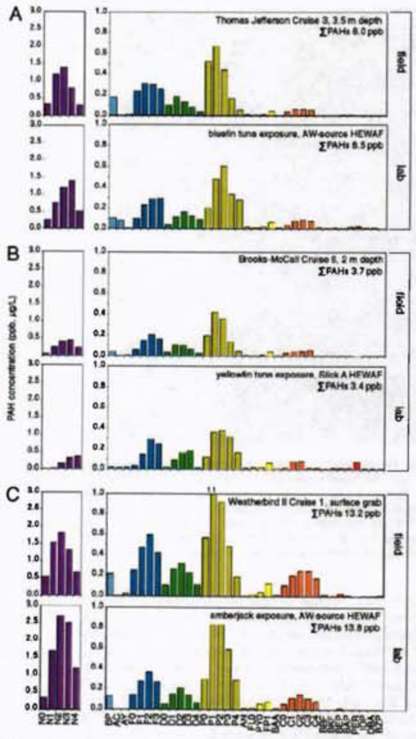

Fig. 1. HEWAF preparations produced environmentally relevant PAH exposures. PAH profiles in HEWAFs used in pelagic embryo exposures are compared with water samples collected during the active spill phase of the *Deepwater Horizon*/MC252 incident. Water samples shown are representative of 78 samples collected during May–July 2010 that had comparable PAH compositions to HEWAFs, 44 of which had ΣPAH levels that exceeded the highest concentrations tested in embryo exposures. These data were generated as part of the NRDA sampling program carried out by the *Deepwater Horizon* oil spill trustee team, and are publicly available (*Methods*). (*A*) Field sample from late June 2010 (*Upper*) and the artificially weathered source oil HEWAF used in a bluefin tuna test (*Lower*). (*B*) Field sample from early June 2010 (*Upper*) and the slick A HEWAF used in a yellowfin tuna test (*Lower*). (*C*) Field sample from late May 2010 (*Upper*) and the artificially weathered source oil HEWAF used in the amberjack test (*Lower*). Abbreviations for PAHs are listed in Table S1 and all values for individual PAHs are provided in Dataset S1. Focal compounds are naphthalenes (N0–N4, purple), fluorenes (F0–F3, blue), dibenzothiophenes (D0–D4, green), phenanthrenes (P0–P4, olive), and chrysenes (C0–C4, orange).

from the HEWAF, most likely because of volatilization, whereas ~25% of the phenanthrenes remained.

Normal Cardiac Development in Tunas and Amberjack. We characterized the timing and general morphological sequence of heart development in unexposed control tuna and amberjack embryos. Heart development was very similar among bluefin and yellowfin tunas and amberjack, both in terms of morphogenetic sequence and timing in relation to developmental stage. There were parallels and contrasts in comparison with the well-described development of the zebrafish heart (39). In all three species, the heart was first visible microscopically in late segmentation-stage embryos (e.g., 24 somites) (Fig. S3*B*) as a cone that was symmetrical along the anterior-posterior axis with a ventrally located base (Fig. S3*D*). At the time the first cardiomyocyte contractions were observed, some left-right asymmetry was already apparent as the cardiac cone began to rotate to position the atrial opening on the left side (Fig. S3*E*). By the free-tail stage (several hours later), the heart showed regular, rapid contractions of both the atrium and ventricle, and was rotated nearly 90° so that the lumen of the atrium was visible in left lateral views (amberjack, Fig. S3 *F* and *G*; yellowfin tuna, Fig. S3 *J* and *K*). After the hatching stage, the heart elongated along the anterior-posterior axis as the head extended and yolk absorbed, bringing the atrium posterior to the ventricle, but still slightly on the left side (amberjack, Fig. S3 *H* and *I*; yellowfin tuna, Fig. S3 *L*–*O*). Cardiac development in bluefin tuna was virtually indistinguishable from yellowfin tuna, and was not photographically documented because of a limited availability of embryos and time constraints for sample processing.

Gross Morphological Defects in Response to MC252 Oil Exposure. Exposures were carried out at temperatures appropriate for broodstock maintenance and routine hatchery rearing for each species (bluefin tuna and amberjack, 25 °C; yellowfin tuna, 27 °C), which generally resulted in high survival rates for unexposed (control) embryos. Bluefin tuna embryos had the lowest control survival at 60 ± 5% ($n = 4$). The control survival rates for yellowfin tuna and amberjack were 72 ± 9% ($n = 6$) and 93 ± 3% ($n = 4$), respectively.

Exposure to each MC252 sample type (source- or surface-collected) produced a virtually identical suite of defects in the pelagic fish embryos (Figs. 2–4) that were consistent with the previously described effects of other crude oils in other fishes, including neritic spawning marine species such as herring. Each pelagic species showed comparable morphological responses, marked by accumulation of pericardial edema, expanding to yolk-sac edema in more severely affected larvae (representative

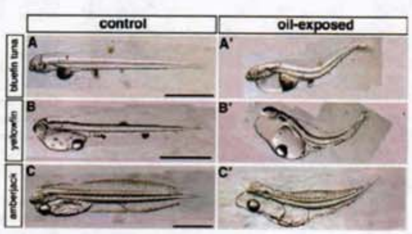

Fig. 2. Gross morphology of hatching stage larvae exposed to MC252 HEWAFs oil during embryonic development. Embryos were exposed from shortly after fertilization to 12–16 h after hatching. Unexposed controls incubated in clean water are shown in *A*–*C*, and oil-exposed (highest-dose tested) in *A′*–*C′*. Representative examples are shown for (*A*, *A′*) bluefin tuna exposed to artificially weathered source oil (1 mg/L oil, 8.5 µg/L ΣPAH). (*B*, *B′*) yellowfin tuna exposed to slick A (2 mg/L oil, 3.4 µg/L ΣPAH). (*C*, *C′*) amberjack exposed to artificially weathered source oil (1 mg/L oil, 13.8 µg/L ΣPAH). (Scale bars, 1 mm.)