and exposure to contaminants could increase such vulnerability by effecting swimming speed (Beauvais et al. 2000), perception (Baker and Montgomery 2001), and behavior (Weis et al. 1999, 2001, Scholz et al. 2000, Wibe et al. 2001).

The Florida pompano *Trachinotus carolinus* was chosen for assessment of acute and sublethal effects of exposure to ethylene glycol because the species occurs in the vicinity of transport pipelines and deepwater wells in the Gulf of Mexico. A marine fish of considerable economic importance to sport and commercial fisheries (Gilbert 1986) the Florida pompano is found in Atlantic and Gulf of Mexico waters from Massachusetts to Brazil (Hoese and Moore 1977). Juveniles are abundant in and near the surf zone during summer months (Bellinger and Avault 1970). Adults can be found in inshore and offshore waters and spawn on the continental shelf (Gilbert 1986).

We addressed our goal in assessing the acute and sublethal effects of ethylene glycol on juvenile Florida pompano swimming performance by identifying a short-term LC50 and a range of lower sublethal concentrations and by testing for changes in U_{crit} following sublethal exposure. The objectives were to provide an estimate of the 24-h LC50, to identify sublethal exposure levels from which individuals could apparently recover after short-term exposures expected in open marine systems (Boehm et al. 2001), and to evaluate sublethal effects by comparing changes in individual swimming performance before and after exposure to ethylene glycol. We found that the mean swimming performance was significantly reduced by 14 % in individuals after a 24-h exposure to a 3.0 % concentration of ethylene glycol and a 17-h recovery period in clean seawater. These results provide a clearer understanding of the possible ecological consequences from sublethal spills or leakage in the marine environment.

2.1 Methods

2.1.1 Collection and maintenance of fish

Juvenile Florida pompano ranging in body mass from 0.5 to 16.0 g were collected for toxicity, behavior, and swimming experiments. Individuals were collected in May-June 1999 (0.5 to 6.0 g), and again in August 1999 (2.0 to 16.0 g), from Port Fourchon Beach and Isle Dernieres, Louisiana, with a 3.05 m (10 ft) beach seine. Fish were held for about 4 weeks in a 5,000-L recirculating seawater system at 30 practical salinity units (psu) before testing. During holding,

the fish were treated with 0.2 mg/L copper (Cutrine-Plus) to eliminate ectoparasites, especially *Amyloodinium* (Cheung 1993). Water quality parameters, including pH, salinity, ammonia level, and copper concentrations were monitored several times per week during acclimation and daily during experiments. Amquel ($HOCH_2SO_3Na$) was added to holding systems to control the ammonia level when it exceeded 0.5mg/L. Fish were fed a commercially produced pellet feed (0.25 g day^{-1} $individual^{-1}$; 45 % protein, 8 % lipid) except within the 15-17 h prior to and during acute toxicity tests, behavioral experiments, and swimming experiments.

2.1.2 Acute toxicity tests

Twenty-four hour static tests were conducted to determine the LC50 of ethylene glycol. Lower concentrations were then tested to allow identification of a sublethal concentration for subsequent swimming experiments. Three LC50 experiments were conducted in a system of eight conical fiberglass tanks. Each tank was filled to 70 L with ultra violet (UV)-sterilized, 30-psu seawater. The tanks were individually aerated and maintained at 25.0±1 °C with aquarium heaters. Four to six logarithmically spaced exposure concentrations plus a control were included in each test, and the exposure concentrations were randomly assigned to the tank array. A 'blank' tank without fish was also included to monitor the mixing and concentration of the chemical over the 24-h. Ten fish were randomly assigned to each of the exposure and control tanks before the addition of ethylene glycol (Fisher Scientific, certified). We maintained a 20-min interval between the sequential dosing of each tank to allow for processing of the fish at the end of the experiment. The activity patterns of the fish in the treatment groups relative to the controls were recorded during the second and third tests and in subsequent behavior tests, and were classified according to the criteria in Table 2-1. Water samples (1.5 mL) for gas chromatographic (GC) analysis, taken periodically from each tank during the second and third tests, showed that the ethylene glycol exposure solutions were accurately estimated, well mixed, and stable throughout the 24-h experiment (Kay 2000).

The initial LC50 range-finding test was conducted on Florida pompano with six ethylene glycol concentrations ranging from 0.56 to 10.00 % volume per volume. In fish from the first experiment, the mean (± SE) BM was 7.6 ± 0.4 g and fork length (FL) was 71.4 ± 1.1 mm. Four exposure concentrations, ranging from 3.59 to 7.16 %, were used in the second experiment. The mean body mass of fish in the second experiment was 8.6 ± 1.1 g, and the mean FL was 72.7 ± 1.3 mm. Because the body size of the fish was consistent between the two experiments, and the experiments were conducted only 6 d apart, we pooled the data to determine LC values with the Environmental Protection Agency's Probit Analysis Program, Version 1.5 (Lazaorchak 1994).
We collected blood samples from each fish to analyze for the effects of ethylene glycol exposure. At the end of each experiment, blood was drawn from the caudal vessels of surviving individuals, anesthesia with tricaine methanesulfonate (MS-222; ≤ 50 mg/L), into heparinized capillary tubes for GC analyses. After a 10-min centrifugation at 12,800±1,200 revolutions per minute (RPM), the plasma samples from individuals of each treatment group was pooled and frozen until GC analyses were performed.

Table 2-1. Behavior classifications used to describe the activity patterns of Florida pompano exposed to several ethylene glycol concentrations for 24 h with a 15-h recovery period following exposure.

Classification	Definition
Normal activity	Swimming activity was similar to control fish
Slightly lethargic	First signs of decreased swimming activity relative to control fish
Severely lethargic	Significantly decreased swimming activity relative to control fish
Severely stressed	No longer able to swim; still operculating
Dead	No longer operculating
Recovering	Initial signs of improvement following exposure, but activity still decreased relative to control fish
Completely recovered	Swimming activity is completely improved and is similar to control fish

2.1.3 Behavior and recovery experiments

Following the LC50 tests, two behavior and recovery experiments were conducted prior to the swimming experiments to identify a sublethal concentration of ethylene glycol from which juvenile Florida pompano could apparently recover. In recovery experiment 1, the mean BM of the fish was not determined, but the fish were similar in size to the second experiment conducted 15 d later. In recovery experiment 2, the mean (± SE) body mass of the fish was 16.9 ± 1.0 g and the mean FL was 93.6 ± 1.7 mm. Exposure of the fish to ethylene glycol was conducted in the same manner as in the LC50 tests. Behavior and mortality were recorded periodically during the 24-h exposure, as well as during a 15-h recovery period in seawater containing no ethylene glycol. At 24 h, fish were switched to clean tanks for further observation. The 15-h recovery period approximated the post-exposure time interval before the swimming tests would be initiated. In the first experiment, three fish per treatment tank were exposed to 0.0, 1.0, 1.25, and 1.5 % concentrations of ethylene glycol. In the second experiment, five fish per tank were exposed to concentrations equivalent to 100, 75, 50, and 0 % of the LC1 (i.e., 4.1, 3.1, 2.1, and 0 % ethylene glycol respectively). Behavior was recorded during both experiments and blood plasma samples for GC analysis were collected and pooled by exposure level from all surviving fish after the second experiment (N = 5 survivors for 0%, 5 for 2.1 %, 4 for 3.1 %, and 3 for 4.1 %).

2.1.4 Swimming chamber design and calibration

Three Blazka-type swimming respirometers were used in simultaneous swimming performance tests. Each unit consisted of two concentric acrylic cylinders, with the inner cylinder having an inside diameter of 20.32 cm. The volume of each unit was 104 L, and the maximum water velocity was 100 cm/s. Water flow within the swimming chamber was generated by a propeller at one end of the inner cylinder and was controlled by a variable-speed DC motor. A plastic grid was placed at each end of the inner cylinder to contain fish and to dampen turbulence in the swimming chamber.

We individually calibrated each swimming chamber to determine the relationship between RPM and water velocity (cm/s) by two separate methods, one intrusive and one unintrusive. In the intrusive method, a Marsh-McBirney model 2000 portable flowmeter was inserted into the swimming chamber near the outflow end of the inner tube. A non-contact tachometer (Monarch model 100) was used to measure RPM of the propeller shaft. Revolutions were increased by 100-RPM increments until a value of 1,700-1,900 RPM was reached. Water velocity (cm/s) was recorded at each increment. The unintrusive method involved video analysis of particles (brine shrimp eggs) moving along the central axis of the inner chamber. A video camera positioned perpendicularly to the flow recorded moving particles as RPM was increased by 100-RPM increments. The velocity (cm/s) of a randomly chosen particle at each corresponding RPM was determined using the Optimas 6.5 Imaging System. The regression relationship between RPM and water velocity for each method was determined independently for each swimming chamber (Kay 2000). The linear models produced from the video analyses:

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

The models were used to solve for the RPM setting at each desired water velocity increment used in the swimming trials.

2.1.5 Measuring critical swimming speed

Critical swimming speed before and after sublethal exposure to ethylene glycol was measured and compared for individual fish (Kolok et al. 1998). To keep track of individuals, 21 juvenile Florida pompano (mean ± SD: 23.1±4.73 g; 102.8±6.21 mm FL) were marked 11 weeks before the start of the experiment. Fish were anesthetized using MS-222 (≤ 50 mg/L) before marking. A unique scheme of colored latex marks (Northwest Marine Technology, Inc.) was applied by injection to the dorsal, caudal, and anal fins of each individual. After injection, the fish were held in the 5,000-L recirculating seawater system.

Several days prior to the experiment, the marked fish were randomly separated into groups of three, which were tested simultaneously in the three swimming chambers. Two of the seven groups were randomly assigned to the control, and the remaining five groups were assigned to

treatments. Each group was randomly assigned to one of seven 60-L, interconnected holding tanks in a recirculating system with a total volume of 550 L. The holding system was equipped with a biofilter and UV sterilizer to maintain water quality. Salinity in the holding system was maintained at 30±1 psu, and temperature was maintained at 25.0±1 °C using aquarium heaters in each tank. Holding tanks were individually aerated with air stone.

The 16-d protocol for the swimming experiment ensured identical fasting, holding, and acclimation periods for each group of fish (Kay 2000). The protocol allowed for a 17-h acclimation to the swimming chambers following the 24-h exposure and included a 41-h fasting period (i.e., 24-h exposure and 17-h acclimation) before pre- and post-exposure swimming trials. All exposures and sham exposures were carried out in one of three round fiberglass tanks, filled to 60 L with 30-psu seawater. The exposure tanks were individually aerated, and the water temperature was maintained at 25.0±1 °C with aquarium heaters. All fish were sham-exposed before the first swimming trial, but only the control groups were sham-exposed before the second swimming trial. Treatment groups were exposed to a 3.0 % ethylene glycol solution before the second swimming trial. To confirm concentrations, we collected water samples for analyses from the exposure tank at the beginning and end of each exposure period to confirm concentrations. All exposed and sham-exposed fish were placed in a bucket of clean sea water for several minutes before being placed in the swimming chambers.

During the 17-h acclimation period, the three swimming chambers were connected to a larger recirculating system that included a UV sterilizer and a biofilter. The entire swimming system was housed in a 25°C temperature-control room. The total recirculating volume of the system was about 765 L. After exposure, each fish was placed in one of the swimming chambers for the acclimation interval at a velocity of 5 cm/s. Before the start of a swimming trial, each swimming chamber was cleared of bubbles and sealed. Individuals were initially assigned randomly to a swim chamber for the pre-exposure swimming trial, but were re-tested in the same swimming chamber for the post-exposure swimming trial.

The experimental protocol tested for changes in the swimming performance of individuals exposed to ethylene glycol by determining pre- and post-exposure critical swimming speeds (U_{crit}) of each individual. The water velocity was set at 10 cm/s at time zero. During the tests, the velocity of each swimming chamber was increased by 10 cm/s every 10 min, with no rest interval between steps. Each swimming test was terminated when the fish could no longer continue swimming and made contact with the back grid for about 2-4 s. A pre- and post-exposure U_{crit} value was calculated for each individual following Brett (1964):

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

where u_i is the highest velocity maintained for the full prescribed period (10 min), u_{ii} is the highest velocity increment, t_i is the time the fish swam in the fatigue velocity interval, and t_{ii} is the prescribed period of swimming. All fish that failed to fatigue after 10 min of swimming at the swimming chamber's maximum speed (100 cm/s) were assigned a U_{crit} value of 100.0 cm/s. After each post-exposure swimming trial, the fish were anesthetized with MS-222 (≤ 50 mg/L), and body masses and fork lengths were measured. Blood was drawn from the caudal vessels into heparinized capillary tubes, and centrifuged in the same manner as the LC50 blood samples. Plasma was combined for the three fish from each group and frozen for GC analysis.

2.1.6 Statistical analysis

The U_{crit} values were expressed in absolute (cm/s) and relative (body lengths per second, BL/s) terms. Statistical analyses were performed on the relative values of U_{crit} to standardize for small variations in individual size. A two-factor analysis of variance (ANOVA) with a randomized block design tested for differences in pre- and post-exposure relative U_{crit} values within and between treatment and control groups. Pre- and post-exposure relative U_{crit} values were paired for each individual, with the fish identification number as the random blocking variable. Differences between the least square means were calculated for all possible combinations of the treatment and control groups. Residuals were tested for normality, and no transformations were required. We used SAS computer software (SAS Institute 1996) to test the model, $Y_{ijk} = \mu + \tau 1_i + \tau 2_j + \beta_k + (\tau 1\ \tau 2)_{ij} + \varepsilon_{ijk}$, where $\tau 1$ is the trial treatment variable (pre or post), $\tau 2$ is the group treatment variable (treatment or control) and β is the random blocking variable (individual fish).

We evaluated individual variation in the performance of treatment fish by testing for the influence of size on absolute U_{crit}. We also used a linear regression analysis (SAS Institute 1996) to test for a relationship between body mass and percentage reduction in the absolute U_{crit} of treatment fish.

2.2 Results

2.2.1 Acute toxicity tests

The 24-h LC50 value was identified for juvenile Florida pompano over a series of experiments that included 11 concentrations ranging from 0% to 10.0 % (volume per volume) ethylene glycol. In the second and third experiments, mortality of all 10 fish was observed at concentrations above 7.16 %, and no sublethal behavioral effects were observed at concentrations below 1.0 %. At concentrations of 1.0-7.16 %, 21 individuals exhibited lethargic behavior, 31 were severely stressed and 10 died. Combined data from the second and third experiments resulted in a 24-h LC50 estimate of 5.63 % (Table 2-2). The lowest experimental concentration at which mortalities were observed was 4.52 %. The simple linear regression of ethylene glycol plasma concentrations on exposure concentrations from eight tanks with surviving individuals was positive and significant ($F=59.167$; $df = 1, 6$; $P<0.0003$; $r^2=0.91$).

Table 2-2. Calculated lethal concentrations (LC; % volume per volume) and associated 95% confidence limits for juvenile Florida pompano exposed to ethylene glycol for 24h.

	Concentration	95 % Confidence limits	
LC level		Lower	Upper
1	4.12	2.776	4.650
5	4.51	3.390	4.955
10	4.74	3.765	5.135
15	4.90	4.036	5.266
50	5.63	5.216	6.084
85	6.47	6.005	7.890
90	6.68	6.157	8.459
95	7.02	6.379	9.397
99	7.69	6.796	11.479

2.2.2 Behavior and recovery experiments

Although slightly to severely lethargic behavior was observed in treatment fish at ethylene glycol concentrations of 1.0% and 1.77 % during the LC50 experiments, sublethal behavioral effects were not observed at any of the concentrations (0, 1.0, 1.25, and 1.50 %) used in the first behavior-recovery experiment. This possible improvement in tolerance may have been due to an increase of 8.93 g in the mean body mass of the fish by the time of this behavioral experiment.

Noticeable differences in behavior were observed between fish exposed to each concentration used in the second behavior-recovery experiment. A 2.1 % concentration was the lowest at which lethargic behavior was observed at 24 h (Table 2-3). A 4.1 % concentration produced severe stress at 24 h and the death of two fish before the end of the 15-h recovery period. At the 3.1 % concentration, the death of one fish was observed only 7 h into the experiment. At 24 h, three of the remaining fish were severely lethargic and disoriented and the fourth was in severe

stress. All four fish, however, improved to a slightly lethargic state by the end of the 15-h recovery period. Because the next highest concentration (4.1 %) had produced no deaths at 24 h, it was concluded that the single mortality at the 3.1 % concentration might have been due to the random inclusion of a stressed, highly susceptible, or unhealthy individual. Therefore, we selected a 3.0 % concentration as the suitable sublethal exposure for the critical swimming experiments.

Table 2-3. Comparative mortality and behavior of juvenile Florida pompano after 24-h exposure to treatment (2.06-4.12 %) and control (0%) levels of ethylene glycol and after a 15-h of recovery in clean seawater. Five fish were exposed to each concentration.

Ethylene glycol Concentration	Mortality and behavior After 24-h exposure	Mortality and behavior After 15-h recovery
0.00	All normally active	All normally active
2.06	All severely lethargic	All slightly lethargic
3.09	3 severely lethargic; 1 severely stressed; 1 dead	All severely lethargic
4.12	2 severely lethargic; 3 severely stressed	2 severely lethargic; 1 severely stressed; 2 dead

Gas chromatographic analyses of initial and final water samples from the exposure tanks showed that analyzed concentrations were within 0.3 % of the calculated concentrations. Ethylene glycol concentrations in blood plasma collected at 15-h post-exposure from all surviving fish exposed to concentrations of 0, 2.1, 3.1 and 4.1 % were 0, 0.37, 0.40 and 0.89 %, respectively, based on GC analysis. The linear regression of plasma concentration on exposure concentration showed a marginally significant increase in plasma concentration as exposure concentration increased ($F=16.156$, $df=2$, $P<0.0567$, $r^2=0.8898$).

2.2.3 Critical swimming speed

A 14 % reduction in relative swimming performance was observed in fish exposed to 3.0 % ethylene glycol. The mean (± SE) critical swimming speed of treatment fish (N=15) was 95.9±1.13 cm/s (9.3±0.17 BL/s) in the pre-exposure trial and 83.0±3.45 cm/s (8.0±0.26 BL/s) in the post-exposure trial. The mean critical swimming speed of control fish (N=6) was 94.1±3.42 cm/s (9.2±0.17 BL/s) in the pre-exposure trial, and 98.2±1.28 cm/s (9.6±0.23 BL/s) in the sham post-exposure trial. These critical swimming speeds are somewhat conservative because fish that did not fatigue after 10 min of swimming at 100 cm/s were assigned a U_{crit} value of 100.0 cm/s. Six of the control fish (three pre- and three post-exposure) and seven of the treatment fish (six pre- and one post-exposure) were assigned a U_{crit} value of 100 cm/s.

Least square means from the ANOVA confirmed the negative effects of ethylene glycol on swimming performance. Pre- and post-exposure U_{crit} differed significantly in the treatment group ($P<0.0002$), but not in the control group ($P<0.3492$). Nevertheless, a slight increase of 0.4

BL/s in the control group may have been due to training or conditioning effects. No difference in pre-exposure U_{crit} was detected between the treatment and control groups (P<0.6693), but a significant difference in post-exposure U_{crit} between the treatment and control groups (P<0.0004) was found. In addition, we detected a significant difference between the pre-exposure performance of the control group and the post-exposure performance of the treatment group (P<0.0058) but not between the pre-exposure performance of the treatment group and the post-exposure performance of the control group (P<0.4843).

Pre- and post-exposure swimming performance varied considerably among individuals (Figure 2-1). Of the 15 treatment fish, only one individual showed an increase in relative U_{crit} of 1.4 BL/s after exposure (Figure 2-2). For treatment fish, performance declined and variability increased after exposure. In the control group, one individual decreased its performance by 0.3 BL/s, three individuals improved, and two individuals outperformed the maximum test velocity in both the pre- and post-exposure trials.

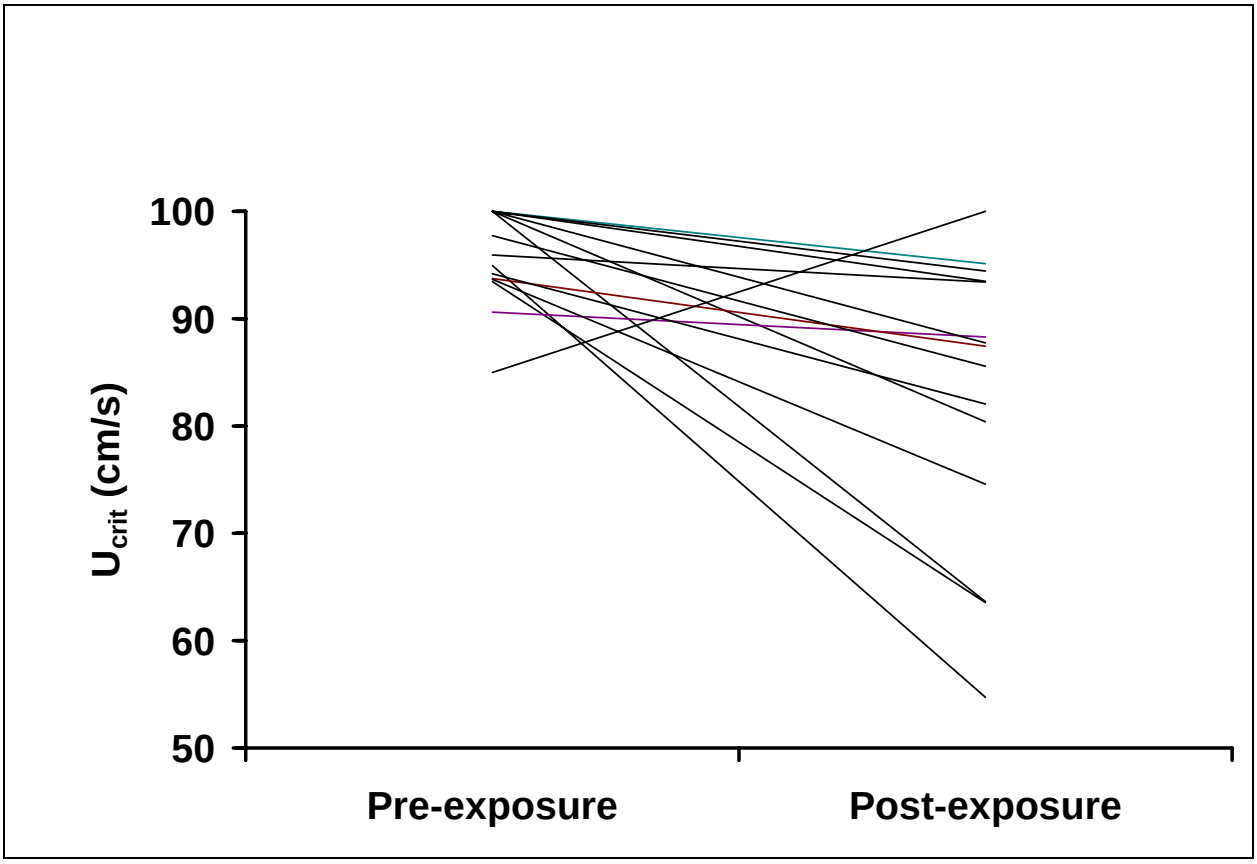


Figure 2-1. Individual variation in the critical swimming speeds (U_{crit}) of juvenile Florida pompano (N=15) before and after a 24-h exposure to 3.0 % ethylene glycol.

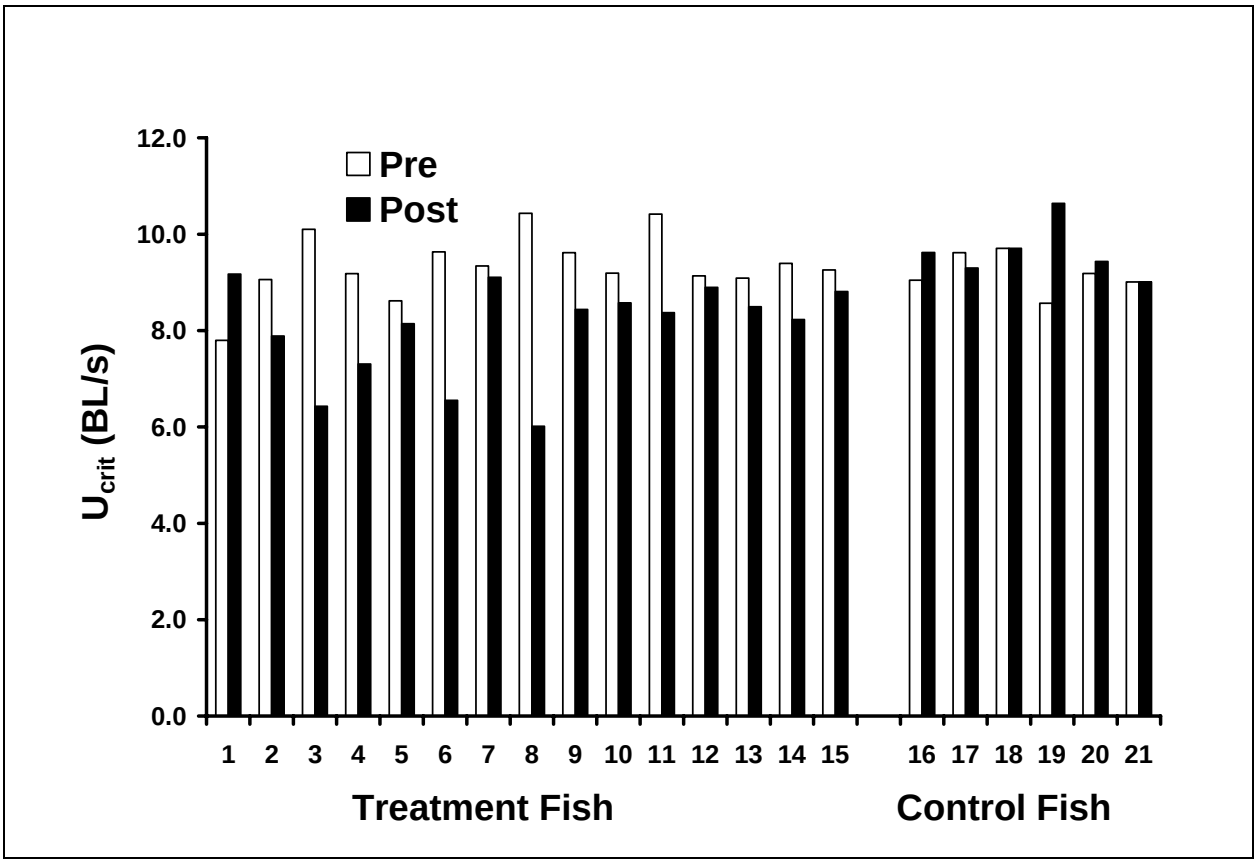


Figure 2-2. Critical swimming speeds (U_{crit}; body length/s) of juvenile Florida pompano before and after a 24-h exposure to 3.0 % ethylene glycol (treatment fish, 1-15) and before and after sham exposure (control fish, 16-21).

The post-exposure reduction (%R) in absolute U_{crit} was not randomly distributed among the exposed fish, but was linearly related to body mass (%R = - 53.5 + [1.7 x body mass], R^2 = 0.3148, df = 13, P<0.0295). Smaller fish experienced a greater percent decrease in swimming performance than did larger fish (Figure 2-3).

Calculated exposure concentrations were verified using GC analysis. Ethylene glycol concentrations did not differ between mean initial and final water samples taken from each 24-h exposure period (*P*<0.0897). Gas chromatographic analysis showed that there was a mean (± SE) ethylene glycol concentration of 3.0±0.04 % for the treatment exposure solutions. Analysis of pooled blood plasma collected approximately 20 h post-exposure (N=5) indicated a mean (± SE) ethylene glycol concentration of 0.3±0.02 % for the exposed fish. No ethylene glycol was detected in the pooled plasma of the control fish (N=2).

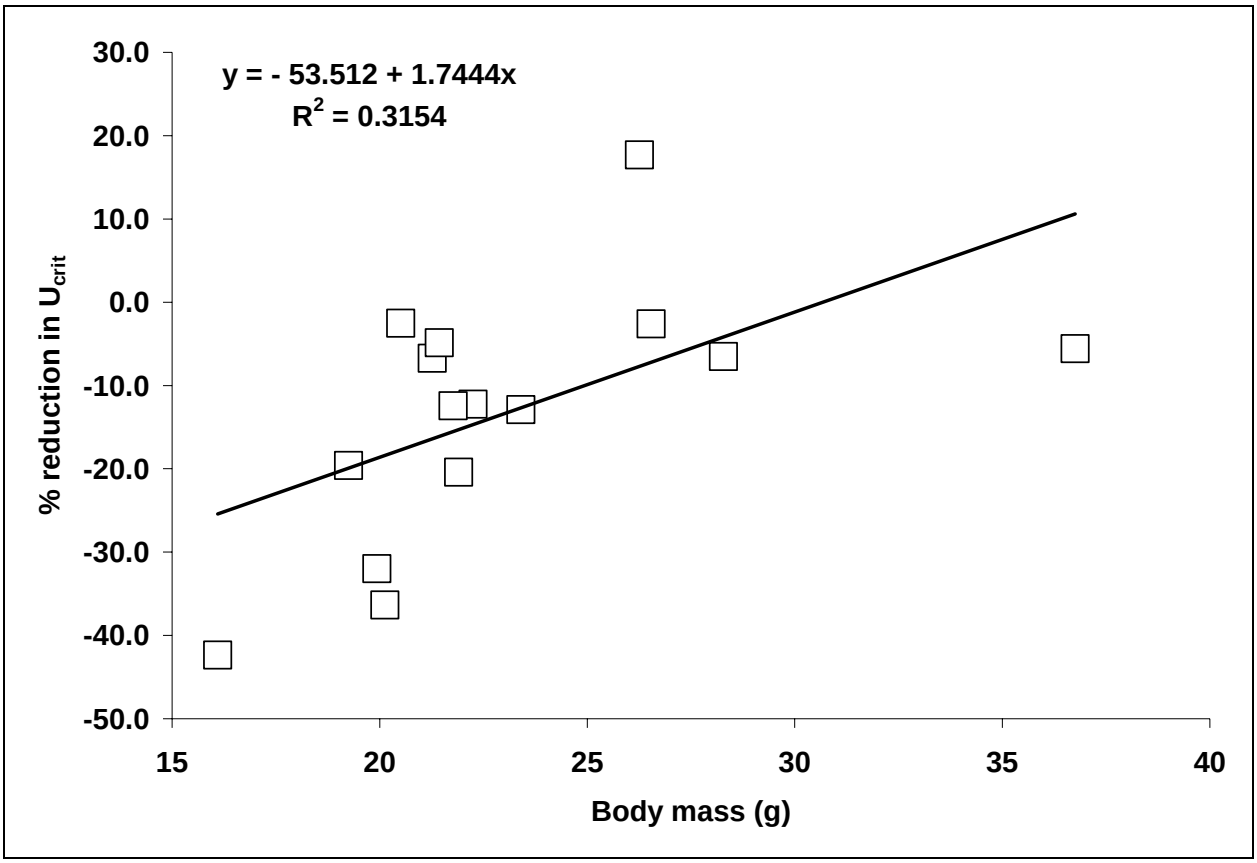


Figure 2-3. The relationship between body mass of Florida pompano (*N*=15) and percentage reduction in critical swimming speed (U_{crit}) after exposure to 3.0 % ethylene glycol.

2.3 Discussion

A 14 % reduction in the relative swimming performance of juvenile Florida pompano was observed in individuals exposed to 3.0 % ethylene glycol for 24 h and allowed to recover for 17 h before testing. Other sublethal effects included lethargic and disoriented behavior at concentrations below 3.0 %. Exposure did not affect the performance of all individuals to the same degree. Variation in critical swimming velocity was negatively correlated with body size and swimming performance of smaller individuals was significantly reduced compared to that of larger individuals.

2.3.1 Individual variation in U_{crit}

Though mean critical swimming speed of the treatment group decreased by 14 % after exposure, considerable individual variation in performance was detected in both the treatment and control groups. Swimming performance studies examining sublethal effects of toxicants commonly compare mean performances of control and treatment groups, but such an approach fails to address within-group variation. Experimental design that addresses within-group variation are valuable because swimming performance of individual fish is repeatable over time (Kolok 1992;

Kolok et al. 1998; Reidy et al. 2000). The two-factor ANOVA with pre- and post-exposure blocks uses this repeatable source of individual variation to analyze treatment effects.

Few prior studies have quantified the effect of a stressor by measuring the pre- and post-exposure U_{crit} of individual fish pre- and post-exposure. In juvenile largemouth bass *Micropterus salmoides* exposed to a 10 ºC decline in water temperature (from 20 ºC to 10 ºC) between swim-performance trials, U_{crit} was reduced by a consistent amount in all individuals (Kolok 1992). Similarly, Kolok and Farrell (1994) found that the U_{crit} of northern pikeminnow *Ptychocheilus oregonensis* after a surgical procedure was reduced from pre-surgery values by a consistent amount in all fish. The results of a study on fathead minnows (Kolok et al. 1998) contrast with those mentioned above. In that study, swimming performance was evaluated before and after an exposure to field-collected sediments contaminated with heavy metals and hexachlorobenzene. Post-exposure U_{crit} varied widely, with some individuals experiencing profound reductions in U_{crit} and others maintaining their performance level regardless of the exposure. Our results resembled those of Kolok et al. (1998), in that the response of individual fish varied greatly, from no effect to a severe effect. This differential response of individuals was not, however, randomly distributed, but was at least partially correlated with body mass (Figure 2-3).

2.3.2 Size effects

Size effects among the Florida pompano used in this study were detected during the toxicity and behavioral tests through behavioral observations made during and after exposure. Behavioral effects of ethylene glycol appeared at lower concentrations in the smaller fish used in the LC50 tests than in the larger fish used in the behavioral experiments. These results suggest that toxicity may decrease with increasing size in Florida pompano, in contrast to results a study on rainbow trout in which ethylene glycol was more acutely toxic to larger individuals (Greene and Kocan 1997). Size effects often vary from one species to another for a given toxicant (Sprague 1990).

It is unclear why ethylene glycol was more toxic to the smaller fish than the larger ones. One possibility is that the rate of ethylene glycol uptake is greater in smaller fish due to their surface-area-volume-ratio. An alternative hypothesis would be that more of the toxic intermediates build up in smaller fish.

2.3.3 Mode of action

The reduction in swimming performance of Florida pompano in this study may have been due to the buildup of ethylene glycol metabolic products. Studies on mammals have shown that ethylene glycol is sequentially oxidized in the liver (Chou and Richardson 1978) and involves several metabolites, with glycolate (glycolic acid) playing a major role in ethylene glycol toxicity. A greater toxicity for metabolic intermediates is also supported by Greene and Kocan (1997). In that study, inhibition of the enzyme alcohol dehydrogenase (the enzyme responsible for the first step of ethylene glycol metabolism) reduced ethylene glycol toxicity in rainbow trout, while inhibition of aldehyde dehydrogenase (the enzyme responsible for the second step in ethylene glycol metabolism) increased ethylene glycol toxicity.

Ethylene glycol may alternatively decrease U_{crit} by evaluating metabolic rate, and decrease the energy available for swimming. Jones (1971) proposed several possible factors, including an inability to exchange enough oxygen at the gills, to deliver enough oxygen to the tissues, to remove metabolic products, or to activate enzymic processes. A reduction in the ability to transfer oxygen across the gills would effectively decrease the scope for active metabolism, thereby reducing the fish's capacity for sustained swimming activity. Our study does not indicate whether gill damage directly influenced the reduction in swimming performance of exposed individuals. Histological analysis showed a definite effect of 10.0 % ethylene glycol exposure, but effects at lower concentrations may have originated from the use of MS-222 before sacrifice (J. Hawke, LSU Veterinary School, personal communication).

2.3.4 Ethylene glycol toxicity

The estimated 24-h LC50 value (5.63 %) for Florida pompano exposed to ethylene glycol cannot be directly compared to 96-h estimates for bluegill sunfish (2.75%; Abdelghani et al. 1990), fathead minnow (6.83 to 7.29 %; Pillard 1995; Greene and Kocan 1997), and rainbow trout (6.08 %; Greene and Kocan 1997; Table 2-4). However, in our study the LC50 was achieved in one-fourth the time at comparable concentrations on much larger individuals indicating that Florida pompano are relatively more sensitive than the minnow and trout tested previously. The toxicity values of different studies can also be compared by using an index of toxicity to estimate 1 h LC50 values (Boehm et al. 2001). Thus, assuming a linear-response model index (i.e., concentration x time), the LC50 for a 1-hr exposure is four to five times lower for Florida pompano, indicating that ethylene glycol was considerably more toxic to the marine species in this study than to some freshwater fishes that have been tested. Tolerance to many toxicants is believed to vary less among fishes (often five- or tenfold) than among other taxonomic groups, such as invertebrates (Sprague 1990).

Table 2-4. Estimated LC50 values (mean +/- SE) for several fish species exposed to ethylene glycol.

Species	Mean body mass (g)	Test duration (h)	LC50 %)	Reference
Bluegill sunfish (*Lepomis macrochirus*)	0.9±0.60	96 h	2.75	Abdelghani et al. 1990
Fathead minnow (*Pimephales promelas*)	(=7 days old)	96 h	7.29	Pillard 1995
Fathead minnow	0.1±0.01 0.1±0.01	96 h 96 h	6.83 6.95	Greene and Kocan 1997
Rainbow trout (*Oncorhynchus mykiss*)	0.7±0.20 1.5±0.50	96 h 96 h	6.08 5.65	Greene and Kocan 1997
Florida pompano (*Trachinotus carolinus*)	8.0±3.20	24 h	5.63	Kay et al. 2000 (present study)

2.3.5 Marine exposures

The high LC50 values of ethylene glycol reported in this and other studies on fishes generally reflect the relatively low toxicity of this chemical. Marine fishes would most likely be exposed to lethal concentrations only in the event of highly localized, large-scale releases into the environment. Marine releases of considerable magnitude have been reported (Boehm et al. 2001). For example, 13,523 L of ethylene glycol leaked into the Gulf of Mexico from an underwater pipeline in June of 1998 (National Response Center 2002). Exposures of marine fishes are likely brief because of the open nature of marine systems and the relatively rapid biodegradation of ethylene glycol (half life ~ 32 d, see Boehm et al. [2001]). Biooxidation of ethylene glycol in the laboratory was 39 % in 5 d and 96 % in 20 d (Conway et al. 1983); dispersion and dilution of ethylene glycol in the marine environment should be even more rapid. Other factors affecting the toxicity of ethylene glycol must also be considered, such as additive or synergistic effects with other chemicals. The toxicity of ethylene glycol increases in freshwater fishes when sublethal levels of thiram, a component of an agricultural seed-protectant, are present (Greene and Kocan 1997), and when it is combined with other chemicals in a deicing agent (Pillard 1995). Boehm et al. (2001) simulated an ethylene glycol spill of 402,902 L (106,447 gal) from a deepwater supply line near the shallow end (water depth, 113 m) and found that the predicted exposure concentrations were an order of magnitude lower than the lowest published 48-h LC50 values (see Pillard [1995]; 34,400 mg/L for a freshwater crustacean, *Ceriodaphnia dubia*). However, we found a relatively lower LC50 for Florida pompanoas well as substantial and significant sublethal effects on swimming performance below the LC1 level (Table 2-2).

2.3.6 Threshold of tolerance

The variation between individuals in the present study suggests that some fish may have a higher threshold of tolerance to ethylene glycol toxicity. Many studies have estimated threshold values for fish exposed to contaminants by testing swimming speed at a range of exposure concentrations (Peterson 1974; Nikl and Farrell 1993). Because this study tested fish at only one ethylene glycol concentration, we were unable to calculate a mean threshold value for this species. Nevertheless, we can estimate, based on significant reduction in swimming speed, that the threshold lies at or below the 3.0 % ethylene glycol concentration (53 % of the estimated LC50 for slightly smaller fish).

Though adverse effects on critical swimming speed were only quantified at an exposure concentration of 3.0 %, Florida pompano exposed to slightly lower concentrations (1.0-2.1 %) exhibited changes in behavioral patterns. Changes in behavior patterns may have ecological implications for individuals, and potentially for populations subject to chronic exposure. Sublethal behavioral effects were not observed at ethylene glycol concentrations below 1.0 % in the LC50 experiments, or below 2.1 % in the behavior and recovery experiments (which used somewhat larger fish). In a preliminary swimming trial conducted prior to this study, a 1.5 % ethylene glycol exposure did not produce an obvious reduction in the critical swimming speeds of two out of three individuals. Future studies may want to examine the effects of ethylene glycol concentrations between 1.5 % and 3.0 % on critical swimming speed to determine a threshold tolerance value this species.

2.3.7 Ecological consequences of sublethal effects

Most studies of petroleum-related contaminants have concentrated on the mortality effects associated with major oil spills (Howarth 1991; Kennicutt et al. 1996; Peterson et al. 1996), but much remains unknown about the effects of low-level exposure on marine organisms. Sublethal effects of contaminant exposure are inherently more difficult to assess and extrapolate to the field. We have shown that individual marine fish can experience reduced swimming performance when exposed to sublethal levels of ethylene glycol. The implications of these results include adverse effects on the ability to perform in ecological situations. While normal cruising speeds of Florida pompano are not near the high critical swimming speeds observed in this study, the capacity for burst swimming and endurance were undoubtedly reduced by ethylene glycol exposure, with direct bearing on the efficiency of prey capture and predator avoidance.

An understanding of sublethal effects is important for the accurate prediction of the impacts to habitat and fish populations because many more organisms are likely to be exposed to sublethal concentrations of contaminants than to lethal concentrations as a spill disperses and because many may experience chronic low levels of one or more contaminants over time. For example, in a study of fishes surrounding oil production platforms, Grizzle (1986) found histological changes in the gills that possibly reflected chronic, low-level exposure to petroleum or petroleum additives.

Extrapolation of sublethal effects from the individual level to the population level remains problematic (Rose et al. 1993), particularly when exposure causing sublethal effects for one size-class are much more toxic (perhaps fatal) to smaller individuals. Mortality can be highly compensatory in fish populations, especially in early life history stages. High levels of mortality from an episodic event, such as a contaminant spill, may not necessarily have a significant effect at the population level, if significant compensation in the mortality process occurs (Houde 1989). For example, individual-based models of contaminant exposures demonstrate that chronic exposures are more likely than episodic exposures to affect recruitment of striped bass *Morone saxatilis* (Rose et al. 1993).

Nevertheless, episodic exposures could have a greater impact on recruitment if they occur at or shortly after a spawning peak or in a discreet nursery area, such as an estuary. Sublethal impacts, such as those documented for swimming performance of Florida pompano, are even more difficult to extrapolate to the population level without sophisticated models and details of exposure conditions (time, contaminant concentration, life stage, and ecological interactions). Currently, deepwater oil production uses ethylene glycol and other pure chemicals and proprietary chemical mixtures as additives to facilitate production. These chemicals and mixtures will also need to be evaluated individually and in concert to achieve an accurate assessment of the potential sublethal and lethal effects of contaminant exposures to marine fish populations. We found a substantial and significant sublethal effect on individual swimming performance from exposure to ethylene glycol concentrations below the LC1 level that is cause for concern.

3.0 TOXICITY AND SUBLETHAL EFFECTS OF METHANOL ON SWIMMING PERFORMANCE OF JUVENILE FLORIDA POMPANO

D. M. Baltz[1,2], E. J. Chesney[3], M. A. Tarr[4], A. S. Kolok[5] and M. J. Bradley[3]

[1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803-7503

[2]Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503

[3]Louisiana Universities Marine Consortium, Chauvin, LA 70344

[4]Department of Chemistry, University of New Orleans, New Orleans, LA 70148

[5]Biology Department, University of Nebraska at Omaha, Omaha, NE 68182

Marine fishes face an ever-changing array of environmental contaminants that may reduce their ability to perform in ecological situations. In the northern Gulf of Mexico, the development of new technologies has allowed expansion of oil and gas production into deep water (> 305 m) and has created the need to evaluate fishery-related risks associated with new practices and chemicals used in petroleum production and transport (Minerals Management Service, www.gomr.mms.gov; Boehm et al. 2001). Marine fishes are potentially at risk from accidental releases of chemical additives used in deepwater wells to prevent the formation of gas hydrates (Anonymous 1996, 2000; Herzhaft and Dalmazzone 2000; Boehm et al. 2001), which can potentially clog pipelines. A variety of chemicals, such as ethylene glycol (Hymel et al. 2002) and methanol, may be transported in small supply lines up to 96.5 km in length, stored in large volumes and used at high rates. Though not responsible for producing direct mortality because of relatively low concentrations or toxicities, sublethal impacts of spills or leaks may affect scope for activity (Cech 1990), swimming performance (Brett 1964, Wedemeyer et al. 1990), growth, reproduction, and survival of individuals (Little et al. 1990). Quantification of sublethal effects at the individual level is an important step in predicting how individuals and populations may respond ecologically to single and multiple contaminants.

The environmental effects of methanol have been widely studied in relation to its use as an industrial organic solvent and reactant in organic procedures (Tephly 1991). Median lethal concentrations (LC50) have been reported for various marine algae and invertebrates (Boehm et al. 2001). The toxicity of methanol to fishes has been assessed on relatively few species, primarily freshwater (Gillette et al. 1952, Nishiuchi and Hashimoto 1967, Buzzell et al. 1968, Juhnke and Luedemann 1978, Linden et al. 1979, Johnson and Finley 1980, Call et al. 1983, Veith et al. 1983, Bengtsson et al. 1984, Ewell et al. 1986, Mayer and Ellersieck 1986, Poirier et al. 1986, Tsuji et al. 1986, Robertson et al. 1988) and two marine or salt-tolerant species,

hooknose *Agonus cataphractus* (Portmann and Wilson 1971) and bleak *Alburnus alburnus* (Tarkpea and Svanberg 1982).

Chemical spills in aquatic systems can lead to environmental effects that range from direct mortality from lethal exposure levels to sublethal effects as contaminants spread and are diluted. Important effects of sublethal exposure on individuals cannot be predicted from toxicity tests alone; however, sublethal effects may be evaluated by measures of individual performance (Sprague 1971) that relate to Darwinian fitness (Plaut 2001). For example, diminished swimming performance can lead to increased risks of mortality or an impaired ability to perform normal life functions (Schreck 1990) and subsequently add to the risk of a decline in a population (Heath 1995). Changes in critical swimming speed (U_{crit}) have been used as a performance indicator in many studies assessing sublethal effects of contaminants on fishes (Beaumont et al. 1995; Heath 1995; Kolok et al. 1998; Nikl and Farrell 1993). Swimming performance has ecological significance and has been shown to affect the ability of larval fishes to avoid predators and catch prey (Zhou et al. 1996; Samson et al. 2001), but the same has not been demonstrated for larger individuals (Plaut 2001). Vulnerability to predation is dynamic and can be affected by exposure to contaminants, resulting in effects on mobility (Beauvais et al. 2000), perception (Baker and Montgomery 2001), and behavior (Weis et al. 1999, 2001; Scholz et al. 2000; Wibe et al. 2001).

The Florida pompano *Trachinotus carolinus* was chosen for assessment of acute and sublethal effects of exposure to methanol because the species occurs throughout the coastal zone in the vicinity of petroleum production structures and transport pipelines. Juveniles are abundant in and near the surf zone during summer (Bellinger and Avault 1970). Adults can be found in inshore and offshore waters and are believed to spawn on the continental shelf (Gilbert 1986). It is also representative of the jack family (Carangidae) with many species that reside near deepwater structures in the Gulf of Mexico and are of considerable economic importance to recreational and commercial fisheries (Gilbert 1986).

We assessed the acute and sublethal effects of methanol on juvenile Florida pompano by identifying a short-term LC50 and a range of lower sublethal concentrations and by testing for changes in U_{crit} following sublethal exposure. The objectives were to estimate the static 24-h LC50, use it to identify sublethal exposure levels from which individuals could recover after short-term exposures expected in open marine systems (Boehm et al. 2001), and then to evaluate within-individual changes in swimming performance before and after sublethal exposure.

3.1 Methods

Collection and maintenance of fish.--Juvenile Florida pompano were collected for experiments in August 1999 from Port Fourchon Beach and Isle Dernieres, Louisiana, with a 3.05 m (10 ft) beach seine. Individual body mass at the time of capture ranged from 2.0 to 16.0 g. Fish were held for at least 4 weeks in a 5,000-L recirculating seawater system at 30 practical salinity units (psu) before testing to allow them to acclimate to captivity and recover from the stress of capture. During their initial quarantine, the fish were briefly treated with 0.2 mg/L copper (Cutrine-Plus) to eliminate ectoparasites, especially *Amyloodinium*, a parasitic dinoflagellate known to cause mass mortality in captive fishes (Cheung 1993). The recirculating holding system was constantly aerated and water quality parameters, including pH, salinity, and ammonia level, were monitored several times a week and daily during experiments, and adjusted

as necessary to maintain suitable conditions. Amquel (HOCH2SO3Na) was added to holding systems to reduce the ammonia level when it exceeded 0.5 mg/L. Fish were fed a commercially produced feed (0.25 g/day per individual; 45% protein, 8% lipid) except within the 17-h prior to and during acute toxicity and swimming experiments.

Acute toxicity and recovery tests.-- The results from a preliminary toxicity experiment were used to select a range of concentrations, 0.99 to 1.51% methanol (volume per volume, % v/v), that was logarithmically divided into the six concentrations, 0.99, 1.07, 1.17, 1.27, 1.38, and 1.51%. A 24-h static LC50 experiment was conducted in a system of eight conical fiberglass tanks. Each tank was filled to 70 L with ultra-violet (UV) sterilized, 30 psu seawater. The tanks were individually aerated, maintained at 25.0±1°C with aquarium heaters, and covered with clear acrylic lids to reduce volatilization of methanol. The exposure concentrations were randomly assigned to the tank array. A 'blank' tank without fish was also included to monitor the mixing and concentration of the chemical over the 24-h period. Ten fish were randomly assigned to each of the exposure and control tanks and they were introduced after the addition of methanol (Fisher Scientific, Certified ACS). The mean (± SE) body mass and fork length (FL) were 33.2 ± 2.4 g and 110 ± 3.1 mm FL. We maintained a 20-min interval between sequential introductions to allow for processing of fish at the end of the experiment. The behavior of the fish in the treatment groups, relative to the controls, was observed and classified following Hymel et al. (2002). Water samples (1.5 mL) for gas chromatography (GC) analysis, taken periodically from each tank during the tests, showed that the methanol exposure solutions were accurately estimated, well mixed, and stable throughout the 24-h experiment. Data from the experiment was used to estimate LC values using the EPA Probit Analysis Program, Version 1.5 (EPA 1994).

We collected blood samples from surviving fish to analyze the effects of methanol exposure. At the end of the experiment, individuals were anesthetized with tricaine methanesulfonate (MS-222; <50 mg/L), blood was drawn from the caudal vessels, and collected into heparinized capillary tubes for GC analyses. After a 10-min centrifugation at 12,800 ± 1,200 revolutions per minute (RPM), plasma samples from individuals of each treatment and control group were pooled and frozen until GC analyses were performed. Prior to all statistical analyses of LC50 data and blood samples, estimated methanol concentrations were measured by GC analysis. Following the LC50 tests, a behavior-recovery experiment was conducted prior to the swimming experiment to identify a sublethal concentration from which juveniles could apparently recover over 15 h in clean seawater (Hymel et al. 2002).

Critical swimming speed.-- Three Blazka-type swimming respirometers were used to simultaneously test swimming performances of 3 fish (Hymel et al. 2002). Critical swimming speed before and after sublethal exposure to methanol was measured and compared for individual fish (Kolok et al. 1998). To keep track of individuals, 24 juveniles (mean ± SE; 20.5 ± 0.9 g body mass; 100.8 ± 1.6 mm FL) were marked two weeks before the start of the experiment. Fish were anesthetized (MS-222 < 50 mg/L) and a unique combination of colored latex marks (Northwest Marine Technology, Inc.) were applied by injection to their dorsal, caudal, and anal fins. After injection, the fish were held in the 5,000-L recirculating seawater system.

Several days prior to the experiment, the marked fish were randomly separated into groups of three, which were tested simultaneously in the swimming chambers. Two of the eight groups

were randomly assigned to the control, and the remaining six groups were assigned to the treatment. Each group was randomly assigned to one of eight 60-L, interconnected holding tanks in a recirculating system with a total volume of 550 L. The holding system was equipped with a biofilter and UV sterilizer to maintain water quality. Salinity in the holding system was maintained at 30 ± 1 psu, and temperature was maintained at 25 ± 1°C. Holding tanks were individually aerated with air stones.

The protocol for the swimming experiment required 27 days to ensure identical fasting, holding, and acclimation periods for each group of fish, and all pre- and post-evolutions occurred at an interval of seven days. The protocol allowed for a 17-h acclimation to the swimming chambers following the 24-h exposure and included a 41-h fasting period (i.e., 24-h exposure and 17-h acclimation) before pre- and post-exposure swimming trials. All exposures and sham exposures were carried out in one of three round fiberglass tanks, filled to 60 L with 30 psu seawater. The exposure tanks were individually aerated, and the water temperature was maintained at 25 ± 1°C. All fish were sham exposed before the first swimming trial, but only the control groups were sham exposed before the second swimming trial. Treatment groups were exposed to a 1.07% methanol solution before the second swimming trial. To confirm concentrations, we collected water samples for analyses from the exposure tank at the beginning and end of each exposure period. All exposed and sham-exposed fish were placed in a bucket of clean seawater for several minutes before being placed in the swimming chambers.

During the 17-h acclimation period, the three swimming chambers were connected to a larger recirculating system that included a UV sterilizer and a biofilter. The total recirculating volume of the system was about 765 L. The entire swimming system was housed in a 25°C temperature-controlled room. After exposure, each fish was placed in one of the swimming chambers for the acclimation interval and the water circulated at a velocity of 5 cm/s. Before the start of a swimming trial, each swimming chamber was cleared of bubbles and sealed. For the pre-exposure swimming trials individuals were randomly assigned to a swim chamber, but were re-tested in the same swimming chamber for the post-exposure trials.

The experimental protocol tested for changes in the swimming performance of individuals exposed to methanol by determining pre- and post-exposure U_{crit} of each individual. At the start of each trial, water velocity was set at 10 cm/s. During the tests, the velocity of each swimming chamber was increased by 10 cm/s every 10 min, with no rest interval between steps. Each swimming test was terminated when the fish could no longer continue swimming and made contact with the back grid for about 2-4 s. A pre- and post-exposure U_{crit} value was calculated for each individual following Brett (1964):

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

where u_i is the highest velocity maintained for the full prescribed period (10 min), u_{ii} is the highest velocity increment, t_i is the time the fish swam in the fatigue velocity interval, and t_{ii} is the prescribed period of swimming. A fish that failed to fatigue after 10 min of swimming at the swimming chamber's maximum velocity (100 cm/s) was assigned a U_{crit} value of 100.0 cm/s. After each post-exposure swimming trial, the fish were anesthetized with MS-222 (< 50 mg/L), and then weighed (g) and measured (FL mm).

Statistical analysis. --The U_{crit} values were expressed in absolute (cm/s) and relative (body lengths per second, BL/s) terms. Statistical analyses were performed on the relative values of U_{crit} to standardize for small variations in individual size. We used the Mixed Procedure in SAS (Littell et al. 1996) to evaluate relative U_{crit} (BL/s) with Group (i.e., treatment and control) and Trial (i.e., pre- and post-exposure performances of an individual) and their interaction (Group*Trial) as fixed factors and individual fish within groups as the randomized factor. Posterior testing was accomplished by comparing differences in least square means among six Group*Trial comparisons. Residuals were tested for normality and no transformations were required. The analysis was performed using SAS computer software (SAS Institute, Version 6.12 1996) to test the model, $Y_{ijk} = \mu + t1_i + t2_j + \beta_k + (t1t2)_{ij} + \epsilon_{ijk}$, where t1 is the 'Trial' variable (pre or post), t2 is the 'Group' variable (treatment or control), t1t2 is the interaction, and β is the random blocking variable (individual fish). We evaluated individual variation in the performance of treatment fish by testing for the influence of size on absolute U_{crit}. We also used a linear regression analysis (SAS Institute 1996) to test for a relationship between body mass and percentage reduction in absolute U_{crit} of treatment fish.

3.2 Results

3.2.1 Acute toxicity and recovery tests

The 24-h LC50 value was identified for juvenile Florida pompano over a series of seven test concentrations determined by GC analysis: 0.00, 0.99, 1.13, 1.22, 1.36, 1.46, and 1.49% (% v/v) methanol. Probit analysis of data from the experiment yielded a 24-h LC50 estimate of 1.28% (Table 3-1). Mortality of all 10 fish was observed at a concentration of 1.49%. The lowest experimental concentration at which mortalities were observed was 1.13%. The simple linear regression (Figure 3-1) of pooled methanol plasma concentrations (% v/v) on exposure concentrations from five treatment levels with surviving individuals and the control was positive and significant ([in plasma] = -0.02 + 0.41[in water]; $R^2 = 0.67$; N = 6, $P < 0.045$).

Table 3-1. Calculated lethal concentrations (LC, % v/v) values and associated 95% confidence limits for juvenile Florida pompano exposed to methanol for 24 h.

	Exposure Concentration	95% Confidence limits	
LC level		Lower	Upper
LC1	0.958	0.757	1.058
LC5	1.044	0.877	1.128
LC10	1.093	0.947	1.168
LC15	1.127	0.997	1.197
LC50	1.284	1.213	1.353
LC85	1.464	1.384	1.632
LC90	1.509	1.419	1.716
LC95	1.580	1.470	1.852
LC99	1.722	1.568	2.143

Noticeable differences in behavior were observed in fish exposed to control and treatment concentrations used in the behavior-recovery experiment (i.e., 0.0, 0.98, 1.07 and 1.17% as verified by GC analysis). Most treatment fish recovered to a normally active state after a 15-h recovery period in clean seawater, but fish in the 1.17% concentration showed residual effects (i.e., minor discoloration). Therefore, we selected 1.07% as the suitable sublethal exposure for the critical swimming experiment.

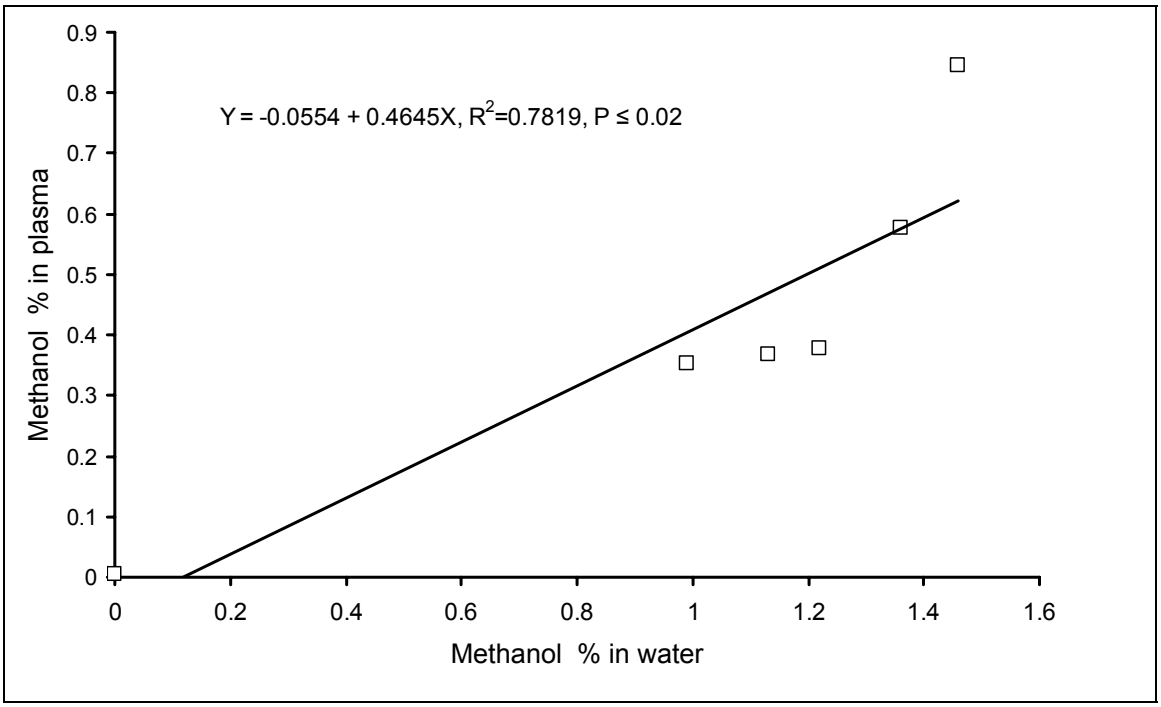


Figure 3-1. The regression of methanol concentration in blood plasma, control, and exposure waters following exposure to concentrations ranging from 0.99 to1.49% v/v. Points are estimates of pooled samples from survivors in control and treatment groups.

3.2.2 Critical swimming speed

The mixed-model analysis of relative U_{crit} (i.e., BL/s) found a significant interaction between pre- and post-exposure trials of control and treatment groups. Two treatment fish were excluded from the analysis: one performed poorly in pre- and post-exposure trials achieving only 1.68 and 1.74 BL/s, and another swam normally in the pre-exposure trial (9.17 BL/s) but later died during exposure. Mean differences for the fixed effects Group ($F = 0.60$, df = 1 & 20, $P > 0.448$) and Trial ($F = 1.03$, df = 1 & 20, $P > 0.3223$) were not significant, but the interaction between Group and Trial was significant ($F = 15.32$, df = 1 & 20, $P < 0.0009$). The least square means for the pre- and post-control trials increased from 8.76 ± 0.26 to 9.11 ± 0.26 BL/s. The least square means for the pre- and post-treatment trials decreased from 9.01 ± 0.16 to 8.42 ± 0.16 BL/s. Only two comparisons of least square means among the six pair-wise were significant. The least square means for the control post-trial (9.11 BL/s) was significantly ($P < 0.0381$) higher than the treatment post-trial (8.42 BL/s). The least square means for the treatment post-trial (8.42 BL/s) was significantly ($P < 0.0001$) lower than the treatment pre-trial (9.01 BL/s) confirming the negative effects of methanol on swimming performance. Although there was no detectable

difference between pre- and post-exposure U_{crit} in the control group ($P < 0.1047$), a slight increase of 0.34 BL/s in the post-exposure trial may have been due to training or conditioning effects.

A 6.5% reduction in relative swimming performance was observed in treatment fish after exposure to 1.07% methanol. This value is a conservative underestimate because one treatment fish that did not fatigue after 10 min of swimming at 100 cm/s was assigned a pretreatment U_{crit} value of 100.0 cm/s. The mean (± SE) absolute U_{crit} of treatment fish (N = 16) was 90.1 ± 1.35 cm/s in the pre-exposure trial and 84.2 ± 1.36 cm/s in the post-exposure trial. The mean absolute U_{crit} of control fish (N = 6) was 87.7 ± 2.54 cm/s in the pre-exposure trial and 91.2 ± 2.75 cm/s in the sham post-exposure trial.

Pre- and post-exposure swimming performance varied considerably among individuals (Figure 3-2). Of the16 treatment fish, only two individuals showed a mean increase in relative u_{crit} of 0.18 and 0.35 bl/s following exposure (Figure 3-2). For treatment fish, mean performance declined between pre- and post-exposure trials. In the control group, four individuals increased their performance by 0.27 to 0.72 bl/s, and two individuals decreased in performance by 0.03 and 0.10 bl/s. Although the post-exposure reduction (%r) in absolute u_{crit} did not impact all individuals to the same degree, variation among treatment fish was unrelated to individual body mass (g) (%r = 6.9 – [0.02 x body mass], n = 16, r = -0.02, $p > 0.05$) and three size groups showed similar declines (Figure 3-3).

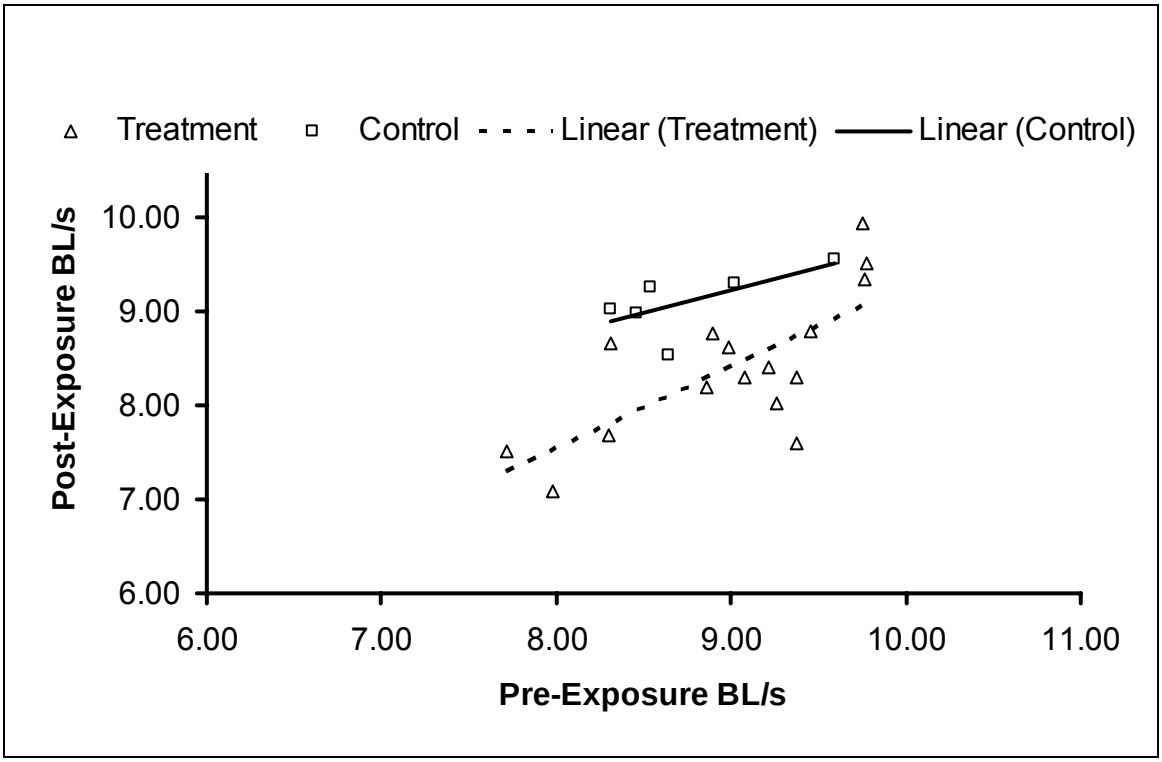


Figure 3-2. Critical swimming speeds, in Body Lengths per second (BL/s), of juvenile Florida pompano before and after a 24-h exposure to 1.07% methanol (treatment fish, N = 16) and before and after sham exposure (control fish, N = 6).

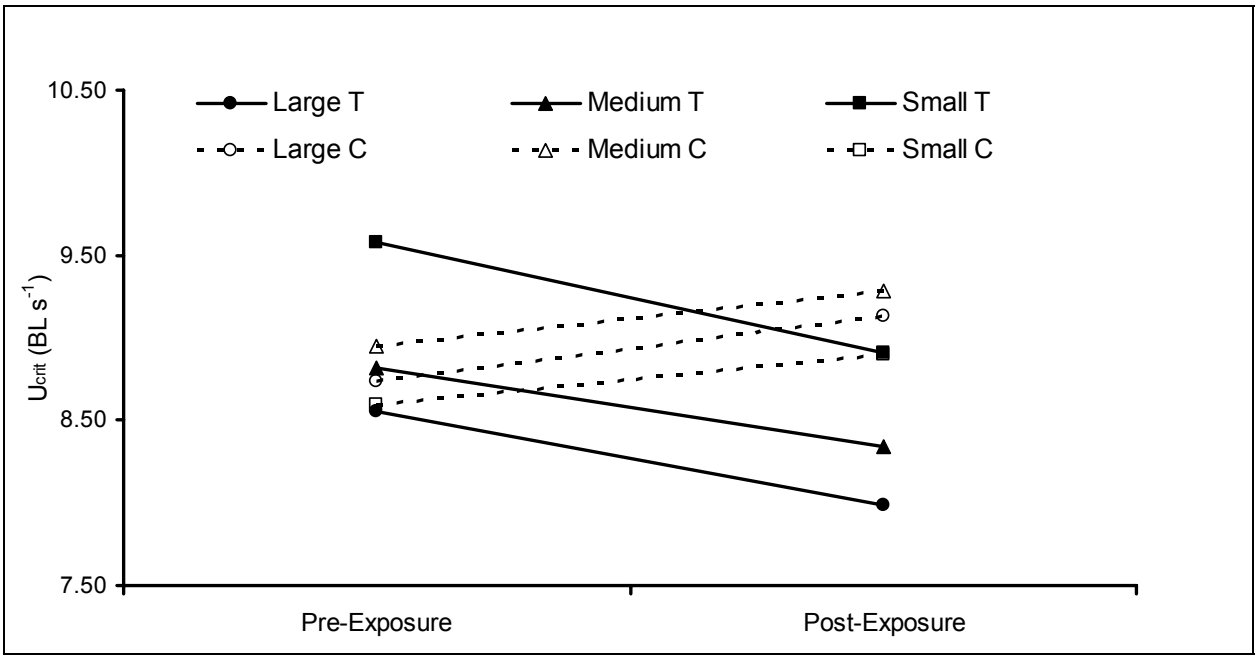


Figure 3-3. Size-related changes in mean critical swimming speeds (BL/s) of juvenile Florida pompano in treatment (T) and control (C) groups before and after exposure and sham exposure to 1.07% methanol for large (T: 106-119 mm, N = 5; C: 103-105 mm, N= 2), medium (T: 100-105 mm, N = 5; C: 100-102 mm, N= 2), and small (T: 85-99 mm, N = 6; C: 92-99 mm, N= 2) individuals.

3.3 Discussion

3.3.1 Methanol toxicity and mode of action

The estimated 24-h static LC50 value (1.28%) for juvenile Florida pompano exposed to methanol cannot be directly compared to many previous studies (Gillette et al. 1952, Nishiuchi and Hashimoto 1967, Buzzell et al. 1968, Juhnke and Luedemann 1978, Linden et al. 1979, Johnson and Finley 1980, Call et al. 1983, Veith et al. 1983, Bengtsson et al. 1984, Ewell et al. 1986, Mayer and Ellersieck 1986, Poirier et al. 1986, Tsuji et al. 1986, Robertson et al. 1988) because of different exposure types and test durations, and neither of the studies in sea water used similar protocols (Portmann and Wilson 1971, Tarkpea and Svanberg 1982). Three of four studies reporting values for 24-h LC50 tests on bluegill (2.41%), fathead minnow (3.75%), medaka *Oryzias latipes* (> 1.26%), and rainbow trout (2.57%) are considerably higher than the 1.28% LC50 for Florida pompano found in this study. Although osmoregulation in fish has been well studied, the interaction between salinity and toxicity of xenobiotics is relatively unexplored (Ferguson and Hogstrand 1998, El-Alfy et al. 2001). To counteract water gain in fresh water and water loss in sea water, freshwater fishes do not drink significantly while marine fishes do (Ferguson and Hogstrand 1998, Moyle and Cech 2000), enhancing the intestinal pathway for uptake of waterborne contaminants. This may account, in part, for the higher

toxicity of methanol in marine fishes. Moreover, Florida pompano also appear to be more sensitive than two other species tested in saltwater, bleak (a euryhaline freshwater species, 3.54%, 96-hr static, tested at 7 ppt) and hooknose (a marine species, 1.26 to 4.17%, 48- and 96-hr renewal). In our study the LC50 was achieved in one-half to one-fourth the time at comparable or lower concentrations indicating that Florida pompano are relatively more sensitive than bleak or hooknose. Toxicity estimates from different studies can be roughly compared with an index of toxicity (IT) to estimate 1-hr LC50 values (Boehm et al. 2001). Thus, assuming a linear-response model index (i.e., concentration x time), the LC50 for a 1-hr exposure is two to twelve times lower for Florida pompano (IT = 30.72), indicating that methanol was considerably more toxic to pompano than to the euryhaline bleak (IT = 340), the marine hooknose (IT = 60 to 400) and most of the freshwater fishes that have been tested (IT = 48 to 357). Only the study on medaka (Tsuji et al. 1986) yielded results similar to those for pompano.

The literature is 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 1979). A more recent study indicates that a second metabolic product, formate, is responsible for the toxicity associated with methanol exposure (Tephly 1991). 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 for prolonged or burst swimming; however, some species are able to oxidate formate into carbon dioxide at a faster rate, and thus have a higher tolerance to methanol exposure (Tephly 1991).

3.3.2 Sublethal effects on U_{crit}

Individuals exposed to a methanol concentration between the LC05 and LC10 levels exhibited sublethal effects on U_{crit} after being allowed to recover in clean seawater for 17 h. A 6.5% reduction (9.01 to 8.42 BL/s) in the relative swimming performance of exposed juveniles contrasts sharply with the 4.0% improved performance of the control group. Thus, the conditioning and/or training effects were surpassed by the significant negative sublethal effects of the methanol exposure.

3.3.3 Individual variation in U_{crit}

Though mean critical swimming speed of the treatment group decreased by 6.5% after exposure, considerable individual variation in performance was detected in both the treatment and control groups. Swimming performance studies examining sublethal effects of toxicants commonly evaluate performances by comparing the means of separate control and treatment groups, but such an approach can overlook within-group variation. Experimental designs that address within-group variation are valuable because swimming performance of individual fish is repeatable over time (Kolok 1992; Kolok et al. 1998; Reidy et al. 2000). Our two-factor ANOVA with pre- and post-exposure measurements used this repeatable source of individual variation for analysis of treatment effects that were controlled for training and conditioning. The design allows both the comparison of pre-treatment versus post-treatment performance in the

same animal and the measurement of sublethal impacts of a contaminant (i.e., methanol), features not commonly seen in toxicological studies.

Few prior studies have quantified the effect of a stressor by measuring the pre- and post exposure U_{crit} of individual fish. In juvenile largemouth bass *Micropterus salmoides* exposed to a 10ºC decline in water temperature (from 20ºC to 10ºC) between swim-performance trials, U_{crit} was reduced by a consistent amount in all individuals (Kolok 1992). Similarly, Kolok and Farrell (1994) found that the U_{crit} of northern pikeminnow *Ptychocheilus oregonensis* after a surgical procedure was reduced from pre-surgery values by a consistent amount in all fish. The results of a study on fathead minnows (Kolok et al. 1998) contrast with those mentioned above. In that study, swimming performance was evaluated before and after an exposure to field-collected sediments contaminated with heavy metals and hexachlorobenzene. Post-exposure U_{crit} varied widely, with some individuals experiencing profound reductions in U_{crit} and others maintaining their performance level regardless of the exposure. Our results resembled those of Kolok et al. (1998) and Hymel et al. (2002), in that the response of individual fish following exposure to methanol (and a recovery period) varied greatly, from no effect on performance to a severe effect. However, in contrast with Hymel et al.'s (2002) finding of a size-dependent effect of ethylene glycol on juvenile Florida pompano, we were unable to detect a size effect for methanol.

3.3.4 Marine exposures

The LC50 values of methanol reported in this and other studies on fishes generally reflect the relatively low toxicity of this chemical. Marine fishes are most likely to be exposed to lethal concentrations only in the event of highly localized, large-scale releases into the environment. Large offshore storage volumes of methanol in the northern Gulf of Mexico (up to 380,800 L) pose significant risk of a spill (Boehm et al. 2001). Marine releases of considerable magnitude have been reported (Boehm et al. 2001). Nine methanol spills were reported from platforms in the northern Gulf of Mexico between March 1994 and January 2002 (National Response Center 2002) and other types of incidents were more common. For example, 39,742 L were released in June 1997 (National Response Center 2002a) and 11,355 L of methanol leaked into the Gulf of Mexico from an underwater injection line in November of 1997 (National Response Center 2002b). Exposures of marine fishes are most likely to be brief because of rapid dispersion and mixing in an open marine system and relatively rapid biodegradation of methanol (half life ~6.2 d, see Boehm et al. 2001). While dispersion and dilution of methanol in the marine environment would be expected to be rapid, other factors affecting the toxicity of methanol must also be considered, such as additive or synergistic effects with other chemicals. Boehm et al. (2001) simulated a methanol spill of 363,360 L into the surface waters over the continental shelf and found that the predicted exposure concentrations were two orders of magnitude lower than the lowest published 96-h LC50 values (see Tarkpea and Svanberg [1982]; 12,539 mg/L) for a benthic marine harpacticoid copepod *Nitroca spinipes*. However, at a comparable concentration, we found a short-term LC50 (24-h, ~12,800 mg/L) for Florida pompano and substantial and significant sublethal effects on swimming performance between the LC05 and LC10 levels (Table 3-1).

3.3.5 Ecological consequences of sublethal effects

Most studies of petroleum-related contaminants have concentrated on the mortality effects associated with major oil spills (Howarth 1991; Kennicutt et al. 1996; Peterson et al. 1996), but much remains unknown about the effects of low-level exposures on marine organisms. Sublethal effects of contaminant exposure are inherently more difficult to assess and extrapolate to the field. We have shown that individual marine fish experience reduced U_{crit} (mostly aerobic) swimming performance when exposed to sublethal levels of methanol or ethylene glycol (Hymel et al. 2002). The implications of these results include adverse effects on the ability to perform aerobically or anaerobically in ecological situations (Plaut 2001). U_{crit} was developed primarily as a measure of aerobic capacity, even though the fish is pushed to use anaerobic metabolism and fatigue. While maximum (*sensu* Sepulveda and Dickson 2000) cruising speeds of Florida pompano are probably not near the high critical swimming speeds observed in this study, the capacity for sprint (s, anaerobic), burst (min, anaerobic), and critical (hr, aerobic) swimming modes (*sensu* Reidy et al. 2000) are all probably affected to some degree by methanol exposure. Each mode has direct relevance to foraging, prey capture, predator avoidance, trawl avoidance, strong currents, migrations, or spawning. However, all three modes of swimming have been tested in only one species, Atlantic cod *Gadus morhua*. Reidy et al. (2000) found that for healthy cod, U_{crit} and sprint performances are positively correlated, but U_{crit} and burst performances are negatively correlated, and sprint and burst performances are uncorrelated. Morphological and physiological tradeoffs among aerobic and anaerobic swimming types in other species and how contaminant exposure affects their sprint, burst, and endurance swimming performances remains unstudied (Plaut 2001, Nelson et al. 2002).

An understanding of sublethal effects is important for the accurate prediction of the impacts to habitat and fish populations because many species may experience chronic low levels of one or more contaminants over time. For example, in a study of fishes surrounding oil drilling platforms Grizzle (1986) found histological changes in the gills that possibly reflected chronic, low-level exposure to drilling contaminants.

Extrapolation of sublethal effects from the individual level to the population level remains problematic (Rose et al. 1993), particularly when sublethal effects vary across size classes. Mortality can be highly compensatory in fish populations, especially when it falls on early life history stages. High levels of mortality from an episodic event, such as a contaminant spill, may not necessarily have a significant effect at the population level, if there is significant compensation in the mortality process (Houde 1989). For example, individual-based models of striped bass *Morone saxatilis* demonstrate that chronic exposures are more likely than episodic exposures to affect recruitment (Rose et al. 1993). Nevertheless, episodic exposures could have a greater impact on recruitment if they occur at or shortly after a spawning peak or in a discrete nursery area (Rose et al. 1993). It is also important to evaluate sublethal effects because they are likely additive (and may be synergistic) and need to be considered when evaluating the effects of contaminant spills. Currently, deepwater oil facilities use methanol and other pure chemicals and proprietary chemical mixtures as additives to facilitate petroleum production. These chemicals and mixtures should be evaluated individually and in concert to explore additive and synergistic effects to achieve an accurate assessment of their potential sublethal and lethal effects on marine fish populations. We found a substantial and significant sublethal effect on individual swimming performance from exposure to methanol concentrations below the LC10 level that should be incorporated into risk assessments.

4.0 SWIMMING PERFORMANCE OF JUVENILE FLORIDA POMPANO AFTER SUBLETHAL EXPOSURE TO ETHYLENE GLYCOL AND METHANOL: SYNERGISTIC EFFECTS

M. A. Stead[1], D. M. Baltz[1, 2], E. J. Chesney[3], M. A. Tarr[4], A. S. Kolok[5], and B. D. Marx[6]

[1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803-7503

[2]Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503

[3]Louisiana Universities Marine Consortium, Chauvin, LA 70344

[4]Department of Chemistry, University of New Orleans, New Orleans, LA 70148

[5]Biology Department, University of Nebraska at Omaha, Omaha, NE 68182

[6]Experimental Statistics, Louisiana State University, Baton Rouge, LA 70803-5606

Although a tremendous amount of research has been directed towards understanding the effects of offshore oil development on fish communities in the northern Gulf of Mexico (Gallaway et al. 1981; Grizzle 1986; Stanley and Wilson 1990; Grossman et al. 1997; Boehm et al. 2001, Hymel et al. 2002), little attention has been paid to sublethal effects associated with low-level, localized discharges of toxicants, which may reduce an individual's ability to avoid predators, feed, reproduce, and resist diseases and parasites. Quantification of the sublethal effects of exposure to one or more contaminants at the individual level is important to our understanding of how populations and communities may respond to these impacts (Weis et al. 1999). Oil and gas platforms are a source of anthropogenic inputs including contaminated waters that are separated from crude oil at the extraction point (i.e., 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 faunas that utilize these structures (Holdway 2002).

Additives such as ethylene glycol and methanol are often transported long distances from shore by underwater pipelines up to 96 km in length and by ships to deep-water production platforms. There they are stored in volumes of up to 300,000 L per platform (Boehm et al. 2001), and are used to prevent the formation of hydrates. Hydrates are solid structures that can permanently clog pipelines (Anonymous 1996; Herzhaft and Dalmazzone 2000). They form when water molecules crystallize around guest molecules in natural gas and other products (Abdelghani et al. 1989; Tephly 1991; Herzhaft and Dalmazzone 2000) due to the cooling effect of seawater at extreme depths.

In this study, critical swimming speed (U_{crit}) was used to quantify the capacity of a marine fish, Florida pompano *Trachinotus carolinus,* for prolonged swimming activity

(Brett 1964). Florida pompano were used as a 'fish model' because they occur from the beach to offshore in the vicinity of petroleum production platforms and pipelines in the northern Gulf of Mexico, they are representative of a number of reef associated species (i.e., family Carangidae), they range widely in the western Atlantic and the Gulf, and they are highly prized commercial and sport fish (Gilbert 1986).

This study extends previous research by testing the combined sublethal effects of 3.0% (v/v) ethylene glycol and 1.07% (v/v) methanol on swimming performance. Swimming performance has been used to determine tolerances of fishes to varying environmental stresses and pollutants (Cairns 1966; Sprague 1971; Nikl and Farrell 1993; Beaumont et al.1995; Heath 1995; Kolok et al. 1998; Hymel et al. 2002). It is associated with the ability of individuals to carry out essential activities, integrates the effects of numerous physiological processes (Beamish 1978), and relates to Darwinian fitness (Plaut 2001). Stress responses are energy draining processes (Schreck 1982; Barton and Schreck 1987) that reduce energy available for other life functions (Schreck 1990). Thus, changes in swimming performance can provide a measure of stress (Cech 1990). Hymel et al. (2002) and Baltz et al. (2005) found significant reductions of 14% and 6.5%, respectively, in critical swimming performance, but the synergistic effects of ethylene glycol and methanol were more profound than the single exposures. Mean performance was synergistically reduced by 41.1% after exposure to both chemicals in combination.

4.1 Materials and Methods

4.1.1 Swimming tunnel design

Three Blazka-type swimming respirometers were used to simultaneously test the swimming performance of three individual fish (Blazka et al. 1960). The swim tunnel 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). The inner chamber had a diameter of 20.3 cm with square plastic grids at both ends to contain the fish and reduce turbulent flow characteristics, and the total volume of each swimming tunnel was 104 L. Propeller speed was adjusted using a hand-held tachometer to set revolutions per minute (RPM) on a variable-speed D.C. motor to obtain desired velocities up to a maximum of 100 cm/s based on three linear regressions of RPM on Velocity (cm/s) developed by Hymel et al. (2002):

Swimming tunnel 1: Velocity = 0.062(RPM) -4.0099 ($r^2 = 0.9452$, $P < 0.001$);
Swimming tunnel 2: Velocity = 0.0627(RPM) -1.1029 ($r^2 = 0.9715$, $P < 0.001$); and
Swimming tunnel 3: Velocity = 0.0571(RPM) +0.0582 ($r^2 = 0.9886$, $P < 0.001$).

4.1.2 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). Throughout 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. Individuals were anesthetized with tricaine methanesulfonate (MS-222; <50 mg/L) 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.

The experimental protocol included identical fasting, exposure, acclimation, and swimming experience for each group and required 18 days. Groups were exposed or sham exposed (i.e., without chemical) in one of three round fiberglass tanks filled with 60 L of 30 ± 1 psu seawater at a temperature of 25 ± 1°C and aerated with two air stones. Fish were acclimated for 17 h in the swimming tunnels following a 24-h exposure and fasted during the entire 41-h period of exposure (24 h) and acclimation (17 h). Swimming performance before and after sublethal exposure to 3.0% ethylene glycol (Hymel et al. 2002), 1.07% methanol (Baltz et al. 2005), or the combination of the two chemicals (3.0% ethylene glycol and 1.07% methanol) was measured for individual fish. These concentrations were selected from dose response curves because they have adverse, but not lethal, effects on swim performance (Hymel et al. 2002, Baltz et al. 2005). 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. Homogenous mixing of treatment chemicals was tested by taking water samples from exposure tanks at the beginning and end of each 24-h exposure period for gas chromatography (GC) analysis. GC analyses and paired t-tests (SAS Institute Version 8) of exposure concentrations showed that there were no significant differences ($P > 0.05$) between initial and final exposure tank concentrations for ethylene glycol, methanol or combination treatments.

Immediately after exposure, groups were placed in a bucket of clean seawater and transferred to swimming tunnels that were set up in a constant temperature room at 25 ± 1°C, and acclimated in clean 30 psu seawater. During the 17-h acclimation, swimming tunnels were connected to a recirculating system that included a biofilter and a UV sterilizer and velocity in each swimming tunnel was set at 5 cm/s. After acclimation, air bubbles were removed and swimming tunnels isolated before the start of the swimming trials. Individual fish were tested and re-tested in the same swimming tunnel during the pre- and post-exposure phases of the swimming trials.

4.1.3 Swimming performance

Swimming performance was tested by determining differences in pre- and post-exposure critical swimming speed for individual fish. Velocities in the swimming tunnels were increased by 10 cm/s every 10 min, without intervening rest periods. Swimming trials ended when the fish fatigued and was 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 were assigned a U_{crit} value of 100 cm/s. Dissolved oxygen in mg/L was measured in each swimming tunnel at the beginning and ending of each swimming trial with a YSI Model 85 handheld oxygen, salinity and temperature meter to verify that the fish were not stressed by low oxygen concentrations.

4.1.4 Plasma samples

At the end of the post-exposure swimming trials, fish were anesthetized using <50 mg/L of MS-222, and Body Mass (BM) to the nearest 0.01 g and Fork Length (FL) to the nearest mm were measured (Table 4-1). Anesthetized 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 blood plasma samples were frozen at –80°C until the GC analysis of ethylene glycol and methanol.

Table 4-1. Individual fork length (fl mm), body mass (g), and pre- and post-exposure u_{crit} (cm/s, bl/s) values for treatment (methanol, ethylene glycol, and combined [m+eg]) and control groups of juvenile florida pompano. Statistical summaries are means (±1se) for size variables and lsmeans for absolute and relative u_{crit} values.

Florida pompano			Pre-exposure U_{crit}		Post-exposure U_{crit}	
Treatment	FL (mm)	Body Mass (g)	cm/s	BL/s	cm/s	BL/s
Methanol	102.00	23.32	96.93	9.50	89.95	8.82
Methanol	98.00	19.29	99.07	10.11	96.97	9.89
Methanol*	107.00	25.67	100.00	9.35	100.00	9.35
Methanol	90.00	15.24	83.85	9.32	74.17	8.24
Methanol	92.00	16.11	94.18	10.24	91.62	9.96
Methanol	91.00	15.50	88.13	9.68	89.03	9.78
Methanol	91.00	14.71	83.88	9.22	82.08	9.02
Methanol	93.00	15.66	90.83	9.77	85.02	9.14
Methanol	94.00	17.11	87.40	9.30	89.07	9.48
Methanol	110.00	26.51	94.93	8.63	91.10	8.28
Methanol	108.00	24.70	98.48	9.12	99.41	9.20
Methanol*	102.00	22.14	100.00	9.80	100.00	9.80
Mean or LSMean	98.17	19.66	92.01	9.47	89.08	9.17
SE	2.12	1.30	5.39	0.51	5.39	0.51
Ethylene glycol	111.00	27.45	93.53	8.43	95.33	8.59
Ethylene glycol	102.00	24.31	100.00	9.80	91.30	8.95
Ethylene glycol	99.00	19.79	100.00	10.10	84.97	8.58
Ethylene glycol	112.00	28.70	74.18	6.62	82.23	7.34
Ethylene glycol	100.00	21.34	100.00	10.00	91.20	9.12
Ethylene glycol	108.00	24.03	91.97	8.52	83.32	7.71
Ethylene glycol	91.00	15.00	92.86	10.20	89.03	9.78
Ethylene glycol	97.00	18.51	91.63	9.45	88.88	9.16
Ethylene glycol	89.00	15.08	92.65	10.41	62.32	7.00
Ethylene glycol	90.00	14.78	81.43	9.05	68.12	7.57
Ethylene glycol	91.00	17.41	89.73	9.86	78.68	8.65
Ethylene glycol	89.00	14.57	95.83	10.77	41.30	4.64
Mean or LSMean	98.25	20.08	91.41	9.44	79.15	8.10
SE	2.48	1.46	4.86	0.44	4.86	0.44
M+EG	106.00	26.81	100.00	9.43	86.05	8.12
M+EG	109.00	24.66	100.00	9.17	65.48	6.01
M+EG	87.00	13.32	83.32	9.58	44.22	5.08
M+EG	89.00	14.44	74.26	8.34	71.25	8.01
M+EG	92.00	14.20	90.48	9.83	72.23	7.85

Florida pompano			Pre-exposure U_{crit}		Post-exposure U_{crit}	
Treatment	FL (mm)	Body Mass (g)	cm/s	BL/s	cm/s	BL/s
M+EG	89.00	16.50	86.03	9.67	6.92	0.78
M+EG	88.00	14.78	86.95	9.88	14.78	1.68
M+EG*	87.00	14.87	86.10	9.90	0.00	0.00
Mean or LSMean	94.29	17.82	90.45	9.32	53.29	5.26
SE	2.65	1.59	5.74	0.56	5.74	0.56
Control	105.00	27.30	94.95	9.04	100.00	9.52
Control	105.00	22.30	99.12	9.44	100.00	9.52
Control*	108.00	25.60	100.00	9.26	100.00	9.26
Control	112.00	27.92	100.00	8.93	96.85	8.65
Control	108.00	25.50	94.05	8.71	99.50	9.21
Control	89.00	16.13	88.08	9.90	59.83	6.72
Control	95.00	16.34	87.08	9.17	91.17	9.60
Control	94.00	16.64	86.78	9.23	91.53	9.74
Control	88.00	16.22	85.10	9.67	92.65	10.50
Control	92.00	16.28	84.43	9.18	82.82	9.00
Control	94.00	17.81	89.06	9.48	92.95	9.89
Mean or LSMean	99.09	20.73	90.66	9.29	90.53	9.25
SE	2.48	1.44	5.10	0.47	5.10	0.47
* Surviving individuals excluded from all statistical analyses						

4.1.5 Statistical analysis

A repeated measures design was used to determine the differences in pre- and post-exposure U_{crit} for the replicated Florida pompano swimming performance tests. Although a 1-way ANOVA used to examine the sizes of individuals randomized among four treatments was unable to detect size differences among treatments (d.f. = 3, 35; F = 0.39; P> 0.76), we included size as a covariate and report U_{crit} in absolute (cm/s) and relative (body lengths per second, BL/s) terms. Statistical analyses of U_{crit} in absolute and relative terms, with and without size (mm FL) as a covariate, yielded similar results. Replicate experiments were conducted in August, 2001 and September, 2001 to increase total sample size and treatments were intermingled to avoid pseudoreplication (Hurlbert 1984). There were two experimental replicates (1 or 2). Exposure tank number (1, 2 or 3) and fish were the experimental units for the chemical/sham treatment and trial time, respectively. The Mixed procedure of SAS (SAS Institute 1996) was used to test the model $Y_{ijklm} = \mu + \rho_i + \alpha_j + \alpha\rho_{ij} + T(\alpha\rho)_{k(ij)} + \tau_l + \alpha\tau_{jl} + F(\rho\alpha T)_{m(ijk)} + \beta L_{ijkm} + \varepsilon_{ijklm}$ for swimming performance tests where ρ_i is the random effect of replication (experiment 1 or 2), α_j is the fixed treatment effect (j=1, 2, 3, 4), $\alpha\rho_{ij}$ is the random interaction of replication by treatment, $T(\alpha\rho)_{k(ij)}$ is the random effect of tank (1, 2 or 3) nested within experiment by treatment, τ_l is the fixed trial effect (pre- or post-exposure), $\alpha\tau_{jl}$ is the fixed interaction between treatment and trial, and $F(\rho\alpha T)_{m(ijk)}$ is the random effect of fish

nested within tank by experiment by treatment, and ß is the slope associated with the covariate length (L) for each fish. The distribution of the model's residuals showed strong symmetric and unimodal behavior. However, due to a few observations in the tails, the Shapiro-Wilk's test rejected normality ($P < 0.01$). As the F-tests are robust for such residual distributions (Glass et al. 1972), we do not present analyses using transformed responses. In post-exposure testing, differences between least-square means (LSMeans) were calculated for all possible treatment comparisons.

Nine fish were excluded from the statistical analysis resulting in a final sample size of 39 individuals. One control and three individuals belonging to the combination treatment group died in the exposure tanks during the pre-exposure (sham) experience. Following the combination exposure (actual experience), one individual died during acclimation to the swim tunnel and another individual would not swim Another three individuals (i.e., 1 control and 2 methanol fish) out-swam the capacity of the swim tunnel during both pre- and post-exposure trials.

4.2 Results

4.2.1 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 4-1).

No detectable difference was observed for the main effect of treatment ($df = 3, 3$, $F = 4.59$, $P > 0.12$); however, the trial ($df = 1, 34$, $F = 29.36$, $P < 0.0001$) and the treatment by trial interaction ($df = 3, 34$, $F = 10.05$, $P < 0.0001$) were highly significant, while the size covariate was not ($df = 1, 34$, $F = 3.53$, $P = 0.0689$). The significant interaction indicated the pre- and post-exposures to ethylene glycol, methanol, the combination, and controls differed widely in effects on swimming performance. The LSMeans from the Mixed procedure (Figure 4-1) showed evidence of a significant difference between pre- and post-exposure swimming performance of pompano exposed to 3.0% ethylene glycol ($P < 0.0071$) 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 ($P > 0.53$) or controls ($P > 0.97$).

Individuals exposed to 1.07% methanol exhibited a 3.2% mean reduction in U_{crit} (Figure 4-1) but the reduction was not detectably different from zero ($P > 0.53$). Mean (±1 SE) pre-exposure U_{crit} was 92.01 ± 5.39 cm/s (9.47 ± 0.53 BL/s) and the post- exposure U_{crit} was 89.08 ± 5.39 cm/s (9.17 ± 0.53 BL/s). Seven of the 10 pompano exhibited a mean decrease in U_{crit} of 5.17%, three fish showed a minor increase of 1.29% in U_{crit} (Figure 4-2).

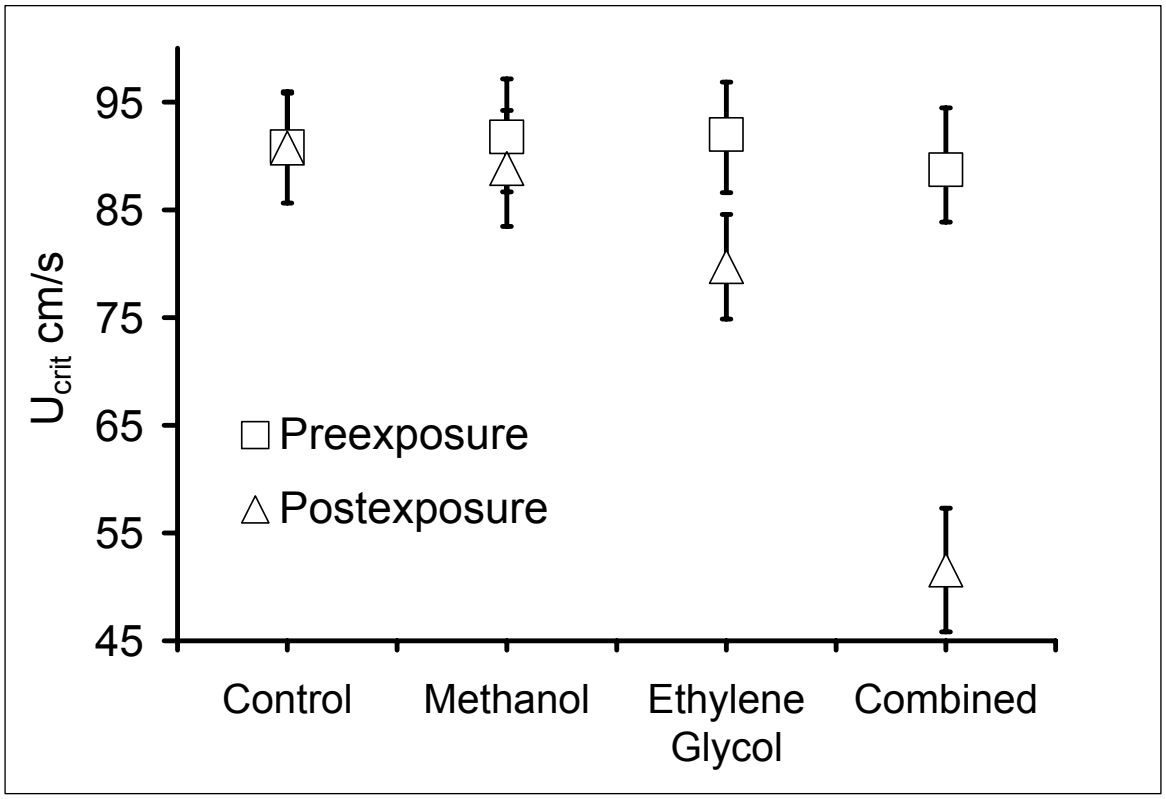


Figure 4-1. Critical swimming speeds (U_{crit}, LSMean (±1 SE)) of juvenile Florida pompano before and after 24-h exposures to 1.07% (v/v) methanol (N = 10), 3.0% (v/v) ethylene glycol (N = 12), the combined chemicals (N = 7), and sham-exposed controls (N = 10).

Individuals exposed to ethylene glycol exhibited a mean 13.4% decrease in U_{crit} (Figure 4-1). Mean (±1 SE) pre-exposure U_{crit} was 91.41 ± 4.86 cm/s (9.45 ± 0.44 BL/s) and mean post-exposure U_{crit} was 79.15 ± 4.86 cm/s (8.10 ± 0.44 BL/s). 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 to ethylene glycol (Figure 4-2).

Individuals exposed to both ethylene glycol and methanol in combination registered the largest drop in performance of all treatment groups (Figure 4-1), a mean decrease in U_{crit} of 42.1%. The mean (±1 SE) pre-exposure U_{crit} of combination exposed fish was 90.45 ± 5.74 cm/s (9.32 ± 0.56 BL/s) and mean post-exposure U_{crit} was 53.29 ± 5.74 cm/s (5.26 ± 0.56 BL/s). A noticeable amount of individual variation in U_{crit} was observed for the combination treatment. 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 4-2).

Individuals in control groups performed at relatively equal levels after both sham-exposures (Figure 4-1) with a mean overall decrease of 0.1%. Mean (±1 SE) pre-exposure U_{crit} was 90.66 ± 5.10 cm/s (9.29 ± 0.47 BL/s) and mean post-exposure U_{crit} was 90.53 ± 5.10 cm/s (9.25 ± 0.47 BL/s). Seven of the 10 control fish exhibited a mean increase in U_{crit} of 5.06%, while three fish showed a mean decrease of 12.38% (Figure 4-2).

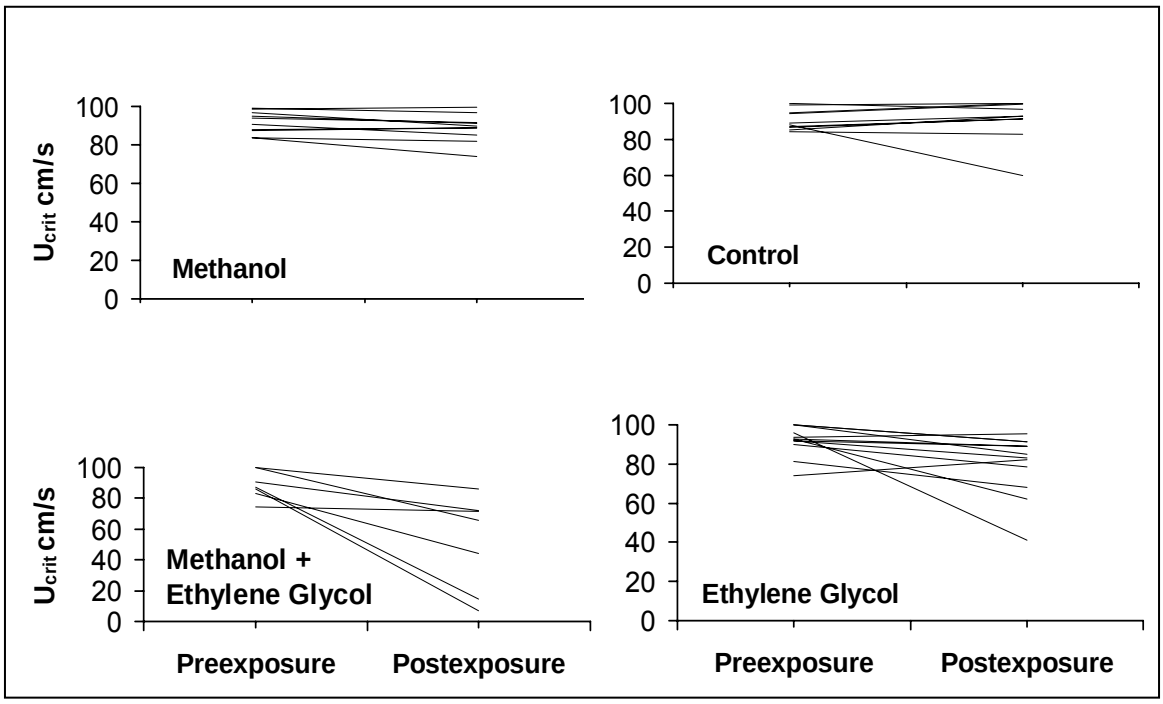


Figure 4-2. Individual variation in the critical swimming speeds (U_{crit}) of juvenile Florida pompano before and after 24-h exposures to 1.07% methanol (N=10), 3.0% ethylene glycol (N = 12), the combined chemicals (N = 7), and sham-exposed controls (N = 10).

4.2.2 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 h of acclimation, up to 100 min of experimental swimming trials, and 0.5 h of postexperimental 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 (±1 SE) ethylene glycol concentration of 0.623 ± 0.04%.

4.3 Discussion

The simultaneous exposure to sublethal concentrations of ethylene glycol and methanol significantly reduced the U_{crit} of juvenile Florida pompano. The 41.1% reduction in performance caused by exposure to the combination of contaminants was profound compared to the reductions exhibited by fish exposed solely to ethylene glycol (a 13.4% reduction) or methanol (a 3.2% reduction). The reduction in U_{crit} for Florida pompano exposed to 3.0% ethylene glycol (a 13.4% reduction) was similar to the 14.0% reduction

observed by Hymel at el. (2002); however, the 3.2% reduction for methanol was somewhat less than the significant 6.5% reduction observed by Baltz et al. (2005). In this study sublethal exposure to 1.07% methanol had a short-term effect on behavior but did little to impair the swimming performance after 17 h of recovery. The lack of detectable level of methanol in the plasma samples suggests that pompano completely metabolized methanol (probably into formaldehyde and formate products) after ~ 20 h of acclimation and swimming (Potts and Johnson 1952; Koivusalo 1970; Tephly 1991); however, methanol was detectable in plasma in similarly exposed individuals that were not exercised (Baltz et al. 2005). In contrast, the mean percentage (±1 SE) of ethylene glycol still present in pompano plasma samples ~ 20 h after exposure, acclimation and exercise was 0.623 ± 0.04%.

4.3.1 Combination exposure

Compared to fish exposed solely to the single concentration of ethylene glycol, performance declined by three fold in fish exposed to the combination of ethylene glycol and methanol. 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 than additive) or antagonistic (less then additive). These interactions occur when one toxicant alters the toxicity of another toxicant present. The synergistic interaction observed in our study was similar to that 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.

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) in humans and may inhibit each other from being completely metabolized and increase the toxic effects after exposure (Dawidek-Pietryka et al. 1998). The metabolic pathways of ethylene glycol and methanol are in fact so similar that treatment for ethylene glycol or methanol poisoning is the administration of ethanol, another ALDH competitor (Dawidek-Pietryka et al. 1998). If the metabolic pathways are similar in pompano, then combining the concentrations of the two would lead to more or less the same number of toxic intermediate compounds as would the doubling of either concentration without mixing them together. In one case the only toxic intermediate would be formaldehyde or glycoaldehyde, while in the other it would be both; nevertheless, if we assume that the toxic intermediates are more or less equally toxic, then we would expect an additive rather than a synergistic effect (i.e., one toxic aldehyde + a second toxic aldehyde probably causes the equivalent toxicity of two aldehydes of the same chemical species). Thus, it is interesting that the metabolites probably increased more or less arithmetically, while the effect on the fish was not arithmetic, but synergistic.

4.3.2 Individual variation

Florida pompano mean U_{crit} decreased after exposure to ethylene glycol, methanol and combination treatments by 13.4, 3.2, and 41.1%, respectively. However, there was considerable individual variation in U_{crit} within treatment and control groups following exposure and sham-exposure (Figure 4-2). 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 et al. 1998). In this and related studies (Hymel et al. 2002; Baltz et al. 2005) 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 stressful treatments. Kolok et al. (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 our study resembled those of Kolok et al. (1998) in that the individual performance in U_{crit} after exposure to ethylene glycol, methanol, and combination treatments varied between slight increases and striking reductions. The variability in this study implies that individual fish may have lower or higher tolerances to ethylene glycol, methanol or combination treatments. This variation may also account in part for the non-significant reduction (3.2%) due to methanol exposure that we found in contrast to earlier, significant findings (6.5%) by Baltz et al. (2005).

4.3.3 Ecological effects

Although Boehm et al. (2001) found that realistic models of chemical spills for ethylene glycol or methanol would result in exposures well below the lowest median lethal concentration (LC50) values for other species found in the literature, pompano appear to be more sensitive to the two chemicals than any species they examined (Hymel et al. 2002; Baltz et al. 2005). An episodic spill of ethylene glycol and methanol could have severe ecological implications at individual, community, or population levels. Nevertheless, it is difficult to forecast how the effects of exposure at the individual level will translate to populations or communities (Weis et al. 1999). Fish populations and recruitment are likely to exhibit compensatory responses (Houde 1989) to an episodic stress or mortality such as a spill; therefore, a spill may not necessarily affect a species at the population level if the exposure is not sustained or widespread (Rose et al. 1993). But a spill during critical periods of life history could reduce recruitment and add an additional stress to populations already burdened by overfishing, reduced habitat quality and other contaminants (Rose et al. 1993).

It is also important to evaluate sublethal effects because they are likely additive (and may be synergistic) and need to be considered when evaluating or forecasting the effects of contaminant spills. Currently, deepwater oil facilities use methanol and other pure chemicals and undisclosed proprietary chemical mixtures as additives to facilitate petroleum production. These pure chemicals and mixtures should be evaluated individually and in concert to explore additive and synergistic effects to achieve an accurate assessment of their potential sublethal and lethal effects on marine fish populations. We found a substantial and significant synergistic effect on individual swimming performance from exposure to methanol concentrations below the LC10 level and to ethylene glycol concentrations below the LC01 level that should be incorporated into risk assessments. An understanding of sublethal and additive and synergistic effects is important for the accurate prediction of the impacts to habitat and fish populations because many species may experience chronic low levels of one or more contaminants over time. For example, in a study of fishes surrounding oil drilling platforms Grizzle (1986) found histological changes in the gills that possibly reflected chronic, low-level exposure to drilling contaminants.

Most studies of petroleum-related contaminants have concentrated on the mortality effects associated with major oil spills (Howarth 1991; Kennicutt et al. 1996; Peterson et al. 1996), but much remains unknown about the effects of low-level exposures on marine organisms. Sublethal effects of contaminant exposure are inherently more difficult to assess and extrapolate to the field. We have shown that individual marine fish experience reduced U_{crit} (mostly aerobic) swimming performance when exposed to sublethal levels of methanol (Baltz et al. 2005) or ethylene glycol (Hymel et al. 2002). The implications of these results include adverse effects on the ability to perform aerobically or anaerobically in ecological situations (Plaut 2001). The U_{crit} procedure was developed primarily as a measure of aerobic capacity, even though the fish is pushed to use anaerobic metabolism and fatigue (K. Dickson, Cal. State Univ. Fullerton, personal communication). While maximum (*sensu* Sepulveda and Dickson 2000) cruising speeds of Florida pompano are probably not near the high pre-exposure U_{crit} values observed in this study, the capacity for sprint (seconds, anaerobic), burst (minutes, anaerobic), and critical (hours, aerobic) swimming modes (*sensu* Reidy et al. 2000) are all probably affected to some degree by contaminant exposure. Each mode has direct relevance to foraging, prey capture, predator avoidance, trawl avoidance, strong currents, migrations, or spawning. However, all three modes of swimming have been tested in only one species, Atlantic cod *Gadus morhua*. Reidy et al. (2000) found that for healthy cod, U_{crit} and sprint performances are positively correlated, but U_{crit} and burst performances are negatively correlated, and sprint and burst performances are uncorrelated. Morphological and physiological tradeoffs among aerobic and anaerobic swimming types in other species and how contaminant exposure affects their sprint, burst, and endurance swimming performances remains unstudied (Plaut 2001, Nelson et al. 2002). What is clear is that exposure to contaminants at sublethal concentrations can reduce swimming performance of fishes and synergistic effects are likely.

5.0 SWIMMING PERFORMANCE OF JUVENILE ATLANTIC SPADEFISH EXPOSED TO SUBLETHAL CONCENTRATIONS OF ETHYLENE GLYCOL

M. A. Stead[1], D. M. Baltz[1, 2], E. J. Chesney[3], M. A. Tarr[4], A. S. Kolok[5], and B. D. Marx[6]

[1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803-7503

[2]Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503

[3]Louisiana Universities Marine Consortium, Chauvin, LA 70344

[4]Department of Chemistry, University of New Orleans, New Orleans, LA 70148

[5]Biology Department, University of Nebraska at Omaha, Omaha, NE 68182

[6]Experimental Statistics, Louisiana State University, Baton Rouge, LA 70803-5606

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 more 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.

5.1 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 a second time 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).

5.1.1 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).

5.1.2 Collection and exposure

Atlantic spadefish were captured by trawls 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 sp*. 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. Throughout 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 ≤ 50 mg/L 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 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.

5.1.3 Swimming performance

Swimming performance was evaluated 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.

5.1.4 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 ≤ 50 mg/L 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 three individuals in 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.

5.1.5 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.

5.2 Results

5.2.1 Swimming performance

The treatment groups exposed to ethylene glycol performed significantly more poorly than control groups (Figure 5-1). 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).

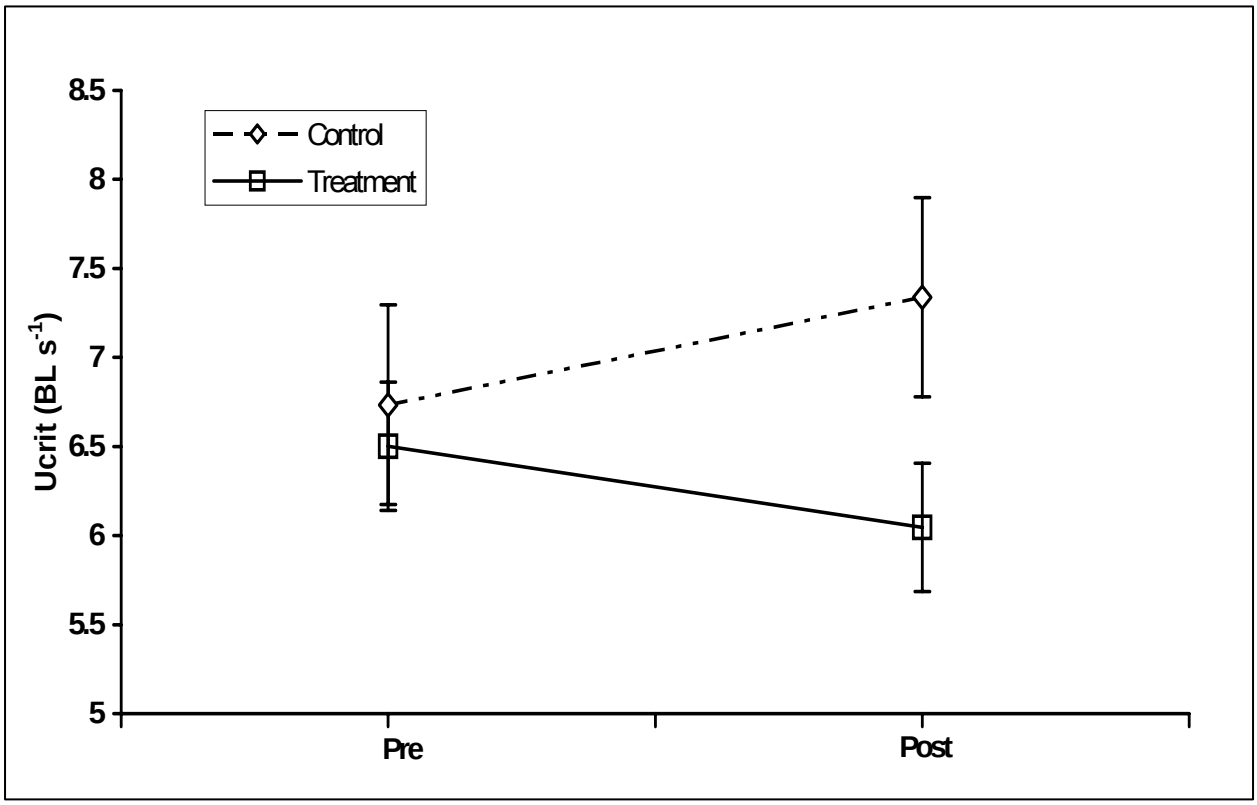


Figure 5-1. Mean (± SE) critical swimming speeds (U_{crit}) of juvenile Atlantic spadefish before and after a 24-hr exposure to 3.0 % ethylene glycol (N = 25) and sham controls (N = 6).

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 (Table 5-1) 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.

Table 5-1. 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 SE	**102.9** **3.40**	**41.5** **4.48**	**66.90** **5.54**	**6.59** **0.36**	**62.44** **5.54**	**6.13** **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 **SE**	**114.7** **5.82**	**56.5** **0.89**	**77.25** **8.24**	**6.73** **0.55**	**82.85** **8.24**	**7.34** **0.55**

Individuals in control groups performed at a higher level after their second sham exposure (Figure 5-1), showing a mean 8.9 % increase in U_{crit} in the second trial. The mean pre-exposure critical swimming of the control groups (Table 5-1) 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 5-2 and 5-3). 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.

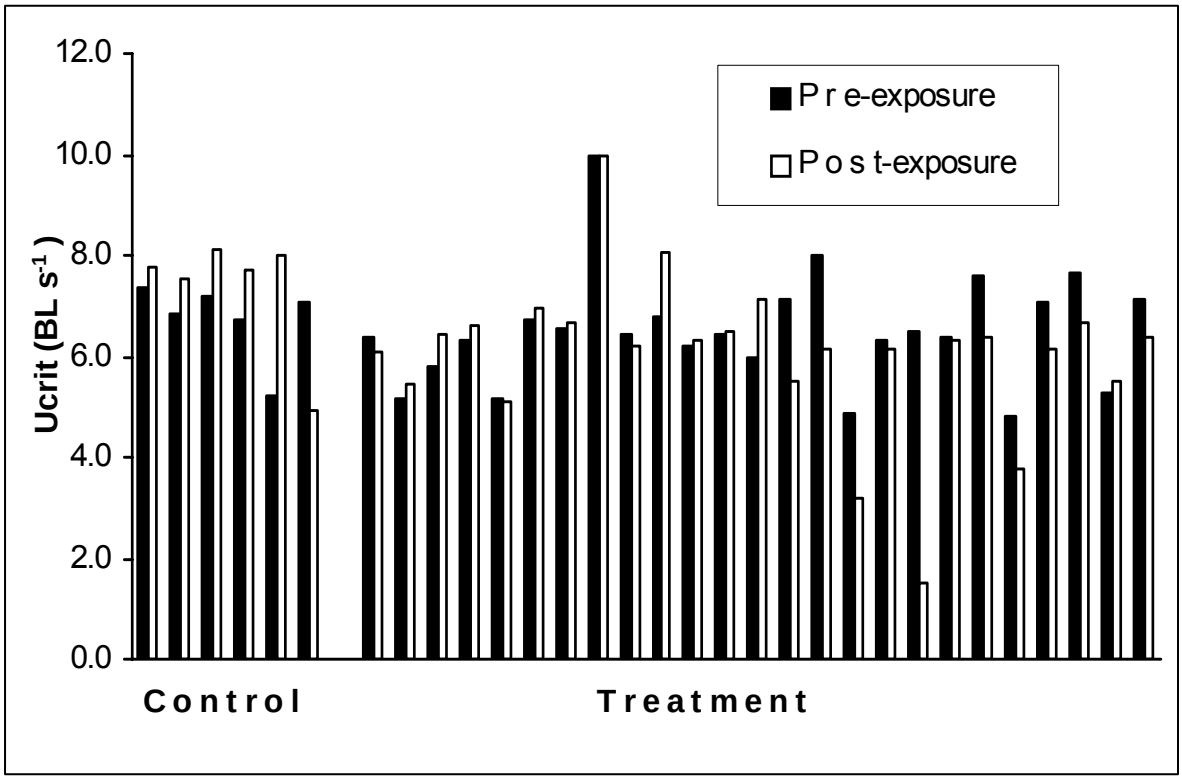


Figure 5-2. Critical swimming speeds (U_{crit}) of juvenile Atlantic spadefish before and after a 24-hr exposure to 3.0 % ethylene glycol (N = 25) and sham controls (N=6).

5.2.2 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 postexperimental processing. Pooled plasma from juvenile Atlantic spadefish treatment groups (N = 10 groups) contained a mean (± SE) ethylene glycol concentration of 0.891 ± 0.09 %.

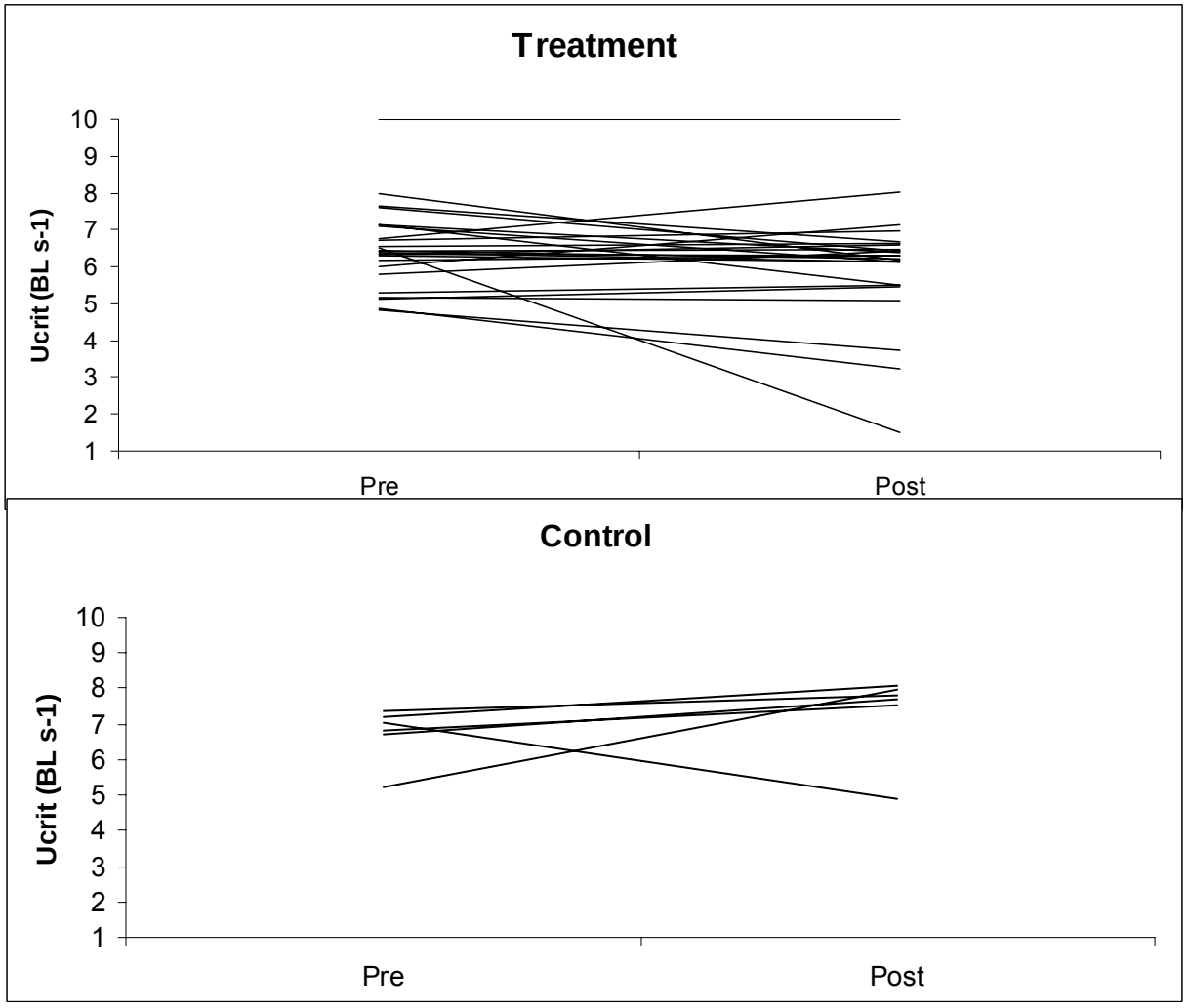


Figure 5-3: Individual variation in the critical swimming speeds (U_{crit}) of juvenile Atlantic spadefish (N = 25) before and after exposure to 3.0 % ethylene glycol and sham controls (N = 6).

5.3 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.

5.3.1 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 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.

5.3.2 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.

5.3.3 Individual variation

Considerable within group variation was observed for individuals in the swimming performance tests (Figure 5-2, 5-3). 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.

5.3.4 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 LC50 decreases linearly with time of exposure, then LC50 values in studies using different test durations can be grossly compared with a toxicity index (concentration x time) (French et al. 1996a; Boehm et al. 2001). Using the index of toxicity (Index) to estimate 1-hr LC50 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.

5.3.5 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.

5.4 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 LC50 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 the 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.

6.0 GENERAL SUMMARY

D. M. Baltz[1, 2] and E. J. Chesney[3]

[1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803-7503

[2]Coastal Fisheries Institute, Louisiana State University, Baton Rouge, LA 70803-7503

[3]Louisiana Universities Marine Consortium, Chauvin, LA 70344

The results of the juvenile Florida pompano study showed that exposure to 1.07 % methanol and 17 hr recovery did little to impair their swimming performance. 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 suggests 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 could have occurred after juveniles were exposed to the combination of ethylene glycol and methanol. This interpretation is similar to that of Greene and Kocan (1997) who found the toxicity of ethylene glycol increases in freshwater fish when sublethal levels of a chemical used in an agricultural seed-protectant (thiram) 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 of humans for ethylene glycol and methanol poisoning is the administration of ethanol, another ALDH competitor (Katarzyna et al. 1998).

Both fish species we tested exhibited a high amount of individual variation in swimming performance after exposure to ethylene glycol or methanol. 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 the 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.

Future studies should concentrate on accumulating more data on the sublethal and lethal effects of ethylene glycol, methanol, and other additives 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 potential impacts on 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 LC50 value of an ethylene glycol and methanol mixture and testing their potential and additive or synergistic effects on the swimming performance of Atlantic spadefish was not possible. LC50 values would allow comparisons of the toxic effects of ethylene glycol and methanol on species with different physiologies and would be important in understanding the overall toxicity of a substance. Answering the question of how the exposure to methanol and the combination of ethylene glycol and methanol would affect a species with different physiology would aid in understanding the overall effect of a localized spill and begin to extend our understanding from the population to community level. Another objective of future studies should be to find LC50 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 et al (2005) were used to find sublethal test concentrations; testing a range of sublethal concentrations on swimming performance would help in identifying the effects of differing exposure ranges.

7.0 LITERATURE CITED

Abdelghani, A. A., A. C. Anderson, G. A. Khoury, and S. N. Chang. 1989. The fate of ethylene glycol in the environment. Louisiana Department of Transportation and Development, State Project 736-12-67, Number 87-1M(B), Baton Rouge.

Anonymous. 1996. Kinetic hydrate inhibitor provides treatment alternative. World Oil December 1996:154-155.

Anonymous. 2000. SINTEF turning problems of gas hydrates to advantage. Offshore 60(4):194.

Baker C. F., and J. C. Montgomery. 2001. Sensory deficits induced by cadmium in banded kokopu, *Galaxias fasciatus*, juveniles. Environmental Biology of Fishes 62:455-464.

Baltz, D. M., E. J. Chesney, M. A. Tarr, A. S. Kolok, and M. Bradley. 2005. Toxicity and Sublethal Effects of Methanol on Swimming Performance of Juvenile Florida Pompano. Transactions of the American Fisheries Society 134:000-000.

Barber, J. T., D. A. Thomas, L. Y. Yatsu, and H. E. Ensley. 1999. The physiological consequences of ethylene glycol-induced changes in the frond structure of *Lemna gibba*. Aquatic Toxicology 45: 253-264.

Barton, B.A. and C.B. Schreck, 1987. Metabolic cost of acute physical stress in juvenile steelhead. Transactions of the American Fisheries Society 116:257-263.

Beamish, F. W. H. 1978. Swimming capacity. Pages 101-187 *in* W. S. Hoar and D. J. Randall, editors. Fish Physiology, Volume 7. Academic Press, New York.

Beamish, F.W.H., 1978. Swimming capacity. In: Fish Physiology vol. 7, edited by W.S. Hoar and D.J. Randall. Academic Press, New York, pp.101-187.

Beasley, V.R. and W.B. Buck, 1980. Acute Ethylene glycol toxicosis: A review. Veterinary and Human Toxicology 22(4):255-263.

Beaumont, M. W., P. J. Butler, and E. W. Taylor. 1995. Exposure of brown trout, *Salmo trutta*, to sub-lethal copper concentrations in soft acidic water and its effect upon sustained swimming performance. Aquatic Toxicology 33:45-63.

Beauvais, S. L., S. B. Jones, S. K. Brewer, and E. E. Little. 2000. Physiological measures of neurotoxicity of diazinon and malathion to larval rainbow trout (*Oncorhynchus mykiss*) and their correlation with behavioral measures. Environmental Toxicology and Chemistry 19:1875-1880.

Bellinger, J. W., and J. W. Avault, Jr. 1970. Seasonal occurrence, growth and length-weight relationship of juvenile pompano, *Trachinotus carolinus*, in Louisiana. Transactions of the American Fisheries Society 99:353-358.

Bengtsson, B. E., L. Renberg, and M. Tarkpea. 1984. Molecular structure and aquatic toxicity - an example with C1-C13 aliphatic alcohols. Chemosphere 13(5-6):613-622

Berry, F. and E.S. Iverson, 1967. Pompano: biology, fisheries and farming potential. Proceedings of the Gulf Caribbean Fisheries Institute. 19:16-128.

Blazka, P., M. Volf, and M. Cepela, 1960. A new type of respirometer for the determination of the metabolism of fish in an active state. Physiologia Bohemoslovaea 9:553-558.

Bliss, C. I. 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26:585-615.

Boehm, P., D. Turton, A. Raval, D. Caudle, D. French, N. Rabalais, R. Spies, and J. Johnson. 2001. Deepwater Program: literature review. Environmental Risks of Chemical Products Used in Gulf of Mexico Deepwater Oil and Gas Operations, volume I: technical report.

Outer Continental Shelf (OCS) Study MMS 2001-011. Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, Louisiana.

Bohnsack J.A., 1989. Are high densities of fishes at artificial reefs the result of habitat limitation or behavioral preference. Bulletin of Marine Science 44(2):631-645.

Brett, J. R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Research Board of Canada 21:1183-1226.

Bridie, A. L., C. J. M. Wolff, and M. Winter. 1979. The acute toxicity of some petrochemicals to goldfish. Water Research 13: 623-626.

Buzzell, J. C. J., R. H. F. Young, and D. W. Ryckman. 1968. Behavior of organic chemicals in the aquatic environment. Part II. - Behavior in dilute systems. Environmental Sanitary Engineering Laboratories, Washington University, St. Louis, Missouri. 81 pp.

Cairns, J. 1966. Don't be half-safe-the current revolution in bio-assay techniques. Proc. 21st int. Waste Conf. Purdue Univ., Engng Extn Ser. No. 121, 559-567.

Call, D. J., L. T. Brooke, N. Ahmad, and J. E. Richter. 1983. Toxicity and metabolism studies with EPA priority pollutants and related chemicals in freshwater organisms. Environmental Protection Agency 600/3-83-095, U. S. EPA, Duluth, Minnesota. 120 pp.

Cech, J. J., Jr. 1990. Respirometry. Pages 335-362 *in* C. B. Schreck and P. B. Moyle, editors. Methods for Fish Biology. American Fisheries Society, Bethesda, Maryland.

Cheung, P. 1993. Parasitic diseases of marine tropical fishes. Page 647 in: Stoskopf, M. K. (ed.), Fish Medicine. W. B. Saunders Co., Philadelphia, PA.

Chou, J. Y. and K. E. Richardson. 1978. The effect of pyrazole on ethylene glycol toxicity and metabolism in the rat. Toxicology and Applied Pharmacology 43: 33-44.

Continental Shelf Associates, Inc., 1982. Study of the effect of oil and gas activities on reef fish populations in the Gulf of Mexico OCS area. OCS Rep. MMS82-010, U.S. Dep. Int., Minerals Manage. Serv., Gulf of Mexico OCS Region, New Orleans, 210 pp.

Conway, R. A., G. T. Waggy, M. H. Spiegel, and R. L. Berglund. 1983. Environmental fate and effects of ethylene oxide. Environmental Science and Technology 17: 107-112.

Dawidek-Pietryka, K., S. Szczepaniak, J. Dudka, and M. Mazur. 1998. In vitro studies of human alcohol dehydrogenase inhibition in the process of methanol and ethylene glycol oxidation. Archives of Toxicology 72:604-607.

Dicks, B.M. 1979. Environmental impact of North Sea oil platforms. In: The marine environment and oil facilities. Institution of Civil Engineers. London. 1-21.

Ditty, J.G., R.F. Shaw and J.S. Cope, 1993. A re-description of Atlantic spadefish larvae, *Chaetodipterus faber* (family: Ephidppidae), and their distribution, abundance, and seasonal occurrence in the northern Gulf of Mexico. Fishery Bulletin 92:262-274.

El-Alfy, A., S. Grisle, and D. Schlenk. 2001. Characterization of salinity-enhanced toxicity of aldicarb to Japanese medaka: sexual and developmental differences. Environmental Toxicology and Chemistry 20:2093-2098.

EPA (U. S. Environmental Protection Agency). 1994. U. S. EPA toxicity data analysis software. (J. M. Lazorchak, editor). U. S. Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio.

Ewell, W. S., J. W. Gorsuch, R. O. Kringle, K. A. Robillard, and R. C. Spiegel. 1986. Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environmental Toxicology and Chemistry 5(9):831-840

Ferguson, E. A., and C. Hogstrand. 1998. Acute silver toxicity to seawater-acclimated rainbow trout: Influence of salinity on toxicity and silver speciation. Environmental Toxicology and Chemistry 17:589-593

Finucane, J.H., 1969. Ecology of the pompano (*Trachinotus carolinus*) and the permit (*T. falcatus*) in Florida. Transactions of the American Fisheries Society. 98(3):478-486.

French, D.P., M. Reed, K. Jayko, S. Feng, H.M. Rines, S.Pavignano, T. Isaji, S. Puckett, A. Keller, F.W. French III, D. Gifford, J. McCue, G. Brown, E. MacDonald, J. Quirk, S. Natzke, R. Bishop, M. Welsh, M. Phillips and B.S. Ingram, 1996a. Validation and Sensitivity Analysis: The CERCLA type A natural resource damage assessment models for coastal and marine environments (NRDAM/CME) and for the Great Lakes environments (NRDAM/GLE) final report, Submitted to the office of Environmental Policy and Compliance. U.S. Dept. of the Interior, Washington D.C., April 1996. Contract No. 14-01-0001-91-C-11/

Gallaway, B. J., L. R. Martin, R. L. Howard, G. S. Boland, and G. D. Dennis. 1981. Effects on artificial reef and demersal fish and macrocrustacean communities. Pages 237-299 *in* B. S. Middleditch, editor. Environmental effects of offshore oil production : the Buccaneer gas and oil field study. Marine Science Vol. 14, Plenum Press, NY.

Gallaway, B.J., L.R. Martin, R.L. Howard, G.S. Boland and G.D. Dennis, 1981. Effects on artificial reef and demersal fish and macrocrustacean communities. In Middleditch, B.S. (ed.), Environmental effects of offshore oil production: The Buccaneer gas and oil field study, p.237-299. Mar. Sci. Vol. 14, Plenum Press, NY.

Gilbert, C. 1986. Species Profiles: Life histories and environmental requirements of coastal fishes and invertebrates (South Florida)—Florida pompano. U.S Fish Wildlife Service Biological Report 82(11.42).

Gillette, L. A., D. L. Miller, and H. E. Redman. 1952. Appraisal of a chemical waste problem by fish toxicity tests. Sewage and Industrial Wastes 24:1397-1401.

Glass, G. V., P. D. Peckham, and J. R. Sanders. 1972. Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. Review of Education Research 42:237-288.

Greene, M. W. and R. M. Kocan. 1997. Toxicological mechanisms of a multicomponent agricultural seed protectant in the rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*). Canadian Journal of Fisheries and Aquatic Sciences 54: 1387-1390.

Gregory, R.T., and C.M. Wood. 1999. Interactions between individual feeding behaviour, growth, and swimming performance in juvenile rainbow trout (*Oncorhynchus mykiss*) fed different rations. Canadian Journal of Fisheries and Aquatic Sciences 56:479-486.

Grizzle, J. M. 1986. Lesions in fishes captured near drilling platforms in the Gulf of Mexico. Marine Environmental Research 18:267-276.

Grossman, G. D., G. P. Jones, and J. S. William, Jr. 1997. Do artificial reefs increase regional fish production? A review of existing data. Fisheries 22(4):17-23.

Hartwell, S.I. and R.G. Otto, 1991. Critical swimming capacity of the Atlantic silverside, *Menidia menidia*. Estuaries 14:218-221.

Hayse, J.W., 1989. Feeding habits, age, growth, and reproduction of Atlantic spadefish *Chaetodipterus faber* (Pisces:Ephippidae) in South Carolina. Fishery Bulletin 88: (1): 67-83.

Heath, A. G. 1995. Water pollution and fish physiology. 2nd edition. CRC Press, Boca Raton, Florida.

Herzhaft, B., and C. Dalmazzone. 2000. Drilling muds for deepwater: hydrates and rheology. Offshore 60(5):54,195.

Hoese, H. D., and R. H. Moore. 1977. Fishes of the Gulf of Mexico. Texas A&M University Press, College Station.

Holdway, D. A. 2002. The acute and chronic effects of wastes associated with offshore oil and gas production on the temperate and tropical marine ecological processes. Marine Pollution Bulletin 44:185-203.

Houde, E. D. 1989. Subtleties and episodes in the early life of fishes. Journal of Fish Biology 35(Supplement A):29-38.

Howarth, R. W. 1991. Assessing the ecological effects of oil pollution from outer continental shelf oil development. Pages 1-8 *in* C. S. Benner and R. W. Middelton, editors. Fisheries and oil development on the continental shelf. American Fisheries Society Symposium 11, Bethesda, Maryland.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54: 187-211.

Hymel, M. K., D. M. Baltz, E. J. Chesney, M. A. Tarr, and A. S. Kolok. 2002. Swimming performance of juvenile Florida pompano exposed to ethylene glycol. Transactions of the American Fisheries Society 131:1152-1163.

Iverson E.S. and F. Berry, 1969. Mariculture: progress and potential. Proceedings of the Gulf Caribbean Fisheries Institute. 21:163-176.

Johnson, G. D., 1978. Development of fishes of the mid-Atlantic Bight, vol. IV. Carangidae through Ephippidae. U.S. Fish Wildl. Serv. Biol. Serv. Prog., 314 p.

Johnson, R.A. and D.W. Wichern. 1988. Applied Multivariate Statistical Analysis. Prentice Hall. Englewood Cliffs, New Jersey.

Johnson, W. W., and M. T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U. S. Department of the Interior, U.S. Fish and Wildlife Service Resource Publication 137, Washington, DC. 98 pp.

Jones, D. R. 1971. The effect of hypoxia and anaemia on the swimming performance of rainbow trout (*Salmo gairdneri*). Journal of Experimental Biology 55: 541-551.

Juhnke, I., and D. Luedemann. 1978. Results of the investigation of 200 chemical compounds for acute fish toxicity with the Golden Orfe Test. Zeitschrift für Wasser -und Abwasser-Forschung 11(5):161-164 (GER, ENG TRANSL).

Katarzyna D.P., S.J.D. Dudka, M. Marek, 1998. In vitro studies of human alcohol dehydrogenase inhibition in the process of methanol and ethylene glycol oxidation. Arch. Toxicol. 72:604-607.

Kay, M. E. 2000. Swimming performance of juvenile Florida pompano, *Trachinotus carolinus*, exposed to ethylene glycol. MS thesis, Louisiana State University, Baton Rouge.

Kennicutt II, M. C., R. H. Green, P. Montagna, and P. F. Roscigno. 1996. Gulf of Mexico operations monitoring experiment (GOOMEX), Phase I: Sublethal responses to contaminant exposure—introduction and overview. Canadian Journal of Fisheries and Aquatic Sciences 53:2540-2553.

Koivusalo, M. 1970. Pages 465-505 *in* J. Tremolieres, editor. International Encyclopedia of Pharmacology and Therapeutics. Section 20, Volume 2 Pergamon Press, New York.

Kolok, A. S. 1992. The swimming performances of individual largemouth bass (*Micropterus salmoides*) are repeatable. Journal of Experimental Biology 170:265-270.

Kolok, A. S., and A. P. Farrell. 1994. Individual variation in the swimming performance and cardiac performance of northern squawfish, *Ptychocheilus oregonensis*. Physiological Zoology 67:706-722.

Kolok, A. S., E. P. Plaisance, and A. Abdelghani. 1998. Individual variation in the swimming performance of fishes: An overlooked source of variation in toxicity studies. Environmental Toxicology and Chemistry 17:282-285.

Lazorchak, J. M., editor. 1994. U.S. Environmental Protection Agency (USEPA) toxicity data analysis software. USEPA, Office of Research and Development. Cincinnati, Ohio.

Linden, E., B. E. Bengtsson, O. Svanberg, and G. Sundstrom. 1979. The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (*Alburnus alburnus*) and the harpacticoid. Chemosphere 8(11/12):843-851 (Author Communication Used, OECDG Data File).

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models, SAS Institute, Cary, North Carolina, 633 pp.

Little, E. E., R. D. Archeski, B. A. Flerov, and V. I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Archives of Environmental Contamination and Toxicology 19:380-385.

Marking, L. L. 1985. Toxicity of chemical mixtures. Pages 164-176 *in* G. M. Rand and S. R. Petrocelli, editors. Fundamentals of aquatic toxicology. Hemisphere, New York, NY.

Mayer, F. L. J., and M. R. Ellersieck. 1986. Manual of acute toxicity: interpretation and database for 410 chemicals and 66 species of freshwater animals. U. S. Department of the Interior, U.S. Fish and Wildlife Service Resource Publication 160, Washington, DC. 505 pp.

McMartin, K. E., J. J. Ambre and T. R. Tephly. 1980. Methanol poisoning in human subjects: role of formic acid accumulation in the metabolic acidosis. American Journal of Medicine 68: 414-418.

Merck Index. 1983. Rahway, N.J., U.S.A. Merk and Co. Inc., 10th ed., pp.550.

Minerals Management Service 2002. Gulf of Mexico region. Minerals Management Service. Available: www.gomr.mms.gov/homepg/offshore/deepwatr/deepover.html. (September 2002).

Moyle, P. B., and J. J. Cech, Jr. 2000. Fishes: An introduction to ichthyology. 4th edition. Prentice Hall. Upper Saddle River, NJ.

National Academy Press. 1988. Artificial reefs and fish aggregation devices. Pages 85-114 in Fisheries technologies for developing countries. report of an Ad Hoc Panel of the Board on Science and Technology for International Development , Office of International Affairs, National Research Council, National Academic Press, Washington, D.C.

National Response Center. 2002a. Incident Report 392483. U.S. Coast Guard. Available: www.nrc.usgc.mil. (September 2002).

National Response Center. 2002b. Incident Report 412292. U.S. Coast Guard. Available: www.nrc.usgc.mil. (September 2002).

National Response Center. August 2002 Report # 412292 (a) and 444025 (b). www.nrc.uscg.mil.

Nelson, G.A. and M.D. Murphy, 2001. A preliminary Assessment of Florida pompano (*Trachinotus carolinus*) in Florida waters. 2001 Preliminary pompano Stock Assessment.

Florida Fish and Wildlife Conservation Commission. Florida Marine Research Institute St. Petersburg, Florida.

Nelson, J. A., P. S. Gotwalt, S. P. Reidy, and D. M. Webber. 2002. Beyond U_{crit}: matching swimming performance tests to the physiological ecology of the animal, including a new fish 'drag strip. Comparative Biochemistry and Physiology Part A 133:289-302.

Nikl, D. L., and A. P. Farrell. 1993. Reduced swimming performance and gill structural changes in juvenile salmonids exposed to 2-(thiocyanomethylthio) benothiazole. Aquatic Toxicology 27:245-264.

Nishiuchi, Y., and Y. Hashimoto. 1967. Toxicity of pesticide ingredients to some fresh water organisms. Sci. Pest Control /Botyu-Kagaku 32(1):5-11 (JPN, ENG Summary).

Parsons, G. R. 1994. Swimming performance of White crappie, *Pomoxis annularis*, the effect of environmental change. Freshwater Fisheries Report Number 136:1-62.

Peterson, C. H., M. C Kennicutt II, R. H. Green, P. Montagna, D. E. Harper, Jr., E. N. Powell, and P. F. Roscigno. 1996. Ecological consequences of environmental perturbations associated with offshore hydrocarbon production: a perspective on long-term exposures in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences 53:2637-2654.

Peterson, R. H. 1974. Influence of fenitrothion on swimming velocities of brook trout (*Salvelinus fontinalis*). Journal of the Fisheries Research Board of Canada 31:1757-1762.

Pillard, D. A. 1995. Comparative toxicity of formulated glycol deicers and pure ethylene and propylene glycol to *Ceriodaphnia dubia* and *Pimephales promelas*. Environmental Toxicology and Chemistry 14: 311-315.

Plaut, I. 2001. Critical swimming speed: its ecological relevance. Comparative Biochemistry and Physiology Part A 131:41-50.

Poirier, S. H., M. L. Knuth, C. D. Anderson-Buchou, L. T. Brooke, A. R. Lima, and P. J. Shubat 1986. Comparative toxicity of methanol and N, N-Dimethylformamide to freshwater fish and invertebrates. Bulletin of Environmental Contamination and Toxicology 37:615-621.

Portmann, J. E., and K. W. Wilson. 1971. The toxicity of 140 substances to the brown shrimp and other marine animals. Shellfish Information Leaflet No. 22 (2nd Ed.), Ministry of Agric. Fish. Food, Fish. Lab. Burnham-on-Crouch, Essex, and Fish Exp. Station Conway, North Wales :12 p.

Potts, A. M., and L. V. Johnson. 1952. Studies on the visual toxicity of methanol. I. The effect of methanol and its degradation products on retinal metabolism. American Journal of Ophthalmology 35:107-113.

Reidy, S. P., S. R. Kerr, and J. A. Nelson. 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. Journal of Experimental Biology 203:347-357.

Richardson, W.J.1995. Documented disturbance reactions. Pages 241-576 in W.J. Richardson, C.R. Greene, C. I. Malme and D.L. Thomson, eds. Marine mammals and noise. Academic Press, San Diego, CA.

Robertson, S. M., A. L. Lawrence, W. H. Neill, C. R. Arnold, and G. McCarty. 1988. Toxicity of the cryoprotectants glycerol, dimethyl sulfoxide, ethylene glycol, methanol, sucrose, and sea salt solutions to the embryos of red drum. The Progressive Fish-Culturist 50(3):148-154.

Rose, K. A., J. H., Cowan Jr., E. D. Houde, and C. C. Coutant. 1993. Individual-based modelling of environmental quality effects on early life stages of fishes: a case study

using striped bass. Pages 125-145 *in* L. A. Fuiman, editor. Water quality and the early life stages of fishes. American Fisheries Society Symposium 14, Bethesda, Maryland.

Samson, J. C., R. Goodridge, F. Olobatuyi, and J. S. Weis. 2001. Delayed effects of embryonic exposure of zebrafish (*Danio rerio*) to methylmercury (MeHg). Aquatic Toxicology 51:396-376.

SAS Institute. 1996. The SAS System for Windows, release 6.12, TS Level 0020. SAS Institute, Cary, North Carolina.

Satchell, G. H. 1984. Respiratory toxicology of fishes. Aquatic toxicology. 2:1-50.

Scholz N. L., N. K. Truelove, B. L. French, B. A. Berejikian, T. P. Quinn, E. Casillas, and T. K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 57:1911-1918.

Schreck, C. B. 1982. Stress and rearing of salmonids. Aquaculture 28:241-249.

Schreck, C. B. 1990. Physiological, behavioral, and performance indicators of stress. Pages 29-37 *in* S. M. Adams, editor. Biological indicators of stress in fish. American Fisheries Society Symposium 8, Bethesda, Maryland.

Sejersted, O. M., D. Jacobsen, S. Overbo and H. Jansen 1983. Acta Med. Acan. 213:105-110.

Sepulveda, C., and K. A. Dickson. 2000. Maximum sustainable speeds and cost of swimming in juvenile kawakawa tuna (*Euthynnus affinis*) and chub mackerel (*Scomber japonicus*). The Journal of Experimental Biology 203:3089-3101.

Sepulveda, C., and K. A. Dickson. 2000. Maximum sustainable speeds and cost of swimming in juvenile kawakawa tuna (*Euthynnus affinis*) and chub mackerel (*Scomber japonicus*). The Journal of Experimental Biology 203:3089-3101

Seyle. H., 1950. Stress and the general adaptation syndrome. British Medical Journal 1950(1):1383-1392.

Sprague, J. B. 1970. Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Research 4:3-32.

Sprague, J. B. 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations. Water Research 5:245-266.

Sprague, J. B. 1990. Aquatic Toxicology. Pages 491-528 *in* C. B. Schreck and P. B. Moyle, editors. Methods for Fish Biology. American Fisheries Society, Bethesda, Maryland.

Stanley, D. R., and C. A. Wilson. 1990. A fishery-dependent based study of fish species composition and associated catch rates around oil and gas structures off Louisiana. Fishery Bulletin 88:719-730.

Stanley, D.R. and C.A. Wilson, 1997. Seasonal and spatial variation in the abundance and size distribution of fishes associated with a petroleum platform in the northern Gulf of Mexico. Can. J. Fish. Aquat. Sci. 54:1166-1176.

Stanley, D.R. and C.A. Wilson. 2000. Variation in the density and species composition of fishes associated with three petroleum platforms using dual beam hydroacoustics. Fisheries Research 47:(2-3):161-172.

Stone, R.B., 1985. National artificial reef plan. NOAA Tech. Mem. NMFS OF-6. Department of Commerce, National Marine Fisheries Service, Washington, D.C. 70pp.

Tarkpea, M., and O. Svanberg. 1982. The acute toxicity of motor fuels to brackish water organisms. Marine Pollution Bulletin 13:125-127.

Tephly, T. R. 1991. The toxicity of methanol. Life Sciences 48:1031-1041.

Tsuji, S., Y. Tonogai, Y. Ito, and S. Kanoh. 1986. The influence of rearing temperatures on the toxicity of various environmental pollutants for killifish (*Oryzias latipes*). Journal of Hygienic Chemistry /Eisei Kagaku 32(1):46-53 (JPN, ENG ABS).

Veith, G. D., D. J. Call, and L. T. Brooke. 1983. Estimating the acute toxicity of narcotic industrial chemicals to fathead minnows. Pages 90-97 *in* W. E. Bishop, R. D. Cardwell, and B. B. Heidolph, editors, Aquatic Toxicology and Hazard Assessment, 6th Symposium, ASTM STP 802, Philadelphia, Pennsylvania.

Wedemeyer, G. A., B. A. Barton, and D. J. McLeay. 1990. Stress and Acclimation. Pages 451-489 *in* C. B. Schreck and P. B. Moyle, editors. Methods for Fish Biology. American Fisheries Society, Bethesda, Maryland.

Weis J. S., J. Samson, T. Zhou, J. Skurnick, and P. Weis. 2001. Prey capture ability of mummichogs (*Fundulus heteroclitus*) as a behavioral biomarker for contaminants in estuarine systems. Canadian Journal of Fisheries and Aquatic Sciences 58: 1442-1452.

Weis, J. S., G. M. Smith, and T. Zhou. 1999. Altered predator prey behavior in polluted environments: implications for fish conservation. Environmental Biology of Fishes 55:43-51.

Wibe, A. E., T. Nordtug, and B. M. Jenssen. 2001. Effects of bis(tributyltin)oxide on antipredator behavior in threespine stickleback *Gasterosteus aculeatus* L. Chemosphere 44:475-481.

Wilson, R. W. and E. W. Taylor. 1993. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. J. Comp. Physiol. 163B: 38-47.

Zhou, T., R. Scali, P. Weise and J.S. Weis. 1996. Behavioural effects in mummichog larvae (*Fundulus heteroclitus*) following embryonic exposure to methylmercury. In: D. E. Hinton, editor. Pollutant responses in marine organisms. Marine Environmental Research 42:45-49.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.

MMS Securing Ocean Energy & Economic Value for America