Supporting Information

Incardona et al. 10.1073/pnas.1320950111

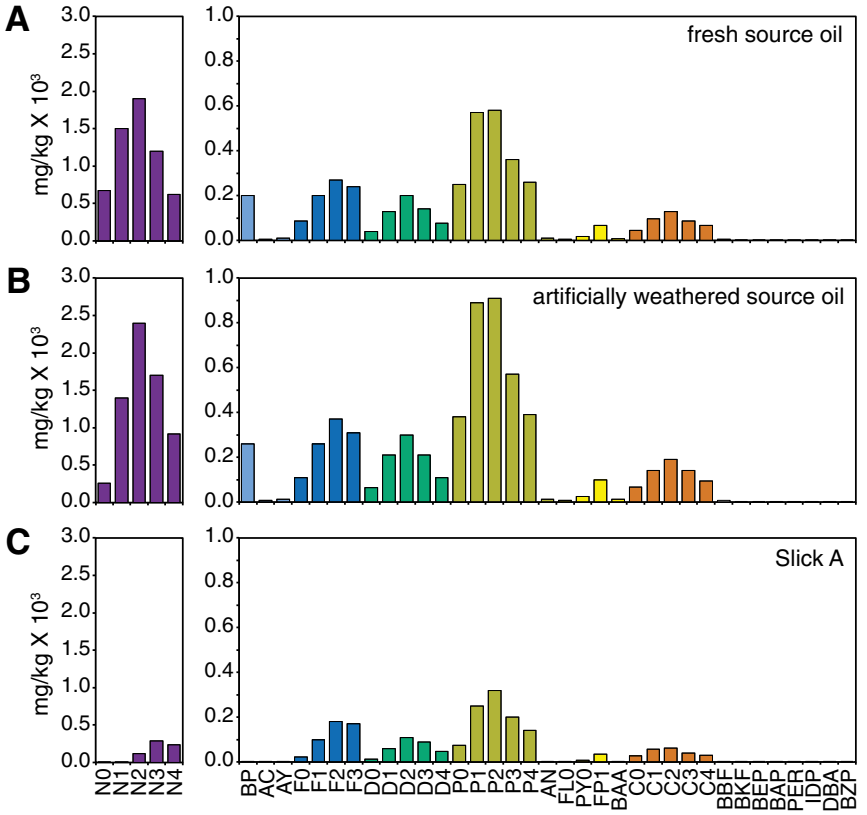


Fig. S1. Polycyclic aromatic hydrocarbon (PAH) concentrations (milligrams per kilogram whole oil) for the MC252 oil samples used in pelagic fish early life stage toxicity tests. (*A*) Fresh, unweathered source oil. (*B*) Artificially weathered (AW) source oil. (*C*) Slick A, a field sample collected from skimmers working to remove surface oil during the active spill phase. Abbreviations for PAHs are listed in Table S1. Focal compounds are naphthalenes (N0–N4), fluorenes (F0–F3), dibenzothiophenes (D0–D4), phenanthrenes (P0–P4), and chrysenes (C0–C4).

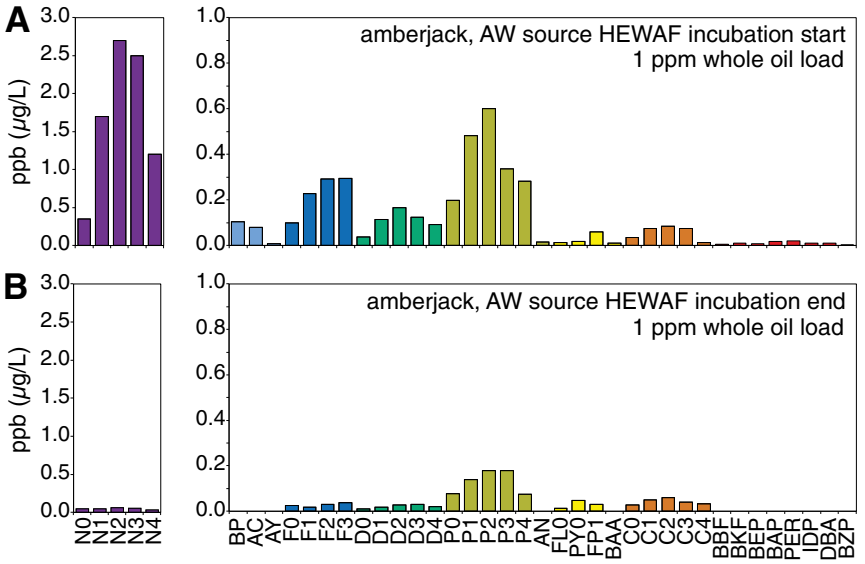


Fig. S2. Measured PAHs in high-energy water-accommodated fractions (HEWAFs) are shown for the highest exposure concentration at the start (*A*) and end (*B*) of amberjack exposure to artificially weathered source oil. Complete datasets for all dilutions are provided as Dataset S1. Detailed abbreviations for PAHs are listed in Table S1; naphthalenes (N0–N4), fluorenes (F0–F3), dibenzothiophenes (D0–D4), phenanthrenes (P0–P4), and chrysenes (C0–C4).

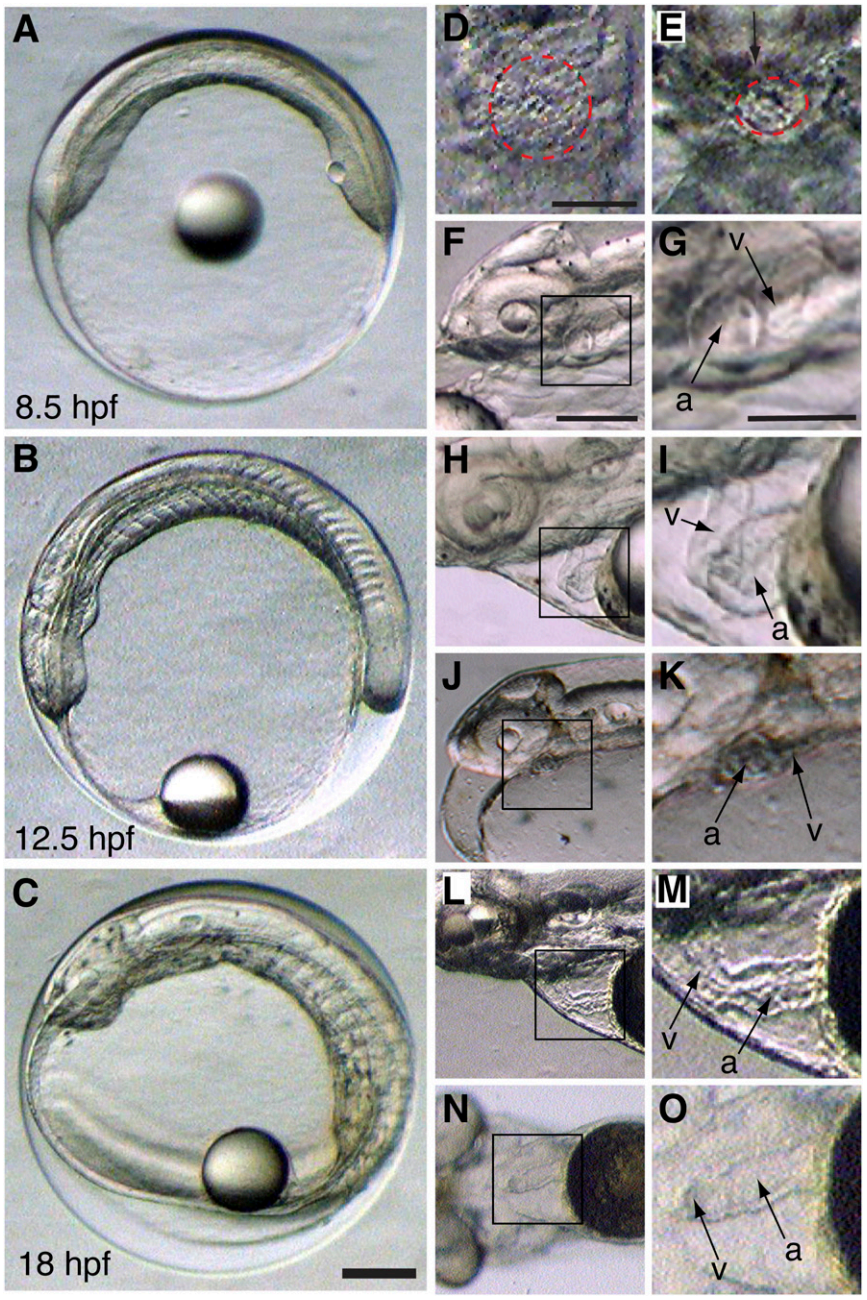


Fig. S3. Normal cardiac development in tunas and amberjack. Control yellowfin tuna embryos at (*A*) 8.5 h postfertilization (hpf) during early segmentation before visible cardiac rudiment, (*B*) 12.5 hpf during late segmentation when the cardiac cone was visible, and (*C*) 18 hpf at the free-tail stage approaching hatch when a regular heart beat was already established. (*D* and *E*) Ventral views of the cardiac cone (outlined by red dashed lines) in yellowfin tuna at 12.5 hpf (*D*) and amberjack at ~14 hpf (*E*) when the cardiac cone was rotated slightly to the left of the midline (arrow indicates midline of the forebrain). (*F–O*) Laterally rotated hearts in newly hatched larvae and posterior extension after hatch in amberjack (*F–I*) and yellowfin tuna (*J–O*). Middle series shows lower magnification views of the pericardial area with *Insets* indicating the hearts, which are enlarged in the paired series to the right; atrium (a) and ventricle (v). (*F* and *G*) Amberjack immediately posthatch and (*H* and *I*) 15 h posthatch (hph). (*J* and *K*) Yellowfin tuna immediately posthatch and 16 hph (*L*, *M*, lateral views; *N*, *O*, ventral views). [Scale bars, 200 μm (*A–C*, *F*, *H*, *J*, *L*, *N*) and 100 μm (*D*, *E*, *G*, *I*, *K*, *M*, *O*).]

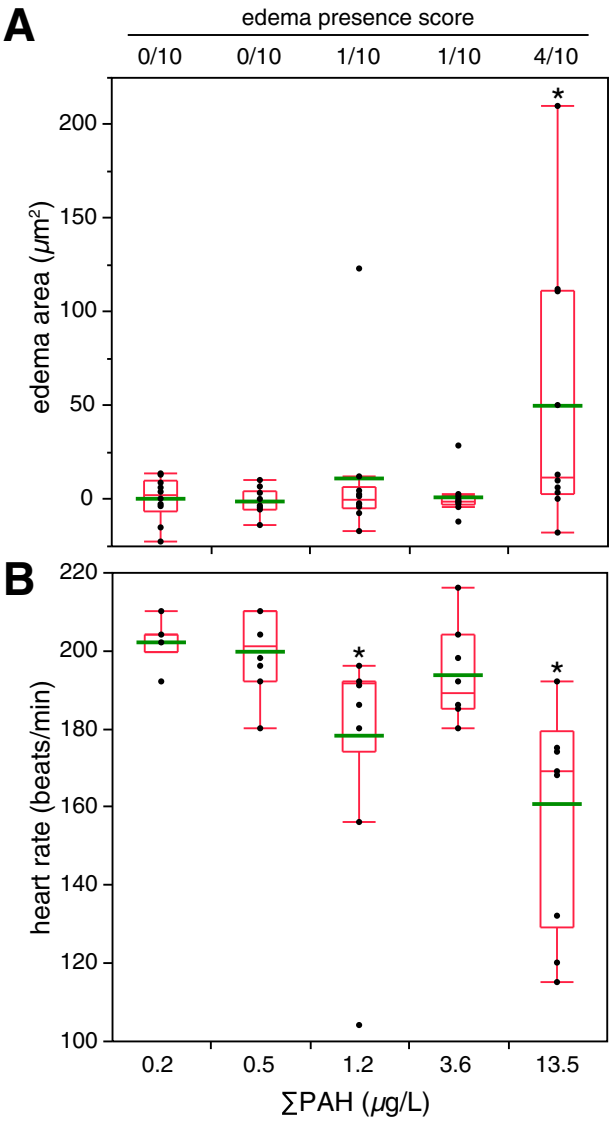


Fig. S4. Dose-dependent cardiotoxicity in amberjack following larger volume MC252 crude oil exposure. Embryos were exposed at a density of 80 embryos per liter in a volume of 7 L, using the same time course as beaker assays. Pericardial edema (*A*) was quantified by measuring the increase in pericardial area above unexposed controls (box-and-whisker plots) and by visual scoring of presence/absence (frequencies above box plots). (*B*) Heart rate measured from digital videos. Box-and-whisker plots encompass the distribution of individual data points. The green line indicates the mean, the ends of the boxes show the 25th and 75th quartiles, and ends of the whiskers show the upper and lower quartiles ± (1.5× interquartile range). Analysis of both pericardial area and heart rate by one-way ANOVA showed a significant effect of oil exposure ($P = 0.02$ and < 0.0001, respectively) and asterisks indicate treatment groups significantly different from control in a post hoc Dunnett's test.

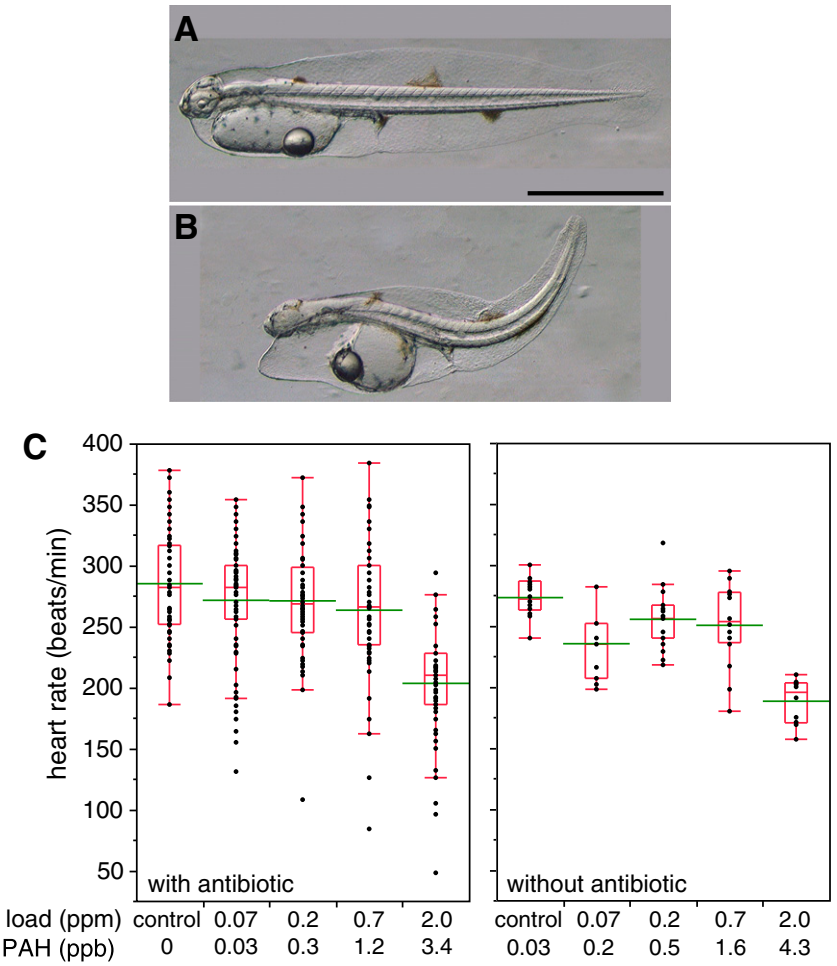
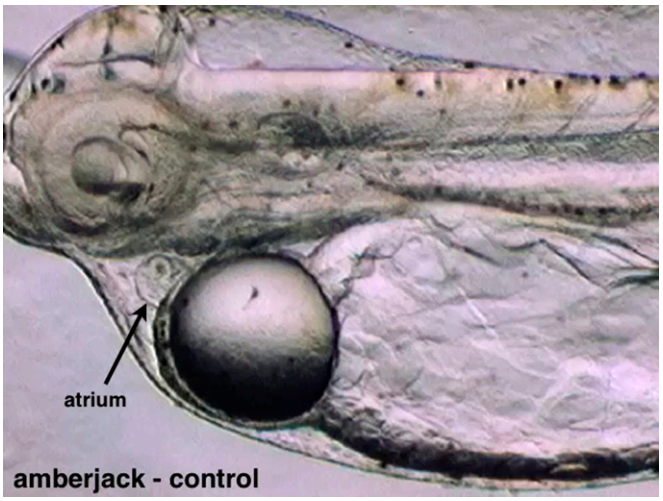


Fig. S5. MC252 oil exposure resulted in identical defects with or without the inclusion of a broad-spectrum antibiotic. Gross morphology of control yellowfin tuna larva (*A*) and exposed to slick A HEWAF with 4.6 μg/L ΣPAH (*B*), both cultured in the presence of 10 mg/L oxytetracycline. (Scale bar, 1 mm.) (*C*) Heart-rate data for both yellowfin tuna exposures without antibiotic (*Left*) and with 10 mg/L oxytetracycline (*Right*). All datapoints are shown for each exposure concentration. The *x*-axis labels indicate nominal oil load at top (mg/L) and measured ΣPAHs at bottom (μg/L). Box-and-whisker plots encompass the distribution of individual datapoints. The green line indicates the mean, the ends of the boxes show the 25th and 75th quartiles, and ends of the whiskers show the upper and lower quartiles ± (1.5× interquartile range). Datapoints outside the whiskers are potential outliers. Data from the no antibiotic treatment represent six replicates; the treatment with oxytetracycline is a single replicate. Data from tests with and without oxytetracycline were analyzed both separately and together, with nearly identical ANOVA and post hoc results.

Table S1. Measured PAHs used to calculate ΣPAH

40-analyte list*	50-analyte list
Naphthalene (N0)	Naphthalene
C1-Naphthalenes (N1)	C1-Naphthalenes
C2-Naphthalenes (N2)	C2-Naphthalenes
C3-Naphthalenes (N3)	C3-Naphthalenes
C4-Naphthalenes (N4)	C4-Naphthalenes
Biphenyl (BP)	Biphenyl
Acenaphthylene (AY)	Acenaphthylene
Acenaphthene (AC)	Acenaphthene
Fluorene (F0)	Fluorene
C1-Fluorenes (F1)	C1-Fluorenes
C2-Fluorenes (F2)	C2-Fluorenes
C3-Fluorenes (F3)	C3-Fluorenes
Anthracene (AN)	Anthracene
Phenanthrene (P0)	Phenanthrene
C1-Phenanthrenes (P1)	C1-Phenanthrenes
C2-Phenanthrenes (P2)	C2-Phenanthrenes
C3-Phenanthrenes (P3)	C3-Phenanthrenes
C4-Phenanthrenes (P4)	C4-Phenanthrenes
Dibenzothiophene (D0)	Retene
C1-Dibenzothiophenes (D1)	Dibenzothiophene
C2-Dibenzothiophenes (D2)	C1-Dibenzothiophenes
C3-Dibenzothiophenes (D3)	C2-Dibenzothiophenes
C4-Dibenzothiophenes (D4)	C3-Dibenzothiophenes
Fluoranthene (FL)	C4-Dibenzothiophenes
Pyrene (PY)	Fluoranthene
C1-Fluoranthenes/Pyrenes (FP1)	Pyrene
Benz(a)anthracene (BAA)	C1-Fluoranthenes/Pyrenes
Chrysene+Triphenylene (C0)	C2-Fluoranthenes/Pyrenes
C1-Chrysenes (C1)	C3-Fluoranthenes/Pyrenes
C2-Chrysenes (C2)	C4-Fluoranthenes/Pyrenes
C3-Chrysenes (C3)	Naphthobenzothiophene
C4-Chrysenes (C4)	C1-Naphthobenzothiophenes
Benzo(b)fluoranthene (BBF)	C2-Naphthobenzothiophenes
Benzo(j+k)fluoranthene (BKF)	C3-Naphthobenzothiophenes
Benzo(e)pyrene (BEP)	C4-Naphthobenzothiophenes
Benzo(a)pyrene (BAP)	Benz(a)anthracene
Perylene (PER)	Chrysene+Triphenylene
Indeno(1,2,3-cd)pyrene (IDP)	C1-Chrysenes
Dibenz(a,h)anthracene (DBA)	C2-Chrysenes
Benzo(g,h,i)perylene (BZP)	C3-Chrysenes
	C4-Chrysenes
	Benzo(b)fluoranthene
	Benzo(j+k)fluoranthene
	Benzo(a)fluoranthene
	Benzo(e)pyrene
	Benzo(a)pyrene
	Perylene
	Indeno(1,2,3-cd)pyrene
	Dibenz(a,h)anthracene
	Benzo(g,h,i)perylene

*The 40-analyte list is used for all ΣPAH reported in the figures and is the conventional list for National Oceanic and Atmospheric Administration publications cited within the text. The 50-analyte list is used for supplemental EC_{50} values reported in Tables 1 and 2, for comparison to other ongoing *Deepwater Horizon* Natural Resource Damage Assessment studies.



Movie S1. Cardiac arrhythmia resulting from MC252 crude oil exposure. The video shows a series of four clips of the beating heart in hatching stage larvae: amberjack (control), amberjack (MC252 crude oil exposed, ∑PAH 13.8 μg/L), yellowfin tuna (control), and yellowfin tuna (MC252 crude oil-exposed, ΣPAH 3.4 μg/L). The atrium is indicated with an arrow. (Total magnification, 63×.) The playback video frame rate was reduced to one-third of the acquisition rate.

Movie S1

Other Supporting Information Files

Dataset S1 (XLSX)