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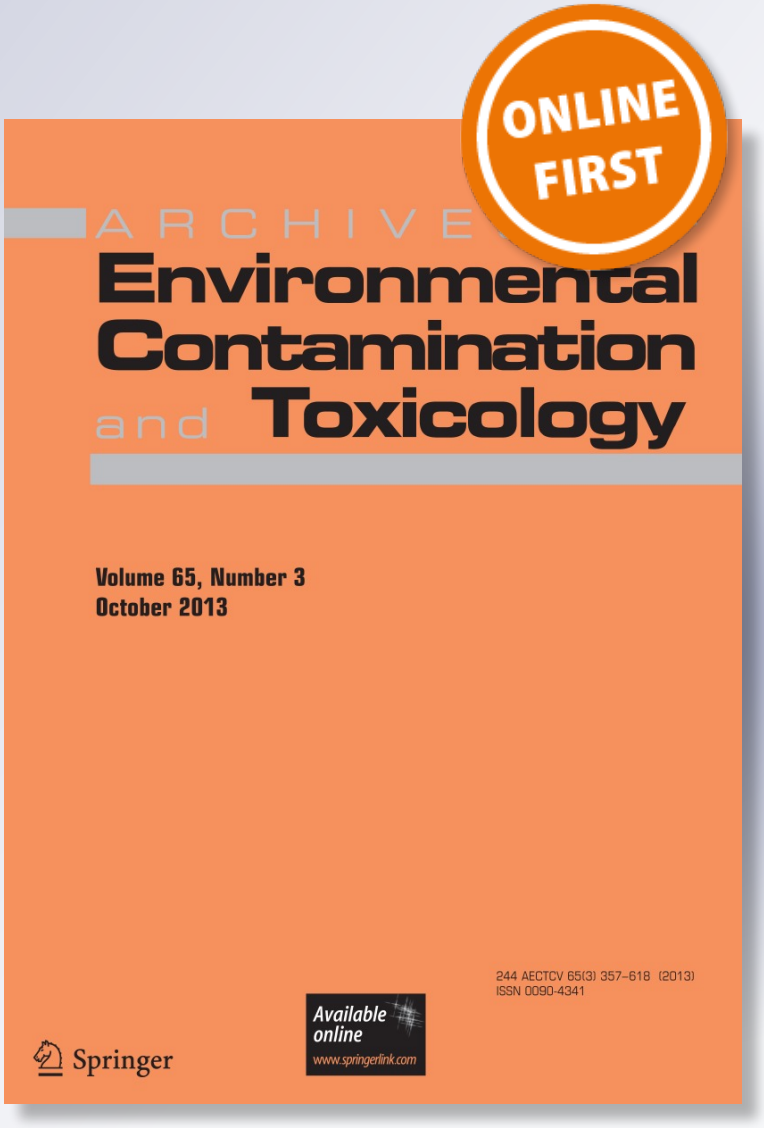

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Short-Term Effects of Military Fog Oil on the Fountain Darter (*Etheostoma fonticola*)

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Abstract Toxicity tests evaluated chronic and sublethal effects of fog oil (FO) on a freshwater endangered fish. FO is released during military training as an obscurant smoke that can drift into aquatic habitats. Fountain darters, *Etheostoma fonticola,* of four distinct life stages were exposed under laboratory conditions to three forms of FO. FO was vaporized into smoke and allowed to settle onto water, violently agitated with water, and dosed onto water followed by photo-oxidization by ultraviolet irradiation. Single smoke exposures of spawning adult fish did not affect egg production, egg viability, or adult fish survival in 21-day tests. Multiple daily smoke exposures induced mortality after 5 days for larvae fish. Larvae and juvenile fish were more sensitive than eggs in 96-h lethal concentration (LC_{50}) tests with FO–water mixtures and photo-oxidized FO. Water-soluble FO components photo-modified by ultraviolet radiation were the most toxic, thus indicating the value of examining weathering and aging of chemicals for the best determination of environmental impact.

This article is dedicated to Tom Smith, who died on August 31, 2013.

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The United States Army manages millions of acres of land and uses a sizable portion as training lands (Doe et al. 1999). Military training scenarios are designed to simulate warfare conditions. Because training operations can alter these lands physically and biologically, the United States military monitors lands to minimize environmental impacts (Williams et al. 2005; Quist et al. 2003). One training activity includes the release of obscurant smokes to simulate visually adverse battlefield conditions. Although overall impacts of warfare on landscapes are widely recognized, training-related effects on freshwater ecosystems are perhaps less so (Francis 2011). The entire range of effects of military obscurant emissions on aquatic habitats is not known.

Of the three more commonly used obscurants, fog oil (FO) is thought to present fewer toxicity issues than hexachloroethane and white phosphorus (Getz et al. 1996). FO oil smoke is generated by injecting a middle distillate mineral oil onto a heated manifold. The expelled oil vapor condenses on contact with air and forms a dense, white cloud. This smoke is actually a mist composed of fine droplets. FO is a complex mixture of aliphatic, olefinic, and naphthenic compounds. These oil types are estimated to contain >1,000,000 organic constituents (Beens et al. 2000) and cannot be completely characterized with current analytical techniques. Furthermore, the composition of FO varies from different sources and even from batch to batch (Langford 2004). Fractionation techniques combined with comprehensive gas chromatography analysis yield primarily long-chain, branched, and cyclic alkanes and olefinic compounds with evidence of only minor amounts of

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polycyclic aromatic hydrocarbons (PAHs) (Kohl et al. 2010).

Studies of obscurant emissions have attempted to identify health hazards (National Research Council [NRC] 1999). As precautions, the United States Army has required decreases in potentially hazardous aromatics and has restricted duration of FO exposure to soldiers. Specific information on FO and FO smoke as an ecological hazard is limited. Some toxicity in aquatic organisms, however, has been shown. Mortalities of *Daphnia magna* were observed downwind of FO smoke generated in field tests (Esarey et al. 2004; Cropek et al. 2008). Aerosolized droplets settling on the water surface formed a quantifiable hydrophobic layer, and *D. magna* died when trapped in this surface film. These mortalities occurred under test conditions of low sunlight exposure.

Ultraviolet (UV) radiation from sunlight can photo-enhance FO toxicity. Components of different oils can be photo-oxidized by UV resulting in transformation of organic compounds to increase their toxicity to aquatic organisms. The influence of sunlight on crude oil inhibited the cyanobacteria *Anabaena doliolum* (Gaur and Singh 2006). Photo-enhanced toxicity of weathered crude oil increased to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis* (Duesterloh et al. 2002), to the tidewater silverside (*Menidia beryllina)* (Little et al. 2000), and to Pacific herring (*Clupea pallasii*) eggs and larvae (Barron et al. 2005). Bluegill (*Lepomis macrochirus*) contaminated with anthracene, a PAH, showed increased mortality rates when moved from a shaded to a sun-exposed area due to photo-sensitization (Bowling et al. 1983). Likewise, UV radiation can photo-oxidize FO to increase organic component solubility and decrease water pH, thus amplifying FO toxicity to the amphipod *Hyallela azteca* (Poston et al. 1988).

For toxicity studies, the United States Environmental Protection Agency (USEPA) 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 because ~80 % of United States Army installations share critical habitat with some 170 federally listed species (United States Army 2000). Listed species may be more sensitive because 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 the short-term effects of FO exposures on survival and reproductive success. These tests explored the potential toxicity of different forms of FO on various life stages. The first objective was to determine if smoke exposures induced mortality in adults or impaired reproductive success as measured by egg production and viability. Second, because sensitivity may vary with life stage, we sought to determine egg and larval sensitivity to smoke from single and multiple FO exposures. The third objective was to create increased water-accommodated fractions (WAFs) of FO to determine 96-h lethal concentration (LC_{50}) mortality rates in eggs and larvae. Our last objective was to evaluate the toxicological effects of photo-oxidized FO on juvenile fish.

Materials and Methods

Forms of FO

The batch of FO in our tests was limited in aromatic content per military specification (NRC 1997) and was the same type used by Cropek et al. (2008). The three physiochemical forms of FO were as follows: (1) a smoke generated by vaporization; (2) a liquid condensed from smoke and then agitated with water; and, finally, (3) the water-soluble portion from a floating film exposed to sunlight.

Smoke was generated by injecting oil onto a manifold heated to 350 °C. The resulting vaporized FO was directed into a 3.66 × 3.66 × 2.44-m^3 chamber where test organisms in 450 mL glass I-Chem jars (Thermo Fisher Scientific, Pittsburgh, PA, USA) filled with USEPA-recommended standardized water ((synthetic moderately hard [SMH] 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 s and then allowed to settle onto the water surface of the jars for 9 h. Preliminary tests determined these times to approximate simulated maximum battlefield training exposures (D.M. Cropek, unpublished data). The FO 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 FO was mixed with SMH water to create WAFs. Difficulty 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, USA). Three hundred milliliters of SMH water plus oil mixtures were individually prepared in 1-quart containers by shaking for 5 min. Renewal mixtures for the static run trials were prepared daily to replace >85 % of the original test volume.

Sunlight exposed FO was prepared by adding 10 mL of FO to 10 L of SMH water in an uncovered 25-L aquarium and then exposed to direct sunlight for 4 days. Three batches were produced and the aqueous underlayer was analyzed for total organic carbon (TOC) and pH. TOC values were 38, 40, and 48 ppm, and pH values were 7.0, 7.8, and 7.0, respectively. A siphon was used to remove the lower aqueous layer containing water-soluble components of photo-oxidized FO without disturbing the floating oil layer above.

Test Fish Production and Shipping

Fountain darters used 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, 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 groundwater (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. Eggs were age 24–48 h postfertilization to accommodate the delay of overnight shipment. Age of larvae was 2–4 days posthatch (total length 3.8–4.4 mm). Fountain darters initially start feeding when they are 2–4 days old and are at a similar physiological stage as <24-h-old fathead minnows are when they are routinely used in toxicological tests. In addition, at this stage larvae show improved resilience to handling. Juveniles were 30-days posthatch, the same age dictated by the USEPA (2002) and ASTM (2003) guidelines for fathead minnows. Adult breeders were 2 years old with standard length of 24–32 mm.

All exposures to FO were performed at the Construction Engineering Research Laboratory (CERL), Champaign, IL, USA. Eggs, juveniles, and adult fish were shipped by commercial overnight delivery in plastic bags in ice chests to CERL from the NFHTC. Water temperature was maintained between 16 and 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 Ziploc® 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 Toxicity to Adults, Eggs, and Larvae

Eggs, larvae, and adult fountain darters were exposed to FO as smoke in toxicity tests. Adults were shipped in EAG water from the NFHTC to CERL. The treatment group consisted of 24 pairs of male and female fish. Each pair was placed in individual jars with 300 mL of SMH water in the fogging chamber and underwent a 120-s fogging and a 9-h deposition dose. A second group of 24 breeding pairs, one pair in each of 24 jars, 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 Ziploc® shipping bags containing one third EAG water and two thirds oxygen by volume. The fish were shipped overnight back to the NFHTC. Arrival back in Texas completed a round trip of ~3 days.

Two breeding pairs were then randomly selected and stocked in each of 24 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 polyvinyl chloride (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 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 nonviable eggs were counted and discarded. Any larvae present also were counted and removed. 8 days after the last eggs were moved into an incubation aquaria, the numbers of viable eggs, nonviable eggs, and larvae present in each aquarium were determined.

The 48 total aquaria (24 for adult breeding pairs 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 and total gas saturation <94 %. Water was exchanged in each aquarium every half hour, and EAG water was added to the reservoir at the rate of ~1 L/min. During the spawning period, fish were daily fed 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 periods.

Fountain darter eggs <24 h old, from FO nonexposed adults, were shipped overnight in EAG water from the NFHTC to CERL. On arrival at CERL, 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 s of smoke production and 9 h of smoke settling time. Two jars containing 30 eggs each plus SMH water and two jars containing 30 eggs each plus EAG water were not exposed to smoke. Eggs infected with fungus were removed daily, and on days 5 and 8 after initiation of trial each jar was inspected and the numbers of viable eggs, nonviable eggs, and larvae 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 performed.

Eggs were also shipped to CERL where they hatched to provide larvae for smoke-exposure tests. Ten 2- to 4-day-old larvae were placed in each of 10 jars containing 300 mL of SMH water. The larvae were exposed to 120 s of FO smoke production and 9 h of smoke retention daily for 7 days. Ten jars each containing ten larvae plus EAG water and ten jars containing ten larvae plus SMH water were not exposed to FO smoke. Larvae were fed live brine shrimp (*Artemia salina*) daily according to modified USEPA (2002) procedures. Brine shrimp were washed several extra times to limit fountain darter mortality associated with ingestion of unhatched brine shrimp eggs. Dissolved oxygen, pH, temperature, alkalinity, hardness, and conductivity were measured according to the procedures described previously. The number of live 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.

Toxicity of Eggs and Larvae to Generated FO WAF

Fountain darter eggs and larvae were used to determine the toxicity of the generated FO WAF. Thirty clear eggs were placed in each of four jars with 300 mL of each of the generated FO–SMH water mixture test concentrations. A range-finding trial was performed 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 nonviable eggs were removed. At the end of 96 h, the number of viable eggs was recorded. Water quality was measured as described previously. 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. Dead larvae were removed daily, and the number of surviving larvae in each jar at the end of 96 h was determined. Water quality was measured as described previously earlier trials.

Toxicity of Eggs, Larvae, and Juveniles to Sunlight-Exposed FO

Static renewal trials with sunlight-exposed FO were used to determine the 96-h LC_{50} values for fountain darter eggs, larvae, and juveniles. Trials for each life stage were run with five dilutions of the water containing photo-oxidized FO components (WSF), 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 as follows: 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 determined final LC_{50} values. Because photo-transformation increased the solubility of FO components, TOC analyses were performed on the original stock solutions and the dilutions of all final tests. Calculations then converted dilution percentages to mg/L to directly compare toxicities with earlier WAF results and among life stages. Eggs, larvae, and juveniles were inspected daily and the dead were counted and removed. Test solutions for 85 % daily replenishments were prepared immediately before 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.

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, Belmont, CA, USA) with no transformations. Statistical significance throughout the experiments was assumed at $p < 0.05$.

Two trials of 7-day 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.

LC_{50} estimates and associated 95 % confidence intervals (CIs) of eggs, larvae, and juveniles exposed to generated FO and photo-oxidized FO were evaluated with trimmed Spearman–Karber tests. This nonparametric procedure was run on USEPA-provided software. Because control survivals were >90 %, no corrections were made for control

mortalities. Significant differences between LC_{50} values were based on proportion overlap ($p < 0.05$) or no overlap ($p < 0.01$) of CIs between mixtures, dilutions, and life stages (Cumming 2009).

Results

FO Smoke

A control was performed with fish in SMH water and fish in EAG. Both controls had >90 % survival. Therefore, there was no confounding effect due to differences in these two water types, and SMH water was used throughout the remainder of this study. Smoke exposure of adults had no effect on survival during or after exposure. All fish survived treatment, shipment back to the NFHTC, and the 21-day spawning period in both the treated and control groups. There also was no effect ($p = 0.23$) of exposure to smoke between treatment and control groups regarding production of viable and nonviable eggs by adults during the same 21-day spawning period (Fig. 1). High variability in egg output between females was examined using female size as a possible external factor that could have influenced the outcome. Inadvertent nonrandom selection of fish for a trial may skew results by larger females producing more eggs. Standard lengths of individual female fish were plotted as linear regressions against the number of eggs produced by each female. No relationship was found.

The response of fountain darters eggs to exposure during the course of 3 days to the FO smoke was similar to the response of the adults. No significant difference was found between survival of the eggs not exposed (i.e., 92 %) and the eggs exposed to FO smoke (i.e., 87 %). However, two extended trials of seven consecutive days of fogging prompted mean larval mortality for the two trials to progress to 15 % on day 6, 30 % on day 7, and 64 % on day 8 (Fig. 2). Mortality in the controls was <10 %. Kaplan–Meier survival functions showed significance between treatments and controls ($\chi^2 = 55.729$, degrees of freedom [*df*] 1, $p < 0.001$) and no significance between exposed treatments ($\chi^2 = 0.742$, *df* 1, $p = 0.389$) and unexposed controls ($\chi^2 = 0.170$, *df* 1, $p = 0.680$).

Generated FO WAFs

Survival in the controls for eggs and larvae was >92 %. Water quality means (±1 SD) in treatment replicates were as follows: 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. Egg mortalities in the treatments ranged from 73 % in 2,400 mg/L to 10 % at 900 mg/L mixtures (Table 1). Mortalities in larval treatments were 100 % in 2,400 and 10 % at 150 mg/L. Larval mortality indicated greater sensitivity compared with eggs. Spearman–Karber calculations of 96-h LC_{50} values with 95 % CIs indicated an almost three-fold greater difference in toxicity for larvae (709.4 mg/L; Table 1) compared with eggs (2105.2 mg/L).

Photo-Transformed Water-Soluble Fractions

Survival in the controls for all life stage tests was >94 %. Means (±1 SD) of water-quality measurements remained within USEPA guidelines: dissolved oxygen 6.7 ± 0.5 mg/L, pH 7.6 ± 0.5, temperature 21.9 ± 0.6 °C, and specific

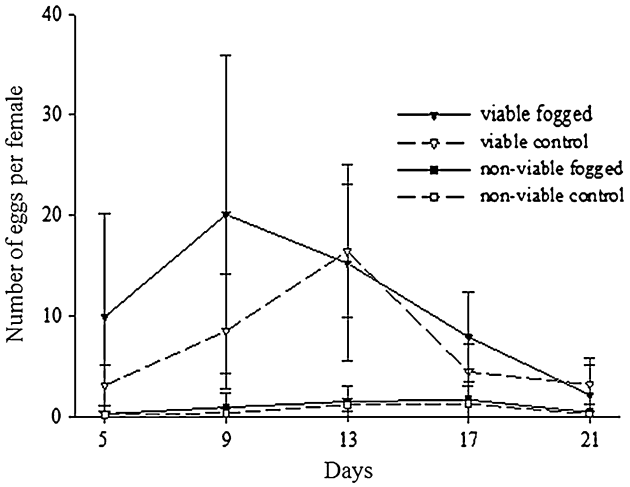

Fig. 1 Mean (±SD) numbers of viable and nonviable eggs produced during 21 days by treatment (single smoke exposure; $n = 24$) and control (unexposed; $n = 24$) groups. Repeated-measures ANOVA indicated there were no treatment effects ($F = 1.546$, *df* 22, $p = 0.2268$)

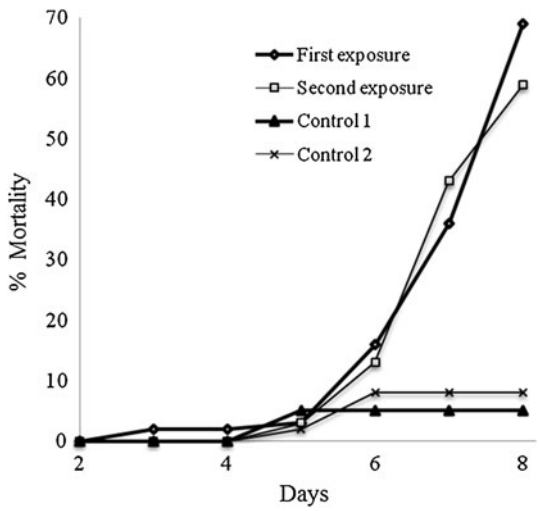

Fig. 2 Cumulative mortalities in two trials of larvae exposed daily to FO smoke (treatment) for 7 days versus unexposed (control) groups

Table 1 96-h LC_{50} values of WAFs and WSFs of FO for different life stages of *E. fonticola*

Life stage	WAF (mg/L)	Mortality (%)	Spearman–Karber LC_{50} (95 % CI)	WSF (mg/L)	Mortality (%)	Spearman–Karber LC_{50} (95 % CI)
Eggs	2,400	72.5	2,150 (2,048–2,257)	20.7	77.5	18.5 (17.9–19.2)
	2,025	40.0		16.1	17.5	
	1,650	35.0		11.5	2.5	
	1,275	25.0		6.9	2.5	
	900	10.0		4.6	2.5	
	0	7.5		0	2.5	
Larvae	2,400	100.0	709 (613–821)	8.7	100	7.4 (7.1–7.7)
	1,200	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)
				8.2	47.5	
				7.2	17.5	
				6.2	7.5	
				5.3	2.5	
				0	2.5	

conductivity 141 ± 12 μmhos/cm. As with the pattern of results of earlier tests with WAFs, larvae were again more sensitive than eggs in exposures to water-soluble fractions (WSFs). Spearman–Karber calculations provided final 96-h LC_{50} (95 % CI) values of 40 % (±2 %) of original stock solution concentration for eggs, 16 % (±1 %) for larvae, and 17 % (±1 %) for juveniles (Table 1). TOC analysis of stock solutions measured 48 mg/L (pH 7.0) and 46 mg/L (pH 7.5) and was used to convert % concentration values to 18.5 mg/L for eggs, 7.4 mg/L for larvae, and 8.2 mg/L for juveniles. Nonoverlapping CIs suggested significant differences in toxic levels between each life stages (Cumming 2009).

Discussion

Our study is the first to quantify lethal and sublethal effects of FO on a freshwater fish. Effects were not always detected with a single exposure but generally were detected after multiple exposures. The preliminary objective was to determine any potential toxicity of foggings through mortality or impaired reproduction. Preliminary exposure trials (A.N. Kohl, D.J. Soucek, T.S. Smith, D.M. Cropek, unpublished data) of fathead minnows, rainbow trout (*Oncorhynchus mykiss)*, and Topeka shiners (*Notropis topeka)* to a single exposure of FO smoke under realistic field conditions found no effect on survival. Likewise in our tests, adult fish that were fogged once incurred no mortality. In addition, egg production and egg viability were not adversely affected.

Because a single fogging produced no adult mortality, multiple fog exposures were performed on eggs as well as on larvae and juveniles, which are considered to be more sensitive life stages. During this study, egg, larval, juvenile, and adult fountain darters were all shipped from Texas to IL, USA. Newly hatched larvae were the only life stage that experienced mortalities during shipment. The larval shipping mortality rate was high enough that the shipping of larvae was stopped. All larvae used in this study were shipped from Texas as eggs and emerged from eggs after arriving in Illinois.

Eggs fogged successively for 3 days had no effect on hatch-out. Daily exposures were limited to 3 days because egg hatch-out begins on day 4. Mortality from smoke was finally induced in larvae after 5 days. Egg and adult tests were not extended to 7 days as with larvae. This would probably have caused mortality as eggs hatch, and it is not known if seven daily exposures would have increased mortality in adults. Overall, raw and generated FO at normal exposure rates did not detectably harm fish considered more sensitive than organisms recommended by USEPA protocols. Because larval mortality occurred after five doses (one dose per day), it is recommended that no more than four fogging events over 4 days should occur near bodies of water during periods when larval fishes could be present. This conservative recommendation is made with the caveat that concentration levels used to

induce mortality in this study are likely much greater than the concentrations that arise in the field under normal use. In addition, the amounts of FO as WAFs also would appear to be improbable under field scenarios because our experiments required extremely high amounts of oil artificially mixed with a high and sustained energy source.

Although these tested mixtures should have difficulty inducing direct mortality in natural settings, indirect routes of FO contamination to fish could exist. Fountain darters likely would not be in direct contact with surface floating oils. Fountain darters, like most darters, lack a swim bladder and typically are associated with benthic substrates or macrophytes. However, their prey might be. *D. magna* and *Ceriodaphnia dubia,* a common food for many larval fishes, incurred mortality in toxicity studies with FO (Cropek et al. 2008). Mortality was thought to be due to physical entrapment and asphyxiation of the organisms within the surface film of oil in the test containers rather than the actual toxicity of the oil. During our study, surface oil was observed on the water during the 7-day smoke exposure and in the greater concentrations of WAFs. Brine shrimp fed to fish during this study probably had come into contact with surface oil, and this could have provided an additional access to the fish through their digestive tracts. Feeding effects from these coated invertebrates and possible accumulated effects on predator fishes, especially surface feeding species, could be of concern. Minimal amounts of FO used near bodies of water could indirectly pose environmental hazards as naturally occurring invertebrates are affected physically or chemically. Other fishes that inhabit other regions of the water column may have more contact with the deposited surface oils and may therefore be at greater risk; however, the UV-weathering process increases the WSF that would directly impact benthos.

Larval fountain darters were more sensitive to FO than eggs in trials with WAFs and photo-oxidized WSFs. FO components transformed by photo-oxidation were shown to be more toxic than the previously tested post-manifold form of FO. Compared 96-h LC_{50} values of WAFs with WSFs fractions indicate that photo-oxidation increased toxicity by $\sim$100 times for eggs and larvae. Various other oils have also been shown to increase in toxicity after exposure to UV with aquatic organisms. A range of light and heavy oils were $\leq$50,000 times more toxic after photo-transformation for *Mulinia lateralis*, a bivalve, and *Mysidopsis bahia*, a mysid shrimp (Kuhn et al. 1997). The toxicity of photolyzed petroleum oils were related to aromatic ring composition and concentration of PAHs. The greater solubility of these photo-oxidized compounds substantially increased the toxicity of weathered crude oil in static renewal tests with *M. bahia* (Cleveland et al. 2000) and increased bioaccumulation factors in copepods by at least 2000 for *M. okhotensis* and 8,000 for *C. marshallae* (Duesterloh et al. 2002). In fishes, photo-toxicity of crude oil increased for *O. gorbuscha* juveniles (Barron et al. 2005), whereas the photo-sensitization of anthracene, a common PAH, increased its toxicity to *L. macrochirus* juveniles (Oris et al. 1984). Oils as complex mixtures, including FO, can have potential environmental consequences in marine and freshwaters either through accumulation and transfer to greater trophic level consumers or through direct toxicity when accidental spills are exposed to daily sunlight. Contaminations could deplete availability of food organisms and disrupt ecosystems. Areas of future research should attempt to identify specific chemical mechanisms and their interactions in aquatic environments.

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