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Effects of Exposure to Low Concentrations of Oil on the Expression of Cytochrome P4501a and Routine Swimming Speed of Atlantic Haddock (*Melanogrammus aeglefinus*) Larvae In Situ

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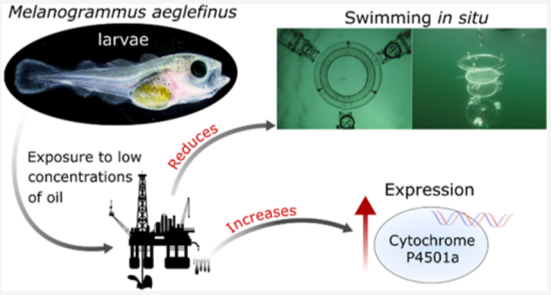
ABSTRACT: Exposure to environmentally relevant concentrations of oil could impact survival of fish larvae in situ through subtle effects on larval behavior. During the larval period, Atlantic haddock (*Melanogrammus aeglefinus*) are transported toward nursery grounds by ocean currents and active swimming, which can modify their drift route. Haddock larvae are sensitive to dispersed oil; however, whether exposure to oil during development impacts the ability of haddock larvae to swim in situ is unknown. Here, we exposed Atlantic haddock embryos to 10 and 80 μg oil/L (0.1 and 0.8 μg $\sum$PAH/L) of crude oil for 8 days and used a novel approach to measure its effect on the larval swimming behavior in situ. We assessed the swimming behavior of 138 haddock larvae in situ, in the North Sea, using a transparent drifting chamber. Expression of cytochrome P4501a (cyp1a) was also measured. Exposure to 10 and 80 μg oil/L significantly reduced the average in situ routine swimming speed by 30−40% compared to the controls. Expression of cyp1a was significantly higher in both exposed groups. This study reports key information for improving oil spill risk assessment models and presents a novel approach to study sublethal effects of pollutants on fish larvae in situ.



KEYWORDS: *larval fish, larval dispersal, oil toxicity, fish behavior, sublethal impacts, oil impact, ecotoxicology*

1. INTRODUCTION

Atlantic haddock (*Melanogrammus aeglefinus*) is a gadoid species of great ecological and commercial relevance, which has supported a large fishery for more than a century.[1] The main European spawning grounds of haddock are located in the northwestern North Sea[2,3] and in the Barents Sea.[4,5] From the spawning areas, haddock eggs and larvae disperse in the plankton toward feeding areas, where they have a higher probability of surviving.[6,7]

In Norway, there is an increasing interest in exploring for oil in regions in which species such as haddock, herring (*Clupea harengus*), and saithe (*Pollachius virens*) spawn and through which their early life history stages disperse.[5,8] In this context, the possibility of adverse effects of exposure to oil on individuals during the early life stages has stimulated significant effort to develop risk assessment tools to assess effects on key fish populations in Norway.[9−12] A key objective in this research is to establish the dose that causes adverse effects[13] and the lowest observed effects threshold concentration.[9]

Crude oil from operational or accidental oil spills significantly affects fish embryos and larvae through complex pathways.[14] These effects include effects on heart development, with consequences on the physiology of the whole organism, abnormal cardiac activity,[15,16] and subtle morphological abnormalities caused by exposure to low oil concentrations of 10−60 μg oil/L (e.g., altered heart shape as observed in Pacific herring (*Clupea pallasi*) and pink salmon (*Oncorhynchus gorbuscha*)).[17] The severity of the morphological abnormalities caused by exposure to oil is correlated with the level of induction of Cytochrome P4501a (cyp1a),[16] which, therefore, represents a suitable and sensitive biomarker of oil toxicity.[18,19]

Research on the impacts of exposure to oil on the early life stages of fish has focused on mortality and morphology. Less research has been conducted on the effects of oil on physiology and behavior. This could result in an underestimation of sublethal effects because any changes in larval physiology and behavior could affect their probability of survival. In gadoid fish, larval behavior during the pelagic dispersal phase plays an

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important role in drift and dispersal route,[20,21] thereby influencing the probability that they reach suitable nursery grounds. Effects of oil exposure on behavior can lead to significant impacts on predator avoidance,[22] predation rates,[23] and potentially feeding rates. Thus, potential sublethal effects of oil on swimming could result in modified dispersal and trophic interactions, with consequent impact on the probability of survival and, therefore, recruitment. Oil could impact larval swimming behavior through, for example, reduced cardiac function,[24,25] heart damage,[26] or an effect on the development of the nervous and/or musculoskeletal systems.[27−30]

Previous studies assessing the effects of exposure to oil on swimming of fish used laboratory-based techniques that quantify swimming through the use of flume tanks. Although these techniques are very valuable to assess the impacts of toxicity on swimming performance, it is difficult to draw conclusions on the effects of oil on swimming in situ from lab-based studies. Here, we applied a novel approach to assess the sublethal effects of toxicity of environmental pollutants on routine swimming of fish larvae in situ. We tested the hypothesis that exposure to environmentally realistic concentrations of oil impacts the swimming behavior of haddock larvae in situ. We exposed Atlantic haddock embryos to two concentrations of crude oil (10 and 80 μg oil/L) for 8 days to test the hypothesis that exposure to low concentrations of oil impacts the swimming behavior of haddock larvae in situ. To assess this, we observed and quantified the swimming behavior of both exposed and control larvae in situ in the Norwegian North Sea using a drifting, transparent circular arena, which is a well-established method to quantify the behavior of fish larvae in situ.[31−37]

2. MATERIALS AND METHODS

2.1. Source of Oil and Exposures. The crude oil used in the exposure was a laboratory weathered blend of crude oil from the Heidrun oil field of the Norwegian Sea (supplied by SINTEF OCEAN, Trondheim, Norway). The weathering and the oil exposure system are described in detail in Nordtug et al.[38] The oil exposures were performed as described in earlier work.[39−41] Crude oil dispersions were introduced into the exposure tanks by a mechanical valve system that allowed systematic dilution of a stock dispersion to nominal oil doses of 10 μg/L (low dose) and 80 μg/L (medium dose) seawater. The oil-exposed embryos were compared to untreated embryos that were cultured in clean seawater.

2.2. Source and Culturing of Haddock Eggs, Larvae, and Juveniles, and Experimental Design. Fertilized Atlantic haddock (*M. aeglefinus*) eggs were collected from brood stock maintained at the Institute of Marine Research (IMR), Austevoll Research Station. The eggs were transferred to indoor egg incubators at 7 ± 1 °C until later transfer to experimental tanks. On day 2 post fertilization (dpf), approximately 18 000 eggs were transferred into each of 12 (three treatments with four replicates each) circular green polyethylene tanks (50 L) in which they were exposed to oil (see below). The water flow through the exposure tanks was 30 L/h, and the water temperature was 8 ± 1 °C. The light regime in the exposure tanks was 12 h light, 12 h dark, and was provided by broad spectrum 2x36W Osram Biolux 965 (Munich, Germany, www.osram.com) dimmable fluorescent light tubes with 30 min smoothed transitions between the light and dark cycles.

The eggs were transferred to the exposure tanks using a 5 mL cylinder with a mesh grid bottom. The cylinder was filled to the top with eggs, which were then quickly transferred to the exposure tanks (7 × 5 mL with eggs into each tank ≈18 000 eggs). At the end of the exposure period, subsamples of the exposed eggs (2 × 5 mL) from each group were transferred to recovery tanks containing clean water. The number of eggs in 5 mL was 2616 ± 202 (estimated by counting all of the eggs in seven replicates of 5 mL from the transfer cylinder). Dead eggs were removed daily from the bottom of the exposure and recovery tanks and counted from digital photos using tools from MATLAB R2012b (The MathWorks, Inc., Natick, MA) (see cumulative egg mortality in Figure S2). The execution pipeline for the computer vision-based automatic counting is described in detail in Duan et al.[42]

The embryos were exposed to oil from 2 to 10 dpf, after which aliquots of 5200 eggs were transferred to new 50 L tanks containing clean seawater. Exposure started after 2 dpf because Atlantic haddock embryos are especially vulnerable to handling during the first 2 dpf. We exposed the embryos for 8 days because we wanted to cover the main embryonic period during which organogenesis occurs in haddock, and also be able to investigate possible delayed oil-induced developmental abnormalities. Morphological differences between treatments were assessed by measuring cardiac function (atrial fractional shortening (AFS), ventricular fractional shortening and silent ventricle), edema (accumulation of fluid), and length of larvae. Those data are reported in Sørensen et al.[39] (Table S5). The light source consisted of two 20 W tungsten halogen light bulbs (12 V) over each tank that provided 300−500 μW/cm^2 at the water surface (IL 1400A photometer, International Light, Inc., Boston, MA). The embryos started to hatch at day 11 dpf, and from 4 days post hatching (dph), natural zooplankton, mainly nauplii of the copepod *Acartia longiremis* harvested from the marine pond system "Svartatjern",[43] was introduced as food for the larvae. To facilitate initial feeding and ensure enough live prey, enriched rotifers (*Brachionus spp*) were also provided until 10 dph. Rotifer culture and enrichment is described in Karlsen et al.[44] To enhance feeding, the tanks were further supplemented with marine microalgae concentrate (Instant Algae, Nanno 3600, Reed Mariculture, Inc., CA) throughout the experiment.[45−47] The larvae were fed zooplankton until use in experiments. The mean water temperature in the culture tanks was 8.8 ± 0.5 °C, and the salinity was 35.0 ± 0.1.

2.3. Water Samples. To verify the actual polycyclic aromatic hydrocarbon (PAH) concentration in the water, water samples (1 L) were taken from each exposure tank at the beginning (day 0) and end (day 8) of the experiment. PAH was extracted from the water samples, as described in Sørensen et al.[48] Water concentrations of PAHs for individual exposures are reported as the mean of the four exposure tanks and the two sampling points. The log-transformed data did not pass the test for homogeneity in variance (Levene's test). Therefore, significant differences in PAH composition relative to controls were tested for using the nonparametric Kruskal−Wallis test, applying the pairwise Wilcoxon rank sum test with Bonferroni correction and $p < 0.05$ set as the level of significance (Figure 2).

2.4. Body Burden of PAHs. Samples of 100 living eggs from three tanks were preserved by flash-freezing in liquid nitrogen. Frozen eggs were stored at −80 °C until further handling. Extraction was performed as described in Sørensen et

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al.[39] Uptake of total PAH (sum of 28 individual PAHs and 24 alkyl PAH clusters) into the embryos was measured in each treatment. Due to large differences in levels of tissue PAH, and thus standard deviation, in the various treatments, the body burden PAH data were log-transformed before statistical testing. The log-transformed data were tested for homogeneity of variance using Levene's test and the Shapiro−Wilk normality test, which yielded p values >0.05. This supported testing for statistically significant differences ($p < 0.05$) using a one-way ANOVA applying Dunnett's multiple-comparison test.

2.5. Expression of *cyp1a*. Groups of 10−20 embryos from each tank were collected for RNA extraction and *cyp1a* analysis at 5 dpf, after 3 days of exposure to oil. This time point represents the developmental stage during which appropriate initiation of Cyp1a-induced xenobiotic metabolism is expected to occur.[41] All embryos collected were observed under a microscope to avoid collection of dead eggs before they were frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated from frozen sets of embryos using Trizol reagent (Invitrogen, Carlsbad, California), according to the procedures provided by the manufacturer. The quantity of RNA was determined using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE), and RNA quality was checked using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Subsequently, cDNA was generated using SuperScript VILO cDNA Synthesis Kit (Life Technologies Corporation), according to the manufacturer's instructions. The cDNA was normalized to obtain a concentration of 50 ng/μL.

Atlantic haddock-specific primers and probes for *cyp1a* and the technical reference *ef1a* (elongation factor 1 α, housekeeping gene) were designed with Primer Express software (Applied Biosystems, Carlsbad, California) according to the manufacturer's guidelines. Primer and probe sequences are presented in Table S2. TaqMan PCR assays were performed in duplicate using 96-well optical plates on an ABI Prism Fast 7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA) with the following settings: 50 °C for 2 min, 95 °C for 20 s, followed by 40 cycles of 95 °C for 1 s and 60 °C for 20 s. No template control, and no reverse transcriptase enzyme control and genomic DNA controls were included. For each 10 μL PCR, 2 μL of cDNA 1:40 dilution (2.5 ng) was mixed with 200 nM fluorogenic probe, 900 nM sense primer, and 900 nM antisense primer in 1xTaqMan Fast Advanced Master Mix (Applied Biosystems, Carlsbad, California). Expression of *cyp1a* was calculated relative to the control samples after normalization to the reference gene (*ef1a*) employing the ΔΔCt method, as described in detail in Bogerd et al.[49] The data were transformed to −log 2 data (−ΔΔCt values) before statistical analysis and tested for homogeneity of variance using Levene's test and the Shapiro−Wilk normality test giving $p > 0.05$. This allowed testing for statistically significant differences ($p < 0.05$) using a one-way ANOVA applying Dunnett's multiple-comparison test.

2.6. In Situ Observations of Swimming Behavior. The swimming behavior of haddock larvae was recorded while they swam in situ in a transparent acrylic circular chamber (DISC, Drifting In Situ Chamber[36,50]) in coastal North Sea (Figure 1). The DISC is attached to a drogue and drifts with the current. The DISC is equipped with a GOPRO camera, a HOBO light and temperature sensor, a GPS, three analog compasses, and a custom Arduino digital compass. When the DISC is placed in the water, the circular arena drifts at a depth of 4 m. An overview of the DISC is shown in Figure S1.

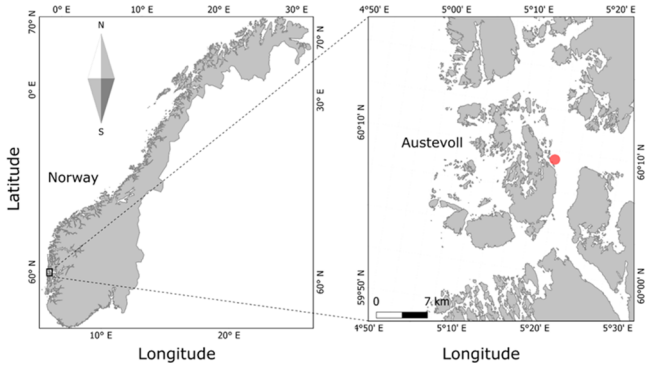

Figure 1. Study area. Location where the behavioral tests on haddock larvae (*M. aeglefinus*) were conducted. The map shows the coastal area where the behavior of the larvae was observed. The red circle indicated the approximate location where the drifting in situ chamber was deployed.

Three of the experimental groups were observed: controls (not exposed to oil, $N = 78$), larvae exposed to 10 μg oil/L (low concentration, $N = 29$), and larvae exposed to 80 μg oil/L (medium concentration, $N = 31$). All of the haddock larvae used in the three groups were between 29 and 35 dph (30−36 days after the end of exposure to oil), and their total length was between 10 and 11 mm in all experimental groups. The observations were made in April−May 2015 in the coastal areas of Bjørnafjorden, Northeast of Austevoll, Norway (60.09 N, 5.28 E).

For each deployment, two haddock larvae from the same experimental group were introduced into the arena when the DISC was held semisubmerged along the side of a small boat. Afterward, the DISC was released and allowed to drift for 15 min. The GOPRO camera placed underneath the chamber recorded the behavior of the larvae for the entire duration of the deployments. However, when extracting data from the videos, the first 5 min of each deployment was considered an acclimation period and only the last 10 min was used to extract the swimming behavior of the larvae.[34,36]

The videos were processed using the DISCR tracking procedure, utilizing R and a graphical user interface provided by ImageJ software.[31,32] The code utilized is available at the web page Drifting In Situ Chamber User Software in R (https://github.com/jiho/discr), released under the GNU General Public License v3.0. The position of each larva was tracked every second for the last 10 min of each deployment (600 data points per larva). Each larva was tracked individually.

The swimming speed of haddock larvae was estimated from the video tracks by dividing the distance that the animal swam in the chamber by time observed (1 s), for the 10 min observation period.[35,36] The average of all of the calculated speeds was considered as the average speed of one larva. This average speed in the DISC is an estimate of routine speed,[51] which is the spontaneous speed of undisturbed larvae over a period of time that varies depending on the study (usually several minutes).[52] As a following step, the average of the routine speeds of all of the individuals for each experimental group (control, exposed to 10 μg oil/L, exposed to 80 μg oil/L) was considered as the routine speed of the group.

2.7. Ethics Statement. The Austevoll Research Station is a certified Research Animal Facility for fish of all developmental

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stages (code 93 from the national Institutional Animal Care and Use Committee (IACUC)) and has permits from the Norwegian Directorate of Fisheries to catch and maintain Atlantic haddock (H-AV 77, H-AV 78, and H-AV 79). No permits are needed for work with embryos and yolk sac larvae. All experiments on feeding haddock larvae and juveniles used in the study were approved by the Norwegian Animal Research Authority (http://www.fdu.no/fdu/, reference number 2012/275334−2). All procedures were performed in accordance with these approvals.

3. RESULTS AND DISCUSSION

3.1. Water Samples and Body Burden of PAHs. The ∑PAH value in the water at the start and end of the exposure period was significantly different from the controls in both exposure groups: $p < 0.01$ (10 μg oil/L exposure) and $p < 0.001$ (80 μg oil/L exposure) (Figure 2A). Accumulated

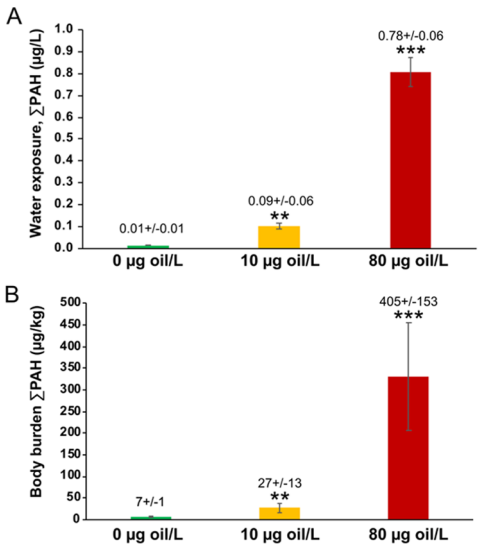

Figure 2. Polycyclic aromatic hydrocarbon (PAH) concentrations. Total PAH concentration (∑PAHs) in the exposure tank water and in Atlantic haddock (*M. aeglefinus*) eggs in control, 10 μg oil/L exposure, and 80 μg oil/L exposure treatments (mean ± SD). (A) The water analysis is given as average concentration in four replicate tanks per group and measured at the start of the exposure 2 days post fertilization (dpf) and at the end (10 dpf). (B) The body burden in the eggs is given as the average concentration in three replicates. The asterisks indicate statistically different from the control fish, "" $p < 0.01$, *** $p < 0.001$.

concentration of ∑PAH in tissue in fish from both exposure groups measured at the end of the 8-day exposure (10 dpf) was higher than in the control group for both the 10 μg oil/L exposed group ($p < 0.01$) and the 80 μg oil/L exposed group ($p < 0.001$) (Figure 2B).

3.2. Detection of cyp1a. Significantly higher levels ($p < 0.001$) of cyp1a were detected in both 10 and 80 μg oil/L exposure groups compared to control (Figure 3), with levels of 5 ± 2 and 86 ±18 fold changes, respectively.

3.3. Impacts of Oil Exposure on Swimming In Situ and Implications for Survival at Sea. Routine swimming speed of haddock larvae decreased with increasing exposure to oil: haddock larvae from the control group ($N = 78$) swam at an average speed of 1.21 ± 0.82 cm/s (mean ± SD), while those from both the low-dose ($N = 29$) and medium-dose (N

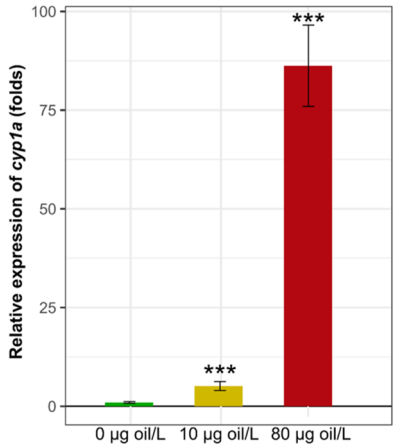

Figure 3. Relative expression of cytochrome P4501a (*cyp1a*). The relative expression of cyp1a in Atlantic haddock (*M. aeglefinus*) embryos in controls ($N = 4$) and groups exposed to 10 μg oil/L ($N = 4$) and 80 μg oil/L ($N = 4$) at 5 days post fertilization (3 days of exposure). Cyp1a expression is reported as relative change (folds) from the control groups. The bars show average ± SD. The asterisks indicate statistical differences relative to control fish ***$p < 0.001$.

= 31) exposure groups swam at significantly lower speeds of 0.83 ± 0.31 cm/s (mean ± SD) and 0.73 ± 0.27 cm/s (mean ± SD) (ANOVA, $p = 0.0006$ and Dunnett's multiple-comparison test) (Figure 4). The swimming speed of larvae

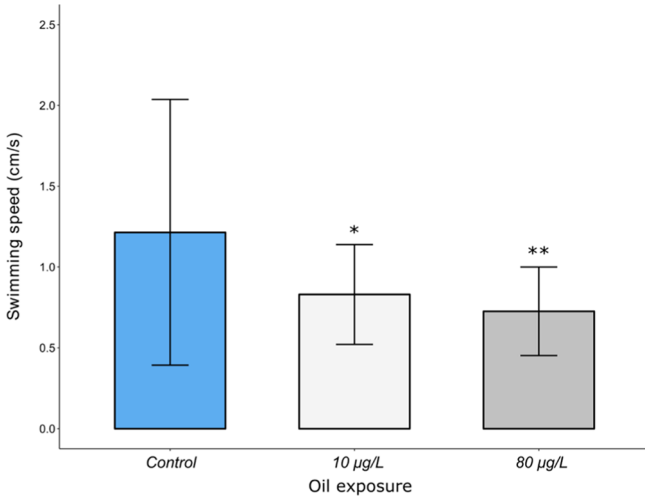

Figure 4. Larval swimming speed. Swimming speed of 29−35 dph Atlantic haddock (*M. aeglefinus*) larvae in situ, control ($N = 78$), and exposed to low-dose (10 μg oil/L; $N = 29$) and medium-dose (80 μg oil/L; $N = 31$) groups. The bar plot presents the mean swimming speed by oil exposure concentration. The T bars are the standard deviations. ANOVA $p = 0.005$; Dunnett's multiple-comparison test *$p = 0.01$; **$p = 0.001$.

from the control group was more variable than that of larvae from either of the oil-exposed groups (Figure 4). All of the larvae in these experiments were 29−35 dph, and their length was not significantly different. Therefore, the differential variability between groups is not because they are different in size but possibly relates to the oil exposure resulting in selection—through differential mortality—for individuals with specific traits.

The routine swimming speed of control haddock larvae observed in this study was consistent with the routine

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swimming speed that 25 dph haddock larvae display in the laboratory at 10 °C, which ranged between 1 and 1.5 cm/s.[53] Exposure to low concentrations of oil 10 and 80 μg oil/L (corresponding to 0.1 and 0.8 μg ∑PAH/L) reduced the swimming performance of the haddock larvae. Individuals that had been exposed to 10 μg oil/L during the embryonic period displayed a swimming speed that was 31% lower than control fish. Higher concentrations of oil caused further reduction in routine speed: exposure to 80 μg oil/L lowered the routine speed by 40% compared to the control group. As haddock larvae are likely to have low true swimming speed in situ,[54] the observed reductions of 30−40% in swimming speed could have a higher ecological relevance compared to species that display higher swimming speeds. For example, tropical reef larvae have in situ swimming speeds that can be >30 cm/s,[52,55] and even if their speed was reduced by 30%, they would still display speeds >20 cm/s. Therefore, larvae of species such as haddock, from cold subpolar regions, could be particularly vulnerable to sublethal effects from exposure to low concentrations of oil.

This reduction of speed after exposure to low doses of oil was also associated with a significant increase in expression of *cyp1a* in both the 10 and 80 μg oil/L exposed groups compared to the controls. The relationship between crude oil toxicity and *cyp1a* induction is not necessarily linear, as PAHs abundant in crude oil can be poor inducers of *cyp1a* expression.[56] In this study, the 10 μg oil/L exposed group showed small but significantly higher *cyp1a* levels during exposure compared to the controls. Although those animals did not exhibit any visible abnormalities, their swimming speed was reduced. This supports the use of *cyp1a* as an effective biomarker for toxicity associated with sublethal effects in fish larvae.

The reduction of swimming speed in situ reported in this study is consistent with the reduction of critical speed (*Ucrit*—the maximum swimming velocity that a fish can sustain until exhaustion) reported in previous studies. For example, exposure to 1.2 ± 0.6 μg/L 1 ∑PAHs at the embryo/larval stage and to 30 ± 7 μg/L ∑PAHs at the juvenile stage caused 37% and 22% decreases in *Ucrit* in juvenile mahi mahi (*Coryphaena hippurus*), respectively.[26] Similarly, exposure to concentrations ranging from 0.2 to 120 μg/L of the water-soluble fraction (WSF) of crude oil reduced *Ucrit* by 11−40% in juvenile Pacific herring (*C. pallasi*).[57] Nevertheless, this reduction of *Ucrit* differs from the reduction of routine speed in situ reported in this study, as the latter has different ecological consequences. At sea, survival during the early life stages is, in part, related to favorable/unfavorable dispersal.[11,58] Dispersal can be passive, if larvae only drift with the currents, or it can be a combination of passive drift and active swimming. Exposure to oil significantly reduced the routine swimming speed of haddock larvae, which could have downstream consequences on larval dispersal, as well as on prey capture and predator avoidance. The degree to which exposure to low concentration of oil could impact dispersal depends on the relative importance of active swimming vs. passive drift. The average speed in the DISC is an estimate of routine speed, which is the spontaneous speed of undisturbed larvae over a period of time that varies depending on the study (usually several minutes).[52] The routine speeds measured in the DISC underestimate true in situ swimming speeds.[36] This is because the routine speed is sensitive to the size of the chamber in which the observations are made: the smaller the chamber, the greater the speed measured underestimates the speed in situ.[59,60] In a confined environment, larvae can swim up to 3−4 times slower than true in situ swimming speeds.[60] On the other hand, in situ sampling of haddock larvae on the shoals of Georges Bank suggests that they may move at a relatively slow speed, ranging between 0.52 and 0.75 cm/s.[54] Thus, for Atlantic haddock larvae, the routine speed measured in the DISC could be closer to the true speed in situ than it is for other species studied with the same approach. Nonetheless, whether the swimming behavior described in this study plays a role in the dispersal of larval haddock at sea—and whether the oil-induced effects observed affect it—remains to be assessed.

There may be detrimental effects of oil on ecologically critical performance abilities—such as swimming in situ—even when there are no obvious observable effects of the exposure on, for example, morphology. Several studies have reported a reduction in the swimming speed of fish early life stages after exposure to crude oil,[26,27,29,61] which could be caused by several factors, including detrimental effects on the nervous system. That is the case in puffer fish (*Takifugu rubripes*) embryos, which did not show observable external morphological changes after exposure to 50 mg/L heavy oil but displayed abnormal organization of the developing central nervous system, especially in the midbrain.[62] This is something that has also been observed in other teleosts exposed to oil.[28,63] The midbrain is responsible for translating sensory input into appropriate motor output[64,65] and, therefore, abnormalities in the midbrain of oil-exposed fish may be linked to their altered behavior. Similarly, six coral reef fish species exposed to sublethal doses of oil showed altered settlement and antipredator behavior, which leads to an increased predator-induced mortality during recruitment.[23]

Effects on swimming behavior could also be linked to reduced cardiac performance.[17,66] Pink salmon (*O. gorbuscha*) and juvenile Pacific herring (*C. pallasii*) that had been exposed to trace levels of crude oil (∑PAH of 45 and 0.23 μg/L, respectively) during the embryonic period developed abnormal hearts and cardiorespiratory function, and this was manifested as reduced swimming speed and metabolic rate.[17] Impacts of exposure to oil on behavior might also depend on oil exposure-induced changes in eye development,[67−69] osmoregulation,[70] excitable tissues, including the central nervous system,[28,62,71] as well as muscle tissue.[41]

The spawning and nursery grounds of fish seasonally support large numbers of the most vulnerable stages and can, therefore, be particularly affected by both accidental high-level acute oil spills and low-level chronic oil exposures related to operational discharges and increased local vessel traffic. Accidental and operational oil spills can affect these vulnerable ecosystems and, therefore, developing risk assessment models in which relevant threshold values for adverse effects are considered is essential. Atlantic haddock eggs are especially susceptible to crude oil exposure due to oil droplet fouling on the chorion increasing the local concentration and prolonging the exposure.[39−41,70] In comparison to Atlantic cod (*Gadus morhua*), the adverse effect threshold causing toxicological response (heart defects, craniofacial malformation) in Atlantic haddock embryos is reduced 10-fold to 0.6 μg ∑PAH/L.[39] However, these studies did not consider delayed mortality or sublethal effects. Altered behavior can translate into reduced survival in the wild, and this and earlier studies highlight the importance of considering behavior when predicting the effect that oil pollution will have on marine organisms.[17,23,29,66] The importance of evaluating delayed effects is also highlighted by

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Heintz et al.[72] After embryonic exposure to crude oil, the surviving juveniles of Pink salmon (*O. gorbuscha*), which appeared healthy, were tagged and released into the marine environment. Survival was evaluated when mature fish returned 2 years later, revealing a 15% decrease in survival in animals exposed to 5.4 μg $\sum$PAH/L.[72] Similarly, although exposure to sublethal concentrations of oil (10 μg oil/L, 0.1 μg $\sum$PAH/L) produced no acute morphological deformities in haddock embryos or larvae, their swimming capacity was reduced. Given the detrimental effects on behavior observed here, we suggest that more research should be conducted to assess the oil exposure threshold that causes adverse effects on survival of haddock early life stages in the wild.

3.4. Novel Approach to Study Sublethal Impacts of Pollutants on Fish Larvae In Situ. In this study, we report subtle effects on in situ swimming behavior of haddock larvae caused by exposure to very low concentrations of oil. These results demonstrate that behavior of fish larvae in the wild can be influenced by exposure to very diluted pollutants, which could lower the probability of survival. This work highlights the importance of in situ studies on larval behavior in ecotoxicology to assess sublethal impacts of pollutants on fish larvae. Future work should use in situ observations to explore sublethal impacts on other ecologically important behaviors such as predator detection/escape, prey detection/capture, and responses to environmental cues important for dispersal and migration. Biophysical coupled models should also be applied to address how behaviors that are affected by oil exposure (in this study, reduced swimming speed) impact pelagic larval dispersal.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c04889.

Details of the behavioral tests conducted with the drifting in situ chamber; real-time qpcr primers and probes; drifiting in situ chamber (DISC); and cumulative egg mortality (PDF)

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A.C. designed the study; collected, analyzed, and interpreted the data; and wrote the paper. C.B.P. designed the study; collected, analyzed, and interpreted the data; and funded the research. H.I.B. designed the study; collected and interpreted the data; wrote the paper; and funded the research. A.B.S. designed the study; collected and interpreted the data; wrote the paper; and funded the research. S.S. collected and analyzed the data. R.B. collected the data and assisted in fieldwork. C.M.F.D. designed the study, collected the data, and wrote the paper. M.F. analyzed and interpreted the data. C.D.P. and V.L. analyzed the data. F.V. designed the study, interpreted the data, and funded the research. E.S. designed the study; collected, analyzed, and interpreted the data; and wrote the paper.

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Notes

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REFERENCES

(1) Pope, J.; Macer, C. T. An Evaluation of the Stock Structure of North Sea Cod, Haddock, and Whiting since 1920, Together with a Consideration of the Impacts of Fisheries and Predation Effects on Their Biomass and Recruitment. *ICES J. Mar. Sci.* **1996**, *53*, 1157−1169.

(2) Daan, N.; Bromley, P. J.; Hislop, J. R. G.; Nielsen, N. A. Ecology of North Sea Fish. *Netherlands J. Sea Res.* **1990**, *26*, 343−386.

(3) Sundby, S.; Kristiansen, T.; Nash, R. D. M.; Johannesen, T. *Dynamic Mapping of North Sea Spawning: Report of the "KINO" Project*; Havforskningsinstituttet, 2017; pp 183.

(4) Bergstad, O. A.; Jørgensen, T.; Dragesund, O. Life History and Ecology of the Gadoid Resources of the Barents Sea. *Fish. Res.* **1987**, *5*, 119−161.

(5) Olsen, E.; Aanes, S.; Mehl, S.; Holst, J. C.; Aglen, A.; Gjøsæter, H. Cod, Haddock, Saithe, Herring, and Capelin in the Barents Sea and Adjacent Waters: A Review of the Biological Value of the Area. *ICES J. Mar. Sci.* **2010**, *67*, 87−101.

(6) Economou, A. N. Food and Feeding Ecology of Five Gadoid Larvae in the Northern North Sea. *ICES J. Mar. Sci.* **1991**, *47*, 339−351.

(7) Heath, M. R.; Gallego, A. Bio-Physical Modelling of the Early Life Stages of Haddock, *Melanogrammus Aeglefinus*, in the North Sea. *Fish. Oceanogr.* **1998**, *7*, 110−125.

(8) Sundby, S.; Fossum, P.; Sandvik, A. D.; Vikebø, F.; Aglen, A.; Buhl-Mortensen, L.; Folkvord, A.; Bakkeplass, K.; Buhl-Mortensen, P.; Johannessen, M.; Jørgensen, M. S.; Kristiansen, T.; Landra, C. S.; Myksvoll, M. S.; Nash, R. D. M. *KunnskapsInnhenting Barentshavet−Lofoten−Vesterålen (KILO)*; Havforskningsinstituttet, 2013.

(9) Carroll, J. L.; Vikebø, F.; Howell, D.; Broch, O. J.; Nepstad, R.; Augustine, S.; Skeie, G. M.; Bast, R.; Juselius, J. Assessing Impacts of Simulated Oil Spills on the Northeast Arctic Cod Fishery. *Mar. Pollut. Bull.* **2018**, *126*, 63−73.

(10) Hjermann, D.; Melsom, A.; Dingsør, G.; Durant, J.; Eikeset, A.; Røed, L.; Ottersen, G.; Storvik, G.; Stenseth, N. Fish and Oil in the Lofoten−Barents Sea System: Synoptic Review of the Effect of Oil Spills on Fish Populations. *Mar. Ecol. Prog. Ser.* **2007**, *339*, 283−299.

(11) Langangen; Olsen, E.; Stige, L. C.; Ohlberger, J.; Yaragina, N. A.; Vikebø, F. B.; Bogstad, B.; Stenseth, N. C.; Hjermann, D. The Effects of Oil Spills on Marine Fish: Implications of Spatial Variation in Natural Mortality. *Mar. Pollut. Bull.* **2017**, *119*, 102−109.

(12) Vikebø, F. B.; Rønningen, P.; Meier, S.; Grøsvik, B. E.; Lien, V. S. Dispersants Have Limited Effects on Exposure Rates of Oil Spills on Fish Eggs and Larvae in Shelf Seas. *Environ. Sci. Technol.* **2015**, *49*, 6061−6069.

(13) Olsen, G. H.; Klok, C.; Hendriks, A. J.; Geraudie, P.; De Hoop, L.; De Laender, F.; Farmen, E.; Grøsvik, B. E.; Hansen, B. H.; Hjorth, M.; Jansen, C. R.; Nordtug, T.; Ravagnan, E.; Viaene, K.; Carroll, J. Toxicity Data for Modeling Impacts of Oil Components in an Arctic Ecosystem. *Mar. Environ. Res.* **2013**, *90*, 9−17.

(14) Hansen, B. H.; Sørensen, L.; Størseth, T. R.; Nepstad, R.; Altin, D.; Krause, D.; Meier, S.; Nordtug, T. Embryonic Exposure to Produced Water Can Cause Cardiac Toxicity and Deformations in Atlantic Cod (Gadus Morhua) and Haddock (*Melanogrammus Aeglefinus*) Larvae. *Mar. Environ. Res.* **2019**, *148*, 81−86.

(15) Incardona, J. P.; Scholz, N. L. The Influence of Heart Developmental Anatomy on Cardiotoxicity-Based Adverse Outcome Pathways in Fish. *Aquat. Toxicol.* **2016**, *177*, 515−525.

(16) Incardona, J. P. Molecular Mechanisms of Crude Oil Developmental Toxicity in Fish. *Arch. Environ. Contam. Toxicol.* **2017**, *73*, 19−32.

(17) Incardona, J. P.; Carls, M. G.; Holland, L.; Linbo, T. L.; Baldwin, D. H.; Myers, M. S.; Peck, K. A.; Tagal, M.; Rice, S. D.; Scholz, N. L. Very Low Embryonic Crude Oil Exposures Cause Lasting Cardiac Defects in Salmon and Herring. *Sci. Rep.* **2015**, *5*, No. 13499.

(18) Goksøyr, A. Use of Cytochrome P450 1A (CYP1A) in Fish as a Biomarker of Aquatic Pollution, In *Toxicology in Transition*, Degen, G. H.; Seiler, J. P.; Bentley, P., Eds.; Springer: Berlin, Heidelberg, 1995; *17*, 80−95.

(19) Kroon, F.; Streten, C.; Harries, S. A Protocol for Identifying Suitable Biomarkers to Assess Fish Health: A Systematic Review. *PLoS One* **2017**, *12*, No. e0174762.

(20) Vikebø, F.; Jørgensen, C.; Kristiansen, T.; Fiksen, Ø. Drift, Growth, and Survival of Larval Northeast Arctic Cod with Simple Rules of Behaviour. *Mar. Ecol. Prog. Ser.* **2007**, *347*, 207−219.

(21) Fiksen, Ø.; Jørgensen, C.; Kristiansen, T.; Vikebø, F.; Huse, G. Linking Behavioural Ecology and Oceanography: Larval Behaviour Determines Growth, Mortality and Dispersal. *Mar. Ecol. Prog. Ser.* **2007**, *347*, 195−205.

(22) Schlenker, L. S.; Welch, M. J.; Meredith, T. L.; Mager, E. M.; Lari, E.; Babcock, E. A.; Pyle, G. G.; Munday, P. L.; Grosell, M. Damsels in Distress: Oil Exposure Modifies Behavior and Olfaction in Bicolor Damselfish (Stegastes Partitus). *Environ. Sci. Technol.* **2019**, *53*, 10993−11001.

(23) Johansen, J. L.; Allan, B. J. M.; Rummer, J. L.; Esbaugh, A. J. Oil Exposure Disrupts Early Life-History Stages of Coral Reef Fishes via Behavioural Impairments. *Nat. Ecol. Evol.* **2017**, *1*, 1146−1152.

(24) Nelson, D.; Heuer, R. M.; Cox, G. K.; Stieglitz, J. D.; Hoenig, R.; Mager, E. M.; Benetti, D. D.; Grosell, M.; Crossley, D. A. Effects of Crude Oil on in Situ Cardiac Function in Young Adult Mahi−Mahi (Coryphaena Hippurus). *Aquat. Toxicol.* **2016**, *180*, 274−281.

(25) Stieglitz, J. D.; Mager, E. M.; Hoenig, R. H.; Benetti, D. D.; Grosell, M. Impacts of *Deepwater Horizon* Crude Oil Exposure on Adult Mahi-Mahi (*Coryphaena Hippurus*) Swim Performance. *Environ. Toxicol. Chem.* **2016**, *35*, 2613−2622.

(26) Mager, E. M.; Esbaugh, A. J.; Stieglitz, J. D.; Hoenig, R.; Bodinier, C.; Incardona, J. P.; Scholz, N. L.; Benetti, D. D.; Grosell, M. Acute Embryonic or Juvenile Exposure to Deepwater Horizon Crude Oil Impairs the Swimming Performance of Mahi-Mahi (Coryphaena Hippurus). *Environ. Sci. Technol.* **2014**, *48*, 7053−7061.

(27) Folkerts, E. J.; Blewett, T. A.; He, Y.; Goss, G. G. Alterations to Juvenile Zebrafish (Danio Rerio) Swim Performance after Acute Embryonic Exposure to Sub-Lethal Exposures of Hydraulic Fracturing Flowback and Produced Water. *Aquat. Toxicol.* **2017**, *193*, 50−59.

(28) Irie, K.; Kawaguchi, M.; Mizuno, K.; Song, J.-Y.; Nakayama, K.; Kitamura, S.-I.; Murakami, Y. Effect of Heavy Oil on the Development of the Nervous System of Floating and Sinking Teleost Eggs. *Mar. Pollut. Bull.* **2011**, *63*, 297−302.

(29) Johansen, J. L.; Esbaugh, A. J. Sustained Impairment of Respiratory Function and Swim Performance Following Acute Oil Exposure in a Coastal Marine Fish. *Aquat. Toxicol.* **2017**, *187*, 82−89.

(30) Vignet, C.; Devier, M.-H.; Le Menach, K.; Lyphout, L.; Potier, J.; Cachot, J.; Budzinski, H.; Bégout, M.-L.; Cousin, X. Long-Term Disruption of Growth, Reproduction, and Behavior after Embryonic Exposure of Zebrafish to PAH-Spiked Sediment. *Env. Sci. Pollut.* **2014**, *R 21*, 13877−13887.

(31) Irisson, J. O.; Paris, C. B.; Leis, J. M.; Yerman, M. N. With a Little Help from My Friends: Group Orientation by Larvae of a Coral Reef Fish. *PLoS One* **2015**, *10*, No. e0144060.

(32) Irisson, J.-O.; Guigand, C.; Paris, C. B. Detection and Quantification of Marine Larvae Orientation in the Pelagic Environment. *Limnol. Oceanogr. Methods* **2009**, *7*, 664−672.

(33) Berenshtein, I.; Kiflawi, M.; Shashar, N.; Wieler, U.; Agiv, H.; Paris, C. B. Polarized Light Sensitivity and Orientation in Coral Reef Fish Post-Larvae. *PLoS One* **2014**, *9*, No. e88468.

(34) Cresci, A.; Paris, C. B.; Foretich, M. A.; Durif, C. M.; Shema, S. D.; O'Brien, C. E.; Vikebø, F. B.; Skiftesvik, A. B.; Browman, H. I. Atlantic Haddock (*Melanogrammus Aeglefinus*) Larvae Have a Magnetic Compass That Guides Their Orientation. *iScience* **2019**, *19*, 1173−1178.

(35) Cresci, A.; Durif, C. M.; Paris, C. B.; Thompson, C. R. S.; Shema, S.; Skiftesvik, A. B.; Browman, H. I. The Relationship between the Moon Cycle and the Orientation of Glass Eels (Anguilla Anguilla) at Sea. *R. Soc. Open Sci.* **2019**, *6*, No. 190812.

https://dx.doi.org/10.1021/acs.est.0c04889
Environ. Sci. Technol. 2020, 54, 13879−13887

(36) Paris, C. B.; Atema, J.; Irisson, J. O.; Kingsford, M.; Gerlach, G.; Guigand, C. M. Reef Odor: A Wake up Call for Navigation in Reef Fish Larvae. *PLoS One* **2013**, *8*, No. e72808.
(37) Kough, A.; Paris, C.; Staaterman, E. In Situ Swimming and Orientation Behavior of Spiny Lobster (Panulirus Argus) Postlarvae. *Mar. Ecol. Prog. Ser.* **2014**, *504*, 207−219.
(38) Nordtug, T.; Johny Olsen, A.; Altin, D.; Meier, S.; Overrein, I.; Henrik Hansen, B.; Johansen, Ø. Method for Generating Parameterized Ecotoxicity Data of Dispersed Oil for Use in Environmental Modelling. *Mar. Pollut. Bull.* **2011**, *62*, 2106−2113.
(39) Sørensen, L.; Sørhus, E.; Nordtug, T.; Incardona, J. P.; Linbo, T. L.; Giovanetti, L.; Karlsen, Ø.; Meier, S. Oil Droplet Fouling and Differential Toxicokinetics of Polycyclic Aromatic Hydrocarbons in Embryos of Atlantic Haddock and Cod. *PLoS One* **2017**, *12*, No. 0180048.
(40) Sørhus, E.; Edvardsen, R. B.; Karlsen, Ø.; Nordtug, T.; Van Der Meeren, T.; Thorsen, A.; Harman, C.; Jentoft, S.; Meier, S. Unexpected Interaction with Dispersed Crude Oil Droplets Drives Severe Toxicity in Atlantic Haddock Embryos. *PLoS One* **2015**, *10*, No. e0124376.
(41) Sørhus, E.; Incardona, J. P.; Karlsen, Ø.; Linbo, T.; Sørensen, L.; Nordtug, T.; Van Der Meeren, T.; Thorsen, A.; Thorbjørnsen, M.; Jentoft, S.; Edvardsen, R. B.; Meier, S. Crude Oil Exposures Reveal Roles for Intracellular Calcium Cycling in Haddock Craniofacial and Cardiac Development. *Sci. Rep.* **2016**, *6*, No. 11058.
(42) Duan, Y.; Stien, L. H.; Thorsen, A.; Karlsen, Ø.; Sandlund, N.; Li, D.; Fu, Z.; Meier, S. An Automatic Counting System for Transparent Pelagic Fish Eggs Based on Computer Vision. *Aquacult. Eng.* **2015**, *67*, 8−13.
(43) van der Meeren, T.; Karlsen, Ø.; Liebig, P. L.; Mangor-Jensen, A. Copepod Production in a Saltwater Pond System: A Reliable Method for Achievement of Natural Prey in Start-Feeding of Marine Fish Larvae. *Aquacult. Eng.* **2014**, *62*, 17−27.
(44) Karlsen, Ø.; van der Meeren, T.; Rønnestad, I.; Mangor-Jensen, A.; Galloway, T. F.; Kjørsvik, E.; Hamre, K. Copepods Enhance Nutritional Status, Growth and Development in Atlantic Cod (*Gadus Morhua* L.) Larvae — Can We Identify the Underlying Factors? *PeerJ.* **2015**, *3*, No. e902.
(45) Naas, K. E.; Næss, T.; Harboe, T. Enhanced First Feeding of Halibut Larvae (Hippoglossus Hippoglossus L.) in Green Water. *Aquaculture* **1992**, *105*, 143−156.
(46) van der Meeren, T.; Mangor-Jensen, A.; Pickova, J. The Effect of Green Water and Light Intensity on Survival, Growth and Lipid Composition in Atlantic Cod (Gadus Morhua) during Intensive Larval Rearing. *Aquaculture* **2007**, *265*, 206−217.
(47) Van Der Meeren, T.; Naas, K. E. Development of Rearing Techniques Using Large Enclosed Ecosystems in the Mass Production of Marine Fish Fry. *Rev. Fish. Sci.* **1997**, *5*, 367−390.
(48) Sørensen, L.; Silva, M. S.; Booth, A. M.; Meier, S. Optimization and Comparison of Miniaturized Extraction Techniques for PAHs from Crude Oil Exposed Atlantic Cod and Haddock Eggs. *Anal. Bioanal. Chem.* **2016**, *408*, 1023−1032.
(49) Bogerd, J.; Blomenröhr, M.; Andersson, E.; van der Putten, H. H. A. G. M.; Tensen, C. P.; Vischer, H. F.; Granneman, J. C. M.; Janssen-Dommerholt, C.; Goos, H. J. T.; Schulz, R. W. Discrepancy Between Molecular Structure and Ligand Selectivity of a Testicular Follicle-Stimulating Hormone Receptor of the African Catfish (Clarias Gariepinus)1. *Biol. Reprod.* **2001**, *64*, 1633−1643.
(50) Paris, C. B.; Guigand, C. M.; Irisson, J.; Fisher, R. Orientation with No Frame of Reference (OWNFOR): A Novel System to Observe and Quantify Orientation in Reef Fish Larvae. *Carribbean Connect.: Implic. Mar. Prot. Area Manage.* **2008**, 52−62.
(51) Cresci, A.; Allan, B. J. M.; Shema, S. D.; Skiftesvik, A. B.; Browman, H. I. Orientation Behavior and Swimming Speed of Atlantic Herring Larvae (Clupea Harengus) in Situ and in Laboratory Exposures to Rotated Artificial Magnetic Fields. *J. Exp. Mar. Bio. Ecol.* **2020**, *526*, No. 151358.
(52) Leis, J. M. Are Larvae of Demersal Fishes Plankton or Nekton? In. *Advances in Marine Biology* **2006**, *51*, 57−141.
(53) Hunt Von Herbing, I.; Keating, K. In *The Big Fish Bang*, Proceedings of the 26th Annual Larval Fish Conference, Browman, H. I.; Browman, H. I.; Skiftesvik, A. B., Eds.; Institute of Marine Research: Bergen, Norway, 2003.
(54) Lough, R. G.; Bolz, G. R. The Movement of Cod and Haddock Larvae onto the Shoals of Georges Bank. *J. Fish Biol.* **1989**, *35*, 71−79.
(55) Leis, J. M.; Sweatman, H. P. A.; Reader, S. E. What the Pelagic Stages of Coral Reef Fishes Are Doing out in Blue Water: Daytime Field Observations of Larval Behavioural Capabilitie. *Mar. Freshwater Res.* **1996**, *47*, 401−411.
(56) Incardona, J. P.; Carls, M. G.; Teraoka, H.; Sloan, C. A.; Collier, T. K.; Scholz, N. L. Aryl Hydrocarbon Receptor-Independent Toxicity of Weathered Crude Oil during Fish Development. *Environ. Health Perspect.* **2005**, *113*, 1755−1762.
(57) Kennedy, C. J.; Farrell, A. P. Effects of Exposure to the Water-soluble Fraction of Crude Oil on the Swimming Performance and the Metabolic and Ionic Recovery Postexercise in Pacific Herring (*Clupea Pallasi*). *Environ. Toxicol. Chem.* **2006**, *25*, 2715.
(58) Hjort, J. *Fluctuations in the Great Fisheries of Northern Europe Viewed in the Light of Biological Research*; Andr. Fred. H0st & Fils: 1914.
(59) Fuiman, L. A.; Smith, M. E.; Malley, V. N. Ontogeny of Routine Swimming Speed and Startle Responses in Red Drum, with a Comparison of Responses to Acoustic and Visual Stimuli. *J. Fish Biol.* **1999**, *55*, 215−226.
(60) Von Westernhagen, H.; Rosenthal, H. Laboratory and In-Situ Studies on Larval Development and Swimming Performance of Pacific Herring Clupea Harengus Pallasi. *Helgol. Wiss. Meeresunters.* **1979**, *32*, 539−549.
(61) Folkerts, E. J.; Blewett, T. A.; He, Y.; Goss, G. G. Cardio-Respirometry Disruption in Zebrafish (Danio Rerio) Embryos Exposed to Hydraulic Fracturing Flowback and Produced Water. *Environ. Pollut.* **2017**, *231*, 1477−1487.
(62) Kawaguchi, M.; Sugahara, Y.; Watanabe, T.; et al. Nervous System Disruption and Concomitant Behavioral Abnormality in Early Hatched Pufferfish Larvae Exposed to Heavy Oil. *Environ. Sci. Pollut. Res.* **2012**, *19*, 2488−2497.
(63) Murakami, Y.; Kitamura, S. I.; Nakayama, K.; Matsuoka, S.; Sakaguchi, H. Effects of Heavy Oil in the Developing Spotted Halibut, Verasper Variegatus. *Mar. Pollut. Bull.* **2008**, *57*, 524−528.
(64) Carrive, P. The Periaqueductal Gray and Defensive Behavior: Functional Representation and Neuronal Organization. *Behav. Brain Res.* **1993**, *58*, 27−47.
(65) Tovote, P.; Esposito, M. S.; Botta, P.; Chaudun, F.; Fadok, J. P.; Markovic, M.; Wolff, S. B. E.; Ramakrishnan, C.; Fenno, L.; Deisseroth, K.; Herry, C.; Arber, S.; Lüthi, A. Midbrain Circuits for Defensive Behaviour. *Nature* **2016**, *534*, 206−212.
(66) Hicken, C. E.; Linbo, T. L.; Baldwin, D. H.; Willis, M. L.; Myers, M. S.; Holland, L.; Larsen, M.; Stekoll, M. S.; Rice, S. D.; Collier, T. K.; Scholz, N. L.; Incardona, J. P. Sublethal Exposure to Crude Oil during Embryonic Development Alters Cardiac Morphology and Reduces Aerobic Capacity in Adult Fish. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 7086−7090.
(67) Lie, K. K.; Meier, S.; Sørhus, E.; Edvardsen, R. B.; Karlsen, Ø.; Olsvik, P. A. Offshore Crude Oil Disrupts Retinoid Signaling and Eye Development in Larval Atlantic Haddock. *Front. Mar. Sci.* **2019**, *6*, No. 368.
(68) Xu, E. G.; Khursigara, A. J.; Magnuson, J.; Hazard, E. S.; Hardiman, G.; Esbaugh, A. J.; Roberts, A. P.; Schlenk, D. Larval Red Drum (Sciaenops Ocellatus) Sublethal Exposure to Weathered Deepwater Horizon Crude Oil: Developmental and Transcriptomic Consequences. *Environ. Sci. Technol.* **2017**, *51*, 10162−10172.
(69) Magnuson, J. T.; Khursigara, A. J.; Allmon, E. B.; Esbaugh, A. J.; Roberts, A. P. Effects of Deepwater Horizon Crude Oil on Ocular Development in Two Estuarine Fish Species, Red Drum (Sciaenops Ocellatus) and Sheepshead Minnow (Cyprinodon Variegatus). *Ecotoxicol. Environ. Saf.* **2018**, *166*, 186−191.
(70) Sørhus, E.; Incardona, J. P.; Furmanek, T.; Goetz, G. W.; Scholz, N. L.; Meier, S.; Edvardsen, R. B.; Jentoft, S. Novel Adverse

Outcome Pathways Revealed by Chemical Genetics in a Developing Marine Fish. *Elife* **2017**, *6*, No. e20707.
(71) Xu, E. G.; Mager, E. M.; Grosell, M.; Hazard, E. S.; Hardiman, G.; Schlenk, D. Novel Transcriptome Assembly and Comparative Toxicity Pathway Analysis in Mahi-Mahi (*Coryphaena Hippurus*) Embryos and Larvae Exposed to Deepwater Horizon Oil. *Sci. Rep.* **2017**, *7*, No. 44546.
(72) Heintz, R.; Rice, S.; Wertheimer, A.; Bradshaw, R.; Thrower, F.; Joyce, J.; Short, J. Delayed Effects on Growth and Marine Survival of Pink Salmon *Oncorhynchus Gorbuscha* after Exposure to Crude Oil during Embryonic Development. *Mar. Ecol. Prog. Ser.* **2000**, *208*, 205−216.

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Environ. Sci. Technol. 2020, 54, 13879−13887