SHORT TERM EFFECTS OF MILITARY FOG OIL ON THE FOUNTAIN DARTER *(ETHEOSTOMA FONTICOLA)*

THESIS

Presented to the Graduate Council of
Texas State University- San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Thomas Ryan

San Marcos, Texas
May 2011

COPYRIGHT

by

Thomas Alfredo Ryan

2011

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

ACKNOWLEDGMENTS

Special gratitude is extended to: Dr. Tom Brandt of the U.S. Fish and Wildlife Service, San Marcos, Texas; Thomas Smith, Dr. Donald Cropek, and Anja Kohl of the U.S. Army Corps of Engineers, Champaign, Illinois; and especially Dr. Timothy Bonner and Dr. Alan Groeger of Texas State University-San Marcos.

This manuscript was submitted on April 25, 2011.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

ABSTRACT

SHORT TERM EFFECTS OF MILITARY FOG OIL
ON THE FOUNTAIN DARTER
(ETHEOSTOMA FONTICOLA)

by

Thomas A. Ryan

Texas State University-San Marcos
May 2011

SUPERVISING PROFESSOR: TIMOTHY BONNER

Fog oil is used by the US military during training exercises to produce obscurant smoke. The smoke enters aquatic systems by settling as an oily residue on water surfaces. Its effects on aquatic biota are largely unknown. Objectives of this study were to assess fish survival and reproduction after exposures to 3 forms of fog oil: surface oil films from precipitating smoke, oil-water mixtures produced by physical agitation, and solutions of dissolved fog oil components photo-transformed by ultraviolet from sunlight.

Surface oil from single smoke releases produced no mortality in adult fish and egg viability and production did not differ from controls ($P > 0.05$) after 21 days. Repeated smoke exposures of 7 consecutive days had 64% mortality in larvae. Endpoints for 96 hour LC50 tests for oil-water mixtures were 709 mg/L in larvae and 2,150 mg/L in eggs. LC50 values for UV-transformed solutions were 7.4 mg/L in larvae and 18.5 mg/L in eggs. Collectively, fog oils as smoke, mixtures with water, and transformed by UV were found to cause mortality in fish but only at elevated concentrations associated with atypical activities, such as up to 7 days of repeated foggings or potentially a contaminant spill.

FOG OIL TOXICITY TESTS

Introduction

The U.S. Army manages millions of acres of land and utilizes a sizable portion as training grounds (USACE 2007). Since training operations alter these lands physically and biologically, the US military monitors training grounds to minimize environmental impacts (Quist et al. 2003; Williams et al. 2005). One use of training grounds is the release of obscurant smokes in open natural areas to simulate visually adverse battlefield conditions. The entire range of effects of these obscurant emissions on aquatic habitats is not known.

Among the three more commonly used obscurants, fog oil has fewer concerns with toxicity than hexachloroethane and white phosphorus (Getz et al. 1996). Fog oil smoke is generated by injecting a middle distillate mineral oil into a heated manifold. The expelled oil vapor condenses upon contact with air and forms a dense, white cloud. This smoke is actually a mist composed of fine droplets. Fog oil is a complex mixture of aliphatic, olefinic, and naphthenic compounds. These oil types are estimated to contain over 1,000,000 organic constituents (Beens et al. 2000) and cannot be completely characterized with current analytical techniques. Furthermore, the composition of fog oil varies from different sources and even from batch to batch (Langford 2004). Fractionation techniques combined with comprehensive gas chromatography analysis

illustrate a domination of long chain, branched, and cyclic alkanes and olefinic compounds with no evidence of polycyclic aromatic hydrocarbons (D Cropek, Construction Engineering Research Laboratory, unpublished data, 2007). Studies of obscurant emissions have identified respiratory health hazards (NRC 1999). As precautions, the Army requires reductions in potentially hazardous aromatics and restricts duration of fog oil exposure to troops. Specific information on fog oil as an ecological hazard is limited. Mortalities of *Daphnia magna* were observed downwind of fog oil smoke generated in field tests, when Daphnia became trapped in the hydrophobic layer formed by settling of the aerosol droplets on the water surface (Esarey et al. 2004; Cropek et al. 2008)

Ultraviolet radiation (UV) from sunlight can photo-enhance fog oil toxicity. Components of different oils are photo-oxidized by UV, transforming organic compounds to more toxic substances. Photo-enhanced crude oil is more toxic than non-weathered crude oil in cyanobacteria (Gaur et al. 2006), crustaceans (Duesterloh et al. 2002) and fish (Little et al. 2000; Barron et al. 2005). Bluegill (*Lepomis macrochirus*) contaminated with anthracene, a polycyclic aromatic hydrocarbon, encountered increased mortality rates when moved from a shaded to a sun exposed area (Bowling et al. 1983). Likewise, ultraviolet radiation can photo-oxidize fog oil to increase organic component solubility and decrease water pH, amplifying FO toxicity to the amphipod *Hyallela azteca* (Poston et al. 1988).

For toxicity studies, the U.S. Environmental Protection Agency recommends the fathead minnow *(Pimephales promelas)* as a test organism (USEPA 2002). As an

alternative, we chose the endangered fountain darter, *Etheostoma fonticola.* An endangered species appeared appropriate since approximately 80% of U.S. Army installations share critical habitat with some 170 federally listed species (U.S. Army 2000). Listed species may be more sensitive since they tend to be more restricted in environmental tolerance. Fountain darters were found to be more sensitive than fathead minnows to copper (Besser et al. 2005) and to interstate highway storm water runoff (Longley et al. 2006). The fountain darter possesses many desirable characteristics for use as a test organism. They are small (< 45 mm in length) and resistant to stress when handled, crowded, or shipped. They are easily maintained and spawn year-round. Culture techniques and testing procedures have been refined in laboratory spawning and rearing studies at the San Marcos National Fish Hatchery and Technology Center (NFHTC); (Brandt et al.1993; Bonner et al. 1998; McDonald et al. 2007).

Our investigations focused on short term effects of exposures on survival and reproductive success. These tests explored the potential toxicity of different forms of fog oil on various life stages of the aquatic organism. The three physio-chemical forms of fog oil were: a smoke generated by vaporization; a liquid condensed from smoke and then agitated with water; and finally the water soluble portion from a floating film exposed to sunlight. The first objective was to determine if smoke exposures induced mortality in adults or impaired reproductive success as measured by egg production and viability. Secondly, since sensitivity may vary with life stage, we sought to determine egg and larval sensitivity to smoke from single and multiple fog exposures. The third objective was to determine 96 hour LC50 toxic endpoints in eggs and larvae of elevated

concentrations of liquid fog oil and water mixed with high energy. Our last objective was to evaluate the toxicological effects of photo-enhanced fog oil on young fish.

Materials and Methods

Forms of Fog Oil

The batch of fog oil in our tests was the same used by Cropek et al. (2008) and met Army requirements of reduced aromatic content (NRC 1997). Smoke was generated by injecting oil into a manifold heated to 350°C. The resulting vaporized fog oil was directed into a 12' x 12' x 8' chamber where test organisms in 450 mL glass I-Chem® jars (Thermo Fisher Scientific, Pittsburgh, PA) filled with water (synthetic moderately hard water (SMR water), pH 7.8, hardness 92 mg $CaCO_3$/L, conductivity 305 μS/cm^2, alkalinity 66 mg $CaCO_3$/L; USEPA, 2002) were placed on the floor. The aerosolized oil was directed into the chamber for 120 seconds and then allowed to settle onto the water surface of the jars for 9 h. Preliminary tests determined these times to approximate simulated battle field training exposures (D. Cropek [CERL], unpublished data, 2007).

The fog oil used to combine with water was derived by collecting condensing smoke on a cold aluminum surface placed 0.3 m from the manifold. This post-manifold fog oil was mixed with Synthetic Moderately Hard (SMH) water SMH water (ASTM 2005) to create water accommodated fractions (WAF). Difficulties of stirring the poorly water-soluble oil with low vortex energy (Maher 1982) sufficient to eliminate surface films dictated an alternative technique. Instead of stirring, the oil and water were violently agitated together in a paint mixer (Red Devil Autosperse, Plymouth, MN 55447). Oil mixtures with 300 mL of SMH water were individually prepared in 1-quart

containers by shaking for 5 min. Renewal mixtures for the static run trails were prepared daily to replace >85% of the original test volume.

Sunlight exposed fog oil was prepared by adding 10 mL of fog oil to 10 L of SMH water in an uncovered 75-L aquarium and then exposed to direct sunlight for 4 d. Solutions containing photo-enhanced water-soluble components were siphoned from underneath the floating oil layers. Three batches were produced and analyzed for Total Organic Carbon (TOC) and pH. Total Organic Carbon values were 38, 40 and 48 ppm, and pH values were 7.0, 7.8, and 7.0.

Test Fish Production and Shipping

Fountain darters utilized in these tests were produced at the NFHTC and were hatchery reared offspring from wild adults collected from the San Marcos River, Hays County, and the Comal River, Comal County, in central Texas. All activities associated with the use of fountain darters were authorized under federal permit TE676811-1 and Texas Parks and Wildlife Department permit SPR-0390-045. Fish were cultured in chilled Edwards Aquifer Ground water (EAG water; temperature 19°C± 2, alkalinity 319 mg/L and hardness 300 mg/L as $CaCO_3$). Spawning was coordinated to produce eggs, larvae, and juveniles of precise and consistent ages for the tests. The eggs were 24 to 48-hour post-fertilization to accommodate the delay of over-night shipment. Age of larvae used was 2 to 4-days post-hatch (total lengths of 3.8-4.4 mm). Fountain darters initially start feeding when they are 2 to 4 days old and are at a similar physiological stage as < 24-hour old

fathead minnows are when they are routinely used in toxicological tests. Also, at this stage larvae show improved resilience to handling. Juveniles were 30-days post-hatch, the same age dictated by USEPA (2002) and ASTM (2005) guidelines for fathead minnows. Adult breeders were 2-years old with standard lengths of 24-32 mm.

All exposures to fog oil were done at the Construction Engineering Research Laboratory (CERL), Champaign, IL. Eggs, juvenile, and adult fish were shipped by commercial overnight delivery (FedEx) in plastic bags in ice chests to CERL from the NFHTC. Water temperature was maintained between 16-21°C during shipment. Frozen gel packs were added to ice chests during warm weather. Each adult male/female pair was shipped with 400 mL of EAG water in a 1-quart plastic freezer bag inflated with oxygen. Eggs and juveniles were bulk shipped in larger plastic bags containing ~3 L of water and inflated to ~9-L volume with oxygen. Larvae were shipped as eggs and allowed to hatch in aquaria at CERL to minimize shipping mortality.

Smoke Exposures on Adults, Eggs, and Larvae

Adult, eggs, and larval fountain darters were exposed to fog oil smoke in toxicity tests. The treatment group consisted of 24 male and female pairs. Each pair was placed in individual jars with 300 mL of SMH water in the fogging chamber and received a 120-second fogging/ 9-hour deposition dose. A second group of 24 breeding pairs were in 24 jars that were not exposed to FO smoke (controls). At the end of the smoke exposure, each pair of treated and control fish were placed back into their original shipping bags

containing 1/3 EAG water and 2/3 oxygen by volume. The fish were shipped overnight to NFHTC which completed a roundtrip of ~ 3 days.

Two breeding pairs were then randomly stocked in each of twenty-four 7 L glass aquaria; 12 aquaria contained treated fish and 12 contained control fish. Each aquarium contained a 10-cm length of 7.6-cm PVC pipe cut lengthwise to be used as spawning substrate by the fish. The PVC spawning substrates were removed and replaced on days 5, 9, 13, 17, and 21. Eggs present on a spawning substrate and the sides of an aquarium were counted and then incubated in a separate adjacent aquarium (a total of 24 incubation aquaria). After the eggs were removed from an aquarium, a siphon tube was used to remove waste from the aquarium bottom. Before additional eggs were added to an incubation aquarium on days 9, 13, 17, and 21, all eggs within an incubation aquarium were inspected and the non-viable eggs were counted and discarded. Any larvae present also were counted and removed. Eight d after the last eggs were moved into an incubation aquaria, the numbers of viable eggs, non-viable eggs, and larvae present in each aquarium was determined.

Forty-eight aquaria (24 for adult fish and 24 for incubating eggs) were placed on top of three 530-L insulate fiberglass reservoir tanks (Living Stream, Frigid Units Inc. Toledo, OH, USA) equipped with 0.5-hp pumps (Hayward Industries, Elizabeth, NJ, USA) and heater/chiller units (Universal Marine Industries, Anmore, BC, Canada) which maintained water temperature at 21.4 ± 0.3°C and total gas saturation below 94%. Water was exchanged in each aquarium every 0.5 h and EAG water was added to the reservoir at the rate of ~ 1 L/min. During the spawning period, the fish were fed daily

live blackworms (*Lumbriculus variegatus*). Standard lengths of the adult fish were determined at the end of the spawning period. No adult mortalities occurred during the shipping, exposure, return shipping and spawning period.

Fountain darter eggs <24 h old were overnight shipped in EAG water from NFHTC to CERL. Upon arrival at CERL, the eggs were individually inspected for viability. Thirty clear eggs were placed in each of four jars with 300 mL of SMH water and exposed daily for three consecutive days to 120 seconds of smoke production and 9 h of smoke retention. Two jars containing 30 eggs each and SMH water and two jars containing 30 eggs each and EAG water were not exposed to smoke. Eggs infected with fungus were removed daily and on days 5 and 8 post initiation of trial, each jar was inspected and the numbers of viable eggs, non-viable eggs, and larvae were recorded. Dissolved oxygen, pH, temperature, and specific conductivity were measured daily in three randomly chosen smoke exposed jars and in all control jars. A second shipment of eggs was sent to CERL and a replicate trial was conducted.

Eggs were also shipped to CERL where they hatched to provide larvae for smoke exposure tests. Ten 2- to 4-day old larvae were each placed in 10 jars containing 300 mL of SMH water. The larvae were exposed to 120 seconds of smoke production and 9 h of smoke retention daily for 7 d. Ten jars each containing 10 larvae and EAG water and ten jars containing 10 larvae and SMH water were not exposed to smoke. Larvae were fed live brine shrimp (*Artemia salina)* daily following modified USEPA (2002) procedures: Additional rinses avoided fountain darter mortality associated with ingestion of un-hatched brine shrimp eggs. Dissolved oxygen, pH, temperature, alkalinity, hardness, and

conductivity were measured following procedures described earlier. The number of alive and dead larvae in each jar was recorded daily through day 8. A second group of larvae hatched at CERL were used to conduct a replicate trial.

Mixtures of Fog Oil and Water to Eggs and Larvae

Fountain darter eggs and larvae were used to determine the toxicity of condensed liquid fog oil mixed with water. Thirty clear eggs were placed in each of four jars with 300 mL of each of the generated fog oil /SMH water mixture test concentrations. A range finding trial was conducted to determine the final testing concentrations of 0, 900, 1275, 1650, 2025, and 2400 mg/L of generated FO. The eggs in each jar were inspected daily and the non-viable ones were removed. At the end of 96 h, the number of viable eggs was recorded. Water quality means in treatment replicates were: dissolved oxygen, 6.0 ± 0.4 mg/L; pH, 7.3 ± 0.4; temperature, 21.8 ± 0.4°C; and specific conductivity, 144 ± 10 µmhos/cm.

Ten 24- to 48-h old larvae were placed in each of four jars with 300 mL of each of the generated FO/SMH water mixture test concentrations. The concentrations tested included 0, 150, 300, 600, 1200, and 2400 mg/L. The larvae were fed brine shrimp daily. Mortalities were removed daily and number of surviving larvae in each jar at the end of 96 h was determined. Water quality was measured as described for earlier trials.

Solutions of Photo-transformed Fog Oil on Eggs, Larvae, and Juveniles

Static renewal trials with sunlight exposed fog oil were used to determine 96-h LC50 toxicity endpoints for fountain darter eggs, larvae, and juveniles. Trials for each life stage were run with five photo-oxidized fog oil dilutions, a SMH water control, and four replicates per dilution. Each replicate received 300 mL of test solution and either 30 eggs, 10 larvae, or 10 juveniles. Dilutions were chosen after preliminary range finding trials were completed. Final dilutions used were: eggs- 0, 10, 15, 25, 35, and 45%; larvae- 0, 11, 13, 15, 17, and 19%; and juveniles- 0, 11, 13, 15, 17, and 19%. Spearman-Karber tests located final LC-50's. As photo-enhancement increased the solubility of fog oil components, Total Organic Carbon analyses were conducted on the original 3 stock solutions. Calculations then converted dilution percentages to mg/ L to order to compare toxicities with earlier water accommodated fraction results and among life stages. Eggs, larvae, and juveniles were inspected daily and mortalities counted and removed. Test solutions for 85% daily replenishments were prepared immediately prior to solution renewal. Larvae and juveniles were fed brine shrimp daily. Dissolved oxygen, pH, temperature, and specific conductivity were measured daily before each static renewal in three randomly chosen treatment jars and in all control jars. Parameter means were: dissolved oxygen, 6.7 ± 0.5 mg/L; pH, 7.6 ± 0.5; temperature, 21.9 ± 0.6°C; and specific conductivity, 141 ± 12 μmhos/cm.

Statistical Analyses

The effects of smoke exposure on adults were evaluated in a repeated measures one-way analysis of variance (ANOVA). The response data of egg output and viability were analyzed using JMP-In®(SAS Institute, Belmont, CA, USA) with no transformations. Statistical significance throughout the experiments was assumed at $P < 0.05$. Two trials of 7 d repeated foggings of larvae were analyzed for significance through Kaplan-Meier estimations of survival functions from mortality data. Group comparisons through tests of equality by Log rank (Mantel-Cox) were performed between exposures, controls, and exposures versus controls. Mean lethal concentration (LC-50) estimates and associated 95% confidence intervals of eggs, larvae, and juveniles exposed to generated fog oil and photo-oxidized fog oil were evaluated with trimmed Spearman-Karber tests. This nonparametric procedure was run on EPA-provided software. Since control survivals were >90%, no corrections were made for control mortalities. Significant differences between LC-50's were based on proportion overlap ($p<0.05$) or no overlap ($p<0.01$) of confidence intervals between mixtures, dilutions, and life stages (Cumming, 2009).

Results

Smoke Exposures on Adults, Eggs, and Larvae

Smoke had no effect on survival of adults during or after the exposure. Treated and control fish survived the initial treatment, shipment back to the NFHTC, and the 21-d spawning period. There was no effect detected between treatment and control groups (p=0.23) of smoke on production of viable and non-viable eggs by the adults over the same 21- d spawning period (Figure 1).

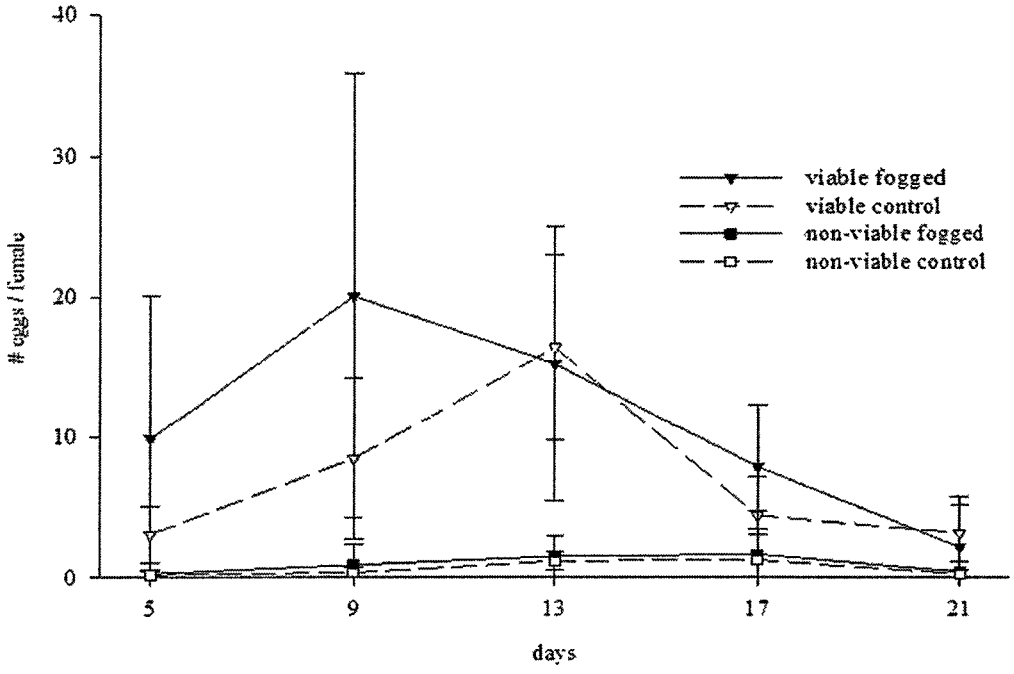

Figure 1. Mean (±S.D.) numbers of viable/ non-viable eggs produced over 21 d by treatment (single smoke exposure; n=24) and control (unexposed; n=24) groups. Repeated measures ANOVA indicates no treatment effects (F = 1.546, DF = 22, p = 0.2268).

Number of fountain darters eggs exposed over 3 d to smoke did not differ (P = 0.13) from the control. Egg survival exposed to FO smoke was 87%, whereas egg survival for the control was 92%. Number of fountain darter eggs exposed for 7 days to fog oil smoke had higher larval mortality, ranging from 15% to 64%, compared to 5 and 8% in the controls (Figure 2).

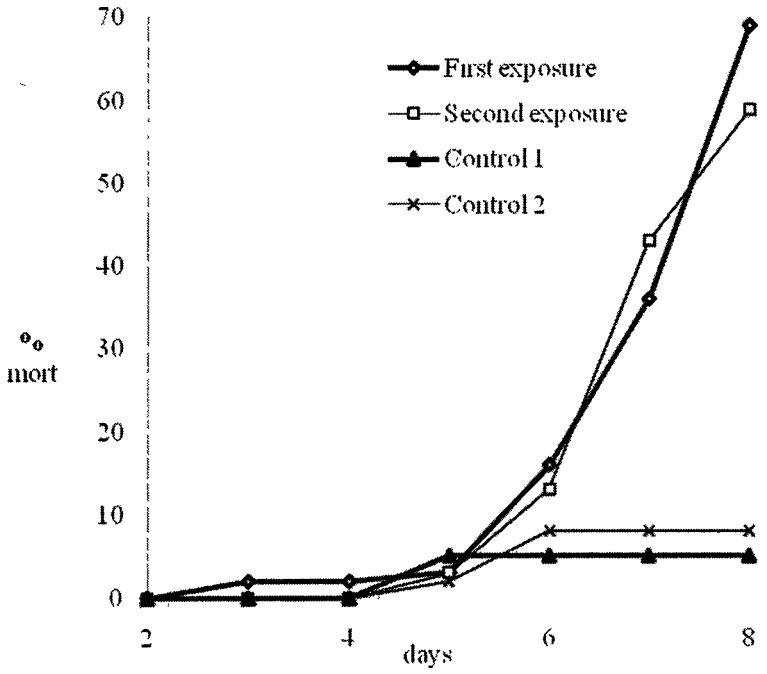

Figure 2. Cumulative mortalities over 7 d of larvae in 2 trials (treatments) daily to fog oil smoke versus unexposed (controls) groups.

Mortality in the controls of the two trials was 5% and 8%. Kaplan-Meier survival functions showed significance between treatments and controls ($\chi 2 = 55.729$, df 1, $P<0.001$).

Mixtures of Fog Oil and Water to Eggs and Larvae

Toxicity of condensed liquid fog oil and water mixtures was greater in larvae than in eggs. Egg mortalities in the treatments ranged from 10% at 900 mg/L to 73% in 2,400 mg/L mixtures to (Table1). Mortalities in larval treatments were 10% at 150 mg/L and 100% in 2,400 mg/L.

Mean survival in the controls for eggs and larvae was 92.5% (± 7.5%). Egg mortalities in the treatments ranged from 72.50% in 2400 mg/L mixtures to 10% at 900 mg/L (Table1). Mortalities in larval treatments were 100% in 2400 mg/L and 10% at 150 mg/L. Spearman- Karber calculations of 96 h LC50 with 95% Confidence Intervals indicated an almost 3X higher difference in toxicity for the larvae (709.4 mg/L; Table 1) compared to eggs (2105.2 mg/L).

Solutions of Photo-transformed Fog Oil on Eggs, Larvae, and Juveniles

Lethal concentrations for 96 h were 40% of stock solution for eggs, 16% for larvae, and 17% for juveniles (Table 1). Non-overlapping Confidence Intervals suggested significant differences ($p< 0.05$) in toxic levels between each life stage (Cumming 2009).

Total organic carbon analysis of stock solutions converted % concentration values to 18.5 mg/L for eggs, 7.4 mg/L for larvae and 8.2 mg/L for juveniles. Survival in the controls for egg, larvae, and juvenile tests was 98%, 94%, and 98%, respectively.

Table 1. 96 hour LC-50 values of water accommodated fractions and water soluble fractions of fog oil for different life stages of *Etheostoma fonticola.*

Life Stage	WAF (mg/L)	% mort	Spearman-Karber LC-50 (95% CI)	WSF (mg/L)	% mort	Spearman-Karber LC-50 (95% CI)
Eggs	2400	72 5	2150 (2048-2257) mg/L	20 7	77 5	18 5(17 9-19 2) mg/L
	2025	40 0		16 1	17 5	
	1650	35 0		11 5	2 5	
	1275	25 0		6 9	2 5	
	900	10 0		4 6	2 5	
	0	7 5		0	2 5	
Larvae	2400	100 0	709 (613-821) mg/L	8 7	100	7 4 (7 1-7 7) mg/L
	1200	87 5		7 8	65 7	
	600	32 5		6 9	31 4	
	300	7 5		6 0	16 7	
	150	10 0		5 1	13 9	
	0	0 0		0	5 6	
Juveniles				9 1	82 2	8 2(7 9-8 5) mg/L
				8 2	47 5	
				7 2	17 5	
				6 2	7 5	
				5 3	2 5	
				0	2 5	

Discussion

Our study is the first to quantify lethal and sub-lethal effects of fog oil on a freshwater fish. Previous studies found no effect on survival to single exposures of smoke to fathead minnows *Pimephales promelas*, rainbow trout *Oncorhynchus mykiss*, and Topeka shiners *Notropis topeka* (Cropek, CERL, unpublished data). While this investigation also produced no adult mortality from a single fogging, lethal effects were eventually obtained by increasing exposure intensities on more sensitive younger fish. The mortality in larvae after 7 d of fogging could suggest caution by Army managers to avoid multiple foggings over successive days, especially during periods of known spawning of fish. But overall, fog oil appears to be relatively benign on fish. Smoke or mixtures at normal rates were difficult to induce direct mortality. The high concentrations needed to provoke mortality appear to be improbable in most in-field scenarios. The amounts of smoke from multiple exposures and of oil mixed artificially with violent and sustained energy under laboratory conditions is probably too high to find naturally.

Where concentrations high enough to be toxic may be probable could be the result of a contaminant spill with sunlight. Fog oil components transformed by UV were demonstrated to be more toxic than previously tested smoke and mixtures. As UV increased toxicity for eggs and larvae ~100X from mixtures to solutions in these tests, concentrations high enough to be toxic could possibly be attained in the field. Various other oils after exposure to UV have also been shown to increase in toxicity with aquatic organisms. A range of light and heavy oils were up to 50,000 times more toxic after

photo-enhancement for *Mulinia lateralis*, a bivalve, and *Mysidopsis bahia*, a mysid shrimp (Pelletier et al. 1997). The photo-toxicity of petroleum oils were related to aromatic ring composition and concentration of polycyclic aromatic hydrocarbons (PAHs). The greater solubility of these photo-enhanced compounds substantially increased the toxicity of weathered crude oil in static renewal tests with *Mysidopsis bahia* (Cleveland et al. 2000) and increased bioaccumulation factors in copepods to 2000 for *Metridia okhotensis* and 8000 for *Calanus marshallae* (Duesterloh et al. 2002). In fishes, photo-toxicity of crude oil increased for *Oncorhynchus gorbuscha* juveniles (Barron et al. 2005) and of anthracene, a common PAH, for *Lepomis macrochirus* juveniles (Oris et al. 1984).

Although not tested here, mortality in natural settings could be of concern through indirect routes of fog oil contamination to fish. Fountain darters may not be in direct contact with surface floating oils as they are exclusively benthic due to evolutionary loss of the swim bladder. Indirectly their prey may be. *Daphnia magna* and *Ceriodaphnia dubia,* a common food for many larval fishes, incurred mortality in toxicity studies with fog oil (Cropek et al. 2008). Mortality was more influenced by physical contact of the organisms to the surface film of oil on the water in the test containers that the actual toxicity of the oil. Surface films of oils are used to kill predatory aquatic invertebrates in fish hatchery nursery ponds. During our study, surface films of oil were observed on water surfaces during exposures of smoke and oil and water. Brine shrimp fed to fish in the study passed through surface oils and could have provided an additional access to the fish through their digestive tracts. Feeding effects from these coated invertebrates could

possibly result in bio-accumulation or bio-magnification having long term physiological effects on fish. Contaminations could also deplete availability of food organisms and pull the rug out from under populations of predator fishes.

References

ASTM (2005) Standard guide for conducting early life-stage toxicity tests with fishes (E 1241-05), pp. 886–913.

Barron MG, Carls MG, Short JW, Rice SD, Heintz RA, Rau M, Di Giulio R (2005) Assessment of the phototoxicity of weathered Alaska North Slope crude oil to juvenile pink salmon. Chemosphere 60:105-110.

Beens J, Blomberg J, Schoenmakers PJ (2000) Proper tuning of comprehensive two-dimensional gas chromatography (GCxGC) to optimize the separation of complex oil fractions. J High Resol Chromatog 23:182-188.

Besser JM, Wang N, Dwyer FJ, Mayer FL, Ingersoll CG (2005) Assessing contaminant sensitivity of endangered and threatened aquatic species: Part II. Chronic toxicity of copper and pentachlorophenol to two endangered species and two surrogate species. Arch Environ Contam and Toxicol 48:155-165.

Bonner TH, Brandt TM, Fries JN, Whiteside BG (1998) Effects of temperature on egg production and early life stages of the fountain darter. Trans Amer Fish Soc 127:971-978.

Bowling JW, Leversee GJ, Landrum PF, Giesy JP (1983) Acute mortality of anthracene-contaminated fish exposed to sunlight. Aquat Toxicol 3:79-90.

Brandt TM, Graves KG, Berkhouse CS, Simon TP, Whiteside BG (1993) Laboratory spawning and rearing of the endangered fountain darter. Prog Fish-Cult 55:149-156.

Cleveland L, Little EE, Calfee RD, Barron MG (2000) Photoenhanced toxicity of weathered oil to *Mysidopsis bahia.* Aquat Toxicol 49:63-76.

Cropek DM, Esarey JC, Conner CL, Goran JM, Smith T, Soucek DJ (2008) Toxicological effects of military fog oil obscurant on *Daphnia magna* and *Ceriodaphnia dubia* in field and laboratory exposures. Ecotoxicol 17:517-525.

Cumming G (2009) Inference by eye: Reading the overlap of independent confidence intervals. Statist Med 28:205-220.

Duesterloh S, Short JW, Barron MG (2002) Photoenhanced toxicity of weathered Alaska North Slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. Environ Sci Technol 36:3953-3959.

Esarey JC, Soucek DJ, Cropek DM, Smith T (2004) Toxicological effects of military smokes and obscurants on aquatic threatened and endangered species. (ADA433710). Defense Technical Information Center, Fort Belvoir, VA.

Gaur JP, Singh AK (2006) Regulatory influence of light and temperature on petroleum toxicity to *Anabaena doliolum.* Environ Toxicol and Water Chem 6:341-350.

Getz LL, Reinbold KA, Tazik DJ, Hayden TJ, Cassels DM (1996) Preliminary assessment of the potential impact of fog oil smoke on selected threatened and endangered species. (A912603) Construction Engineering Research Laboratory, Champaign, IL.

Langford RE (2004) Introduction to Weapons of Mass Destruction: Radiological, Chemical, and Biological. John Wiley & Sons, Hoboken, NJ.

Little EE, Cleveland L, Calfee R, Barron MG (2000) Assessment of the photoenhanced toxicity of a weathered oil to the tidewater silverside. Environ Toxicol Chem 19:926-932.

Longley G, Guerrero J, Castillo V III (2006) Water-quality characterization of storm-water runoff from I-35 in San Marcos, Texas. Report no. 2006-1 Edwards Aquifer Research & Data Center, Texas State University, San Marcos.

Maher WH (1982) Preparation and characterization of water soluble fractions of crude and refined oils for use in toxicity studies. Bull Environ Contam Toxicol 29:268-272.

McDonald DL, Bonner TH, Oborny EL, Brandt TM (2007) Effects of fluctuating temperatures and gill parasites on reproduction of the fountain darter, *Etheostoma fonticola*. J Freshwater Ecol 22:311-318.

National Research Council (NRC): Committee on Toxicology—Subcommittee on Military Smokes, Obscurants (1997) Toxicity of military smokes and obscurants, Vol. 1. National Academy Press, Washington, DC.

National Research Council (NRC): Subcommittee on Military Smokes and Obscurants (1999) Toxicity of military smokes and obscurants. Vol.3. National Academy Press, Washington DC.

Oris JT, Giesy JP, Allred PM, Grant DF, Landrum PF (1984) Photo-induced toxicity of anthracene in aquatic organisms: an environmental perspective. Studies Environ Science 25: 639-658.

Pelletier MC, Burgess RM, Ho KT, Kuhn A, McKinney RA, Ryba SA (1997) Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environ Toxicol Chem 16: 2190-2199.

Poston TM, Bean RM, Kalkwarf DR, Thomas BL Clark ML, Killand BW (1988) Photooxidation products of smoke generator fuel (SGF) no. 2 fog oil and toxicity to *Hyallela azteca.* Environ Toxicol and Chem 7:753-762.

Quist MC, Fay PA, Guy CS, Knapp AK, Rubenstein BN (2003) Military training effects on terrestrial and aquatic communities on a grassland military installation. Ecol Applications 13: 432-442.

United States Army (2000) U.S. Army conservation technology program plan; environmental technology management plan for user requirement #1, impacts of military operations on T&E species. Washington, DC.

United States Army Corps of Engineers (2007) Preserving the environment. www.usace.army.mil/whatwedo/text.html (accessed 2007).

United States Environmental Protection Agency (2000) Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136) (EPA 821-B-00-004) United States Environmental Protection Agency, Office of Water, Washington, DC.

United States Environmental Protection Agency (2002) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 4th ed. (EPA-821-R-02-013) United States Environmental Protection Agency, Office of Water, Washington, DC.

Williams LR, Bonner TH, Hudson JD, Williams MG, Leavy TR, Williams CS (2005) Interactive effects of environmental variability and military training on stream biota of three headwater drainages in western Louisiana. Trans Amer Fish Soc 134: 192-206.

VITA

Thomas Alfredo Ryan was born in Hawthorne, Nevada on April 16, 1960, the son of F. Thomas Ryan and Margarita C. Ryan. After completing his work at Reno High School in Reno, Nevada, he attended Texas A&M University in College Station, Texas and the Universidad de Compostela, in Santiago de Compostela, Spain. In 1983 he entered the United States Peace Corps as a fisheries volunteer stationed in the Amazon River Basin. During the following years he was employed in commercial aquaculture in Ecuador, Honduras, Indonesia, and Belize. In January 2005 he entered the Graduate College of Texas State University- San Marcos.

Permanent Email Address: oliver.moose@gmail.com

This thesis was typed by Thomas A. Ryan.