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Vessel speed restrictions reduce risk of collision-related mortality for North Atlantic right whales

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Abstract. Collisions with vessels are a serious threat to a number of endangered large whale species, the North Atlantic right whale (*Eubalaena glacialis*) in particular. In late 2008, the U.S. National Oceanic and Atmospheric Administration issued mandatory time-area vessel speed restrictions along the U.S. eastern seaboard in an effort to mediate collision-related mortality of right whales. All vessels 65 feet and greater in length are restricted to speeds of 10 knots or less during seasonally implemented regulatory periods. We modeled mortality risk of North Atlantic right whale when the vessel restrictions were and were not in effect, including (1) estimation of the probability of lethal injury given a ship strike as a function of vessel speed, (2) estimation of the effect of transit speed on the instantaneous rate of ship strikes, and (3) a consideration of total risk reduction. Logistic regression and Bayesian probit analyses indicated a significant positive relationship between ship speed and the probability of a lethal injury. We found that speeds of vessels that struck whales were consistently greater than typical vessel speeds for each vessel type and regulatory period studied; a use-availability model fit to these data provided strong evidence for a linear effect of transit speed on strike rates. Overall, we estimated that vessel speed restrictions reduced total ship strike mortality risk levels by 80–90% with levels that were closer to 90% in the latter two of the four active vessel speed restriction periods studied. To our knowledge, this is the most comprehensive assessment to date of the utility of vessel speed restrictions in reducing the threat of vessel collisions to large whales. Our findings indicate that vessel speed limits are a powerful tool for reducing anthropogenic mortality risk for North Atlantic right whales.

Key words: Bayesian risk analysis; endangered whales; *Eubalaena glacialis*; right whale; ship strike; speed restrictions; use-availability; whale-vessel collisions.

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INTRODUCTION

Violent collisions involving vessels and whales are a growing concern for marine resource managers. The outcome for the whale is often death or serious injury, including fractured bones, hemorrhaging, or propeller lacerations (Moore et al. 2004, Campbell-Malone et al. 2008). The occurrence of vessel strikes is a threat to a number of endangered large whale species (Clapham et al. 1999, Waring et al. 2011). In U.S. waters alone, tens of large whale deaths per year are ascribed to vessel strikes (Henry et al. 2012, van der Hoop et al. 2012), and globally the

number may be in the hundreds of deaths each year (Laist et al. 2001, Jensen and Silber 2003, Van Waerebeek et al. 2007). Not all dead whales are detected (particularly in offshore waters), and the cause of death for carcasses that are recovered cannot always be determined due to decomposition (Henry et al. 2012). Thus, the actual number of whales that succumb to vessel collisions is likely far higher than reported.

The North Atlantic right whale (*Eubalaena glacialis*) is particularly vulnerable to vessel strikes. In a population that contains fewer than 500 individuals, an average of about two known deaths have been documented each year for at least the last decade (Waring et al. 2011, Henry et al. 2012). This anthropogenic threat has slowed the recovery of this highly depleted species (Knowlton and Kraus 2001, Kraus et al. 2005, NMFS 2005).

A number of approaches have been taken to reduce the threat of vessel strikes to right whales. These actions include mariner awareness-raising programs and modifications of customary vessel operation practices that include vessel speed reductions and changes in vessel routing patterns (Vanderlaan and Taggart 2009, Silber et al. 2012).

Vessel speed has been identified as a contributing factor in the occurrence and severity of vessel collisions with various marine vertebrates (Laist and Shaw 2006, Hazel et al. 2007), large whale species in particular (Laist et al. 2001, Jensen and Silber 2003, Pace and Silber 2005, Vanderlaan and Taggart 2007). Impact forces involved in a collision increase with increasing vessel speed (Wang et al. 2007, Campbell-Malone et al. 2008, Silber et al. 2010) and the probability of death or serious injury of a whale involved in a collision increases as vessel speed increases (Pace and Silber 2005, Vanderlaan and Taggart 2007, Wiley et al. 2011). Gende et al. (2011) found that the encounter distance between whale and vessel is also influenced by vessel speed such that higher vessel speeds may increase the probability of a strike occurring. These various findings have prompted the use of vessel speed restrictions as a means of diminishing the threat of vessel strikes to endangered marine mammal species in various locations (NPS 2003, Laist and Shaw 2006, Tejedor et al. 2007).

To address the threat of vessel strikes to North Atlantic right whales, the U.S. National Oceanic and Atmospheric Administration's National Marine Fisheries Service (NMFS) issued regulations that limit vessel speeds in certain locations along the U.S eastern seaboard (NOAA 2008). The speed limits are in effect seasonally in prescribed areas (''seasonal management areas'', or "SMAs"). The SMAs were designed to correspond with the timing and locations of right whale migration, feeding, and nursery activities where they co-occur with high vessel traffic densities (typically near sizable port entrances and vessel traffic bottlenecks), while also minimizing economic impact to the maritime transport industry (Fig. 1). While in a management zone, all vessels 65 feet and greater in length are required to travel at 10 knots or less (speed over ground). Sovereign (e.g., U.S. military) vessels are exempted from the regulations.

It can be difficult to determine with certainty if vessel speed limits and related management actions are achieving their intended objective of reducing whale strikes, particularly in the relatively short period since their enactment (Pace 2011, Silber and Bettridge 2012). Studies have used risk reduction models to assess the relative effectiveness of various vessel routing measures (Vanderlaan and Taggart 2009, Vanderlaan et al. 2009, van der Hoop et al. 2012). Others have provided estimates of vessel strike risk reduction resulting specifically from NOAA's vessel speed restrictions (Lagueux et al. 2011, Wiley et al. 2011). However, estimates arising from the latter studies were obtained by examining only limited aspects of the restrictions both temporally and geographically. Further, most assessments of risk to date have been made by simulating whale and vessel movement to quantify strike rates. Although this approach is useful for determining how likely a whale is to come in close proximity to a vessel, it cannot be used to account for whale avoidance behavior that can prevent vessel collisions.

In this paper, we attempt to model the effect of mandatory vessel speed restrictions along the U.S. east coast on comprehensive North Atlantic right whale mortality risk. This includes an assessment of risk associated with different years and management regimes (i.e., vessel speed restrictions in/not in effect). Our analysis includes three components: (1) estimation of the probability of lethal injury given a ship strike at different

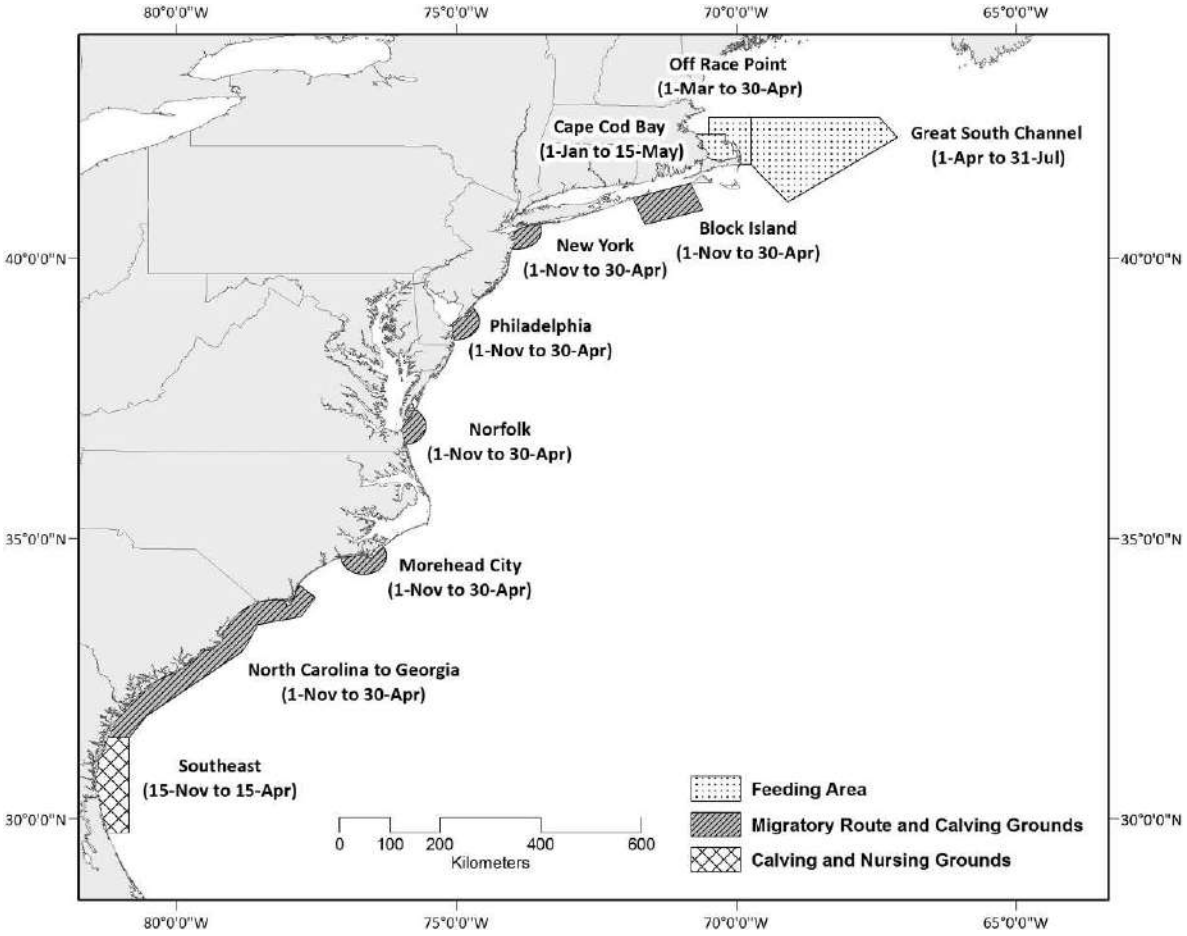

Fig. 1. Times and locations of vessel speed restriction seasonal management areas (SMAs) for North Atlantic right whales along the U.S. east coast.

vessel speeds; (2) estimation of the effect of transit speed on the instantaneous, per capita rate of ship strikes; and (3) a consideration of total risk reduction. The first component involves analyzing a dataset of ship strikes roughly twice the size as in previous work (e.g., Vanderlaan and Taggart 2007), while the second involves fitting a Bayesian model to describe the differences in observed ship speeds for vessels that struck whales from those which may or may not have struck whales. This latter approach differs conceptually from previous approaches to quantifying strike rates in that the effect of vessel speed on instantaneous strike rate is explicitly estimated via a statistical model. Finally, we jointly analyze all of these data sources to produce an estimate of mortality risk that simultaneously accounts for all sources of uncertainty.

Methods

Lethality of whale strikes

To explore the relationship between vessel speed and the lethality of vessel strikes, we examined records of known vessel strikes of whales in which sufficient information was provided to indicate with certainty both the speed of the vessel at the time of the strike and the severity of injury or fate (e.g., death resulted) of the whale involved in the collision. Records included all large whale species and all geographic areas worldwide.

In compiling vessel strike data for our analysis, we relied on the same data used in related

studies by Pace and Silber (2005) and Vanderlaan and Taggart (2007). The latter study used published sources (Laist et al. 2001, Jensen and Silber 2003) that detailed the historical record of vessels striking large whales ($n = 47$). Pace and Silber (2005) used these same data in addition to unpublished records of vessel/whale strikes ($n = 5$) not used by Vanderlaan and Taggart (2007). We began our compilation with the data set ($n = 52$) used by Pace and Silber.

By reviewing scientific literature and canvassing information from various stranding programs and data sources, we then compiled additional vessel strike records that occurred after the Pace and Silber (2005) study had concluded in May 2005, or were not previously documented in the Pace and Silber analysis. We included only those cases in which both the vessel speed and the fate of the whale were known with certainty. This yielded a total of 38 records not analyzed in previous studies. Unique records that met criteria for evaluation were derived from Neilson et al. (2012) for Alaskan waters ($n = 7$); NMFS' National Marine Mammal Stranding databases for the U.S. northeast ($n = 10$), northwest ($n = 2$), and southwest ($n = 7$) regions, national program ($n = 5$), and the Hawaiian Islands Humpback Whale National Marine Sanctuary ($n = 7$). A total of 90 records meeting the criteria identified above were used in our analysis. These data included records through September 2012.

For each record we recorded a binary response variable for whether injuries were lethal/not lethal, using the same criteria as in previous studies (e.g., Vanderlaan and Taggart 2007, Andersen et al. 2008). Records in which the whale was known to have died (e.g., carcass observed) or a severe injury was described (e.g., blood in the water, open or bleeding wounds observed) were classified as "lethal" (Vanderlaan and Taggart 2007). Individuals that were known to have survived (for example, where there were subsequent sightings of the living whale), who exhibited no apparent injury, or only minor injuries (e.g., visible non-bleeding wound, or no report of blood) were recorded as non-lethal "0" responses (i.e., we assumed these whales did not die as a result of the encounter). In making these determinations, we adopted the same classification of records utilized by Pace and Silber (2005), Vanderlaan and Taggart (2007), and Neilson et al. (2012). This left $n = 30$ records for which we made new injury determinations.

In total, our data set consisted of roughly double the number of observations previously used to estimate the relationship between vessel speed and the lethality of ship strikes (see, e.g., Vanderlaan and Taggart 2007), so we hoped to substantially increase precision of parameters describing the strike speed-mortality relationship. Two different analyses were performed. First, we analyzed the data using a simple logistic regression model where severity of injury ($Y_i = 1$, lethal injury; $Y_i = 0$, non-lethal injury) is modeled as a Bernoulli response variable with success probability M_i, where

$$\text{logit}(M_i) = \beta_0 + \beta_1 x_i.$$

Here, M_i gives the probability of a lethal injury for strike i, and x_i gives the speed (in knots) of the vessel involved in the collision. We provide estimates from this approach for historical consistency; several authors have used this formulation when addressing mortality associated with ship strikes (Pace and Silber 2005, Vanderlaan and Taggart 2007, Lagueux et al. 2011). For this approach, we conducted analysis with the 'glm' function in the R statistical language (R Development Core Team 2012).

Second, to integrate the relationship between ship speed and mortality into our comprehensive mortality risk analysis, we conducted a Bayesian probit regression analysis, which is similar to a logistic regression but uses the probit link function in place of the logit. In particular, we considered the model

$$\text{probit}(M_i) = \beta_0 + \beta_1 x_i. \quad (1)$$

The probit link function leads to some computational advantages when conducting Bayesian analysis; in particular, one can construct a collapsed Gibbs sampler as suggested by Albert and Chib (1993) to sample regression parameters. Defining $\mathbf{X}$ to be the design matrix where

$$\mathbf{X}' = \begin{bmatrix} 1 & 1 & \cdots & 1 \\ x_1 & x_2 & \cdots & x_N \end{bmatrix}$$

and augmenting the parameter space with $\tilde{Y}_i$ values for each observation, the algorithm proceeds as follows:

(1) Update $\tilde{Y}_i$ values according to a truncated normal distribution. If $Y_i = 1$, sample $\tilde{Y}_i \sim$ Normal ($[\mathbf{X}\beta]_i$, 1) with the constraint that $\tilde{Y}_i > 0$; if $Y_i = 0$, sample $\tilde{Y}_i \sim$ Normal ($[\mathbf{X}\beta]_i$, 1) with the constraint that $\tilde{Y}_i < 0$.
(2) Update the vector of regression parameters (in this case $\boldsymbol{\beta}' = [\beta_0\ \beta_1]$) according to $\boldsymbol{\beta} \sim$ MVN $((\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\tilde{\mathbf{Y}}, (\mathbf{X}'\mathbf{X})^{-1})$, where MVN denotes the multivariate normal distribution. This formulation implies a flat, improper prior distribution for the regression coefficients.

Posterior predictions of mortality probability at pre-specified vessel speeds can then be produced by sampling from

$$M_k = \Phi(\mathbf{X}_k\beta) \quad (2)$$

where $\Phi(Z)$ denotes the cumulative distribution function of the standard normal distribution evaluated at Z, and $\mathbf{X}_k$ gives the design vector associated with predictions (e.g., $\mathbf{X}_k = [1\ x_k]$). We used this algorithm to sample the posterior distribution of model parameters and make posterior predictions; 11,000 such values were simulated, and we discarded the first 1,000 as a burn-in. We provide R code to conduct this analysis as an online supplement.

Strike rate analysis

In an analysis of vessel encounter rates with humpback whales, Gende et al. (2011) provided evidence that the likelihood of vessel-whale encounters increases with vessel speed. Others have used simulation to model the likelihood of whale-vessel intersections given assumptions about whale and vessel movement (e.g., Vanderlaan and Taggart 2007, van der Hoop et al. 2012). However, the degree to which whales are likely or able to move to avoid vessels of varying speeds has heretofore been a subject of uncertainty.

To investigate the relationship between whale strike rates and vessel speeds, we compared the speeds of vessels that struck whales to a larger population of vessel speeds. From a statistical perspective, these data sources are similar to use-availability data as commonly modeled in animal resource selection studies (see, e.g., Manly et al. 2002), where speeds that resulted in whale strikes can be viewed as "use" and random vessel speeds can be viewed as "availability."

For this analysis, we obtained randomly selected vessel speeds in SMAs along the U.S. east coast summarized for analysis by speed and vessel type (i.e., cargo, passenger, sovereign vessel types). Vessel operations in SMAs were monitored using the Automatic Identification System (AIS), a safety-at-sea navigation tool that transmits very high frequency (VHF) radio signals. All vessels 300 gross tons or greater making international voyages are required by the International Maritime Organization's (IMO) International Convention for the Safety of Life at Sea to maintain functioning and operational AIS capabilities. The same requirement applies to nearly all vessels 65 feet or greater sailing in U.S. waters. An AIS signal is transmitted several times per minute and contains both static (e.g., ship name, call sign, and hull specifications) and dynamic information that is unique to that particular voyage. Dynamic information includes vessel location, heading, and speed, and is automatically incorporated into the AIS signal by a global positioning system (GPS). Due to its signal transmission rate, AIS provides a detailed, continuously sampled, and precise record of vessel operations for a nearly complete census of vessels subject to the speed limits. Additional information about the function and characteristics of the AIS can be found in Aarsæther and Moan (2009) and Tetreault (2005); a description of methods used to acquire and parse AIS data for this study can be found in Silber and Bettridge (2010 and 2012).

Using the U.S. Coast Guard (USCG) network of AIS receivers, we obtained vessel operations data from 9 December 2008 to 31 July 2012. We randomly selected one speed value per SMA vessel transit. This sample was restricted to speeds that were >2 knots because AIS transmitters may continue to operate while vessels are at anchor or while in port. To generate a random population of such vessel speeds, we resampled these speeds with replacement, weighting each observation by the number of AIS records available per transit.

For analysis of instantaneous per capita strike rates, we limited strike records to the U.S. east coast and to vessel types that were comparable to the categories available in the AIS data. Strike records were derived from published large whale

ship strike databases (Jensen and Silber 2003) and those maintained by NMFS stranding personnel. We restricted analysis to cargo vessels ($n = 1$ strike), passenger vessels ($n = 1$ strike), and to sovereign vessels (e.g., USCG operated vessels ($n = 10$ strikes)). Strike records were obtained over a wider time frame than AIS data; $n = 6$ were from 2000–2009, $n = 4$ were from 1990–1999, and $n = 2$ records came from 1950–1980. There is little indication that the speeds of vessels changed appreciably even over this relatively long horizon. We did not include strikes with small private vessels or whale watching vessels as these types of vessels were seldom identifiable in the AIS database. Although it may have been possible to isolate random transit speeds associated with particular whale watching vessels, we anticipated that these would not adequately represent the activities being conducted when whale strikes occurred (since whale watching vessels are actively searching for whales during portions of their transits). Since the fate of whales was not necessary for analysis of strike rates, we included records where the fate of the animal was unknown. A simple comparison of strike speeds from our vessel strike database to transit speeds randomly sampled from our AIS database in different regulatory periods suggested that strikes occurred when vessels, in each vessel category studied, were traveling faster than average vessel speeds (Fig. 2).

To formalize the relationship between vessel speed and strike rates, we start by defining the per capita, instantaneous rate at which whales are struck, λ_i. In particular, we express it as a function of vessel speed, x_i, with a log link. We considered both linear and quadratic functional forms for the effect of vessel speed on strike rate:

$$\text{Model 1: } \log(\lambda_i) = \alpha_0 + \alpha_1 x_i \quad (3)$$

$$\text{Model 2: } \log(\lambda_i) = \alpha_0 + \alpha_1 x_i + \alpha_2 x_i^2.$$

Letting $[X|Y]$ denote the conditional distribution of X given Y, we can describe the likelihood of observing strike speeds $\mathbf{x}$ for a particular combination of vessel type and regulatory period as

$$[\mathbf{x}|\mathbf{y} = 1, \alpha] = \frac{[\mathbf{y} = 1|\mathbf{x}, \alpha][\mathbf{x}]}{\int [\mathbf{y} = 1|\mathbf{x}, \alpha][\mathbf{x}] d\mathbf{x}}$$

where $y = 1$ if a particular vessel speed resulted in a strike. Here, $[\mathbf{y} = 1 \,|\, \mathbf{x}, \boldsymbol{\alpha}]$ is the joint probability that the observed vessel strike speeds resulted in strikes, and can be given as

$$[\mathbf{y} = 1|\mathbf{x}, \boldsymbol{\alpha}] = \prod_{i=1}^{S} 1 - \exp(-\lambda_i).$$

The component $[\mathbf{x}]$ denotes the probability density function for vessel speeds independent of whether or not those vessels struck whales. We follow Lele and Keim (2006) in approximating the denominator as

$$\int [\mathbf{y} = 1|\mathbf{x}, \alpha][\mathbf{x}] d\mathbf{x} \approx \frac{1}{B} \sum_{j=1}^{B} [\mathbf{y} = 1|\mathbf{x}_j^*, \alpha]$$

where $\mathbf{x}_j^*$, $j \in 1, 2, \ldots, B$ denotes a randomly sampled vessel speed from our transit database. This formulation has the advantage that we can use the empirical distribution of transit speeds as opposed to a fitted model, a desirable property since observed vessel speeds often were multimodal and/or bore little resemblance to parametric distributions. We set $B = 10{,}000$ in subsequent analysis.

The parameter α_0 controls the proportion of vessel speed observations that result in reported whale strikes. This parameter is not identifiable using the previous setup; however, inference can still be drawn regarding α_1, the effect of vessel speed on strike rates (Lele and Keim 2006). We used maximum likelihood to estimate parameters for the linear and quadratic models for strike rates, employing AIC (Burnham and Anderson 2002) for model selection. We then used the model with highest support in a Bayesian analysis of strike rates, imposing diffuse Normal(0,100) prior distributions on regression parameters (i.e., the α values). For this purpose, we used Markov chain Monte Carlo (Gelman et al. 2004) to sample from the joint posterior. When implementing this approach, we used separate data models for $[\mathbf{x}]$ for each combination of vessel type (cargo, passenger, sovereign) SMA speed restriction active/inactive period. An R script to conduct this analysis is provided in the Supplement.

Joint risk analysis

To estimate a total risk reduction value, we again sampled AIS data transmitted between 9 December 2008 and 31 July 2012 by cargo, tanker,

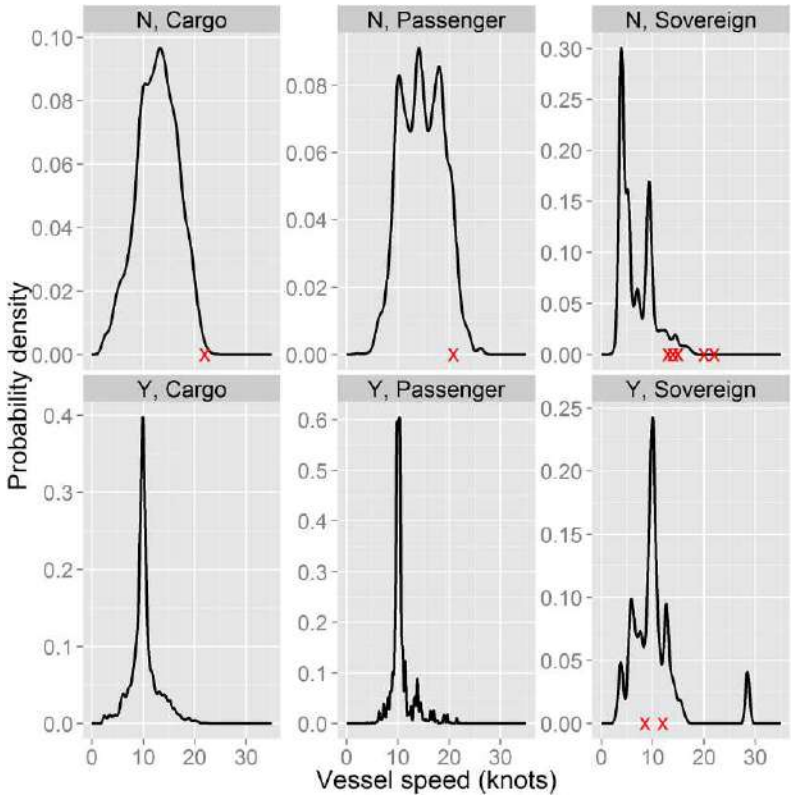

Fig. 2. A comparison of randomly sampled ship speeds from our Automatic Identification System (AIS) database (black kernel densities) to speeds at which vessels struck whales (represented by X's). Data are partitioned by vessel type ("Cargo", "Passenger", or "Sovereign") and by whether the data point occurred during periods when Seasonal Management Area (SMA) speed restrictions were ("Y") or were not ("N") in effect. Each panel represents a distribution of 10,000 randomly sampled ship speeds which are used to define separate availability datasets in the strike rate analysis (strike rate itself is only modeled as a function of ship speed).

and passenger vessels with lengths of 65 feet or greater (a total of tens of millions of individual speed records). We analyzed vessel speed information for 73,319 trips in SMAs at times in which speed restrictions were in effect and for 68,099 trips in the same geographic areas defined by SMAs when restrictions were not in effect. A single mean speed was computed for each trip. Vessel speed analyses were limited to transits that were at least one nautical mile in length, had at least five AIS records, and an average transit speed of >2 knots.

We formulated alternative expressions for relative mortality risk associated with R time periods with different management regulations. Assuming that the risk of mortality is temporally and spatially homogeneous within time period r, the probability that a single whale, chosen at random, is lethally injured can be given as

$$p_r = 1 - \exp(-T_r h_r)$$

where T_r gives the time interval and h_r gives a constant hazard rate associated with period r. The hazard rate h_r is fundamental to survival analysis (cf. Cox and Oakes 1984) and measures instantaneous mortality risk. In practice, we expect variability in h_r over time and space, but little information exists to quantify changes in spatial distributions of whales over the entire east coast. Assuming that this distribution remains relatively constant, comparisons of constant h_r over different management regimes may still prove illuminating. In particular, the relative risk of morality in management period r relative to some reference period 0 may be written as

$$R = h_r/h_0 \quad (4)$$

with values of $R > 1$ indicative of increased risk associated with a management action, and $R < 1$ indicative of reduced risk. In practice, there is considerable uncertainty in the hazards associated with each period because of uncertainty about mortality and strike rates, so that R is best viewed as a probability density function. Further, managers may be interested in different functional forms of h_r since these may provide different interpretations of the effect of management actions.

Ultimately, the mortality hazard throughout the management area (i.e., over all SMAs) in a given time interval is the sum of independent hazards associated with different transits (which are at different speeds and of different lengths). If we wish to directly compare the realized mortality risk in different management periods (i.e., speed regulation in effect/not in effect), we can approximate the mortality over each management period using a single, constant hazard during regulation period r:

$$h_r = \sum_{t=1}^{N_r} \lambda_{tr} D_{tr} M_{tr}/T_r.$$

Here, λ_{tr} is the instantaneous striking hazard for transit t in regulation period r (assumed here to be constant for the entire transit), D_{tr} is the duration of the transit, and M_{tr} gives the probability that a whale is lethally injured given that it is struck during transit t in period r. This formulation describes actualized change in mortality risk, but is dependent upon N_r, the number of transits in regulation period r, as well as the duration of such trips. Thus, changes in the total number of transits over time (or the duration of such transits) will affect interpretation of R. This formulation is also problematic for right whales because vessel speed regulations were temporally staggered based on location (Fig. 1) so T_r is not well defined.

Although absolute increases and decreases in risk can be of interest, managers may also be interested in standardized risk, or changes in risk associated with a management action while controlling for variables not under control of management. For instance, if the number and durations of transits varied markedly between regulation periods due to extrinsic factors, realized risk may give an unclear picture of the effects of regulations. In this case, managers may still be interested in changes in mortality risk that would have resulted had the number of transits remained constant. To make this estimate, we suggest calculating relative risk using

$$h_r^* \propto \sum_{t=1}^{N} \lambda_{tr}^* \Delta_{tr}^* M_{tr}^*$$

where λ_{tr}^*, Δ_{tr}^*, and M_{tr}^* are random draws for strike rate, vessel transit duration, and mortality probability (see below). We refer to risk computed using this approach as standardized risk.

Since we have empirical data on the length and speed of transits by management period and models for how whale mortality (M) and strike rate (λ) change as a function of transit speed (Eqs. 1 and 3) it is a relatively simple matter to calculate comprehensive risk reduction associated with speed restrictions. To properly account for uncertainty in these relationships, we computed a posterior distribution for the standardized risk ratio R (Eq. 4) by incorporating uncertainty in the estimated lethality-vessel speed relationship and the estimated strike rate-speed relationship. Again letting $[X|Y]$ denote the conditional probability distribution of X given Y, and bold symbols denote vectors of parameters, we start by symbolically writing the joint posterior distribution of R and transit speed and mortality parameters given the data as

$$[R, \mathbf{M}, \Delta, \beta, \theta, \alpha_1 | \mathbf{S}, \mathbf{Y}, \mathbf{Z}, \mathbf{x}] = [R|\mathbf{M}, \Delta, \alpha_1][\mathbf{M}|\theta, \beta][\beta|\mathbf{Y}][\Delta|\mathbf{Z}, \theta][\theta|\mathbf{S}][\alpha_1|\mathbf{x}].$$

Here, $\mathbf{S}$ denotes vessel speed data for different management periods, $\mathbf{Z}$ denotes transit length data, and $\mathbf{Y}$ denotes strike/mortality data as analyzed in our Bayesian probit analysis. The posterior distribution depends on simulated vessel speed values θ, which are assumed to be distributed according to $[\boldsymbol{\theta}|\mathbf{S}]$, and simulated transit durations, Δ. The latter depend upon both the empirical distribution of transit length values $\mathbf{Z}$ and upon vessel speed (given a particular transit length Z_i, and speed θ_i, duration can be calculated as Z_i/θ_i). Posterior predictions of lethality at different vessel speeds associated with the component $[\mathbf{M}|\boldsymbol{\theta}, \boldsymbol{\beta}][\boldsymbol{\beta}|\mathbf{Y}]$ may be generated via Eq. 2, while posterior values of α_1 can be sampled from the strike rate analysis.

Given these values, we express the standardized risk ratio as

$$[R|\mathbf{M},\Delta,\lambda] = \frac{\sum_{t=1}^{19,345} \lambda_{tr}^{*}\Delta_{t}^{*}M_{tr}^{*}}{\sum_{t=1}^{19,345} \lambda_{t0}^{*}\Delta_{t}^{*}M_{t0}^{*}} = \frac{\sum_{t=1}^{19,345} \exp(\alpha_1^{*}\theta_{tr}^{*})\Delta_{t}^{*}\Phi(\beta_0^{*}+\beta_1^{*}\theta_{tr}^{*})}{\sum_{t=1}^{19,345} \exp(\alpha_1^{*}\theta_{t0}^{*})\Delta_{t}^{*}\Phi(\beta_0^{*}+\beta_1^{*}\theta_{t0}^{*})}. \quad (5)$$

Note that the non-identifiable strike rate parameter α_0 cancels out of the above expression. As correlation between transit lengths and vessel speeds was low (Pearson correlation coefficient $\rho = -0.03$), we used independent probability density functions for both quantities. In particular, we drew vessel speed values $\boldsymbol{\theta}$ from the AIS-generated empirical distribution of vessel speeds within sampling regime r, and simulated Δ_t^* based on draws from the empirical distribution of observed transit lengths $\mathbf{Z}$ over the whole study period. We selected the limits of summation, 19,345, because it was the average number of transits occurring within a six month period. As results were somewhat sensitive to high speeds outside the range of strike rate and/or mortality analyses, we replaced any randomly selected transit speed above the 99th percentile of transit speeds (22.5 knots) with a value of 22.5 knots.

To summarize comprehensive risk ratios, we generated 10,000 posterior predictions using Eq. 5. Separate predictions were made for each year (1–4) of the study and for control and treatment periods for each strike rate scenario. We also analyzed pooled control and treatment data (pooling over years).

Results

Using logistic regression analysis, we detected a significant positive relationship between ship speed and the probability of a lethal injury ($\hat{\beta}_1 = 0.217$; SE 0.058; $p < 0.001$); the intercept was estimated as $\hat{\beta}_0 = 1.905$ (SE 0.821). The Bayesian probit analysis produced an almost identical relationship to the logistic regression analysis (Fig. 3), with posterior means of $\beta_0 = -1.067$ (SE 0.452) and $\beta_1 = 0.124$ (SE 0.030). As with logistic regression, there was substantial evidence for a positive effect of vessel speed on strike lethality (the posterior sample for β_1 was greater than zero for all realizations). Owing to several new observations of serious injury vessel strikes at lower vessel speeds (e.g., one each at 2 and 5.5 knots), the relationship between lethality and strike speed was less extreme than the one produced by Vanderlaan and Taggart (2007) and used in previously published risk analyses (Fig. 3).

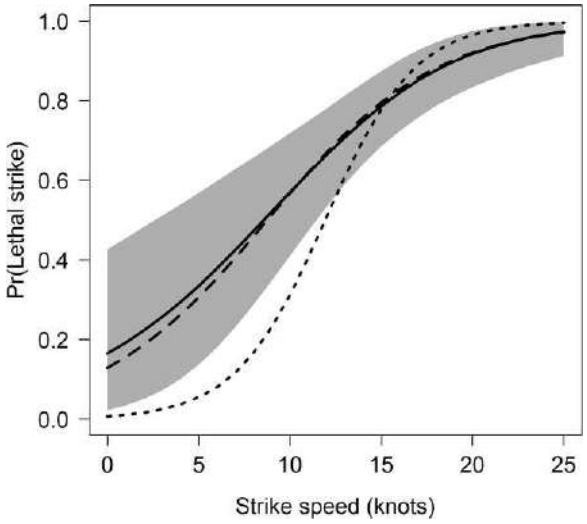

Fig. 3. Probability of a lethal whale strike given strike speed. The dashed line gives predictions from a logistic regression, the solid line gives posterior mean estimates from a Bayesian implementation of probit regression, and the dotted line gives logistic regression estimates reported by Vanderlaan and Taggart (2007). The gray area represents a 95% credible interval from the Bayesian analysis.

The speeds of vessels that struck whales were consistently greater than typical vessel speeds for each vessel type and regulatory period (Fig. 2). Accordingly, maximum likelihood fits of the use-availability model for strike speeds provided strong evidence for a linear effect of transit speed on strike rates ($\hat{\alpha}_1 = 0.49$, SE = 0.09); however, there was insufficient evidence to support a quadratic effect ($\Delta AIC = 1.1$). We therefore used a linear formulation for the effect of transit speed

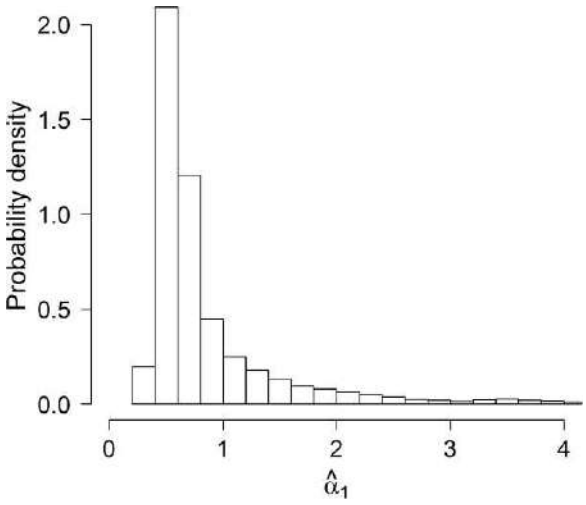

Fig. 4. Estimated posterior density function for α_1, the effect of vessel speed on the log of the instantaneous rate at which vessels strike whales. A value greater than zero indicates that instantaneous strike rate increases with vessel speed.

on strike rates in our Bayesian analysis. The posterior distribution for $\hat{\alpha}_1$ was substantially greater than zero (Fig. 4), providing further evidence that strike rates increase as a function of vessel speed.

Estimates of comprehensive risk reduction suggest a large decrease in standardized mortality risk associated with vessel speed restrictions (Fig. 5). In particular, control periods (i.e., when SMAs were not in effect) all had similar risk levels, while treatment periods (i.e., when SMAs were in effect) resulted in a risk reduction of 80–90%. Examining individual years separately (Fig. 5), it appeared that risk reduction was on the order of 80% for the first 2 years of vessel speed restrictions, and closer to 90% for the final 2 years of regulation. Pooling over years and simply comparing risk between treatment periods when speed regulations were in effect versus control periods when regulations were not in effect, the posterior mean mortality risk level in treatment periods was 14% of that in control periods (95% credible interval 5.6–29.0%), representing an 86% reduction.

Discussion

Various measures, focused primarily on changes in vessel routing patterns and reductions of vessel speed, have been employed to reduce the threat of vessel collisions with North Atlantic right whales. Routing changes that result in lowered co-occurrence of vessels and whales is the most desirable approach in most settings (Silber et al. 2012, van der Hoop et al. 2012), and several studies have provided estimates of vessel strike risk reduction afforded by established routing modifications (Firestone 2009, Vanderlaan and Taggart 2009, Vanderlaan et al. 2009, Lagueux et al. 2011). However, changing vessel routes is not always feasible due to navigational safety constraints, particularly in coastal waters.

Arguments for lowering vessel speed to limit the threat of fatal vessel collisions with both large whales (Laist et al. 2001) and manatees (*Trichechus manatus*) (Laist and Shaw 2006) first appeared in the early- and mid-2000s. These assertions were bolstered by risk reduction analyses (Pace and Silber 2005, Vanderlaan and Taggart 2007) and helped prompt use of speed restrictions in a number of locations (NPS 2003, Tejedor et al. 2007), the most extensive of which occur along the U.S. eastern seaboard. NOAA's vessel speed limits have been the subject of legal (Norris 2008, Firestone 2009), economic (Silber and Bettridge 2012), and risk reduction analyses (Lagueux et al. 2011, Wiley et al. 2011). Estimates of risk reduction to date have been applied to limited areas and times and relied on previously published logistic regression curves. Risk reduction values provided here include the full geographic scope of the vessel speed restrictions over a multi-year period using quantified vessel speeds, new whale strike data, and novel analyses. We believe this to be the most comprehensive assessment to date of the utility of vessel speed restrictions in reducing the threat of vessel collisions with large whales.

Our analysis highlights the importance of accounting for the combined effects of ship speed on (1) the rate at which vessels strike whales, and (2) the probability of mortality given that a whale is struck. In particular, we have shown that vessel speed is positively related to both components. To our knowledge, this is the first time that a use-availability model has been used to analyze the effect of vessel speed on the rate of whale strikes. Although simulation analyses (e.g., by modeling whale and vessel movement) can

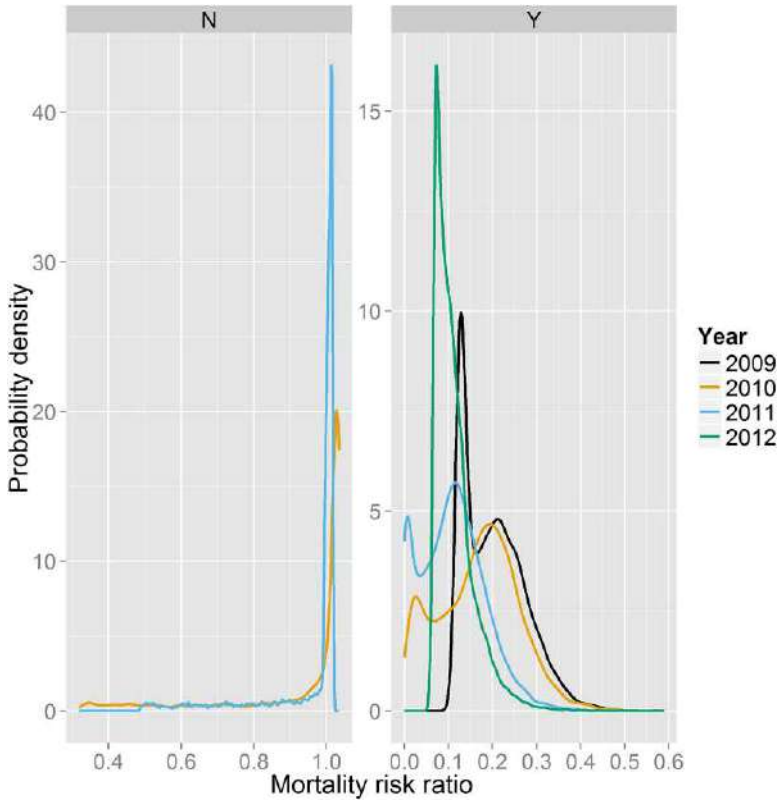

Fig. 5. Posterior predictive densities for comprehensive mortality risk ratio associated with transit speed restrictions in different years and management regimes. The left panel gives results for control periods ('N') while the right panel shows risk ratios when speed restrictions were in effect ('Y'). A ratio less than one indicates reduced risk relative to the control period in 2009.

provide some guidance as to likely functional forms for the relationship between vessel speed and the likelihood of a whale coming into close proximity with a vessel, it is difficult to use these analyses to reliably predict the probability of a collision because of uncertainty about fine scale nature of whale avoidance behavior. For instance, little is known about whale reaction, if any, to approaching vessels, particularly in the near-field. We view our analysis as an improvement in this regard, in that it allows one to explicitly estimate the effect of vessel speed on instantaneous strike rate. The obvious limitation of this approach is the small sample size associated with whale strike speeds, particularly when limited to vessels for which we had reliable control (availability) data. Nevertheless, with just 12 data points there appeared to be ample indication that strike rates increased with vessel speed. By contrast, if one fixes strike rates to be constant and simply uses the mortality curve to account for changes in mortality risk, it is actually possible to arrive at an (erroneous) increase in mortality risk, simply because slower vessel speeds increase transit times (and thus exposure of whales to vessels). This emphasizes the importance of simultaneously accounting for the effects of vessel speed on whale mortality and on strike rates.

The present analysis does not account for potential reductions in whale mortality attributable to changes in vessel routing regimes. For instance, previous analysis of vessel routing measures designed to lessen vessel occurrence in or near right whale aggregation areas (Lagueux et al. 2011, van der Hoop et al. 2012) suggested that there were substantial decreases in strike rates in at least portions of the range of North Atlantic right whales. In fact, Areas To Be Avoided and modifications to Traffic Separation

Schemes and other routing changes were made in the range of this species during the same period as vessel speed restrictions were introduced (Silber et al. 2012), albeit in targeted localized areas such as the Bay of Fundy, and waters off Georgia, Florida, and New England. We do not currently have data sufficient to account for the effects of management actions based on vessel routing across the entire east coast; however, we note that proportional changes to strike hazards result in an equivalent change to our risk ratio (Eq. 5). For instance, if vessel routing restrictions decreased the strike rate hazard by half, then the risk ratio in Eq. 5 would also be reduced by half. This suggests that our standardized risk ratio likely underestimates the true level of risk reduction accompanying the full suite of implemented management actions. However, we believe the risk ratios we provided here are valuable because it allows us to isolate the effects of a particular management action (in this case, transit speed regulations).

Our finding that vessel strike risk was lowest in the latter two of the four active periods studied is consistent with a measurable increase in vessel trips that comported with the required speed limits in years three and four, particularly as citations and fines were issued at the outset of year three (G. K. Silber, J. D. Adams, S. Bettridge, and B. Sousa, *unpublished manuscript*). This substantial shift in behavior observed across the entire regulated community helps explain, and contributes to, increased risk reduction in the latter two periods of our study.

We note the disparity of records of known vessel strikes by vessel type. Although cargo vessels represent the vast majority of vessels utilizing U.S. east coast ports and are the type most strongly represented in our AIS database, we were only able to obtain a single cargo vessel whale strike record for which strike speed was recorded. In contrast, sovereign vessels account, proportionally, for much higher numbers of recorded vessel strikes than other vessel types (Fig. 2). However, we wish to strongly emphasize that sovereign vessels are much more likely than other vessel types to report a struck whale because they are required to do so by internal protocol, and are obliged by conditions of U.S. Endangered Species Act Section 7 consultations to endeavor to reduce vessel strikes of whales by posting dedicated lookouts, traveling at reduced speeds when traversing active SMAs when and where feasible and when not jeopardizing vital or national security missions, and reporting when a whale strike has occurred. In addition, due to the sheer size of most commercial cargo and passenger vessels (which may be substantially larger than many sovereign vessel classes), these types of vessel operators are rarely aware that a collision with a whale has occurred. Nevertheless, it is important to note that our overall inference about the effect of ship speed on vessel strike rates could be biased if there were a statistical interaction between ship speed and ship type on vessel strike rates (that is, if whales respond to increasing ship speed differently among vessel types). Unfortunately, we do not have data sufficient to test this assumption, but believe it is the safest (and statistically parsimonious) to proceed with the assumption that transit speed affects strike rates similarly regardless of vessel type. A large number of additional reports of transit speed for non-sovereign vessel whale strikes would likely be necessary to relax this assumption.

Indications are that expansion will occur in the commercial maritime transport industry (Corbett 2004, Dalsøren et al. 2007), the cruise industry, offshore energy development, and other maritime sectors, thereby increasing risk of vessel strikes to whales. Conversely, factors such as restrictions on air-borne emissions from large vessels and the recent economic downturn may result in reduced vessel speeds (Khan et al. 2012) or fewer vessel trips (McKenna et al. 2012), which could reduce the likelihood of whale strikes. Nonetheless, the threat is likely to remain a concern as maritime transport and other activities increase and as whale populations grow in some locations. Our analysis suggests that vessel speed restrictions will likely remain a key tool for reducing anthropogenic mortality risk and promoting recovery of endangered large whale species.

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Supplemental Material

Supplement

R code to implement mortality risk analysis for North Atlantic right whales as a function of vessel speed (*Ecological Archives* C004-006-S1).

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ERRATUM

In the first paragraph of the *Results* section in the paper by Conn and Silber ("Vessel speed restrictions reduce risk of collision-related mortality for North Atlantic right whales"; *Ecosphere* 4:43), there was a typographical error in the intercept of the logistic regression model, $\hat{\beta}_0$. The value should have been −1.905, not 1.905. The remaining statistics (including standard error) are correct.

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EDITORIAL

PAHs and fish—exposure monitoring and adverse effects—from molecular to individual level

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Polycyclic aromatic hydrocarbons (PAHs) are a diverse family of more than one hundred compounds, containing at least two aromatic rings. In addition to parent compounds, the PAH family also includes substituted derivatives, bearing one or several alkyl groups, sulfur, or oxygen. In the environment, PAHs are ubiquitous and present as very complex mixtures. They can also be associated with metallic and/or other organic compounds. The composition of PAH mixtures depends on their origin. There are two major types of such PAH mixtures, petrogenic and pyrogenic, which enter the environment through different routes. Petrogenic mixtures originate from oils, including natural oil seeps. They enter the aquatic environment due to harbor activity or soil runoff or as a consequence of oil spills. Pyrolytic mixtures result from the incomplete combustion of organic matter, including fossil fuel, entering aquatic environments through deposits of atmospheric emissions directly into water or soil, followed by soil erosion and runoff. Directly linked to human activity, the release of PAHs into the environment has increased over the last few decades. As an example, the amount of PAHs released into the atmosphere has dramatically increased from under 50,000 tons in 1987 (Eisler 1987) to over 500,000 tons in 2004 (Zhang and Tao 2009).

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PAHs are hydrophobic molecules and are found mainly associated with suspended particulate matter in water. They tend to accumulate in sediments over time. Consequently, sediments are major sinks for PAHs and can also act as secondary sources of contamination in aquatic systems (Hylland 2006).

A large number of publications have reported the toxic effects of PAHs, observed on wild-caught fish after oil spills and after experimental exposures. Studies looking at the former revealed the toxic potential of PAHs and possible mechanisms, while work examining the latter confirmed the toxicity of PAHs, and provided very detailed information on underlying molecular mechanisms. However, in the second case, experimental designs mostly involved waterborne exposure to individual PAHs, and only a handful of studies investigated exposure via sediment and/or trophic transfer to complex PAH mixtures. Mechanisms identified include the triggering of the aryl hydrocarbon receptor (AhR) pathway and downstream molecular cascades. This pathway is, for example, involved in the activation of detoxifying enzymes such as Cyp1 (Billiard et al. 2002).

The ConPhyPoP project ("Contamination et Physiologie des Poissons exposés aux Polluants"; CES 09_002) has been funded by the French National Research Agency (ANR) to investigate contamination and effects of PAHs on early life stages or in whole life cycle experiments in three model fish species: zebrafish *Danio rerio*, Japanese medaka *Oryzias latipes*, and rainbow trout *Oncorhynchus mykiss*. The ConPhyPoP project had two main goals: to characterize the early effects of mixtures approaching situations occurring in the environment to develop early indicators suitable for pollution monitoring by hydrophobic compounds such as PAHs and to identify long-term effects on future fish performances. Besides articles produced as part of the ConPhyPoP project, this Special Issue benefits from the input of many other external contributions, also focused on these two topics.

The first three papers of this issue describe the optimization and use of a sediment-contact assay to assess the toxicity of hydrophobic pollutants. In the first, a reference artificial sediment was developed and validated using three model PAHs: fluoranthene, benz[a]anthracene, and benzo[a]pyrene (Le Bihanic et al. 2014a). Japanese medaka embryos are incubated on the artificial sediment spiked with different concentrations of the studied compounds. Classical developmental endpoints are then investigated. The main advantage of this approach is the known composition of the sediment matrix. In addition, it is totally safe for fish embryos and presents relatively high sorption capacities for hydrophobic compounds. In the two following papers, this approach was applied to evaluate the toxicity of three PAH fractions representative of different situations or sources: a pyrolytic mixture extracted from Seine sediment, a crude light oil (Arabian light), and a heavy oil (Erika oil) in two fish species, medaka and rainbow trout. In rainbow trout, *Oncorhynchus mykiss*, the degree and spectrum of toxicity were shown to vary according to the extract considered. The concentration and proportion of methylphenanthrenes and methylanthracenes appeared to drive the toxicity of the three PAH fractions tested. The minimal concentration causing developmental defects was as low as 0.7 $\mu g\ g^{-1}$ (sum of PAHs), indicating the high sensitivity of the assay with rainbow trout embryos and validating its use for toxicity assessment of particle-bound pollutants (Le Bihanic et al. 2014b). In medaka, the three PAH fractions were shown to delay hatching, to induce deformities, to disrupt larvae swimming activity, and to damage DNA at environmental concentrations. Differences in toxicity levels were observed and are likely related to differences in PAH proportions in the different extracts studied, in particular content in alkylated PAHs and low molecular weight PAHs (Le Bihanic et al. 2014c).

Sediments are generally considered as sinks for persistent organic pollutants and a possible secondary source of contamination for aquatic species. To elucidate the effects of sediment-bound organic pollutants such as PAHs, juvenile rainbow trout were exposed to three resuspended natural sediments with different contamination levels (Hudjetz et al. 2013). Significant differences in bile metabolite concentrations as well as in 7-ethoxyresorufin O-deethylase (EROD) induction compared to controls were observed for all exposure scenarios. The ratio between 1-hydroxypyrene in bile from fish exposed to the three different contamination levels correlated well with the ratio of pyrene concentrations in corresponding sediments and suspended particulate matter. In contrast, hepatic lipid peroxidation and micronuclei formation showed a less conclusive link with contamination. The results of this study clearly demonstrate that firmly bound PAH from aged sediments can become bioaccessible upon resuspension under flood-like conditions and are readily absorbed by aquatic organisms such as rainbow trout.

Oil spills are another major generator of PAHs in certain aquatic environments. In their study, Sturve et al. (2014) reported that eelpout exposed to a bunker oil—both in the field and in controlled laboratory conditions—showed elevated levels of EROD activity and DNA adducts, as well as an increased level of PAH metabolites. Likewise, Danion et al. (2014) showed that sea bass (*Dicentrarchus labrax*) juveniles chronically exposed to a water-soluble fraction of Arabian light exhibited a significant increased EROD activity in their liver, along with a drop in SOD activity, and a greater gill Glutathione content.

Two other articles report analysis of molecular responses after exposure to PAHs and other chemicals. In one case, mature polar cods are exposed to oil (the same Arabian light oil mentioned above), which is either mechanically or chemically dispersed. The authors report reduced O_2 consumption by permeabilised cardiac muscle fibers, indicative of inhibition of complexes I and IV of the respiratory chain in polar cod exposed to mechanically dispersed oil. They also show that dispersant did not increase oil toxicity (Dussauze et al. 2014). In the second article, European flounder juveniles were exposed through diet to a mixture of PAHs and PCBs. Short-term exposure led to expression of detoxification biomarkers, DNA damage, and deregulation of the immune system. Detoxification process biomarkers remained activated for the highest concentration after 2 weeks of recovery (Dupuy et al. 2014).

A number of articles refer to experiments carried out based on ConPhyPoP project specifications, with long-term exposure of zebrafish to the same three environmental PAH mixtures as described above. Exposures were performed at the PEP platform (http://wwz.ifremer.fr/pep) through spiked diets from the first meal and up to more than 1 year. Their effects on survival and growth were monitored throughout the exposure period (Vignet et al. 2014c). This article also report that jaw morphology disruptions observed after embryonic waterborne exposure to AhR agonists also occurred when exposure began later in the subjects' development. This can contribute to growth impairment in addition to observed digestive metabolism disruptions. A second article describes behavioral disruptions in juveniles and adults and shows modification of mobility, a reduction in exploratory activity, and an increase in anxiety levels (Vignet et al. 2014b). A third article describes carcinogenesis occurring in the same fish and revealed a time- and dose-dependent increase in neoplastic disorders with bile duct described as the main target. Paradoxically, no DNA damage has been observed with the comet and micronucleus assays. This apparent discrepancy may be due to the fact that it has been assayed in blood, following an overnight starvation, while a kinetic analysis revealed a quick but transient induction of *cyp1a* expression in hours following feeding (Larcher et al. 2014). A common finding of these three articles is that all three mixtures tested produced disruptions at varying degrees of severity. Heavy oil produced the greatest disruption, followed by light oil, while pyrolytic extract was the least disruptive. This ranking is consistent with that resulting from

the short-term exposures described above. This underlines the major role played by alkylated (particularly methylated PAH) derivatives in the toxicity of these mixtures.

The toxicity of PAH derivatives has also been evaluated in two other articles featured in the Special Issue, oxygenated PAHs and methylpyrene. In one article, the authors exposed Japanese medaka embryos to sediment spiked with pyrene and methylpyrene and revealed similar responses after exposure to both compounds, leading to embryotoxicity characteristics of AhR activation, as well as some gene activation at relevant environmental concentrations (Barjhoux et al. 2014). In addition, methylpyrene also activated the expression of genes involved in cell cycle control, oxidative DNA damage repair, and the retinoid pathway. Oxy-PAH can also be detected in aquatic sediments but knowledge about their toxicity is currently very limited. A screen of seven oxy-PAHs revealed that these pollutants can induce developmental abnormalities, including jaw, heart, and tail cartilage anomalies in exposed Japanese medaka embryos (Dasgupta et al. 2014). Certain oxy-PAHs were also shown to significantly increase DNA damage as revealed by the comet assay. Comparisons between the genotoxic potential of these oxy-PAHs, their corresponding parent PAHs, and the potent mutagenic PAH, benzo[a]pyrene, indicated similar potency. This study pointed out the need for additional data about the environmental occurrence and biologic effects of oxy-PAHs.

Finally, three articles tackle the question of long-term and trans-generational effects. In one, the authors exposed zebrafish embryos to a sediment spiked with a mixture of three PAHs and bred fish until adulthood in clean medium. This early and short-term exposure produced a late disruption of growth and a trend toward a lower reproductive ability. In addition, adults displayed lethargic and/or anxiety-like behaviors. This latter behavior was also identified in offspring (F1) at the larval stage (Vignet et al. 2014a). A second one reports an increase of cardiac frequency in larvae originating from fish exposed to pyrolytic mixture. This is associated to an increase in ATPase, cardiac transporting *atp2a2a* (Lucas et al. 2014). In the third article, Clark et al. pursued their study of a killifish population exposed to PAHs for several decades (Atlantic Wood Industries Superfund; AW). This population had developed remarkable resistance to the acute effects and teratogenesis caused by AhR agonists. In this article, they compared sensitivity to AhR inducers of AW F1 and F2 embryos to embryos obtained from a control unexposed population. In this way, they revealed an impressive resistance of F1 and F2 embryos to express cardiac teratogenesis and to activate expression of *cyp1* genes. It is noteworthy that despite strong resistance to cardiac teratogenesis, repression of *cyp1* and EROD activation is intermediate in AW F2 compared to AW F1. The authors conclude resistance to cardiac teratogenesis in AW fish is conferred by multiple factors, not all of which appear to be fully genetically heritable (Clark et al. 2013).

Taken together, these articles increase our knowledge of the effects of PAHs in fish at several developmental stages and via a number of different routes of exposure. They all lead to the conclusion that whatever the route and the mixture used, exposure to environmental concentrations of PAHs reduces fish fitness and participation in recruitment (i.e., their ability to contribute to the next generation). They also pave the way for new important research, such as that examining mixtures of PAHs and PAHs associated with other chemical and non-chemical stressors, e.g., photoactivation by UV, as well as the need for studies on the effects of PAH derivatives and long-term and trans-generational effects. Considering the major adverse effects of PAHs on reproduction success and development and survival of early life stages, we believe that it is of paramount importance to investigate the effects of PAHs on recruitment and dynamics in fish populations.

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Xavier Cousin (left) is a *Chargé de Recherches* (researcher) from INRA working at the Ecotoxicology laboratory (Ifremer La Rochelle). He received his Ph.D. in Life Sciences in 1995 from the University Pierre et Marie Curie (Paris 6). He worked on fish physiology with a focus on embryonic development and the way it influences later fish performances. He has authored 39 peer-reviewed publications. He joined the Ifremer Ecotoxicology laboratory in 2006 to develop integrative research approaches dealing with the assessment of ontogenesis and physiology of fish exposed to chemicals (mainly organic pollutants) and physiological stressors. He coordinated the ANR ConPhyPoP project and has been involved in several other National and European projects. He is particularly interested in the molecular mechanisms underlying stressor effects and the way early embryo-larval disruptions can shape later life history traits. He is head of zebrafish facilities at the Ifremer Fish Ecophysiology Platform (http://wwz.ifremer.fr/pep_eng/) in La Rochelle, with a number of projects currently underway.

Jérôme Cachot (right) is a professor of environmental toxicology at the University of Bordeaux, based at the EPOC Laboratory (UMR 5805 CNRS-University of Bordeaux). He obtained his Ph.D. in Ecotoxicology in 1998 from the University of Aix-Marseille II. He also worked for several years at the Laboratory of Ecotoxicology (University of Le Havre), focusing on the mutagenic and carcinogenic effects of organic pollutants on fish and mollusks. In 2007, he moved to Bordeaux and initiated a new research program dealing with the effects of mixtures of pollutants on the embryo-larval development of marine and fresh water fish and mollusk species. He is the author of 34 peer-reviewed publications. To date, he has supervised 8 Ph.D. students and has been involved in 25 national and international research projects in the field of aquatic toxicology.

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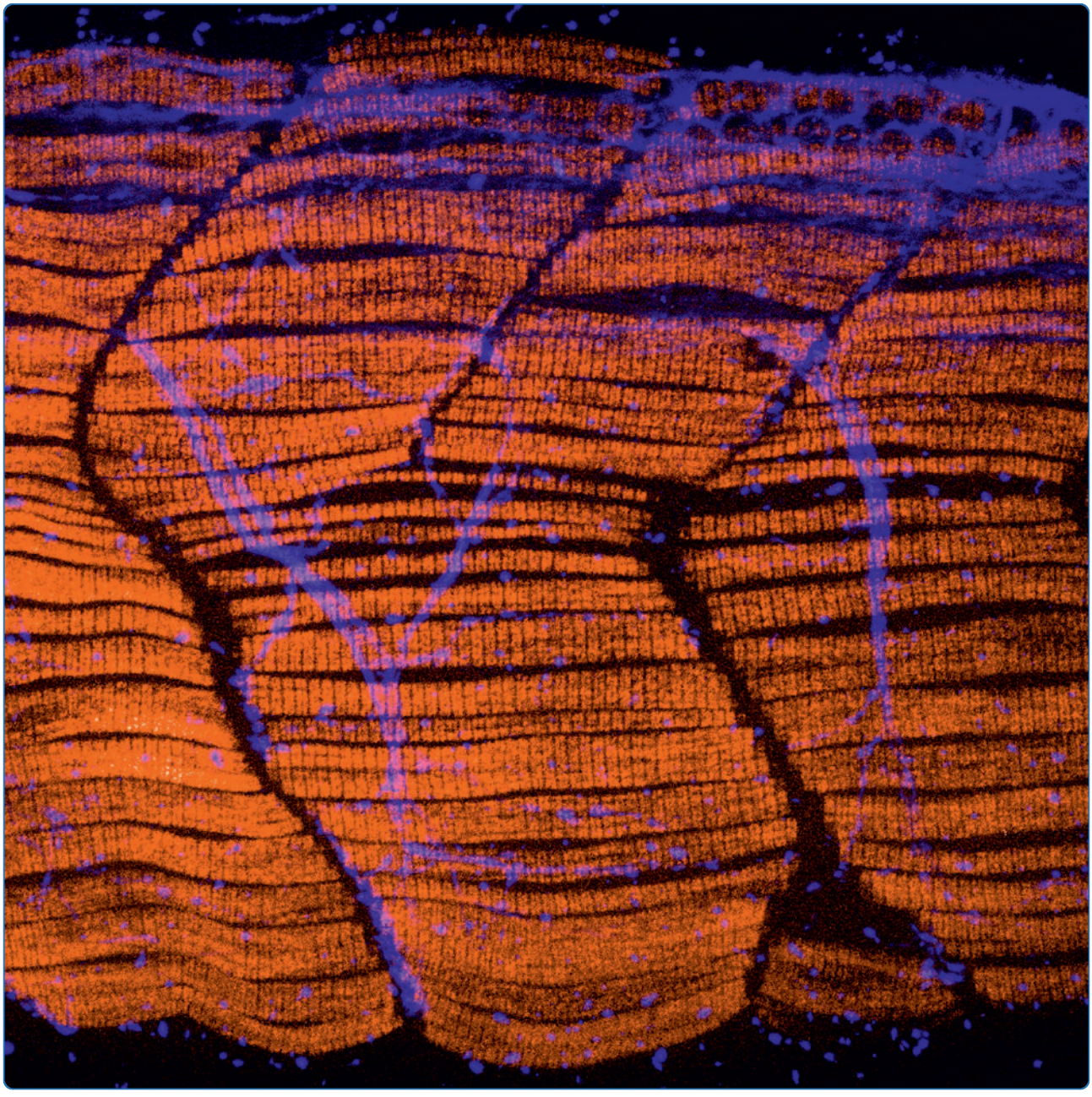

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RESEARCH ARTICLE Open Access

Macondo crude oil from the Deepwater Horizon oil spill disrupts specific developmental processes during zebrafish embryogenesis

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Abstract

Background: The Deepwater Horizon disaster was the largest marine oil spill in history, and total vertical exposure of oil to the water column suggests it could impact an enormous diversity of ecosystems. The most vulnerable organisms are those encountering these pollutants during their early life stages. Water-soluble components of crude oil and specific polycyclic aromatic hydrocarbons have been shown to cause defects in cardiovascular and craniofacial development in a variety of teleost species, but the developmental origins of these defects have yet to be determined. We have adopted zebrafish, *Danio rerio*, as a model to test whether water accumulated fractions (WAF) of the Deepwater Horizon oil could impact specific embryonic developmental processes. While not a native species to the Gulf waters, the developmental biology of zebrafish has been well characterized and makes it a powerful model system to reveal the cellular and molecular mechanisms behind Macondo crude toxicity.

Results: WAF of Macondo crude oil sampled during the oil spill was used to treat zebrafish throughout embryonic and larval development. Our results indicate that the Macondo crude oil causes a variety of significant defects in zebrafish embryogenesis, but these defects have specific developmental origins. WAF treatments caused defects in craniofacial development and circulatory function similar to previous reports, but we extend these results to show they are likely derived from an earlier defect in neural crest cell development. Moreover, we demonstrate that exposure to WAFs causes a variety of novel deformations in specific developmental processes, including programmed cell death, locomotor behavior, sensory and motor axon pathfinding, somitogenesis and muscle patterning. Interestingly, the severity of cell death and muscle phenotypes decreased over several months of repeated analysis, which was correlated with a rapid drop-off in the aromatic and alkane hydrocarbon components of the oil.

Conclusions: Whether these teratogenic effects are unique to the oil from the Deepwater Horizon oil spill or generalizable for most crude oil types remains to be determined. This work establishes a model for further investigation into the molecular mechanisms behind crude oil mediated deformations. In addition, due to the high conservation of genetic and cellular processes between zebrafish and other vertebrates, our work also provides a platform for more focused assessment of the impact that the Deepwater Horizon oil spill has had on the early life stages of native fish species in the Gulf of Mexico and the Atlantic Ocean.

Keywords: Deepwater Horizon, crude oil, zebrafish, embryonic development, cardiovascular, cartilage, neural crest, peripheral nervous system, somitogenesis, muscle

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Introduction

More than 200 million gallons of crude oil were released from the Macondo Well in the Gulf of Mexico during the 2010 Deepwater Horizon oil spill [1,2]. The oil gushed from approximately 5,000 feet below the surface creating underwater plumes of oil and slicks on both the ocean floor and surface. The oil's total vertical exposure to the water column suggested it had the potential to impact a diversity of ecosystems in the Gulf of Mexico and Atlantic Ocean from coastal wetlands to coral reefs to deep-water benthic communities [3,4]. In fact, many thousands of invertebrate and vertebrate carcasses have been discovered and attributed to the Macondo oil, and due to the wide-spread and hidden nature of this disaster, species mortality rates are hypothesized to be at least 50 times underrepresented [4,5].

Of particular concern is how embryos and larvae will be affected by this oil spill, as organisms in these early life stages are typically unable to flee a contaminated area and lack the tissue mass and mature detoxification systems necessary to withstand toxic insults. Furthermore, loss of whole clutches of embryos and larvae can have catastrophic consequences for population dynamics over long time periods [6]. The National Oceanic and Atmospheric Administration (NOAA) reported an unusually high number of stillborn and newborn dolphins that began washing up on the Gulf shores starting in January 2011 [7]. While only a small number of these dead dolphins had obvious signs of oil exposure, the timing suggests an association between the gestational period of dolphin embryonic and fetal development with the Deepwater Horizon blowout and oil spill [4]. There are 52 taxa of native fish species also known to spawn in the Gulf of Mexico such as tuna, red snapper and yellowfin groupers [8], and unfortunately little is known about how the Macondo crude oil might directly impact early development of dolphins or these fish species. It will be critically important to identify the molecular targets of crude oil toxicants that effect embryonic development to provide greater predictive power in the risk assessment of this and future spills.

Exposure to crude oil and/or the water-soluble, polycyclic aromatic hydrocarbons (PAH) that make up much of the oil have been shown to cause cardiovascular abnormalities and pericardial edema in topsmelt and Pacific herring embryos, as well as additional defects in jaw and spinal cord development in the crimson-spotted rainbow fish [9-11]. Whether oil from the Macondo well can cause similar defects in embryonic development needs to be determined. The majority of research using native Gulf fish species to determine the effects of crude oil on embryonic development has been limited to the analysis of mortality and gross morphological changes. However, the relatively recent characterization of zebrafish (*Danio rerio*) as a laboratory fish model system has revealed significant insights into the developmental mechanisms governing embryogenesis [12,13]. Zebrafish provide researchers with the consistent accessibility of genetically defined lines of embryos and a battery of reliable genetic, molecular and cellular techniques for higher resolution analysis, benefits that are providing significant advantages to the use of zebrafish in toxicology. The small adult fish size, large embryo clutches, *ex utero* development, and transparent embryo and larval stages of zebrafish enable cost effective maintenance of many fish, reproducible sample sizes, simple application of toxin treatments, and easy evaluation of end-point toxicity [14-18]. The use of zebrafish to assay drug and pollutant toxicity has already provided insights into the developmental and molecular mechanistic roles of metals [19,20], dioxins [21-24], pesticides [25,26], endocrine disruptors [27,28], alcohols [29-31], chemotherapies [32-34] and specific pharmaceutical compounds many of which were assessed via high-throughput screening [35].

Examination of the effect of crude oil and its more common PAHs on zebrafish embryos has shown developmental abnormalities that include cardiac edema, reduced jaw development, curvature of the spine, hemorrhaging, reduced larval heart rate and cardiac arrhythmia [6,36-39]. While these varied deformations are clearly attributed to crude oil exposure, it is unknown what early embryonic processes at the cellular or molecular levels are directly affected. Further investigations utilizing zebrafish to elucidate the molecular mechanisms that interact with and mediate crude oil effects could guide future preventative measures to oil spills and their clean up procedures, as well as yield new strategies to combat the teratogenesis associated with oil spill exposure.

In the present study, we sought to determine whether water-soluble components of the crude oil from the Deepwater Horizon oil spill could disrupt normal embryonic and larval development in zebrafish. Embryos were exposed to water accumulated fractions (WAFs) made from crude oil sampled from the riser insertion tube attached to the Macondo well during the 2010 oil spill. Using an array of high resolution assays to characterize a variety of discrete developmental processes, we show that the Macondo oil does not cause general and wide-spread toxicity but rather disrupts specific developmental processes, some of which have never been reported previously for any crude oil source. We show that WAF-induced deformations in cardiovascular and craniofacial development, observed in this and likely other studies [6,36-39], are associated with reductions in specific rostral migratory streams of cranial neural crest cells. Surprisingly, Macondo oil exposure also generated novel phenotypes in apoptosis, sensory and motor axon pathfinding, somitogenesis and muscle fiber type development, which provide causative

evidence for the locomotor swimming behaviors exhibited by WAF-treated embryos. Lastly, we demonstrate that dose and timing of WAF exposure are equally important for the induction of cell death and muscle specific phenotypes. Our results suggest that crude oil components may interact with important molecular mechanisms to influence embryogenesis. The severe teratogenic effects in zebrafish associated with the Deepwater Horizon oil are cause for concern if native fish species in the Gulf of Mexico responded similarly. Finally, our findings provide a more comprehensive phenotypic map to assess pollution effects on development.

Results

Gross morphological deformations

Water accumulated fractions (WAF) of the Macondo crude oil were vortex mixed in embryo medium at a 1:10 dilution in accordance with conventional methods [40]. Analysis of different mixing procedures and serial dilution experiments were performed and are described in Additional files 1 and 2. Based on our dose response analysis we conducted all embryo treatments at the 100% concentration of the vortex-mixed, 1:10 WAF stock solution, which will from here on be referred to as WAF. Only during our analysis of locomotor behavior was 50% WAF solution used. These WAF preparations serve to model the portions of the Macondo crude oil sampled from the riser insertion tube during the Deepwater Horizon oil spill that are capable of dissolving in embryo medium. It is important to acknowledge that according to communications with BP during the period of 2 and 3 May 2010 when this sample was being collected Nalco EC9323A defoamer was being applied topside and methanol with VX9831 oxygen scavenger/catalysts solution injected subsea. While we cannot rule out the possibility that these additional agents in the vicinity of the spill site could be present in this sample, it is highly unlikely due to the method of direct sampling through the riser tube.

Embryos exposed to WAF starting at 3.5 hours post-fertilization (hpf) until a maximum of 5 days post-fertilization (dpf) caused an array of morphological phenotypes that included dorsal tail curvature, cardiac edema, cyst formation, reduced head structures and brain hemorrhages (Figure 1). More specifically, WAF-treated embryos showed moderate (Figure 1F) to severe cardiac edema (Figure 1C-E, arrows) that, in some cases, also included yolk sac edema (Figure 1C, right most arrow). In addition, a dorsal curling of the tail and caudal cyst formation were variably observed within a treated clutch of embryos but were consistently present across treatments (Figure 1E, F, double arrows; Figure 1C, D, F, arrowhead). Qualitatively, the overall size of the brain and eyes were often reduced, which was most apparent in the ventral jaw structures (Figure 1G, H, brackets). Equally obvious was the average 28% of embryos with brain hemorrhages present in the forebrain, midbrain, or hindbrain regions (Control 0.0%, n = 452; WAF, n = 441; Figure 1H-J, arrowheads). While the eyes of some WAF-treated embryos were noticeably smaller, the mean measurements of the area and perimeter of the retina and lens showed only subtle, but statistically significant, reductions in size (Figure 1K-M). These varied, but relatively specific, morphological deformations suggest that certain embryonic processes may be affected by exposure to Macondo crude oil.

Chemical analysis of water accumulated fraction

To determine what components of the crude oil were released into the WAF mixture and thus exposed to the embryos during experimental treatments, WAF samples were analyzed within one hour of being made using solid phase microextraction (SPME, 100 mm polydimethylsiloxane) and gas chromatography mass spectrometry (GCMS, Agilent 7890A GC/5975C MSD). Chemical analysis confirmed the presence of *n*-hexane, toluene, xylene, benzene, naphthalene and ethylbenzene that were reported by BP to be present in this source oil B. This analysis also revealed a variety of additional components, of which the most prominent were aromatics and alkanes (Figure 2A), as observed in chemical analyses of Gulf of Mexico waters after the Deepwater Horizon blowout [41-43]. Aromatic concentrations were similar over the four WAF solutions sampled (Figure 2A). We interpret the large variation in heavier alkane concentrations (Figure 2A) to the inclusion of a non-aqueous phase, into which these poorly soluble components fractionate. Such partitioning of these less-soluble components into deep-plume oil droplets [42] and into the surface slick [43] was observed in the Gulf of Mexico after the BP Oil Spill. For a full detailed report of the chemical analysis obtained see Additional file 2.

To determine how component concentrations changed with time, samples from a WAF solution were taken every five hours from petri dishes maintained under the same conditions as the treated zebrafish embryos. As demonstrated by a few selected representative components, there is a dramatic drop-off in concentrations with time (Figure 2B). For aromatics (blue), the drop-off is less extreme for the heavier napthalenes (75 to 80% drop in five hours), compared to the lighter alkylated benzenes (93 to 95% drop in five hours). The initial concentrations for the heavy alkanes (red) are beyond reported hydrocarbon solubilities, such that the WAF solution is likely to have been initially fully saturated with these poorly soluble components (as discussed further in Additional files 1 and 2). Assuming initial saturation, the drop-off for tetradecane is similar to the heavier napthalenes, at 75% within five hours. These results suggest that while embryos are initially

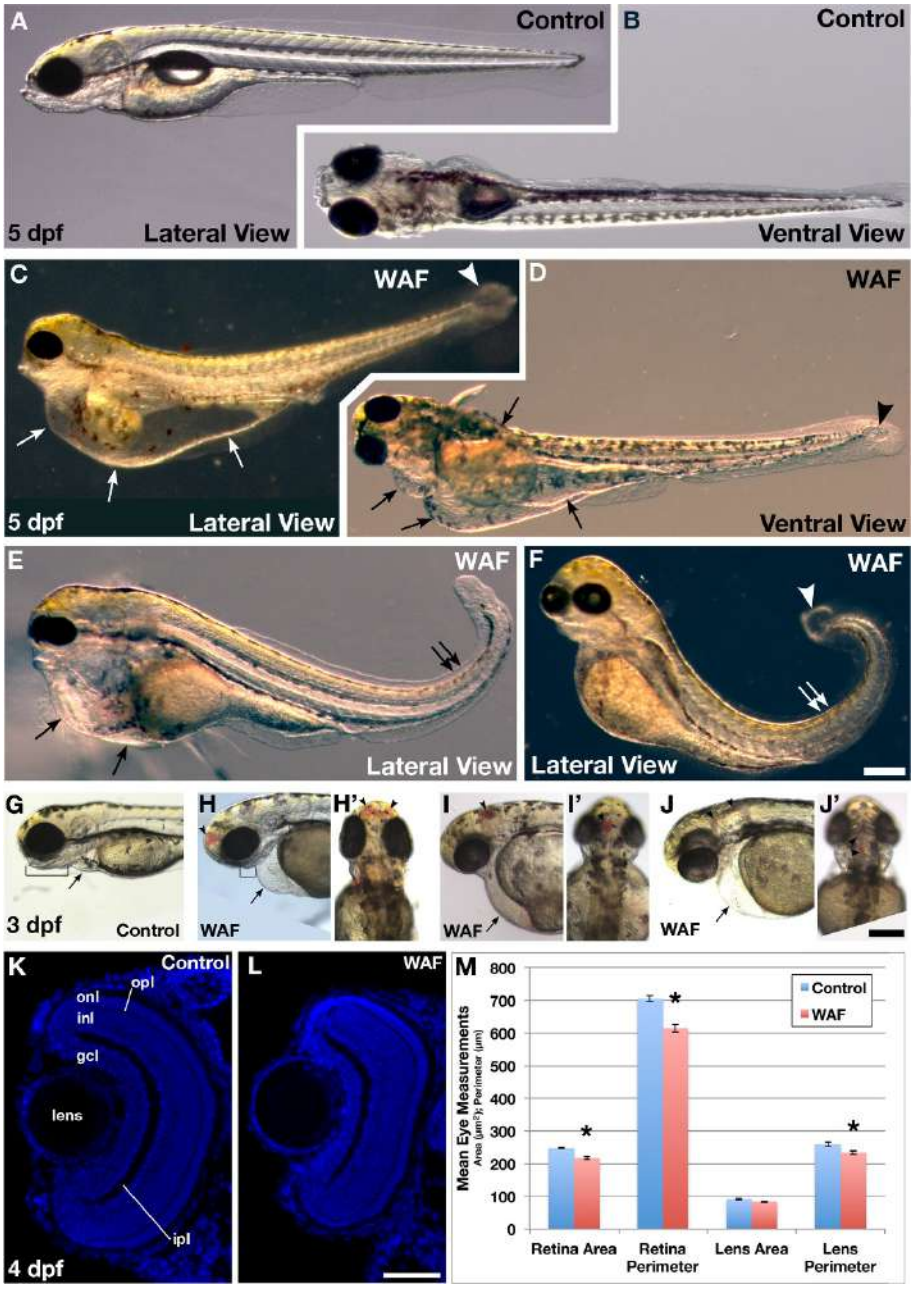

Figure 1 Exposure to Macondo crude oil-derived WAFs induced diverse gross morphological deformations in zebrafish embryos. (**A-F**) Lateral and ventral views of live untreated control (A, B) and WAF-treated embryos (C-F) at 5 dpf. Severe cardiac and yolk edema (C, D, E, arrows), dorsal tail curvature (E, F, double arrows), and cysts at the tip of the tail (C, D, F, arrowhead) were visible. (**G, H**) WAF-treated embryos (H) had reduced jaws compared to controls (G, brackets). (G-**J**) At 3 dpf cardiac edema was evident in WAF-treated embryos (arrows), and 28% of embryos had hemorrhaging in the forebrain, midbrain and hindbrain (arrowheads). Lateral (G, H, I, J) and dorsal views (H', I', J'). (**K-M**) Retinal architecture appeared normal in control and WAF-treated embryos (K, L) but there was a slight reduction in the area and perimeter of WAF-treated retinas (M). Except for lens area, the size reductions were statistically significant (M, asterisks; *t*-tests: lens area, $P = 0.015$; lens perimeter, $P = 0.007$; retina area $P < 0.0005$; retina perimeter, $P < 0.0005$). Scale bars: 200 μm, F, J'; 50 μm, L. Abbreviations: gcl, ganglion cell layer; ipl, inner plexiform layer; inl, inner nuclear layer; opl, outer plexiform layer; onl. outer nuclear layer.

exposed to high levels of hydrocarbon compounds, within the 5- to 10-hour window, they are experiencing hydrocarbon concentrations that approach levels observed in underwater plumes in the Gulf of Mexico [41,42] and those along the Louisiana marshes capable of altering gene expression in adult killifish [44]. For example, within 10

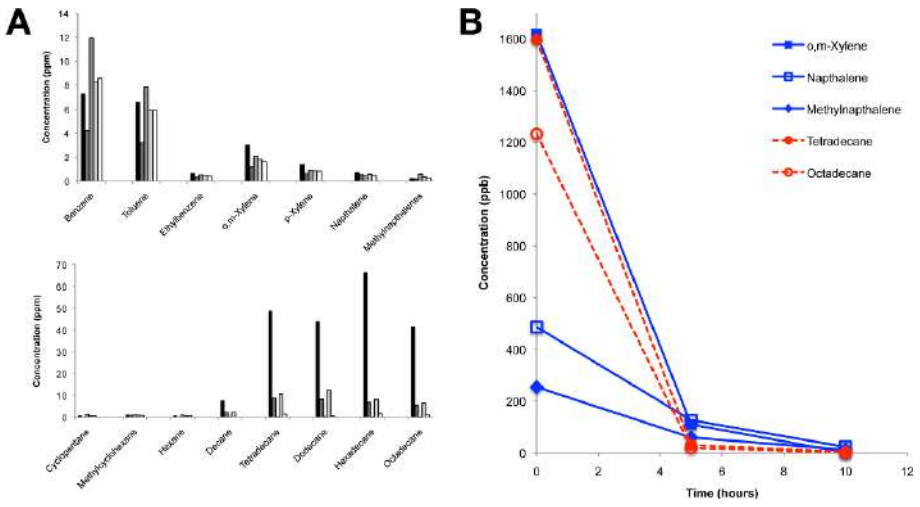

Figure 2 Compound composition of Macondo crude oil-derived WAFs. **A**) Concentrations of selected components in samples taken from four different WAF experiments. The most prominent components were aromatics and alkanes. See Supplementary Information for details. **B**) Selected aromatic (blue) and alkane (red) WAF components decreased in concentration over a 10-hour test period.

hours, our concentrations of toluene, ethylbenzene and total xylenes fall below ranges observed in underwater plumes [42].

Cell proliferation and cell death

We next wanted to characterize the gross morphological phenotypes we observed at higher resolution to glean better insight into whether exposure to Macondo oil affected specific developmental processes during zebrafish embryogenesis. WAF-treated embryos exhibit subtle reductions in brain and eye size and changes to the symmetric elongation of the tail (Figure 1), all of which could indicate reductions in cell numbers. No obvious greying of tissue, indicative of cell necrosis, was ever observed in WAF treated embryos; reduction in tissue size could be attributed to either reduced cell proliferation or increased programmed cell death.

Embryos treated with WAF from 3.5 hpf to 30 hpf were immunolabeled for Phosphorylated Histone H3 (PH3) to visualize all cells undergoing mitosis [45]. Pooled averaging of three replicate WAF treatments did not show significant changes in the number of PH3-positive cells as compared to untreated controls (Figure 3A, B; control, 79.9; WAF, 76.2; *t*-test, $P = 0.18$). To control for the presence of outlying values, the lack of statistical significance was confirmed with a non-parametric test (Mann-Whitney, $P = 0.0511$). If mitotic rates are not altered in WAF-treated embryos, then reductions in tissue size may be due to increased apoptosis or programmed cell death.

To test whether cells were dying by apoptosis we immunolabeled embryos for Activated Caspase 3, which is a marker for cells undergoing programmed cell death [46]. Pooled averaging of four replicate experiments did show a statistically significant increase in the number of dying cells positive for Activated Caspase 3 along the trunk in WAF-treated embryos as compared to controls (Figure 3C; control, 1.94; WAF, 12.9; *t*-test, $P = 0.0001$). Apoptotic cells were present both inside and outside the spinal cord (Figure 3D-F). Interestingly, despite using the same crude oil source for each WAF preparation, the number of apoptotic cells in WAF-treated embryos decreased with each successive replicate experiment (Figure 3C). In the first treatment an average of 38.25 Activated Caspase 3-positive cells were seen in WAF-treated embryos (Control, 3.9; *t*-test $P = 0.0001$), and in the second treatment this average dropped to eight cells (Control, 2.05; *t*-test $P = 0.0062$). Furthermore, in the third and fourth replicate experiments the number of apoptotic cells in WAF-treated embryos was no longer statistically different from their respective controls (Rep3: control, 1.1; WAF, 3.05 *t*-test $P = 0.0966$; Rep4: control, 0.7; WAF, 2.15; *t*-test $P = 0.1151$). A total of 20 embryos were examined for each condition in each separate replicate experiment.

The prevalence of WAF-induced cell death during the initial experiments suggests our crude oil sample was changing and becoming less potent over time. Chemical analysis did show much of the aromatics and alkanes in the WAF drop off dramatically within five hours after exposure to the embryos (Figure 2B), thus it is possible that our crude oil source sample experienced an incremental loss of some compounds over the course of storage. To test this hypothesis directly, we treated embryos with WAF from 3.5 hpf to 18.5 hpf and then replaced this solution with a freshly mixed WAF solution and continued the experiment until 30 hpf. This WAF refreshing

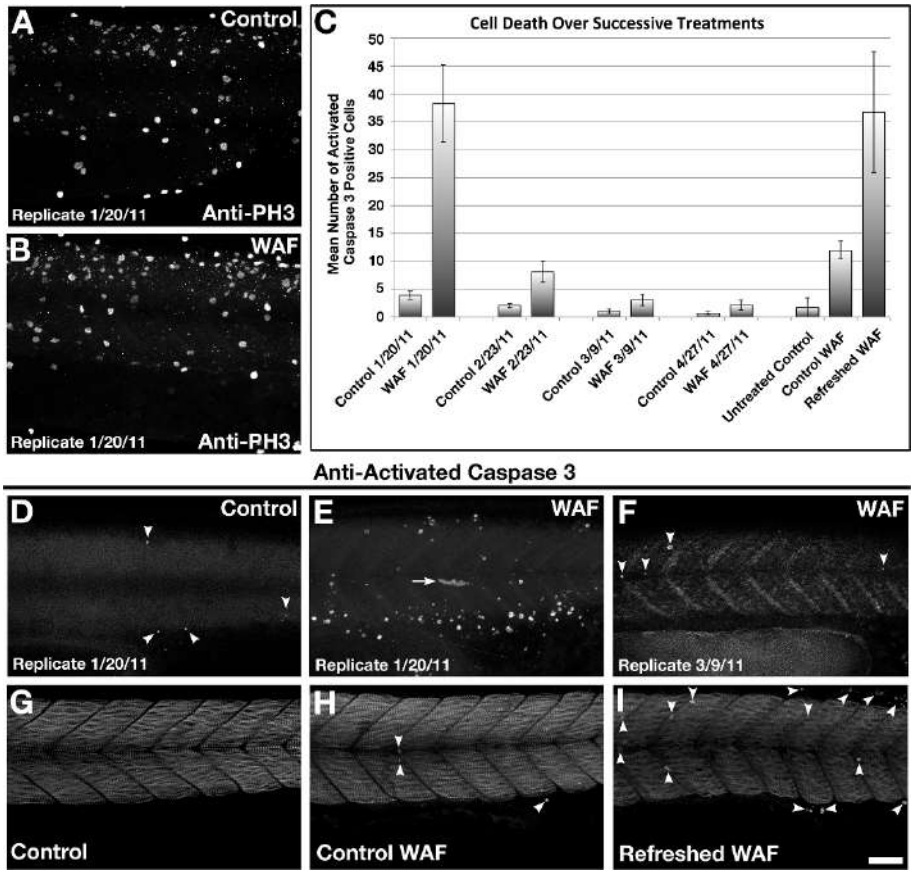

Figure 3 Macondo crude oil exposure did not affect cell proliferation but did induce programmed cell death. (**A**, **B**) Phospho-Histone H3 labeling of cells in mitosis were unaffected in 30 hpf WAF-treated embryos (B). (**C**) Quantification of anti-Activated Caspase 3-positive cells in 30 hpf control and WAF-treated embryos over 4 replicates and a WAF-refreshing procedure. The number of apoptotic cells decreased with each successive replicate, but increased following application of freshly-mixed WAF. (**D-F**) Activated caspase-3 labeled 30 hpf control (D, arrowheads) and WAF-treated embryos (E) from experiments in January 2011, and a WAF-treated embryo from an experiment in March 2011 (F, arrowheads). There was a significant decrease in the number of apoptotic cells in WAF-treated embryos between January (E) and March (F). (**G-I**) Representative images from the refreshed WAF experiments (arrowheads denote positive anti-Caspase 3 cells). Embryos were either untreated (G), exposed to the same WAF from 3.5 hpf to 30 hpf (H), or exposed to WAF from 3.5 hpf to 15 hpf and then exposed to a fresh WAF solution from 15 hpf to 30 hpf (I). Embryos in the refreshed WAF group (I) partially recovered the cell death phenotype of earlier replicates (E). (A, B, D-I) Lateral trunk views centered on somites 14 to 21. Scale bar 50 μm, A, B, D-I.

protocol did show a statistically significant increase in the number of Activated Caspase 3 cells (36.74 cells; n = 42) as compared to untreated (1.75 cells; n = 40) or non-refreshed WAF treated controls (11.97 cells; n = 38) (*Kruskal-Wallis Test* $P < 0.0005$) (Figure 3G-I). In fact, the number of apoptotic cells in the refreshed WAF experiment was similar to the initial WAF experiment (Rep1, 38.25 cells; $P = 0.2276$) (Figure 3C). This result suggests refreshing the WAF solution returned the cell death-inducing properties of the crude oil.

Circulation and vasculogenesis

We have demonstrated that embryos treated with WAF derived from the Macondo Oil exhibit cardiac edema and hemorrhaging in the brain (Figure 1). We next wanted to determine whether the Macondo Oil WAF likewise interferes with the proper development and physiology of the circulatory system that has been demonstrated previously for other crude oil types and components [9,10,36-39,47,48]. Closer examination for the presence of blood through whole-mount hemoglobin stained control and WAF-treated embryos showed a qualitative reduction in the amount of blood cells in treated embryos (Figure 4A-D). This reduction was particularly obvious in the vasculature of the pharyngeal arches (Figure 4A, B, brackets; C, arrowheads, D). Importantly, significant hemoglobin staining was still present in the common cardinal vein distal to and including the heart, but abruptly ended in the heart or bulbus artery prior to filling the aortic arches (Figure 4A-D, arrow).

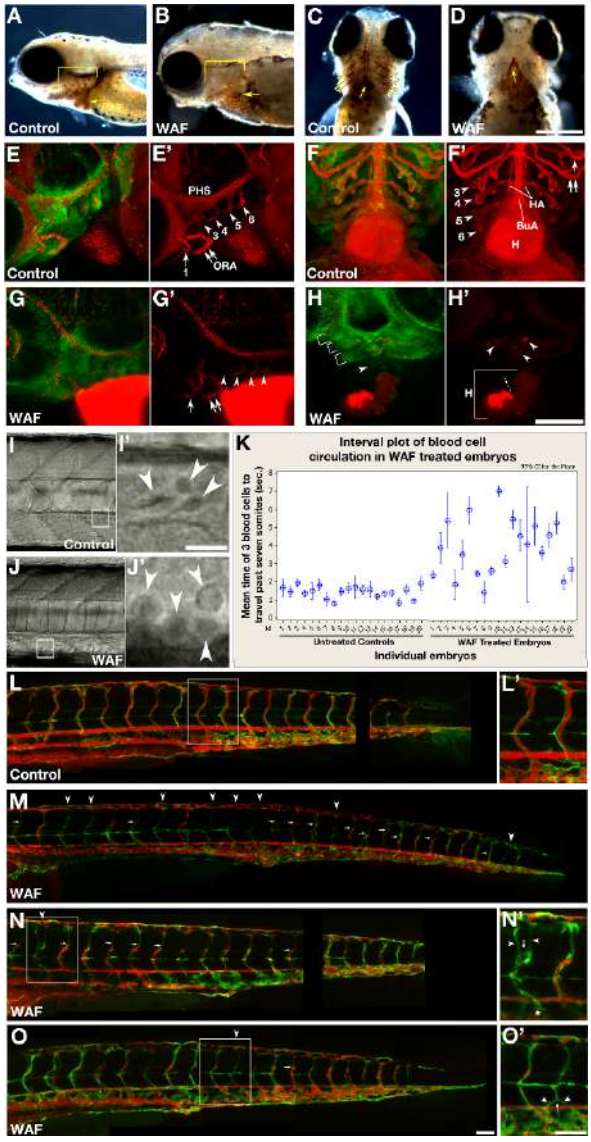

Figure 4 Defects in head and trunk vascular development result in reduced circulatory function. (**A-D**) Hemoglobin staining revealed a reduction in the amount of blood cells in 3 dpf WAF-treated embryos, notably in the vasculature of the pharyngeal arches (A, B, brackets, lateral view; C, D, arrowheads, ventral view), with staining abruptly ending in the heart or bulbus artery prior to filling the aortic arches (arrow). (**E-H'**) Microangiography analysis with QTracker 655 fluorescent quantum dots (red) injected into 3 dpf *tg[fli:eGfp]* transgenic larvae to visualize endothelial cells associated with the vasculature (green). Endothelial vasculature in moderately affected WAF-treated embryos (G) was comparable to controls (E), however in severe cases posterior arch vasculature was lost and circulation was reduced (H, brackets, arrowhead, H', arrowheads). (E', F', G', H') Arrowheads and arrows denote the specific blood vessels associated with the pharyngeal arches. Accumulation of quantum dots in the heart atrium suggests reduced flow into the ventricle (H', dashed line). (**I-K**) Real time analysis of the flow speed of individual blood cells (I-J', arrowheads) over a 7-somite distance in the dorsal aorta. WAF-treated embryos have reduced blood circulation (K, right half of graph). (**L-O'**) Intersegmental blood vessels had reduced circulation of quantum dots as demonstrated by either a complete absence of flow (M-O, arrowheads) or truncated flow (M-O arrows). Ectopic branching and vascular remodeling was evident in some segments devoid of circulation (N', O'). Abbreviations: BuA, bulbus arteriosus; H, heart; HA, hypobranchial artery; ORA, opercular artery; PHS, primary head sinus. Numbers and affiliated arrowheads in E' and F' represent the first through sixth aortic arch. Scale bars = 200 μm, A-D; 100 μm, E-H'; 50 μm, I, J, N', O'; 20 μm, I', J'; 50 μm, L-O.

The reduced blood volume specifically beyond the heart could be due to diminished hematopoiesis, improper vessel formation or altered heart function. To better discern between these possibilities, we conducted angiography with fluorescent quantum dots in 72 hpf live embryos treated with WAF starting at 3.5 hpf. This procedure was done in *tg[fli:eGfp]* transgenic larvae, which allowed for visualization of green endothelial cells

as the red quantum dots flowed throughout the circulatory system, illuminating both the extent of circulatory function and blood vessel anatomy [49]. Imaging of the aortic arches revealed that in some cases the endothelial vasculature was present and capable of supporting circulation (Figure 4E, G, arrows and arrowheads), while in more severe cases the more posterior arch vasculature was lost and circulation was significantly reduced in the remaining vasculature (Figure 4F, H). In addition, there was often a build-up of the quantum dots in the heart atrium paired with a reduced flow of these quantum dots into the ventricle (H', dashed line). These results suggest that the endothelial vasculature and proper heart development and function are all variably compromised in WAF-treated embryos. Further supporting these results, real time imaging of blood flow through the dorsal aorta showed that individual blood cells from 60 different WAF-treated embryos (3 replicates) took, on average, 2.68 times longer to travel the distance of 7 somites (Figure 4I-K; Control, 1.44s; WAF, 3.85s; *t*-test, $P = 0.0001$).

Continued analysis of quantum dot circulation and endothelial vasculature in the trunk revealed additional phenotypes involving intersegmental blood vessel development. The most prevalent phenotype was varied frequency of reduced circulation of fluorescent quantum dots through the intersegmental blood vessels, such that vessels showed either a complete absence of flow (Figure 4L-O, arrowheads), truncated flow (Figure 4L-O, arrows), or normal circulation. Interestingly some vessels devoid of any quantum dots often showed ectopic branching and an overall improper vessel pattern (Figure 4N', O'). These phenotypes were consistent over three separate experimental replicates with at least 10 embryos imaged per replicate. As a whole, these results suggest the Macondo crude oil is capable of causing specific deformations in vasculogenesis in both the trunk and head of zebrafish that leads to reduced circulatory function.

Craniofacial development

Due to the vascular defects associated with pharyngeal arches, we next examined whether exposure to Macondo crude oil similarly causes defects in craniofacial development, a phenotype known to occur with specific PAH exposure [11,36]. We treated embryos from 3.5 hpf to 4 dpf and then performed Alcian Blue staining to visualize cartilage [50]. Consistent with head vasculature phenotypes, WAF-treated larvae showed a range of head and jaw cartilage phenotypes (Figure 5A-F). WAF-treated embryos had a general size reduction in all cartilage elements with a significant lack of anterior extension of the most prominent jaw elements (Meckel's and ceratohyal cartilage) (Figure 5B, C, E, F). The most severely affected embryos showed dramatic reduction in all of the pharyngeal arch cartilage elements (ceratobranchial) with a complete loss of the most posterior three arches as compared to untreated control larvae (Figure 5A, B, E). In addition, we report here for the first time, in response to any crude oil treatment, a lack of anterior midline fusion of the basihyal cartilage (Figure 5E, arrowhead). Less severely affected embryos, however, did not display this basihyal phenotype (Figure 5F).

Because of the corresponding deformations in both endothelial and cartilage cell development in the head, we next hypothesized that these phenotypes may be derived from an earlier defect in the differentiation or migration of neural crest cells, which are a common progenitor cell contributing to pharyngeal arch cartilage, smooth muscle of the pharyngeal arch arteries as well as portions of the heart such as the arterial pole and endocardial cushions [51-56]. To test this we conducted whole-mount *in situ* hybridizations for *crestin* transcripts in WAF-treated and control embryos at 27 hpf. *crestin* is a known marker of neural crest cells during their early specification, delamination from the dorsal neural tube, and subsequent migration into the characterized pathways of the trunk and head [57]. Interestingly, embryos treated with WAF from 3.5 hpf to 27 hpf of development showed qualitative reductions in *crestin*-labeled cells, specifically in the anterior migratory streams known to populate the pharyngeal arches (Figure 5G-L; H, J, L, arrowheads and bracket). In contrast, the amount and position of *crestin*-positive cells in the trunk qualitatively appeared normal (Figure 5G, I, K, insets).

To confirm that deformations in pharyngeal arch development were associated with defects in early cranial neural crest populations, we examined the presence of rostral migratory streams of neural crest cells in *tg(fli:GFP)* transgenic embryos and whether they appropriately expressed the cell specification marker *dlx2* [58,59]. A total of 76% of WAF treated embryos (n = 83) showed a specific loss of one of the more posterior pharyngeal arches (Figure 5M, N, arrows) (control = 8.6%; n = 83; $P < 0.0005$). Importantly, despite normal *dlx2* expression in the forebrain, 89.4% of WAF-treated embryos (n = 94) showed a significant reduction of *dlx2* expression in all of the rostral migratory streams with a near complete absence in the presumptive posterior-most arch locations (Figure 5O, P, bracket) (control = 15.6%; n = 122; $P < 0.0005$). These findings suggest that Macondo crude oil is compromising the early development of cranial neural crest cell differentiation, which results in reduced posterior pharyngeal arches and the more visible defects in the heart, arch vasculature and craniofacial development.

Locomotor behavior

During our many WAF treatments it was clearly evident embryos displayed irregular swimming behaviors. The

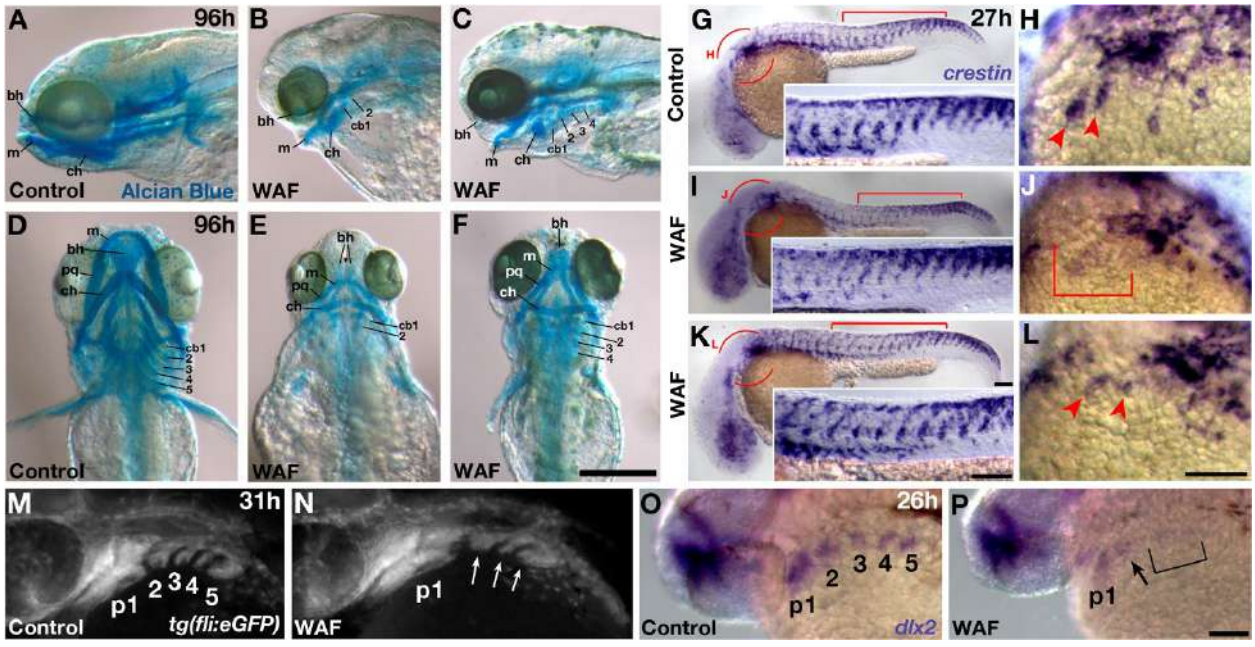

Figure 5 Craniofacial defects induced by Macondo crude oil exposure were correlated with defects in neural crest development. (**A-F**) Alcian blue staining of head and jaw cartilage in 4 dpf control (A, D) and severely (B, E) or moderately (C, F) affected WAF-treated embryos. WAF-treated embryos had a variable reduction in the size of all cartilage components, notably a lack of anterior extension of jaw elements and a dramatic reduction in posterior pharyngeal arches (B, C, E, F). (**G-L**) Whole mount *in situ* hybridization of *crestin* expression in neural crest cells. *crestin* expression is normal in the trunks of control and WAF-treated embryos (G, I, K, bracket; circles in G, I, K represent magnified view in H, J, L). However, *crestin* expression was variably reduced specifically in the anterior migratory streams, an area of cells that will populate the pharyngeal arches (H, J, L, arrowheads and bracket). (**M, N**) Cranial neural crest forming pharyngeal arches (p1-5) at 31 hpf as visualized by *fli* driven expression of GFP. One of the posterior-most pharyngeal arches is missing in WAF treated embryos (N, arrows) as compared to controls (M, 3, 4, 5). (**O, P**) *dlx2* expression in the region of pharyngeal arches is reduced in 26 hpf WAF-treated embryos (P, p1, arrow, bracket) as compared to controls (O). *dlx2* expression is nearly lost in the most posterior regions of the presumptive pharyngeal arches (P, bracket) despite robust expression still seen in the forebrain. Abbreviations: bh, basihyal cartilage; cb1-5, ceratobranchial branches; ch, ceratohyal; m, Meckel's; pq, palatoquadrate. Scale bars = 200 μm, A-F; 100 μm, G-L.

locomotor escape response to stimuli is an important survival behavior that develops later in embryogenesis. Interestingly, previous studies examining the effect of PAHs on zebrafish swimming behavior did not reveal any significant phenotypes [36]. Therefore, we systematically tested whether exposure to Macondo crude oil WAF impacted swimming patterns and escape responses. To do this, we recorded the swimming behavior of individual 48 hpf larvae with a high-speed video camera (1,000 frames/second) following the administration of a specific touch stimulus [60]. WAF-treated embryos demonstrated abnormal swimming behavior and a failure to escape based on multiple criteria. WAF-treated embryos showed reduced sensitivity to touch stimuli, as demonstrated by 70% response rate for WAF-treated embryos as compared to a 99% response rate for untreated control embryos (n = 100 trials from 10 embryos each). When a response was produced in WAF-treated embryos they showed a significantly reduced frequency of body bends (Control, 39.10Hz; WAF, 18.82Hz; n = 10 each; *t*-test, $P < 0.01$) and swam for less time than untreated control embryos (Control, 875.8mS; WAF, 282mS; n = 10 each; *t*-test, $P = 0.01$) (Figure 6). The presence of locomotor behavior phenotypes suggests that there could either be a problem with neural transmission or a developmental problem resulting from an anatomical deformation in the nervous or musculature systems.

Neuronal development in the central and peripheral nervous system

Development of locomotor movement in response to touch stimuli requires a complex neural network that begins with an elaborate meshwork of Rohon Beard sensory axons at the periphery that originate from the dorsal neural tube. These bipolar sensory neurons make connections with a variety of interneurons that function to relay signals to Mauthner neurons, which serve as the major motor control center in the hindbrain. Mauthner neurons then send signals back down the spinal cord to stimulate coordinated motor neuron activation to achieve the alternating contraction of skeletal muscle required for swimming behaviors [61-63]. In order to determine what

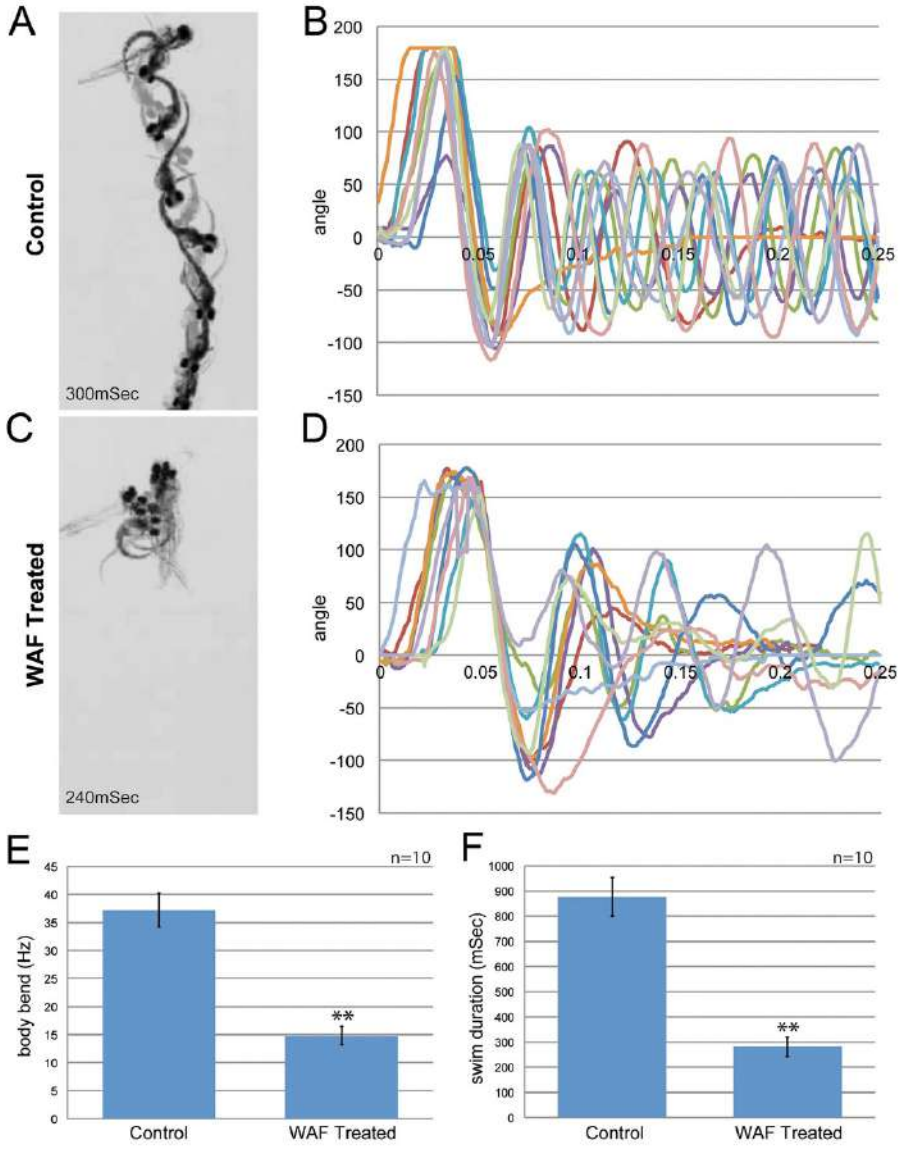

Figure 6 Macondo crude Oil exposure impaired escape behavior by 48 hpf. (**A**) Individual frames from high-speed video recordings are shown for control larvae. The images are overlaid in 20 mS intervals and the duration of the response captured within the field is indicated. (**B**) Kinematics traces are shown for 10 escape responses each for control larvae. 0° indicates a strait body and positive and negative angles represent body bends in opposite directions. The time is indicated in seconds. (**C**) Image overlays for a WAF-treated larva escape response illustrates the failure to clear the field that was frequently observed. (**D**) Kinematic traces for WAF-treated larvae reveal reduced, abnormal body bend frequencies. (**E**) Quantification of body bend frequencies. (**F**) Quantification of the duration of escape responses reveals that WAF-treated larvae respond for shorter periods of time. Asterisks in E and F indicate statistically significant differences (n = 10, $P < 0.01$).

underlying developmental processes may be contributing to the visible defects in locomotor behavior, we used specific antibody markers to visualize each cellular step in the locomotor neural circuit.

Anti-Acetylated Tubulin (AT) labels all axonal pathways in the zebrafish as well as specifically marks the somas of Rohon Beard sensory neurons [64]. Anti-Islet-1 labels both the nuclei of Rohon Beard Sensory neurons and motorneurons, anti-Gaba labels a series of interneurons, and the 3A10 antibody marks Mauthner neurons in the hindbrain [65-67]. Surprisingly, using this spectrum of neuronal markers we found only one consistent and specific anatomical deformation that was restricted to the peripheral projections of sensory axons. The Mauthner neurons that serve as the central mediators for startle-reflex behavior in the hindbrain, showed absolutely no difference in number or axonal projections between WAF-treated and untreated control embryos

(Figure 7A, B; n = 60) [67-69]. Likewise, no statistical difference was seen in the average number or position of Gaba-positive DoLa, CoSA, VeLD or KA interneurons (Figure 7C, D; Control, 41.1 cells, n = 60; WAF, 41.3 cells, n = 59; *t*-test, $P = 0.855$).

Investigation of motorneuron development did not reveal major differences in their cell differentiation. Specifically, anti-AT labeling of primary motor axons did not show consistent defects over the length of the trunk (Figure 7E, F, arrows). It should be noted that while occasional errors in motor axon pathfinding were detected, they were always associated with an affiliated muscle patterning defect that we describe in detail later. Quantification of three separate replicate experiments of Islet1 labeling for primary and secondary motorneurons at 30 hpf showed only minor reductions in the number of motorneurons, with only one of the three replicates actually exhibiting statistical significance (Figure 7G, H, lower bracket; Rep 1: 16% reduction (control n = 11, WAF n = 20), *t*-test, $P = 0.0001$; Rep 2: 4.6% reduction (control n = 18, WAF n = 18), *Mann-Whitney test*, $P = 0.456$; Rep3: 6.6% reduction (control n = 20, WAF n = 19), *Mann-Whitney test*, $P = 0.043$). These results suggest that in some limited way motorneuron number may be impacted by exposure to Macondo oil WAF. However, based on the relatively normal pattern of primary motor axons, very modest overall reductions in number, variable significance and time period of analysis correlating with the middle of secondary motorneuron birth [65], we attribute these minor decreases in motorneuron populations more likely to a mild developmental delay in treated embryos rather than any biologically relevant effect from the oil.

Similarly, no qualitative differences were seen in the position or morphology of Rohon Beard sensory neurons as detected with anti-AT labeling (Figure 7E, F asterisks). Quantification of Rohon Beard sensory neuron numbers with anti-Islet1 showed little to no effect (Figure 7G, H, upper bracket). Similar to motorneurons, only one of the three replicates revealed a statistically significant difference (Rep 1: 2% increase (control n = 11, WAF n = 20), *t*-test, $P = 0.72$; Rep 2: 6.3% reduction (control n = 18, WAF n = 18), *t*-test, $P = 0.184$; Rep 3: 15.6% reduction (control n = 20, WAF n = 19), *t*-test, $P = 0.0001$). Again, based on the normal morphology and position of Rohon Beard cells, with no statistical difference present in the first two replicates, and only a small effect observed in the third replicate, we conclude Rohon Beard sensory neuronal number to be unaffected by Macondo oil. However, analysis of the meshwork of sensory axonal projections in the periphery was consistently both visually and

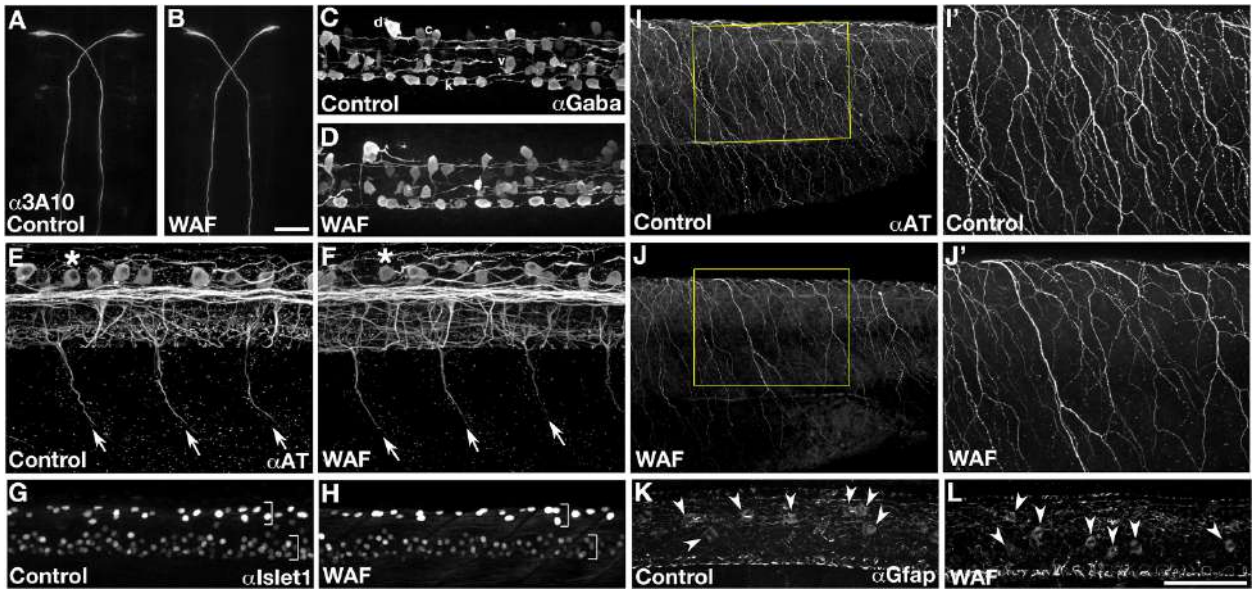

Figure 7 Macondo crude oil exposure caused specific deformations in the peripheral but not central nervous system. (**A-L**) 30 hpf control and WAF-treated embryos labeled by immunohistochemistry. (A, B) 3A10 labeled Mauthner neurons in the hindbrain were normal. (C, D) Distribution of Gaba-positive interneurons in the spinal cord were not impacted by WAF treatment (d, DoLa; c, CoSa; v, VeLD; k, KA). (E, F) Anti-Acetylated tubulin (AT) labeling of primary motor axons (arrows) or Rohon Beard sensory neuronal somas (asterisk) were correctly positioned in WAF-treated embryos (lateral view of trunk and spinal cord). (G, H) Qualitatively, Islet1 labeling for primary and secondary motor neurons (lower bracket) and Rohon Beard sensory neurons (upper bracket) were positioned normally. (I, J) The branching pattern of AT-labeled sensory axonal projections along the trunk epidermis was significantly reduced (I', J', magnified views of boxed area in I, J). (K, L) Anti-Gfap labeled radial glia somas in WAF-treated embryo spinal cords (L) were correctly positioned in the ventricular zone (arrowheads) and similar in number to controls (K). (A, B) Dorsal views of the hindbrain. (C-L) Lateral views of the spinal cord (C, D, E, F, G, H, K, L) and trunk (I, J). Scale bars = 50 μm, A-L.

quantitatively significantly reduced (Figure 7I, J, Control axonal labeling intensity = 1274.1 pixels, n = 55; WAF axonal labeling intensity = 883.9 pixels, n = 59; *t*-test, $P = 0.0001$). These results suggest most of the anatomical development of the locomotor circuitry is unaffected by Macondo crude oil WAF with the exception of subtle but consistent deformations in the amount and/or branching dynamics of sensory axonal projections.

While the overall number of many key neuronal cell types was normal in WAF-treated embryos, we wanted to confirm that radial glial cells, which serve as the neural stem cell population in the developing spinal cord, were also unaffected [70-72]. Using an antibody to Glial fibrillary acidic protein (Gfap) to mark radial glia we show that radial glial cell staining throughout the spinal cord appeared normal (data not shown). Most relevant was the normal number of Gfap-positive cell somas located at the ventricular surface of the neural tube (Figure 7K, L, arrowheads; Control, 5.99 cells, n = 40; WAF, 5.67 cells, n = 40; *t*-test, $P = 0.582$). Anti-Gfap labeling fills radial glial cell bodies when they are undergoing mitosis at the ventricular zone (Johnson and Barresi, in preparation; [73]). Normal numbers of such dividing radial glia indicates that exposure to Macondo crude oil WAF does not affect neural stem cell proliferation during embryogenesis.

Somitogenesis and muscle fiber type development

Because our analysis of the nervous system showed specific deformation restricted to the axonal projections of the peripheral nervous system, it was possible that these defects were secondary to deformations in the paraxial mesodermal environment through which these axonal projections need to navigate. During somite development, radially migrating slow muscle precursor cells provide key guidance cues to the simultaneously outgrowing motor axons [74]. Therefore, any defects in the proper specification, migration and/or position and integrity of slow muscle fibers could influence the development of the peripheral nervous system. We exposed embryos from 3.5 hpf to 48 hpf in Macondo crude oil WAF and assayed for F59 labeling, which preferentially recognizes slow muscle myosin in zebrafish [75,76]. As with most assays, we discovered embryos displaying a range of slow muscle development phenotypes from no observable defects to the loss of muscle fibers and segment boundary errors. Interestingly, similar to what was observed for our cell death analysis over time, embryos displaying slow muscle phenotypes were extremely severe during initial treatments but these phenotypes decreased significantly over the course of several months of repeated experiments using the same crude oil source for WAF preparation (Figure 8).

Initial experiments in November 2010 showed clear defects that represented several distinguishable phenotypes in the treated embryos (Figure 8A-D). The first was the sporadic instance of defects in proper somite boundary formation, such that a segment boundary in a portion of one somite would be absent as represented by slow muscle fibers extending from the anterior boundary of one somite to the posterior boundary of the neighboring somite (Figure 8B, C, arrowhead). Most interestingly, double labeling for motor axons showed pathfinding errors in the affected somite that were directly correlated to the somite/slow muscle defect (Figure 8B, asterisk, arrow). These results suggest that Macondo crude oil may not only be affecting slow muscle fiber development but also somitogenesis, the process by which segment boundaries are established in the paraxial mesoderm [77-79]. The second distinguishable phenotype was the sporadic loss and disorganization of slow muscle fibers in the superficial monolayer (Figure 8D), which suggests impairments in slow muscle differentiation and subsequent migration in WAF-treated embryos.

Later experiments revealed somitogenesis defects with corresponding motor axon pathfinding errors, but these deformations were not as dramatic as compared to our first experiments (Figure 8E-G) and subsequently absent from the final replicates (Figure 8H-M). Later experimentation still showed extremely sporadic loss and disorganization of the slow muscle monolayer (Figure 8H-M). Interestingly, high resolution imaging of regions showing reductions in slow muscle fibers revealed muscle phenotypes resembling muscle degeneration [80-85]. Variable sized fragments of slow muscle myosin were found present in locations devoid of morphologically normal slow-twitch fibers (Figure 8I, J, red). In contrast to the somite boundary defects, motor axon pathfinding appeared normal in areas showing these missing or degenerating muscle fibers (Figure 8I, J, green). Lastly, these phenotypes continued to diminish in severity upon our last round of treatment (Figure 8K-M).

The sporadic nature of muscle phenotypes both in a clutch of treated embryos and within an affected embryo paired with the gradual loss of these phenotypes over time suggests that the causative agent in the crude oil is not particularly potent and somehow loses its activity over time. Therefore, in an attempt to reproduce the severity of the muscle and somitogenesis defects observed initially, we exposed embryos from 3.5 hpf to 48 hpf in WAF that was refreshed every 15 h. Similar to our results of increased apoptosis in refreshed WAF treatments, more severe muscle deformations were present in refreshed WAF-treated embryos as compared to untreated controls and non-refreshed WAF-treated embryos (Figure 9). The sporadic nature of these deformations was consistent, but the severity was now qualitatively similar to our initial experiments. Remarkably, somitogenesis defects were detected again, showing slow muscle fibers crossing in

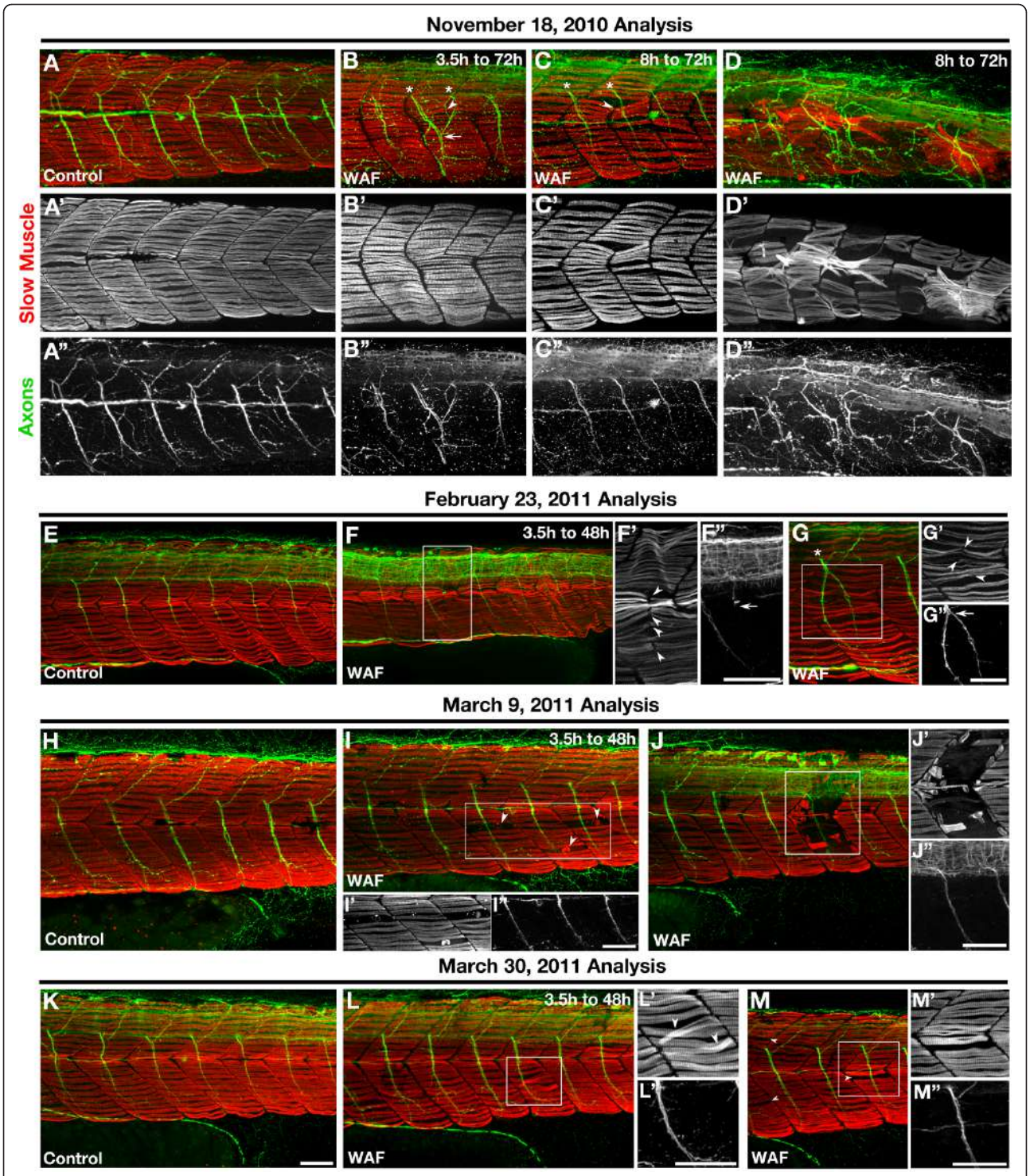

Figure 8 The severity of deformations in slow-twitch skeletal muscle development decreased with each experimental replicate. (**A-M**) Lateral views of F59 labeled Myosin heavy chains in slow-twitch muscle fibers (red) and anti-Acetylated tubulin labeled motor axons (green) in the embryonic trunk at 72 hpf (A-D) and 48 hpf (E-M). (A-D) Initial Macondo crude oil WAF treatments beginning at either 3.5 hpf (B) or 8 hpf (C, D) and ending at 72 hpf showed severely defective neuromuscular phenotypes, such as improper somite boundary formation (B, C, arrowhead) or slow muscle loss and disorganization (D). Somite boundary defects associated with the middle or ventral portion of the somite correlated with motor axon pathfinding errors (B, asterisks, arrow; D). (E-M) Subsequent WAF treatments beginning at 3.5 hpf and ending at 48 hpf induced somitogenesis (F, G; F, G', arrowheads), slow muscle (I-M', arrowheads), and motor axon pathfinding (F, G; F", G", arrows) defects, however the severity of these defects decreased over time with each experimental replicate from November to March. (A-M) Primed letters represent single channel images of slow muscle (single prime) or axon (double prime) labeling for the whole image (A-D) or just the boxed regions (F, G, I, J, L, M). Scale bars = 50 μm, A-M".

locations that would normally have a somitic boundary (Figure 9D, D', arrowheads) as well as the presence of irregularly shaped somites (Figure 9D', arrows). In addition, significant slow muscle loss and potential degeneration was also observed following this WAF refreshing protocol. Specifically, slow muscle myosin fibrils were seen in varying stages of degeneration (Figure 9E). This type of dose-response approach confirms that some component(s) within the Macondo crude oil WAF do directly disrupt somitogenesis and slow muscle development.

Discussion

By all standard measures the Deepwater Horizon blowout caused the worst oil spill the Gulf of Mexico has ever experienced, and recent news reports suggest oil might still be leaking from the Macondo well into the Gulf waters [4,86-89]. To truly assess the total impact of this disaster it will be critical to monitor the fauna and flora of the Gulf of Mexico and Atlantic Ocean for years to come. To make this assessment efficient and thorough, researchers will need to know the range of phenotypes expected from exposure to Macondo oil; however, collecting this information from fish growing within these waters may be particularly challenging. If native species encountered the Macondo oil from the Deepwater Horizon spill during their embryonic or larval stages, these organisms may have died shortly after exposure or were eaten by predators, limiting the number of specimens available for study. Moreover, collection procedures for critical stages of embryonic development and reliable

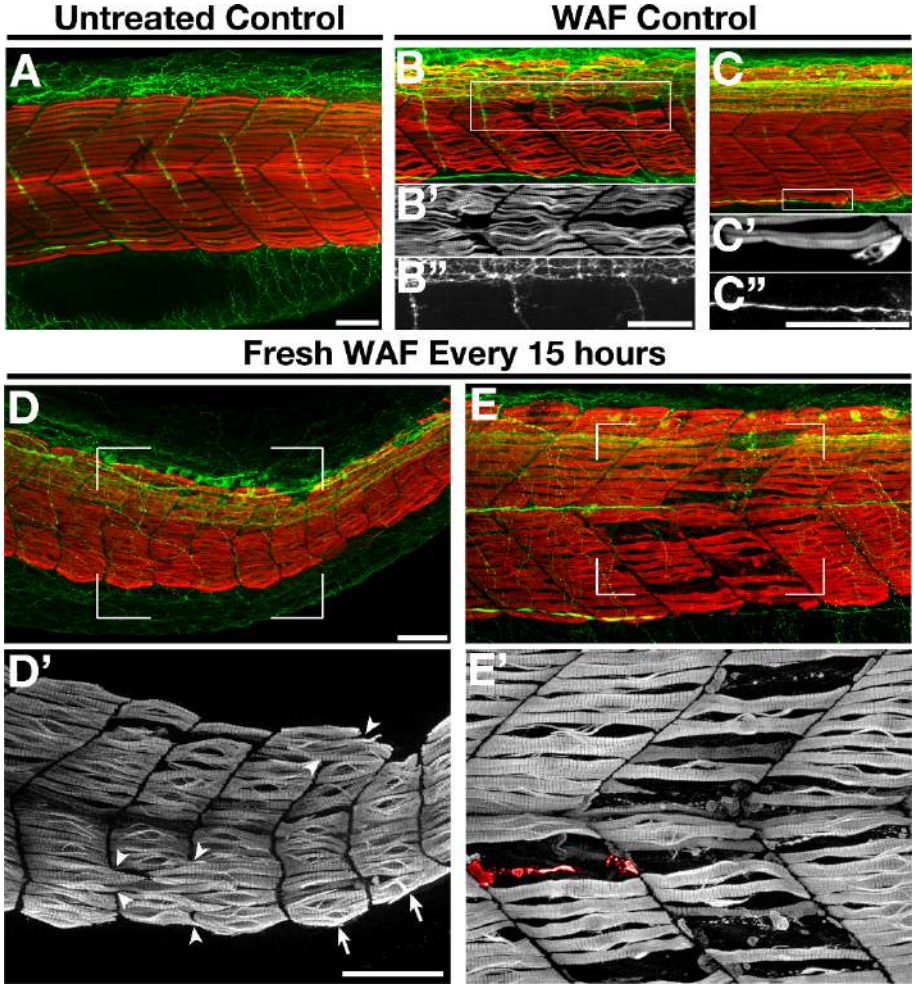

Figure 9 Repeated application of freshly mixed WAF reproduced the severe skeletal muscle phenotypes. (**A-E**) Lateral views of the trunk of an untreated control embryo (A), WAF-treated embryos from 3.5 hpf to 48 hpf (B, C), and embryos exposed to freshly mixed WAF every 15 h (D, E). WAF-treated controls showed mild slow muscle defects (B', C'), while embryos treated repeatedly with refreshed WAF displayed severe somite and slow muscle phenotypes (D', E'). As seen in earlier experiments, slow muscle fibers spanned presumptive boundaries (D', arrowheads), somitic shape was irregular (D', arrows), and slow muscle degeneration was evident (E, a representative degenerating myofibril is pseudo-colored red). Scale bars = 50 μm, A-E'.

endpoint assays are limited for the analysis of native species from the Gulf of Mexico. In this study we used the tractable zebrafish model system to determine whether water-soluble components of the Macondo oil collected from the riser during the oil spill could directly impact the embryonic development of a bony fish. WAF made from the Macondo oil did not cause wide spread toxicity or even cause significant problems with the earliest developmental processes required by the embryo to progress through cleavage, gastrulation and neurulation. This is surprising, as these early stages of development are arguably the most vulnerable to any environmental teratogen. Rather, our data support a model in which Macondo crude oil WAF cause specific developmental deformations that exhibit both spatial and temporal selectivity.

Many fish species, such as the commercially relevant Atlantic bluefin tuna, red snapper and gag grouper all lay their eggs in the open waters of the Gulf of Mexico near the site of the Deepwater Horizon platform [90-93]. The produced embryos are carried by water currents to the shallows of the Gulf shores to develop into larvae before swimming back into the open ocean as juvenile fry. Unfortunately, the Macondo oil from the Deepwater Horizon disaster was similarly carried along current driven paths to the shores and throughout the Gulf of Mexico [41]. Historically most oil spills have occurred at or near the surface of the water, which would result in limited hydrocarbon dissolution, particularly for more volatile hydrocarbons that are quickly lost to the atmosphere. However, the Deepwater Horizon oil spill is unique in its deep sea origin, which provided the crude oil more time and exposure to the water column creating underwater plumes mostly composed of water-soluble fractions of C_1-C_3 hydrocarbons and aromatic compounds [42,43]. Therefore, understanding the impact that water accumulated fractions may have on the development of native species is particularly relevant for this disaster. In fact, our results demonstrate that the hydrocarbon concentrations that produced phenotypes in zebrafish embryos are comparable to the levels detected in the underwater plumes [42,43] and along the Louisiana marshlands that affected gene expression in adult killifish [44]. It is known that interactions between crude oil and various environmental factors, such as temperature, salinity and pressure, will impact hydrocarbon dissolution and PAH uptake [94,95]; however, while our WAFs were largely made in fresh water, the similar ranges of hydrocarbon concentrations recently documented in the Gulf waters following the spill suggest our experiments can provide real insight into the potential risks faced by native species that spawned in the Gulf of Mexico during and after this oil spill.

We observed three broad phenotypes following treatment with Macondo oil WAF. The first observation was a mild but consistent reduction in embryo size paired with changes in head and trunk morphology, and the second phenotype was a compromised cardiovascular system. Both of these broad effects are consistent with responses reported previously to a variety of crude oil sources and specific PAHs [6]. However, we report here for the first time that the Macondo oil WAF does dramatically reduce touch sensitivity and impair proper swimming behavior. Using a variety of molecular and cellular labeling procedures paired with high resolution microscopy we were able to further elucidate the developmental origins behind these three broad observations.

Defects in size and shape through induction of apoptosis

WAF-treated zebrafish embryos had visible reductions and morphological changes in the size and shape of the head and trunk. These phenotypes could be explained by a reduction in cell proliferation or increase in programmed cell death. We found there was no reduction in the number of mitotic cells (Figure 3A, B), which was further confirmed by no change in the number of dividing neural stem cells (Figure 7K, L). These data suggest cell division rates were unaffected by Macondo crude oil WAF. However, we did detect a statistically significant increase in the amount of apoptosis (Figure 3C-I), supporting a cell death regulatory role for some component in the crude oil. This component could actively induce cell death or play a role in the repression of a survival factor. There are relevant data to support this hypothesis; crude oil, fuel oils, or specific PAHs have been documented to up-regulate known apoptotic proteins in juvenile cod, to increase programmed cell death in cultured dolphin renal cells, and to trigger apoptotic DNA fragmentation in ovarian and liver cells of the juvenile channel catfish [96-98]. We observed an increase in apoptotic cells present inside and outside of the nervous system (Figure 3), suggesting activation of programmed cell death was not necessarily tissue specific. Systematic chemical analysis in zebrafish could help discern which components of the Macondo oil are responsible for induction of programmed cell death and whether induction is cell type specific.

Selective impairment of neural crest cells is at the 'heart' of the problem

While increased cell death may play a role in the defects contributing to reductions in embryo size and shape, reductions in head size may be more directly associated with a lack of proper jaw formation. Defects in craniofacial development in response to crude oil or specific PAHs have been demonstrated previously [36]. The most prevalent and severe craniofacial defect we observed in response

to Macondo crude oil WAF was the preferential loss of posterior pharyngeal cartilage elements (Figure 5A-F). As one might expect, the vasculature associated with these same pharyngeal arches was also reduced (Figure 4A-H). Most crude oils, or their components, cause cardiac edema, heart morphogenesis defects, and reduced circulatory function during embryogenesis of several fish species [9,36,38,47]. We hypothesized that the defects we observed in pharyngeal cartilage, vasculature development and heart morphogenesis were linked by an earlier disruption in the proper development of cranial neural crest cells, which are contributing precursors for all of these tissues.

Neural crest cells function as multipotent stem cells that actively delaminate from the dorsal neural tube and migrate along separate pathways across the entire anterior-posterior axis of an organism. During migration to their final destinations neural crest cells differentiate into a variety of cell types, some of which contribute to the development of pigment cells, peripheral nervous system, head cartilage, endothelial and smooth muscle vasculature, and portions of the heart [52-56]. Interestingly, recent investigations in amniotes have suggested pharyngeal arches and the heart are derived from the same "vagal" domain of neural crest cells lying at the intersection between the head and trunk axial positions (reviewed in [52]). This is of particular relevance since the most significant phenotypes we observed were restricted to the pharyngeal arch cartilage, arch vasculature and heart (Figure 4). Importantly, cardiac neural crest cells specifically migrate through the posterior pharyngeal arch pathways on route to the developing heart fields, where they differentiate into smooth muscle and endothelial cell derivatives that contribute to the morphogenesis of the outflow tract, septum, and valves of the heart [51,55].

We discovered that Macondo WAF-treated embryos show a specific reduction in one of the posterior arches, and a similar restricted reduction was seen in both *crestin* and *dlx2* gene expression by cranial neural crest cells associated with the posterior pharyngeal arches (Figure 5G-P). Initially cranial neural crest cells migrate in three streams toward the presumptive arches, and the posterior-most stream undergoes a branching process to form three additional streams that establish the posterior most pharyngeal arches [99]. Therefore, we hypothesize that the causative toxins in the Macondo WAF may be affecting neural crest cell specification as it relates to their ability to carry out this branching step during posterior pharyngeal arch development. This spatially restricted defect in neural crest development is a remarkably targeted effect by crude oil, which suggests there are equally specific molecular pathways directly influenced by the components of the Macondo WAF; such as, the known neural crest regulators Wnt, Fibroblast growth factor, or the Bone Morphogenic Protein signaling pathway [51,100,101]. These pathways may be activated directly or independent of the *aryl hydrocarbon receptor 1 and 2*, which has been shown to be required for cardiac edema and heart morphogenesis defects in response to selective PAHs [37].

Despite the apparent lack of neural crest defects in the trunk of WAF-treated embryos, there was reduced circulation in the intersegmental blood vessels, and those vessels devoid of any circulation often showed excessive vascular branching (Figure 4L-O'). These elaborate and often forked branching patterns (Figure 4N', O', arrow) were reminiscent of an angiogenic process called intussusception, in which a vessel splits along its longitudinal axis and undergoes vascular remodeling [102,103]. While intussusception has not yet been described in zebrafish, others have demonstrated mouse retinal vasculature responds to hypoxic conditions by forming characteristic "vascular loops" [104]. In WAF-treated embryos vascular remodeling is seen only in intersegmental blood vessels that are devoid of circulation, which could represent an extreme but focused hypoxic event during which vascular looping might be observed (Figure 4N', bracket). We, therefore, hypothesize that the vascular remodeling observed in WAF-treated embryos is an indirect effect caused by reduced circulation in the intersegmental blood vessels due to a primary disruption in early neural crest-mediated heart development and function.

Defects in patterning the peripheral nervous system impacts locomotor escape behaviors

Like most teleosts, zebrafish larvae evolved early stereotypical swimming patterns in response to touch stimuli that enable fast escape locomotor behaviors [105,106]. The inability to properly react to touch would have adverse consequences to larva survival. We observed that larvae exposed to the Deepwater Horizon crude oil had significantly reduced sensitivity to touch and disorganized swimming patterns relative to untreated controls (Figure 6). A previous study that examined the effects of specific PAHs on zebrafish locomotor behaviors did not detect any irregularities [36], suggesting that either the Macondo crude oil WAF possesses a unique component or a particularly toxic combination of known components impaired proper locomotor function. This could have unfortunate implications for the species of the Gulf of Mexico and Atlantic Ocean where even subtle reductions in larval or adult escape responses can be deadly. There is some precedent for this, as delayed escape behaviors have been documented in fiddler crabs in response to chronic exposure to No. 2 fuel oil polluting the sediment of Wild Harbor in Buzzards Bay, MA [107]. We sought to determine the developmental origin of the locomotor phenotype in WAF-treated embryos by assessing the anatomical organization of the neural circuitry and skeletal muscle necessary to respond to touch and yield functional swimming behaviors.

Considering the complexity of the locomotor system, we were surprised to only detect specific deformations in the peripheral nervous and muscular systems; the neurons and astroglia within the central nervous system were normal in quantity and position (Figures 7, 8).

Similar to our findings of touch response defects, the peripheral sensory and motor axon defects and slow muscle patterning phenotypes, to the best of our knowledge, have never previously been documented. Specifically, Macondo crude oil WAF caused reduced sensory axonal branching along the entire trunk, and sporadic motor axon pathfinding errors directly associated with corresponding deformations in slow muscle fiber development. Reductions in sensory neuronal branches likely cause reductions in touch responses [108,109], which suggests that WAF-induced reductions in sensory axonal arbors contribute to the reduction in touch sensitivity. However, when a touch response was elicited in a WAF-treated larva, they exhibited disorganized swimming behaviors. While problems in sensory branching may contribute to these swimming errors, the cause is more likely a problem with stimulus transduction controlled by the downstream neural circuitry and muscular output [110].

WAF-treated embryos exhibit deformations in motor axon pathfinding and slow muscle development (Figure 8). These specific defects could definitely lead to impaired muscle contractions and swimming behaviors. Early muscle contractions have been shown to be required for the proper ventral trajectory of pathfinding sensory axons and their ability to exhibit appropriate self-avoidance behaviors to establish the mesh-like pattern of sensory branching [111]. However, this is not likely a significant influence as muscle and motor axon defects were not consistently seen throughout the trunk of WAF-treated embryos unlike the sensory axonal defects, nor were longitudinal pathfinding errors or significant axon to axon contact seen that are characteristically found following muscle contraction loss [111].

Motor axon pathfinding errors were only found in somites that exhibited corresponding slow muscle patterning defects, which strongly suggests motor axon pathfinding errors are not direct effects of the oil but rather indirect phenotypes in response to inappropriate guidance cues derived from earlier problems with somitogenesis and slow muscle patterning. This is in contrast to the sensory neuron branch reductions that are uniformly present throughout the trunk and, thus, likely a direct affect of exposure to the Macondo crude oil. Slow muscle cells have been shown to provide critical axon guidance cues that direct the proper pathfinding of motor axons [74,112]. This primary defect in early skeletal muscle development can be interpreted as two separable processes, in which there are changes to the highly stereotypical pattern of segmentation and then improper slow muscle positioning.

Within the musculature of the trunk we observed losses of somitic boundaries as well as the occurrence of inappropriate boundaries within the same somitic region (Figure 8B, C, F, G as examples). At this point we can only speculate how Macondo crude oil might be affecting somitogenesis. Segmentation in vertebrates is controlled by the precise coordination of a Notch-Delta mediated molecular clock that determines when a boundary will form and this process is paired with opposing anterior and posterior morphogenic gradients of Retinoic acid and Fibroblast growth factor that define the location of a segment boundary [77,79,113-116]. It is possible that some component within the Macondo crude oil might be impacting this somite molecular clock mechanism; however, it is also plausible that our WAF treatments were affecting the terminal step in boundary formation that involves the process of epithelialization and formation of the myotendinous junction rather than the timing of somitogenesis [117,118].

After a segment boundary has formed in the paraxial mesoderm of zebrafish, "adaxial" slow muscle precursor cells located adjacent to the notochord undergo substantial morphogenesis and movement to the outer-most edge of a somite to form a monolayer of elongated, slow-twitch muscle fibers [76,119,120]. While alterations in proper somite boundary formation would yield irregularly elongated slow muscle fibers, it has not been documented to cause alterations in the medial to lateral positioning of fibers, change the parallel positioning of the slow muscle array, nor cause early slow muscle loss [118]. Therefore, we hypothesize that in addition to somite formation defects, Macondo crude oil WAFs may be independently affecting some aspect of slow muscle specification and migration.

Hedgehog signaling is required for proper slow muscle cell specification and Cadherin cell adhesion molecules are required for proper slow muscle morphogenesis and movement, and their loss causes slow muscle positioning phenotypes similar to what we observed following WAF treatments [120-122] (Figure 8D, L, M as examples). While these conclusions related to muscle development are speculative, they provide a basis for a series of future experiments aimed at analyzing the effects of specific Macondo crude oil compounds on somitogenesis and muscle development, as well as examining the role of the Aryl hydrocarbon receptor signaling pathway in these processes [37].

Aside from these early somitogenesis and muscle fiber type patterning defects, a separate and later forming slow muscle degeneration phenotype was also variably present in some WAF-treated embryos. We found a number of embryos treated with the Macondo oil WAF

had sporadic muscle fiber breaks and single sided detachment from the lamina, which subsequently exhibited cell death morphologies (Figure 8I, J). We interpret this phenotype as a specific, WAF-mediated muscle degeneration, as it is nearly identical to the muscle pathology seen in zebrafish genetic models of muscular dystrophy [80-85]. Importantly, this late muscle degeneration defect does not have corresponding motor axon pathfinding errors because it occurs after motor axons have already successfully reached their target cells. While these varied early and late muscle phenotypes have real consequences for embryonic health and locomotor function, the sporadic nature of these muscle phenotypes suggests they cannot fully account for the consistent errors in swimming behavior exhibited by WAF-treated embryos.

The abnormal locomotor behaviors exhibited by WAF-treated larvae could be due to defects in central nervous system function. Our level of analysis did not reveal any neuroanatomical defects; however, fine mapping of neural circuits or analysis of neuronal network activity could help elucidate specific cellular and molecular mechanisms disrupted by crude oil exposure. Moreover, it will be equally important to characterize the quality of myelin wrapping by oligodendrocytes and schwann cells, which contribute significantly to how well signals are conducted between neurons.

Phenotypic changes over time

An unexpected finding of our research was the phenotypic reduction in certain defects over successive and identical experimental replicates. Specifically, apoptotic cell death and skeletal muscle phenotypes decreased in severity over the course of multiple experiments, while the phenotypes associated with neural crest development and sensory neuronal branching remained consistent. This observation suggests that different components within the Macondo crude oil might cause these phenotypes, and that some specific component(s) changed over the course of the use and storage of our Macondo crude oil sample. Importantly, we were able to reproduce the severity of both cell death induction and the severity of all the skeletal muscle phenotypes by exposing embryos to freshly mixed WAF preparations several times over the course of a single experiment. This confirms that Macondo crude oil is responsible for these phenotypes and, while the unidentified compound has reduced potency over time, it is still present and capable of impairing embryonic development. A recent study demonstrated that dissolved PAHs, rather than oil particles, are toxic to zebrafish embryos and cause edema, hemorrhaging, developmental delays, and abnormalities in cardiac function [38]. We propose a model in which the more readily dissolved PAHs are responsible for the neural crest derived phenotypes that lead to cardiac and craniofacial deformations, whereas the cell death and skeletal deformations may be caused by novel Macondo oil components, such as smaller ringed hydrocarbons that are more easily released from the WAF as a gas, or much heavier hydrocarbons that readily fall out of solution. Our findings will enable logical candidate approaches to analyze the role of individual components of Macondo oil in regulation of specific developmental processes during fish embryogenesis.

Conclusions

These studies demonstrate that water-soluble components of Macondo crude oil cause specific teratogenic effects on developing zebrafish embryos. While exposure to Macondo oil WAFs did yield similar defects in cardiovascular and craniofacial development as with other crude oil types, we show this is likely due to an earlier impairment in the development of cranial neural crest cells. In addition, irregular locomotor escape responses in Macondo oil WAF treated larvae may be in part due to specific reductions in sensory axon branching as well as deformations in somite and slow muscle development. These results shed new light on a variety of specific developmental processes never before associated with crude oil exposure that provide a framework for future studies to define the molecular mechanisms governing crude oil teratogenesis. These novel phenotypes suggest that Macondo crude oil sampled during the Deepwater Horizon oil spill is toxic to zebrafish embryonic development, and, based on the high conservation of developmental mechanisms *Danio rerio* shares with other vertebrate species, the Macondo crude oil will similarly impact the embryonic development of native teleost species known to spawn in the Gulf of Mexico. We predict these findings can be used as a guide to initiate more efficient and systematic analyses of native species that may have been impacted by the original Deepwater Horizon oil spill and those species that could be affected by future leakage from the Macondo well.

Methods

Sampled Macondo crude oil

A one-liter sample of Macondo crude oil, MC 252 (Source Oil B; ID #: 20100617-121) from the Deepwater Horizon oil spill was provided by British Petroleum (BP) on September 8, 2010. This oil sample was collected from the riser insertion tube on the Enterprise by Entrix samplers during May 2-3, 2010. BP acknowledged that during sampling of this crude oil, Nalco EC9323A defoamer was being injected topside, while methanol with 10,000 ppm VX9831 oxygen scavenger/catalysts solution was being injected subsea. While the presence of these additional agents in the sample cannot be ruled out, it is unlikely they were incorporated into this crude oil sample

due to the method of sampling directly through the riser tube. It was further acknowledged by BP that there was a variable amount of water in the oil samples collected in this manner. Material safety data sheets for the Source Oil B crude oil sample stated its major constituents were n-hexane, toluene, xylene, benzene, naphthalene, ethylbenzene, and hydrogen sulfide.

Water accommodated fraction (WAF)

Following previously accepted mixing procedures, we created water accumulated fractions (WAF) of Macondo oil with autoclaved E3 embryo medium [40,123]. Specifically, the 1-liter container of Macondo crude oil was slowly inverted 20 times before collecting a sample to ensure consistent sampling over the course of the study. Oil dilutions of 1:10 in embryo medium were prepared in cap-sealed glass bottles that allowed for 20% air volume. Initial gas chromatography mass spectrometry (GCMS) analysis of WAF exposed to plastics detected n-butyl phthalate in solution, which is a plasticizer speculated to cause developmental effects on its own (data not shown, [124]). Therefore, only glass pipettes and glass petri dishes were used with WAF solutions. For initial characterization, WAF stock solutions were mixed with a magnetic stir bar in two different ways: (1) WAF stock was stirred at a slow, non-vortex inducing mixing speed [40]; and (2) WAF stock was stirred at a rate sufficient to produce a vortex equivalent to one-third the volume of the solution. Both solutions were mixed for 24 h, followed by a 2 h resting period before only the aqueous WAF phase was sampled. Unless otherwise noted, the embryos in the following experiments reported here were exposed to a 100% WAF solution derived from the 1:10 (crude oil to embryo media) vortex-mixed stock solution.

WAF chemical analysis

To determine what the chemical composition was of the resulting WAF solutions made in embryo medium, Solid phase microextraction (SPME,100 mm polydimethylsiloxane) and gas chromatography mass spectrometry (GCMS, Agilent 7890A GC/5975C MSD) were performed. WAF samples were analyzed within one hour after the mixing procedure was completed. The SPME fiber was immersed for six minutes in a 15 mL vial of diluted WAF (25-fold dilution), with constant stirring, before insertion into the GC (helium carrier gas; splitless mode for first six minutes; 220°C inlet; oven at 35°C for four minutes, ramped 8°C/minute to 275°C, held at 275°C for three minutes). For consistency among WAF samples, standards for selected components (cyclopentane, methylcyclohexane, n-hexane, n-decane, n-tetradecane, benzene, toluene, xylenes, naphthalene, anthracene, phenanthrene, all from Supelco) were also made in diluted embryo medium (25-fold dilution). Benzene-d6 (Supelco) was added as an internal standard. An expanded explanation of the chemical analysis is provided in Additional files 1 and 2.

Embryo collection and treatment

Fish lines were maintained in the ALAAC accredited Smith College Zebrafish Facility using standard husbandry techniques ([125]; Zebrafish International Resource Center). Fertilized eggs from wild type embryos (AB strain) and *Tg[fli:eGfp]* transgenic fish (TU/AB background, provided by the Lawson Lab, UMass-Medical School) were collected, washed in embryo medium, and incubated at 28.5°C. Unless otherwise stated, embryos were exposed to WAF solutions starting at 3.5 hours post-fertilization (hpf) and maintained in WAF solutions until a maximum of 5-days post fertilization (dpf). Control embryos were maintained in oil-free embryo medium and grown in a separate 28.5°C incubator to eliminate potential exposure to WAF gases. All treatments were carried out with either 20 mL of solution in 100 mm glass petri dishes or 10 mL of solution in 50 mm glass petri dishes. Prior to fixation or live embryo imaging, embryo chorions were removed by chemical digestion with Pronase (2 mg/ml, Sigma-Aldrich, St Louis, MO, USA) for three to five minutes.

Behavioral analysis

In an effort to perform a more conservative assessment of locomotor behavior, analysis was carried out using a 50% WAF solution. Embryos exposed to either 50% or 100% WAF had similar locomotor responses (data not shown). To characterize larval swimming behavior at 48 hpf, a light touch stimulus was applied to the head of larvae. The minimum stimulus required to elicit a response was determined using pressure-specific Von Frey filaments. The touch-response was recorded using a high-speed camera (Fastec Imaging, San Diego, CA, USA) at 1,000 frames per second (fps). A 35 mm lens (Nikon, Melville, NY, USA) was used for magnification. To illustrate the responses, single frames taken at 20 mS intervals were overlaid in Adobe Photoshop (San Jose, CA, USA).

For kinematic analysis, the head-to-tail angles were calculated for each frame using automated software developed by the Downes Lab (Biology Department, University of Massachusetts, Amherst, MA, USA) [126]. In brief, pixel density was used to identify three landmarks along the larval body: the tip of the nose, the border between the yolk extension, and the tip of the tail. These three points form an angle, which was plotted over time using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). To calculate body bend frequency, a full body bend was defined as two intervals of more than 50 degrees of opposite directions. To determine the duration of a swimming episode, we measured the beginning of a swimming episode until the final time the body was straightened to within 20 degrees of being straight (defined as 0 degrees).

Blood, circulation and vascular analysis

Visualization of blood in 3 dpf embryos was accomplished by staining hemoglobin with *o*-Dianisidine as previously described [127]. Blood circulation rates were determined by recording the amount of time a single blood cell took to travel through the dorsal aorta from somite 8 to somite 14. A total of three blood cells were recorded and their travel times averaged for each fish. Individual blood cells were easily visualized in live larvae at 3 dpf using an Olympus stereo microscope (Center Valley, PA, USA).

Microangiography was performed as previously published [49,128] and described in detail online [129]. Briefly, 3 dpf live control and WAF treated *tg[fli:eGfp]* larvae were anesthetized with Tricaine (MS222, Argent Chemical Laboratories, Inc, Redmond, WA, USA) to prevent skeletal muscle contractions, mounted ventral side up in 5% methylcellulose, and microinjected with Qtracker 655 non-targeted quantum dots (Invitrogen, Life technologies, Carlsbad, CA, USA) into the sinus venosus. Larvae were then laterally embedded in 0.75% agarose on glass bottom petri dishes (MatTek, Ashland, MA, USA), and imaged with an inverted Leica SP5 Laser Scanning Confocal Microscope (Lieca, Buffalo Grove, IL, USA) with resonant scanning. Live cell imaging of the fluorescently labeled green endothelial cells and red quantum dot-filled vasculature was captured at 20X using bi-directional scanning at 8,000 hz, 16-times line averaging at a 1024 × 1024 resolution, and with a 1.7 × digital zoom. Z-stacks with an optical slice thickness of 1 μm were collected of the entire trunk or portions of the head. Z-stack analysis and maximum intensity projections were performed using *Volocity* software (PerkinElmer, Waltham, MA, USA). Adobe Photoshop was used to align projections and reconstruct the entire trunk vasculature of individual larva as illustrated in Figure 4L-O.

Immunohistochemistry and imaging

Embryos were fixed in 4% phosphate-buffered formaldehyde, except for anti-Islet1 labeling, which were fixed in 4% formaldehyde, 0.05% glutaraldehyde, 5 μM EGTA, 5 μM $MgSO_4$ and 0.1% Triton-X-100. Primary antibodies used were mouse IgG_{2b} anti-Acetylated Tubulin (AT, 1:800; Sigma); rabbit anti-Glial fibrillary acidic protein (Gfap, 1:500; Dako, Carpinteria, CA, USA), mouse IgG_1 anti-Phospho-Histone H3 (PH3, 1:1000, Cell Signaling Technology, Inc., Danvers, MA USA), mouse IgG_1 anti-slow muscle myosin (F59, 1:10; Developmental Studies Hybridoma Bank, University of Iowa, Department of Biology, Iowa City, Iowa, USA), rabbit anti-Gamma-aminobutyric acid (Gaba, 1:1000; Sigma), mouse IgG_1 anti-3A10 (1:50, Developmental Studies Hybridoma Bank), and rabbit anti-activated Caspase 3 (1:500; BD Pharmigen, San Jose, CA, USA). All primary antibodies were diluted in blocking solution (see below). Secondary antibodies used were Alexa-594 goat anti-rabbit IgG, AlexaFluor-594 goat anti-mouse IgG_1, AlexaFluor-647 goat anti-mouse IgG_{2b}, and AlexaFluor-488 goat anti-mouse IgG_1, all obtained from Invitrogen and diluted 1:200 in blocking solution. Immunocytochemistry was carried out as previously described [130] with minor modifications. Embryos were first dehydrated at -20°C for 20 minutes with 100% MeOH followed by 7 minutes with 100% Acetone. Further antibody penetration was achieved with protein digestion using Proteinase-K (10 μg/ml) for 2 minutes to 25 minutes depending on the age of the embryos. Embryos were incubated in blocking solution (0.5 g BSA; 25 ml Phosphate buffered saline, 0.1% Triton-X-100; 5% normal goat serum; 1% DMSO) prior to both primary and secondary antibody application.

Fluorescent, whole-mount images of fixed preparations were captured using the Zeiss AxioImager epifluorescent compound microscope with ApoTome for structural illumination. Embryos were dissected into separate trunk and head segments. Trunks were laterally mounted on a microscope slide, while heads for 3A10-labeled embryos were mounted dorsal side up. Imaging was performed with either 40× or 20× objectives and Z-stacks were collected with an averaging of 2 and 0.53 μm or 0.8 μm optical slice distance, respectively. An AxioCam Mrc (Carl Zeiss, Thornwood, NY, USA) was used to capture images on the AxioImager, and processing of Z-stacks and maximum intensity projections was done using Axiovision software (Carl Zeiss, Thornwood, NY, USA).

Whole-mount *in situ* hybridization at for *crestin* and *dlx2* mRNA expression was completed as previously described [131]. Alcian Blue staining was performed to visualize cartilage development at 4 dpf and conducted as previously described [50]. *crestin, dlx2, tg(fli:Gfp)* labeled pharyngeal arches and Alcian Blue labeling were imaged on a Zeiss Lumar stereo microscope using an AxioCam HR and Axiovision software.

All figures were constructed using Adobe Photoshop. Any adjustments to image brightness, contrast or other properties were done to the entire image and identically performed between controls and WAF-treated data.

Quantitation and statistics

Data collection was obtained in at least 20 embryos per 3 separate replicate experiments for all analyses. One exception was made for the microangiography live embryo imaging where labor intensive microinjection and live embryo mounting restricted analysis to 10 embryos per control and WAF-treated for each replicate. However, at least eight separate locations were imaged along the anterior to posterior axis of each embryo. Cell counts for PH3, Caspase, Gaba, Islet1, and Gfap were done on maximum intensity projections and quantified using the "counting tool" in Adobe Photoshop CS Extended version. Sensory

neuron processes were quantified in Image J [132]. Briefly, 8-bit images were converted into binary images to calculate pixel density, and the average number of pixels for each group was analyzed for statistical significance. The results for each replicate were pooled and means calculated for most statistical analyses except where noted in the results. The two-sample T-Test was used to compare treatments with a stringency of $P < 0.01$ for all analyses, except when the data did not fit a normal distribution, in which case a non-parametric analysis (Kruskal-Wallis or Mann-Whitney tests) was performed. Statistical significance for *fli:Gfp* labeled arch formation and *dlx2* expression was determined using a Fisher's Exact Test.

Additional material

Additional file 1: Initial assessment of water accumulated fractions. This file provides greater explanation of the methods and results associated with our WAF mixing procedure, as well as initial assessments of the effects of different WAF concentrations on the development of zebrafish gross morphology.

Additional file 2: Expanded WAF chemical analysis. This file provides a detailed list of all the compounds and concentrations discovered following gas chromatography mass spectrometry analysis of our WAF. *Supplemental Table S1*: GCMS parameters for Agilent 7890A GC/5975C MSD. *Supplemental Table S2:* Aqueous solubilities and Henry's constants for selected hydrocarbons. *Supplemental Table S3:* Most prominent GCMS peaks in WAF. *Supplemental Figure S1:* Relative detection efficiencies of the SPME/GCMS method for selected aromatics and alkanes. *Supplemental Figure S2:* Total ion chromatogram. *Supplemental Figure S3:* Total ion chromatograms for the aqueous portion of a WAF sample and for a sample that includes some of the oily layer.

Abbreviations

AT: Acetylated Tubulin; BP: British Petroleum; dpf: days post-fertilization; fps: frames per second; Gaba: Gamma-aminobutyric acid; GCMS: gas chromatography mass spectrometry; Gfap: Glial fibrillary acidic protein; hpf: hours post-fertilization; NOAA: National Oceanic and Atmospheric Administration; PAH: polycyclic aromatic hydrocarbons; PH3: Phosphorylated Histone H3; SPME: solid phase microextraction; WAF: water accumulated fraction.

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Authors' contributions

TS participated in experiment design and played a role in carrying out many of the experiments in this study with particular efforts on all cardiovascular, craniofacial, and neural crest analysis. AU contributed significantly to initial experimental design of this study as well as carried out most of the initial replicates. TF carried out the behavioral analysis of touch responsiveness and the kinematics of swimming. DP carried out a majority of the analysis of WAF chemical composition. SC carried out a majority of the refreshing WAF treatments and experiments as well as performed many of the statistical analyses. DO assisted in a majority of embryo treatments as well as supported immunocytochemistry experiments. RB provided assistance on all immunocytochemistry, embryo treatments and imaging on the AxioImager. GD designed, monitored and participated in the behavioral analysis. SH designed, monitored and participated in the chemical analysis of WAF components. RS assisted in data collection for the analysis of cell death. MCL performed the initial experiments on slow muscle development. KH designed and supported the statistical analysis of collected data. LK supported many of the final experiments of this work contributing directly to the acquisition of *fli:GFP* and *dlx2* data. MB conceived of the study, substantially contributed to the design, coordination, and implementation of many of the experiments, with particular contributions to microangiography procedures, and cartilage and neural crest imaging. MB participated in all data and statistical analysis and drafted the manuscript. All authors read, contributed feedback to, and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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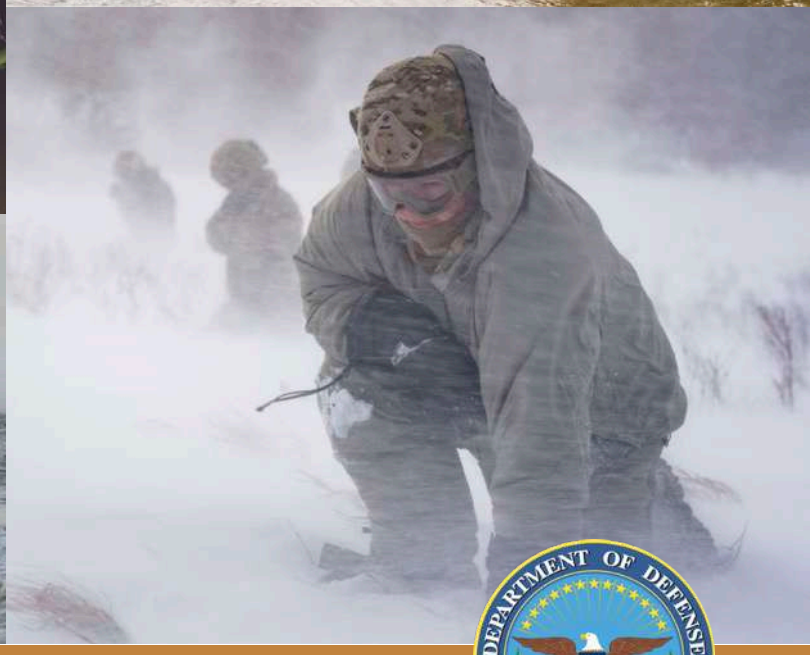

To the National Security Council

Department of Defense Climate Risk Analysis

EXECUTIVE SUMMARY

Climate change is reshaping the geostrategic, operational, and tactical environments with significant implications for U.S. national security and defense. Increasing temperatures; changing precipitation patterns; and more frequent, intense, and unpredictable extreme weather conditions caused by climate change are exacerbating existing risks and creating new security challenges for U.S. interests. The risks of climate change to Department of Defense (DoD) strategies, plans, capabilities, missions, and equipment, as well as those of U.S. allies and partners, are growing. Global efforts to address climate change – including actions to address the causes as well as the effects – will influence DoD strategic interests, relationships, competition, and priorities. To train, fight, and win in this increasingly complex environment, DoD will consider the effects of climate change at every level of the DoD enterprise.

The DoD Climate Risk Analysis (DCRA) responds to requirements specified in Executive Order (EO) 14008, "Tackling the Climate Crisis at Home and Abroad."[1] The DCRA is organized as follows:

- Section I introduces key security implications of climate change to DoD, including DoD's role supporting whole-of-government and international efforts in concert with allies and partners.
- Section II reviews DoD climate policy and responsibilities, highlighting key documents.
- Section III presents a review of climate hazards, risks, and security implications. *Sections on specific regions have been identified as Controlled Unclassified Information (CUI) and not releasable to the public. These sections were removed to allow this to be a publicly-releasable document.*
- Section IV outlines how DoD will incorporate consideration of climate into relevant strategy, planning, and processes.
- Section V describes interagency scientific and intelligence products and experts, which could support future analyses of climate risk, as well as expected funding for exercises, wargames, analyses, and studies related to climate change.
- Section VI concludes the DCRA.

The DCRA is an important step towards integration of climate change considerations at DoD. To understand specific climate effects on plans, resourcing, operations, and missions, DoD Components will include climate considerations in relevant risk analyses, leveraging high-quality data, scenarios, and analytical tools tailored to DoD needs. Working within the whole-of-government, and in coordination with allies and partners, DoD will strive to prevent, mitigate, account for, and respond to defense and security risks associated with climate change.

[1] EO 14008, Section 103(c) requires the Secretary of Defense to develop "an analysis of the security implications of climate change (Climate Risk Analysis) that can be incorporated into modeling, simulation, war-gaming, and other analyses."

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FOREWORD

To keep the nation secure, we must tackle the existential threat of climate change. The unprecedented scale of wildfires, floods, droughts, typhoons, and other extreme weather events of recent months and years have damaged our installations and bases, constrained force readiness and operations, and contributed to instability around the world.

Climate change touches most of what this Department does, and this threat will continue to have worsening implications for U.S. national security.

To meet this complex challenge, the Department of Defense (DoD) is integrating climate change considerations at all levels, including in our risk analyses, strategy development, planning, modeling, simulation, and war gaming.

The DoD Climate Risk Analysis (DCRA) is a critical step for incorporating climate change security implications at a strategic level. As the global and cross-cutting consequences of climate change increase the demands on the Department, the DCRA provides a starting point for a shared understanding of the mission risks of climate change—and lays out a path forward.

For example, climate considerations will be included in key DoD documents, such as the forthcoming National Defense Strategy, which guides the ways that DoD meets national security challenges. Coupled with the Climate Adaptation Plan, which will help the Department operate under changing climate conditions, the DCRA reflects the Department's focus on confronting climate change.

Climate change presents serious risks, but DoD, along with the entire U.S. government, as well as our allies and partners, is determined to address this common threat. The Department will work to prevent, mitigate, and respond to the defense and security risks associated with climate change. By doing so, we will ensure that we continue to fulfill our mission of defending the United States.

Lloyd J. Austin III, Secretary of Defense

I. INTRODUCTION

"There is little about what the Department does to defend the American people that is not affected by climate change."

— Secretary of Defense Austin, Statement Released in January 2021

Climate change is reshaping the geostrategic, operational, and tactical environments with significant implications for U.S. national security and defense. Increasing temperatures; changing precipitation patterns; and more frequent, intense, and unpredictable extreme weather conditions caused by climate change are exacerbating existing risks and creating new challenges for U.S. interests. Without adaptation and resilience measures, climate hazards, particularly when combined with other stressors, are likely to contribute to political, economic, and social instability around the world. In many cases, the physical and social impacts of climate change transcend political boundaries, increasing the risk that crises cascade beyond any one country or region. Box 1 provides definitions of key terms used in this document.

BOX 1. DEFINITIONS OF KEY TERMS

Adaptation - Adjustment in natural or human systems in anticipation of or response to a changing environment in a way that effectively uses beneficial opportunities or reduces negative efforts. (DoD Directive (DoDD) 4715.21)

Climate Change - Variations in average weather conditions that persist over multiple decades or longer that encompass increases and decreases in temperature, shifts in precipitation, and changing risk of certain types of severe weather events. (DoDD 4715.21, Joint Publication (JP) 1-02)

Climate Change Mitigation - Measures to reduce the amount and speed of future climate change by reducing emissions of heat-trapping gases or removing carbon dioxide from the atmosphere. (U.S. Global Research Program)

Hazard - A condition with the potential to cause injury, illness, or death of personnel; damage to or loss of equipment or property; or mission degradation. (DoD Dictionary, 2021)

Resilience - The ability to anticipate, prepare for, and adapt to changing conditions and withstand, respond to, and recover rapidly from disruptions. (DoDD 4715.21)

Risk - Probability and severity of loss linked to threats or hazards and vulnerabilities. (DoDD 3020.40)

The risks of climate change to DoD strategies, plans, capabilities, missions, and equipment, as well as those of our allies and partners, are growing. Therefore, analyses based on historical frameworks will not be sufficient to prepare for future risks complicated by a changing climate. To train, fight, and win in this increasingly complex strategic, operational, and tactical environment, DoD will consider the effects of climate change at every level of the DoD enterprise. The Department will consider how crises exacerbated by climate change are likely to increase demand for defense missions and impact critical supply chains, infrastructure, and readiness. Mission success will depend on planning and operational adaptability that account for climate-related complexities and contingencies, and on forces, equipment, and capabilities engineered to adapt to and withstand more extreme environments. Box 2 includes examples of the security implications of climate change both at home and abroad.

BOX 2. EXAMPLES OF SECURITY IMPLICATIONS OF CLIMATE CHANGE AT HOME AND ABROAD

- For the homeland, extreme weather events exacerbated by climate change have caused hardships for millions of Americans, and have the long-term potential to undermine training capability and readiness. Extreme events have cost the United States billions of dollars in damages in recent years, such as at Tyndall Air Force Base and Marine Corps Base Camp Lejeune.
- In the Arctic, climate change is dramatically altering the natural environment and creating a new frontier of geostrategic competition.
- In the Indo-Pacific, sea-level rise and more extreme weather events complicate the security environment, place key DoD warfighting infrastructure and surrounding communities at risk, and challenge local capacity to respond. For example, the United States has important defense assets located in Guam, the Marshall Islands, and Palau, all of which are vulnerable to these hazards. Additionally, competitors such as China may try to take advantage of climate change impacts to gain influence.

Even with aggressive international and whole-of-government action to mitigate future climate change, many effects to the physical environment are now unavoidable and will continue to shape our security environment. DoD will adapt to and mitigate the impacts of these changes to the climate as outlined in the DoD Climate Adaptation Plan (CAP) as well as the Sustainability Report and Implementation Plan (SRIP). The CAP details pathways to achieve an end-state where "DoD can operate under changing climate conditions, preserving operational capability and enhancing the natural and manmade systems essential to the Department's success."

There is evidence that climate change is making hurricanes stronger and more destructive.

DoD plays an important role in the whole-of-government effort to address climate change security risks, which includes working closely with allies, partners, and multilateral institutions to mitigate future climate change and adapt to those changes that are unavoidable. The challenges posed by climate change demand on-going analysis of evolving risks as well as investments in resilience, international development, and governance. As many areas of U.S. Government (USG) response are primarily the responsibility of civilian agencies, DoD will work closely with state and local governments and other parts of the Federal Government, including the Department of State, U.S. Agency for International Development (USAID), the Department of Homeland Security (DHS), intelligence agencies, and science agencies. DoD will often be in a supporting role to other agencies in working with local stakeholders and governments to counter climate-related risks to U.S. national security.

U.S. allies, partners, and competitors are assessing the implications of climate change on their respective strategic objectives. Malign actors may try to exploit regional instability exacerbated by the impacts of climate change to gain influence or for political or military advantage. Global efforts to address climate change – including actions to address the causes as well as the effects – will influence DoD strategic interests, relationships, and priorities. Cooperation with international partners to address the security implications of climate change can strengthen alliances and partnerships. Building awareness of how other nations are preparing for climate change is critical to understanding the risks and opportunities across strategic, operational, and tactical environments.

II. DOD CLIMATE POLICY AND RESPONSIBILITIES

The foundation for DoD's climate policy is based on U.S. policy, statutes, executive orders, international agreements, and administrative guidance. A list of relevant documents is provided in Appendix 1. Both the 2010 and 2014 Quadrennial Defense Reviews considered the impacts of climate change on DoD. The 2014 DoD Climate Change Adaptation Roadmap, building off of a previous 2012 roadmap, identified climate change as a national security threat and detailed vulnerabilities to a changing climate. Statutory requirements include Section 335(b) of the National Defense Authorization Act for Fiscal Year 2018 (Public Law 115-91), which stated that "climate change is a direct threat to the national security of the United States and is impacting stability in areas of the world both where the United States Armed Forces are operating today, and where strategic implications for future conflict exists." The statute includes the sense of Congress that DoD "must ensure that it is prepared to conduct operations both today and in the future and that it is prepared to address the effects of a changing climate on threat assessments, resources, and readiness," and that installations should consider climate damage in their master plans.

Last updated in 2018, DoD Directive (DoDD) 4715.21, "Climate Adaptation and Resilience," states that DoD must assess and manage risks associated with the impacts of climate change on DoD missions and installations and strengthen resilience to those impacts. Given that climate change has implications for nearly all that DoD does, the CAP describes how no Component can "opt out." Looking forward, DoD will consider all the strategic implications of climate, as well as continue to assess the ways climate impacts DoD installations, operations, and planning.

DoDD 4715.21 also describes responsibilities related to climate in the Department. The Under Secretary of Defense for Policy (USD(P)) is responsible for developing policies, plans, programs, forces, and posture needed to implement the DoD strategy including, as appropriate, adapting actions to increase resilience to climate change. In doing so, USD(P) responsibilities include defining strategic climate risks and those that might contribute to demand for defense missions, including consideration of climate into relevant strategic and operational documents; interfacing with interagency efforts related to the links between climate and stabilization; supporting USAID humanitarian assistance and disaster response; leading international engagements including security cooperation; and overseeing mission assurance. USD(P)'s staff also convene a group of DoD subject matter experts to share information and inform strategic thinking about the links between climate change, resource competition, and other aspects of environmental security.

Additionally, DoDD 4715.21 states that the Under Secretary of Defense for Acquisition & Sustainment (USD(A&S)) leads the development and oversees the implementation of DoD policy on climate change adaptation and resilience. These efforts support the delivery and sustainment of secure and resilient capabilities to the Warfighter, to include energy, climate, and water resilience and adaptation to the effects of climate change. Recent USD(A&S) climate-focused documents and tools include the CAP, an evaluation of resilience measures installations can deploy to reduce vulnerability to climate hazards ("*DoD Installation Exposure to Climate Change at Home and Abroad*," 2021), and the DoD Climate Assessment Tool (DCAT).

Many other offices play critical roles related to climate change. DoDD 4715.21 provides details on roles specifically related to climate change adaptation and resilience, including roles for additional Office of the Secretary of Defense (OSD) staff, as well as the Chairman of the Joint Chiefs of Staff, Combatant Commanders, and other DoD Components.

In January 2021, President Biden signed EO 14008, "Tackling the Climate Crisis at Home and Abroad," which elevates climate considerations to be "an essential element of United States foreign policy and national security" and highlights the urgency of tackling climate change to "avoid the most catastrophic impacts." The DCRA is in response to the requirements specified in Section 103(c) of EO 14008. The Secretary of Defense's March 2021 Memorandum established the OSD Climate Working Group to (1) coordinate Department responses to EO 14008 and subsequent climate- and energy-related directives, and (2) track implementation of climate- and energy-related actions and progress against future goals.

III. CLIMATE HAZARDS, IMPACTS, AND SECURITY IMPLICATIONS

Across the globe, climate change is contributing to an array of hazards including higher temperatures; changing precipitation patterns; and more frequent, intense, and unpredictable extreme weather conditions. These climate hazards can lead to impacts due to natural or social vulnerabilities, which have security implications for DoD. Some security implications may result directly from climate change, but many result from direct or indirect impacts of climate change (Figure 1). For example, the climate hazard of changing precipitation patterns is expected to cause more frequent and intense droughts in certain regions of the world. Primary impacts of drought include reduced water availability. Secondary impacts include reduced agricultural yields, which, in certain situations, could contribute to migration. Particularly when climate hazards converge and compound, there will likely be unprecedented challenges for governments to respond. For example, drought increases the chance of wildfires, which, in turn, contribute to more frequent and severe flooding; combined, these hazards can compound exponentially on populations.

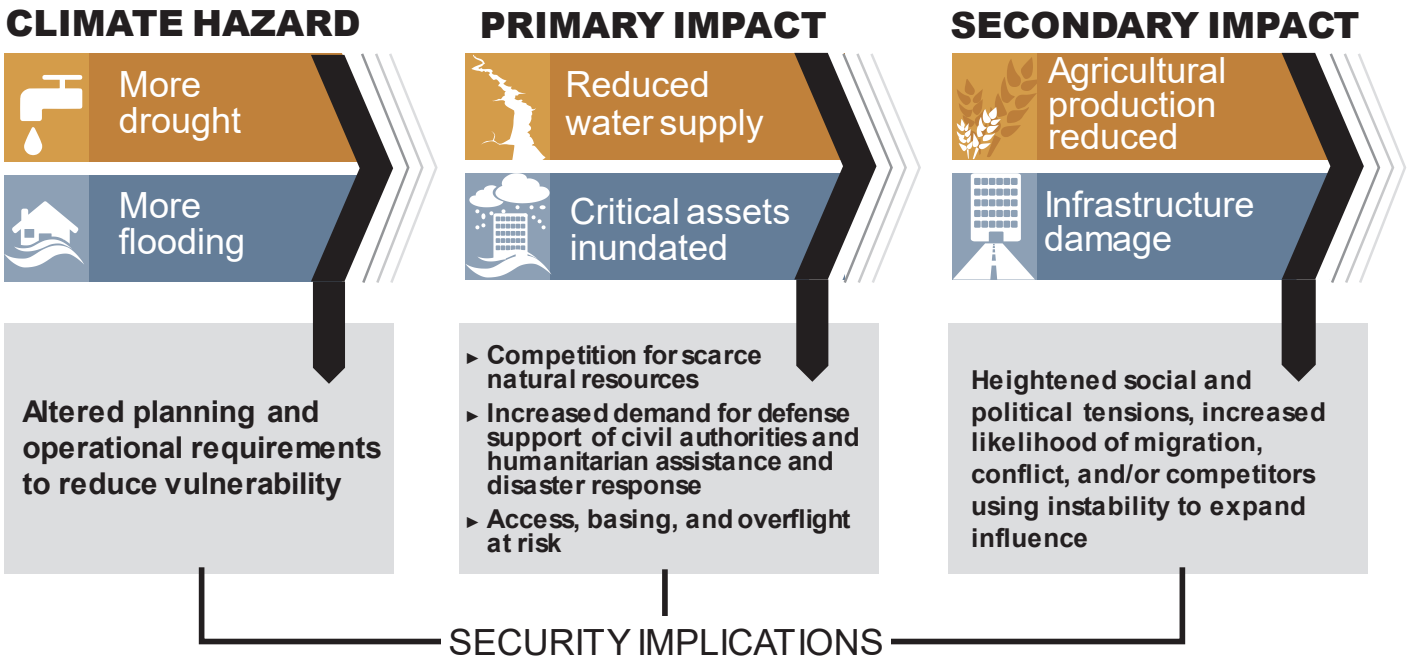

Figure 1. Examples of connections between climate hazards, primary and secondary climate impacts, and security implications.

As the frequency and intensity of these hazards increase, impacts are likely to expand competition over regions and resources, affect the demands on and functionality of military operations, and increase the number and severity of humanitarian crises, at times threatening stability and security. Climate change is one of many factors that contribute to instability and conflict; resilience and strong governance responses can reduce the likelihood of climate hazards having security implications. However, in worst-case scenarios, climate change-related impacts could stress economic and social conditions that contribute to mass migration events or political crises, civil unrest, shifts in the regional balance of power, or even state failure. This may affect U.S. national interests directly or indirectly, and U.S. allies or partners may request U.S. assistance.

While the effects of climate change are global, specific hazards, impacts, and risks associated with climate change will differ by region. The majority of climate hazards are not new; however, climate change is altering the frequency, intensity, and location of the hazards, contributing to vulnerability and compounding risks. Additionally, when climate change intersects with other forms of environmental degradation, such as deforestation and erosion, the impact can be even greater. Climate impacts, such as increased competition over scarce resources, are likely to contribute to internal tensions within countries, as well as external tensions between countries. As the likelihood of multiple converging extreme events increases with climate change, risks can compound and put enormous pressure on any government's capacity to respond, increasing the possibility of cascading security impacts. Box 3 provides examples of some of these crosscutting risks.

BOX 3. CROSSCUTTING CLIMATE CHANGE RISKS

While certain climate hazards and impacts are specific to particular regions of the world, many are cross-cutting, cascading, and/or global. Some examples include:

- Shifts in agricultural production in one region can impact global food prices and availability, contributing to food shortages, protests, and instability in other parts of the world.
- Impacts to marine ecosystems and resources have implications for fisheries and food security across the globe, which could become a source of friction.
- As temperatures and precipitation patterns shift, distribution and range of vector-borne diseases, such as malaria, will change.
- Increasingly unpredictable rainfall related to climate change could make it harder to resolve disputes over transboundary rivers such as the Nile and Mekong Rivers.

DoD supports the US Government Assistance to Haiti after Hurricane Matthew in 2016.

- Climate change that exacerbates insecurity and instability in one region may disrupt nomadic population movements and/or contribute to temporary or permanent migration that impacts other regions.
- Global supply chains are at risk to extreme weather events exacerbated by climate change. For example, the 2011 floods in Thailand disrupted production of components for global companies including computer disk drives and cars.
- Policy responses to climate change could also have unintended consequences and become sources of dispute, such as policies that impact supply chains or critical minerals.

Figure 2 illustrates some representative climate change hazards and potential impacts on DoD missions around the world.

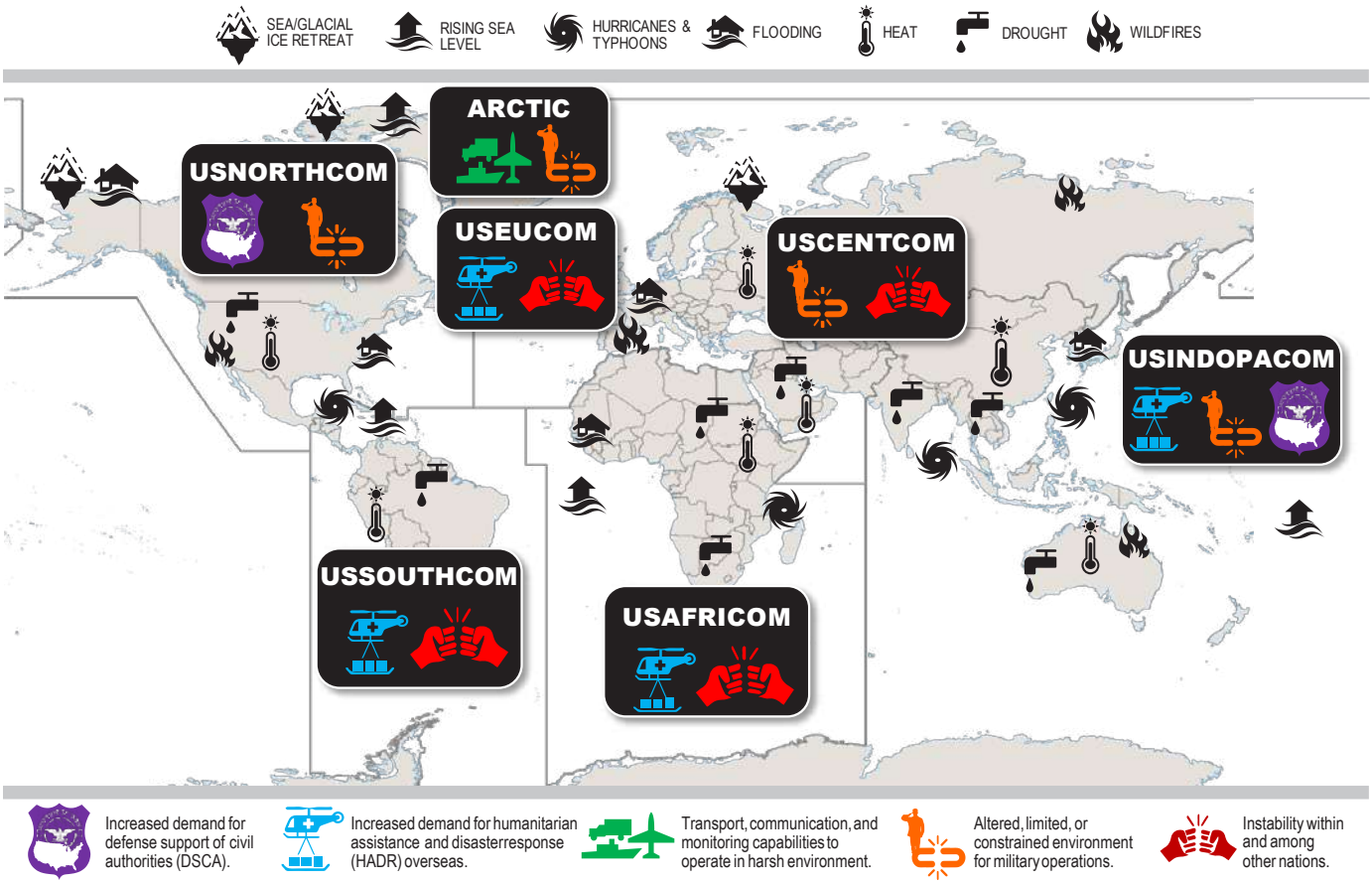

Figure 2. Selected regional hazards worsened by climate change (key to symbols on the top row above map) and identified security implications (key to symbols on the bottom row below the map). This map illustrates some of the key risks by region, but is not comprehensive of all risk.

The regional sections have been identified as Controlled Unclassified Information (CUI) and not releasable to the public. These sections were removed to allow this to be a publicly-releasable document.

IV. INCORPORATING CLIMATE RISK

DoD will integrate the security implications of climate change into key strategic documents, programs, and international partner engagements. DoD will also consider how to integrate climate considerations into DoD educational institution curriculums.

A. KEY DOD DOCUMENTS

Strategic documents signed by the Secretary or other senior DoD leaders guide DoD Components, formalize priorities to direct resource allocation, and drive action to accomplish desired ends. As required by EO 14008, Section 103(d), DoD will incorporate climate considerations into the National Defense Strategy (NDS); Defense Planning Guidance (DPG); the Chairman's Risk Assessment; and other relevant strategy, planning, and programming documents and processes. A report on progress of this integration is due annually to the National Security Council starting in January 2022. Climate change will be integrated into several of these documents through the office of the Assistant Secretary of Defense for Strategy, Plans, and Capabilities under the USD(P). In addition to the guidance in EO 14008, Section 103(d), DoD will also include consideration of climate across all relevant strategy, planning, force management, force employment, force development, and budget documents as discussed below and summarized in Figure 3.

Figure 3. Examples of DoD documents in which climate will be incorporated.

As the capstone strategic guidance for DoD, the NDS will examine ways in which climate change affects the security landscape and defense missions, activities, and resources. The NDS will also identify priorities and outcomes and direct the integration of climate considerations into nested strategy documents, including the National Military Strategy.

The Secretary of Defense updates the NDS at least every four years. Under Section 113 of Title 10 U.S. Code U.S.C., the NDS must include: DoD's priority missions; assumed force planning scenarios; assumed strategic environment, including the most critical and enduring threats, as well as strategies for countering threats; a strategic framework for prioritization of threats and risks; the roles and missions of the armed forces; elements of the defense program necessary to support the strategy; and a five-year plan for major investments that support the strategic framework. In the intervening years, the Secretary is required to assess the NDS implementation and any needed revisions. The 2021 Interim National Security Strategic Guidance clearly identifies climate change as one of the most significant threats facing the United States. As an important element of the strategic environment, now and in the future, climate change will be integrated into the NDS.

Section 113 also requires the Secretary to provide guidance on the preparation and review of contingency and campaign plans, which is provided through Contingency Planning Guidance (CPG) and the Guidance for Employment of the Force (GEF).

The CPG addresses preparation and review of contingency and campaign plans, including homeland defense and military support to civil authorities. The 2021 CPG includes prioritization of severe weather challenges to war plan development, and future CPGs will include consideration of other aspects of climate change.

A Texas Army National Guard UH-60 Black Hawk helps fight wildfires.

The GEF includes a prioritization of forces and resources; prioritization of contingency and campaign plans; prioritization of global, regional, and functional policy objectives; policy and strategic assumptions; guidance on global posture and global force management; security cooperation priorities; and nuclear policy. Climate change will be integrated into the GEF through the identification of priorities and planning, including global, regional, and functional considerations. The GEF provides a set of strategic assumptions that reflect the implications of climate change, such as impacts on competition over resources, likelihood of increased demand for humanitarian assistance and disaster response (HADR), and vulnerabilities of allies and partners' in each Combatant Command (CCMD). A new GEF will be released in spring 2022 and will reflect climate guidance.

B. INTERNATIONAL PARTNER ENGAGEMENT

Tackling climate change is a USG priority, and DoD will incorporate climate considerations into its engagements with allies and partners. For example, DoD worked closely with NATO allies to develop a Climate Change and Security Agenda and subsequent Action Plan in June 2021. There are opportunities for DoD to share its climate tools, resources, and experts, and to learn from partners and allies who have climate change mitigation and adaptation expertise. The DoD CAP also describes the importance of coordination with allies and partners in its Line of Effort 5 "Enhance Adaptation and Resilience Through Collaboration."

There are many ways for the Department to integrate climate considerations into international partner engagements, including supporting interagency diplomacy and development initiatives in partner nations; providing humanitarian assistance amid climate-affected crises; countering malign actors who seek to exploit climate change to gain influence; and sharing best practices. Some specific tools, funds, and programs, which are summarized in Appendix 2, include: the DCAT; Overseas Humanitarian, Disaster, and Civic Aid (OHDACA) funds; USINDOPACOM's Pacific Environmental Security Partnership (PESP); DoD Regional Centers for Security Studies; U.S. Army Corps of Engineers (USACE) technical assistance to partner countries on climate resilience; the State Partnership Program (SPP) as a forum for disaster-response planning and communication; the Institute for Security Governance engagements building global partner resilience; and the Defense Institute of International Legal Studies (DIILS) advising, education, training engagements, and courses.

V. INFORMING AND FUNDING FUTURE CLIMATE RISK ANALYSES

The DCRA serves as a first step towards inclusion of the security implications of climate change across the DoD enterprise. Tailored analyses of climate risk will be needed to inform specific modeling, simulation, and exercises. The following subsections detail scientific and intelligence information, as well as relevant interagency groups, that can help support future analyses of climate hazards, impacts, risks, and security implications for DoD.

A. SCIENTIFIC INFORMATION

DoD relies on data, science, and evidence in decision-making, including planning, policy, and decisions related to climate risk. Risk analyses are not meant to be fixed in time; rather, they must be frequently updated through an iterative process that captures evolving risks and new scientific advances. To understand specific climate effects on plans, resourcing, operations, and missions, DoD Components will include climate considerations in relevant risk analyses, leveraging high-quality data, scenarios, and analytical tools tailored to DoD needs.

Coast Guard crews train in high surf to ensure they are prepared to respond to any maritime emergency during rough weather conditions.

Current DoD-specific resources for climate data, analysis, and assessments include, but are not limited to, the DCAT; the U.S. Army Climate Assessment Tool (ACAT); the U.S. Air Force 14th Weather Squadron within the 557th Weather Wing under Air Combat Command; and the Climatology Division at Fleet Numerical Meteorology and Oceanography Center. DoD relies on authoritative scientific data and modeling provided by USG science agencies. Improvements in predictive capacity of short- to medium-term weather anomalies are needed to have scientific information at time scales required for DoD strategy and planning. DoD will validate its selection and use of climate data and projections internally and through consultation with scientific agencies and other partners such as DoD's Climate Working Group, DoD's Strategic Environmental Research and Development Program (SERDP), Federally Funded Research and Development Centers (FFRDCs), and the U.S. Global Change Research Program (USGCRP)—(of which DoD is a member)—within the White House Office of Science and Technology Policy (OSTP). DoD will continue to review and assess how to integrate climate science, data, and scenarios into Department documents and processes.

B. INTELLIGENCE PRODUCTS

There are various classified and unclassified U.S. intelligence products regarding the security risks associated with climate change, including Annual Threat Assessments, the quadrennial Global Trends reports, and the 2016 National Intelligence Council White Paper. In 2020, consistent with 50 U.S.C. § 3060, the Director of National Intelligence established the Climate Security Advisory Council (CSAC), bringing together the Intelligence Community and Federal Science Agencies to advance insights on the national security impacts of climate change. As required by EO 14008, Section 103(b), the Intelligence Community is developing a National Intelligence Estimate (NIE) on the national and economic security impacts of climate change. Both the CSAC and the climate NIE will be important resources to inform future DoD climate security risk analyses.

C. FUNDING

The Department intends to prioritize funding DoD Components in support of exercises, wargames, analyses, and studies of climate change impacts on DoD missions, operations, and global stability. The President's FY 2022 Budget request included funds to incorporate climate risk into exercises, wargames, analyses, and studies. Appropriated funds will be allotted via existing governance structures including the Combatant Commander Exercise Engagement and Training Transformation program and the Wargaming Incentive Fund. Box 4 provides a list of examples of climate risk that could be included in future modeling, simulation, and wargaming.

BOX 4. EXAMPLES OF CLIMATE RISK TO BE INCORPORATED INTO MODELING, SIMULATION, AND WARGAMING

- Sensor operations: changes in the operating environment due to temperature extremes or extended rainfall; loss of effectiveness based on climate conditions.
- Information operations: how changes in "normal" climate cycle affect operations.
- Aircraft performance (fixed wing and rotary wing): loss of payload capacity, range, and loiter time based on increased temperatures.
- Wildfires: loss of range or accuracy due to extreme weather conditions.
- Ground mobility: extended monsoon season and trafficability; impact on medical evacuation and resupply by ground means.
- Naval operations: underway replenishment in altered sea conditions; loss of capability/efficiency in fuel transfer (e.g., ship to shore).
- Non-combatant Evacuation Operations: conflict exacerbated by climate change impacts; embassy security or evacuation considerations.
- Access, basing, and overflight constraints.
- Exercises: extreme weather-related cancellations and effects on readiness.
- Threat icons: integrate irregular threat icons that represent non-state actors, transnational criminal organizations, or other unofficial competitors motivated to disrupt operations during an exercise or wargame.
- Critical infrastructure: climate-related delays, disruption, and/or degradation of DoD to produce, package, repair, and distribute materiel and ammunition and its effects on readiness and/or operations.
- Degradation of Joint Force performance due to extreme weather events associated with climate change.
- Consideration of the impact of increased demand for HADR and DSCA.

VI. CONCLUSION

"No nation can find lasting security without addressing the climate crisis."

— Secretary of Defense Austin, April 2021

The DCRA provides an initial review of the security implications of climate change specific to DoD missions and identifies strategy, planning, and other documents that will incorporate consideration of climate change. Guidance will be included in Secretary-level strategic documents, such as the NDS, DPG, and GEF, as well as other DoD core guidance documents to drive prioritization of climate considerations throughout the Department. The authors thank DoD and interagency counterparts for reviewing and contributing to this document. The DCRA provides a critical step towards integration of climate change considerations at every level of the DoD enterprise. Working within the whole-of-government, and in coordination with allies and partners, DoD will work to prevent, mitigate, account for, and respond to defense and security risks associated with climate change.

APPENDIX 1. CLIMATE-RELATED STATUTES, DOD ISSUANCES AND POLICIES, AND EXECUTIVE ORDERS

Climate-Related Statutes

10 U.S.C. § 118, Quadrennial Defense Review
10 U.S.C. § 2802, Military Construction Projects
10 U.S.C. § 2864, Master plans for major military installations
10 U.S.C. § 2902, Strategic Environmental Research and Development Program Council
15 U.S.C. § 2904, National Climate Program
50 U.S.C. § 3060, Climate Security Advisory Council

Climate-Related Issuances

DoDD 3020.40, Mission Assurance
DoDD 4715.21, Climate Change Adaptation and Resilience
DoD Instruction (DoDI) 3020.45, The Mission Assurance Construct
DoDI 3200.21, Sustaining Access to the Live Training Domain
DoDI 4170.11, Installation Energy Management
DoDI 4715.03, Natural Resources Conservation Program
DoD Manual 4715.03, Integrated Natural Resource Management Plan

Climate-Related DoD and Departmental Policies and Guidance

Air Force Instruction (AFI) 32-1015, Integrated Installation Planning
AFI 90-2001, Mission Sustainment
Air Force Manual (AFMAN) 32-7003, Environmental Conservation
Air Force Severe Weather/Climate Hazard Screening and Risk Assessment Playbook
Army Climate Resilience Handbook
Army Directive 2020-08, U.S. Army Installation Policy to Address Threats Caused by Changing Climate and Extreme Weather
Army Memorandum Consideration of Greenhouse Gas Emissions and the Effects of Climate Army Regulation 210-20, Real Property Master Planning for Army Installations
Climate Adaptation for DoD Natural Resource Managers Report
DoD Installation Exposure to Climate Change at Home and Abroad, April 2021
Marine Corps Order (MCO) 11000.5, Facilities Sustainment, Restoration and Modernization Program
MCO 11000.12, Real Property Facilities Manual, Facilities Planning and Programming
Office of the Chief of Naval Operations Instruction 11010.40A, Encroachment Management Program
NAVFAC Climate Change and Installation Adaptation and Resilience Planning Handbook
Unified Facilities Criteria (UFC) 2-100-01 Installation Master Planning

Recent Climate-Related Executive Orders (EOs)

EO 13985, Advancing Racial Equity and Support for Underserved Communities Through the Federal Government, January 20, 2021
EO 13990, Protecting Public Health and the Environment and Restoring Science to Tackle the Climate Crisis, January 20, 2021
EO 14008, Tackling the Climate Crisis at Home and Abroad, January 27, 2021
EO 14013, Rebuilding and Enhancing Programs to Resettle Refugees and Planning for the Impact of Climate Change on Migration, February 4, 2021
EO 14017, America's Supply Chains, February 24, 2021
EO 14027, Establishment of the Climate Change Support Office, May 7, 2021
EO 14030, Climate-Related Financial Risk, May 20, 2021

APPENDIX 2. EXAMPLES OF TOOLS, FUNDS, AND PROGRAMS THAT SUPPORT INTERNATIONAL PARTNER CLIMATE RESILIENCE.

Note: This list is intended to provide some examples but is not exhaustive.

- The DoD Climate Assessment Tool (DCAT) uses data from past weather events and projections of some future climate changes to provide high-level assessments of exposure to climate change for critical infrastructure. To date, the Department has used the DCAT on hundreds of domestic installations and a selection of overseas installations (see the DoD *Installation Exposure At Home and Abroad* report for more information about these assessments and the DCAT). Many of these locations are important for operational plans and ongoing operations. The Department will incorporate the results of these exposure assessments into military construction requests, partnership activities, and relationships with allies. DoD is committed to share the DCAT with allies and partners to help build climate resilience.
- One of the Department's most effective tools for helping partners cope with extreme events exacerbated by climate change is through its Overseas Humanitarian, Disaster, and Civic Aid (OHDACA) appropriation and associated Title 10 authorities. The majority of the annual OHDACA funding supports steady-state humanitarian assistance projects, including those related to the effects of climate change. DoD-supported projects include sponsoring disaster assessments to identify gaps; training our partners to conduct disaster planning and disaster management; assisting partners with disaster response exercises; constructing disaster warehouses, emergency operations centers, and hurricane shelters; and building up a partner's health capacity. Joint DoD and USAID disaster risk reduction efforts in Bangladesh, the Philippines, and Nepal continue to be encouraging examples of ways to reduce adverse effects of extreme weather events on vulnerable populations.
- The Pacific Environmental Security Partnership (PESP), an initiative of USINDOPACOM, brings together a network of environmental security partners with an interest in cooperation and capacity development. Their annual event, the Pacific Environmental Security Forum, has been held for over a decade. This could be an example for other CCMDs.
- DoD Regional Centers for Security Studies build partners' capacity to forecast, assess, and mitigate the security impacts of climate change by conducting bilateral and multilateral research, workshops, subject matter exchanges, and training and education activities for U.S. and partner military and civilian participants. For example, the Daniel K. Inouye Asia-Pacific Center for Security Studies teaches climate security modules in every resident course and includes climate change security impacts in partner exercises and gray zone games.
- Under its Title 10 authorities, the U.S. Army Corps of Engineers (USACE) provides technical assistance to certain partner countries and inter-governmental organizations on climate resilience and risk mitigation. For example, USACE supports Mekong River Commission climate resilience efforts in the Mekong basin and collaborates with the United Nations on climate risk-informed water management, including flood and drought monitoring and forecasting in Southern Africa.
- The State Partnership Program (SPP) partners State National Guard organizations with 92 countries and provides an opportunity for DoD and partner-nation governments to coordinate on all aspects of force readiness. It also provides a forum for disaster-response planning, ensuring the United States remains a partner of choice. The SPP provides a channel of communication for discussion of military implications of climate change and mitigation efforts with partner and allied nations.
- The Institute for Security Governance builds global partner resilience capacity via engagements specifically designed to enhance partner whole-of-government, all-hazards readiness, and resilience to face the full range of crises and emergencies. These engagements incorporate consideration of issues directly related to climate security such as: comprehensive risk assessment, energy security, secure and sustained provision of essential goods and services, civil preparedness, and governance under degraded or de-stabilizing conditions.
- The Defense Institute of International Legal Studies (DIILS) builds global partner legal capacity via advising, education, training engagements, and courses. Some of these address legal issues directly related to climate security and destabilizing conditions, such as those associated with military support to civilian authorities for border security or disaster response operations, Civilian Harm Mitigation (CHM) efforts, rights and obligations associated with refugees and displaced persons, maritime security and interdiction operations, and hybrid warfare and malign influence operations.

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Review

Threats to seabirds: A global assessment

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ABSTRACT

We present the first objective quantitative assessment of the threats to all 359 species of seabirds, identify the main challenges facing them, and outline priority actions for their conservation. We applied the standardised Threats Classification Scheme developed for the IUCN Red List to objectively assess threats to each species and analysed the data according to global IUCN threat status, taxonomic group, and primary foraging habitat (coastal or pelagic). The top three threats to seabirds in terms of number of species affected and average impact are: invasive alien species, affecting 165 species across all the most threatened groups; bycatch in fisheries, affecting fewer species (100) but with the greatest average impact; and climate change/severe weather, affecting 96 species. Overfishing, hunting/trapping and disturbance were also identified as major threats to seabirds. Reversing the top three threats alone would benefit two-thirds of all species and c. 380 million individual seabirds (c. 45% of the total global seabird population). Most seabirds (c. 70%), especially globally threatened species, face multiple threats. For albatrosses, petrels and penguins in particular (the three most threatened groups of seabirds), it is essential to tackle both terrestrial and marine threats to reverse declines. As the negative effects of climate change are harder to mitigate, it is vital to compensate by addressing other major threats that often affect the same species, such as invasive alien species, bycatch and overfishing, for which proven solutions exist.

1. Introduction

Seabirds are one of the most threatened groups of birds (Croxall et al., 2012; BirdLife International, 2018). They are also regarded as good indicators of the health of marine ecosystems (Piatt and Sydeman, 2007; Parsons et al., 2008), and have a key role in marine ecosystems, with an overall consumption of biomass of the same order of magnitude as global fisheries landings (Brooke, 2004; Cury et al., 2011). They occur across all oceans, from coastal areas to the high seas, and are easier to study than most other marine animals because they are readily visible at sea and depend on land to breed, allowing for a better understanding of their population trends and of their threats.

The latest assessment of the global threat status of seabirds, using the International Union for Conservation of Nature (IUCN) Red List criteria, revealed that 31% of all seabird species are globally threatened (i.e. Critically Endangered, Endangered or Vulnerable; 110 of 359 species), and another 11% (40 species) are Near Threatened (NT) (BirdLife International, 2018; Fig. A1, Appendix 3). Additionally, almost half of all species (47%) have declining population trends (BirdLife International, 2019).

Some of the drivers of these declines are threats faced at the colonies, such as invasive alien species (Spatz et al., 2014, 2017), whereas others operate at sea, including incidental mortality (bycatch) in fisheries, and overfishing (Žydelis et al., 2009; Anderson et al., 2011; Grémillet et al., 2018). Well-documented cases of bycatch as a threat driving severe declines in seabird populations include those of albatross species, with long-term studies conducted at South Georgia in the south Atlantic Ocean identifying bycatch in longline or trawl fisheries as the primary cause of the 40–60% population declines observed over the last 35 years (Pardo et al., 2017). Bycatch is also identified as a key threat to the Balearic Shearwater *Puffinus mauretanicus* (Arcos, 2011). Species of small petrels such as Mascarene Petrel *Pseudobulweria aterrima* and Fiji

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Petrel *Pseudobulweria macgillivrayi* are on the brink of extinction due to predation by introduced mammals at their breeding colonies (Riethmuller et al., 2012; Rodríguez et al., 2019), and some are already possibly extinct (e.g. Jamaican Petrel *Pterodroma caribbaea*, Guadalupe Storm-petrel *Hydrobates macrodactylus*; Tobias et al., 2006). Depletion of food resources around seabird colonies due to intense fishing or changes in oceanographic conditions has also resulted in population declines of the African Penguin *Spheniscus demersus* in the Benguela Current region (Pichegru et al., 2009), of the Black-legged Kittiwake *Rissa tridactyla* in the North Sea (Carroll et al., 2017) and of several species in the area of the Humboldt Current (Barbraud et al., 2018).

Most previous reviews of threats to seabirds have focused on the causes of declines of specific groups, e.g. albatrosses (Phillips et al., 2016), petrels (Rodríguez et al., 2019), penguins (Borboroglu and Boersma, 2013; Trathan et al., 2015), or on the impact of a single threat, e.g. longline or gillnet bycatch (Anderson et al., 2011; Žydelis et al., 2009). The only global review to date was based on data up to 2010 and focused only on globally threatened seabirds (Croxall et al., 2012). However, to understand the conservation status of this group worldwide, it is important to assess the anthropogenic and natural pressures affecting all species, since many relatively abundant and widespread species of Least Concern on the IUCN Red List are now also in decline (e.g. Little Auk *Alle alle*, Fort et al., 2013; Chinstrap Penguin *Pygoscelis antarcticus*, Korczak-Abshire et al., 2012; Arctic Tern *Sterna paradisaea*, Burnham et al., 2017).

We present the results of the first quantitative review of the threats affecting all seabird species globally. We used data from > 900 publications and a standardised assessment approach (the IUCN Red List Threats Classification Scheme; IUCN, 2012; Salafsky et al., 2008), aiming to: a) identify the main ongoing drivers of population declines of seabirds globally; b) provide the first systematic appraisal of the overall impacts of each threat on multiple species; c) quantify the number of individual seabirds exposed to each threat; and d) highlight some of the challenges and priority actions needed and to improve the conservation status of seabirds.

2. Materials and methods

2.1. Selection and categorisation of species

We followed the taxonomy used by BirdLife International for the IUCN Red List (del Hoyo et al., 2014; BirdLife International, 2019) and considered seabirds to be those species for which a large proportion of the population rely on the marine environment for at least part of the year (Croxall et al., 2012). This criterion was met by 359 extant species (list available in Appendix 1). We grouped species based on taxonomy: albatrosses; large petrels and shearwaters; gadfly petrels (genera *Pterodroma* and *Pseudobulweria*); storm-petrels; other small petrels; penguins; auks; skuas; terns; gulls; frigatebirds and tropicbirds; gannets and boobies; cormorants and pelicans; sea ducks and allies; phalaropes (Appendix 1). We also split species into “pelagic” and “coastal” based on the definition provided by Croxall et al. (2012): “pelagic seabirds” are those that primarily use marine deep water (typically > 200 m in depth), or neritic, continental shelf water; and “coastal seabirds” are those that primarily use inshore waters (typically < 8 km from the shoreline; see Appendix 1). The global population trend of each species was also used in some analyses (using two categories: declining versus stable/increasing/unknown; BirdLife International, 2019).

2.2. Data sources and threats classification

For the first time, threats were systematically assessed for all 359 seabird species. We undertook a detailed review of the seabird threat data, held by BirdLife International, which are used to support the global status assessment of bird species for the IUCN Red List (BirdLife International, 2019), and the consistency of threat scoring between species was rigorously checked. We collected data through a combination of expert consultation (in collaboration with the respective seabird IUCN Species Survival Commission (SSC) Specialist Groups) and a comprehensive bibliographic search for studies reporting threats to each seabird species. In a first stage, we consulted summary species accounts published in the Handbook of the Birds of the World Alive (HBW Alive, 2018), supplemented by regional accounts from the Birds of North America (BNA online, 2018), New Zealand Birds Online (NZ Birds Online, 2018) and the Australian Government Species Profile and Threats Database (Department of the Environment and Energy, 2018). Secondly, we conducted searches in Web of Knowledge and Google Scholar, first using the *species name* (scientific name and common name separately) + “threat”, and then using the *species name* and each threat named in the results of the preceding search. For species listed under the Agreement on the Conservation of Albatrosses and Petrels (ACAP), the ACAP Secretariat and relevant working groups reviewed the revised threat codings, and for penguins, the IUCN SSC Penguin Specialist Group performed this role, allowing the incorporation of additional literature and unpublished data. Overall, information from over 900 publications (each referenced to the appropriate species in the factsheets available on the BirdLife Data Zone; BirdLife International, 2019) was used to revise the ‘threats’ texts that form part of the IUCN Red List factsheets and assessments (BirdLife International, 2019).

Threats were classified using the IUCN Red List Threats Classification Scheme version 3.2 (Salafsky et al., 2008; IUCN, 2012). This scheme defines threats as “the proximate human activities or processes that have impacted, are impacting, or may impact the status of the taxon being assessed. Direct threats are synonymous with sources of stress and proximate pressures” (IUCN, 2012). In other words, and in the context of this study, a threat is a human activity or other process that affects the current conservation status of a species by causing a population or range reduction.

Each threat was coded initially using the IUCN Red List Threats Classification Scheme, down to Level 3 (the most detailed classification level) where possible (IUCN, 2012). For each threat, we assessed: 1) timing (i.e. ongoing; past but likely to return; past and unlikely to return; future); 2) scope (i.e. the proportion of the total population

Table 1
System for scoring impact of threats (from Garnett et al., 2018). Values within parentheses represent the percentage of the total population affected (scope) and the known or likely rate of population decline caused by the threat over three generations (severity). Impact values are the average of the product of the extremes of scope and severity in each interval (mean [min (scope)*min (severity)/100, max (scope)*max (severity)/100]).

Scope/severity	Very rapid declines (> 30%)	Rapid declines [20–30%]	Slow but significant declines or causing/could cause fluctuations [5–20%]	Negligible declines (< 5%)
Whole (> 90%)	63	23.5	11.8	2.9
	Very high	High	Medium	Low
Majority [50–90%]	51.6	17.9	9.7	2.4
	Very high	High	Medium	Low
Minority (< 50%)	24.9	7.4	4.8	1.2
	High	Medium	Medium	Low

affected: minority (< 50%); majority (50–90%); whole (> 90%)); and 3) severity (i.e. the rate of population decline caused by the threat within its scope: very rapid; rapid; slow but significant; negligible; causing/could cause fluctuations) (IUCN, 2012 and Table 1). Each threat at the most detailed level can be recorded only once against a species, with the exception of 'Invasive & other problematic species, genes & diseases', for which an entry for each problematic species is possible. As one threat can have different timing and severity across the range of a species, the following convention was applied: 'Ongoing' threats were prioritised over 'Future' threats, which were prioritised over 'Past' threats. Hence, a slow, ongoing reduction was coded in preference to a rapid, past reduction. Stresses, which are the mechanism by which a threat directly or indirectly impacts the species, such as species mortality or ecosystem degradation, were also recorded as part of the IUCN threat assessment approach (IUCN, 2012). Further relevant detail beyond that required for the IUCN assessment was also noted when available, in particular the type of fishing gear and the scale of the fishery (small versus large) associated with the impact of bycatch. Overall, this process resulted in the compilation of 1637 records of threats to 359 seabird species.

The IUCN Red List Threats Classification Scheme was developed to be applied across all species of plants, animals and fungi, and thus often lacks resolution when applied to a specific group. For example, bycatch and overfishing, two frequent threats to seabirds (Croxall et al., 2012), are allocated the same threat code under the IUCN scheme (Level 1 = Biological Resource Use, Level 2 = Fishing & harvesting aquatic resources, and Level 3 = Unintentional effects). We therefore refined the threats classification by splitting: 1) "Biological resource use" into: "Bycatch", "Overfishing", "Disturbance", "Hunting/trapping" and "Logging & wood harvesting"; 2) "Invasive and other problematic species, genes & diseases" into "Invasive alien species" and "Diseases"; 3) "Agriculture & aquaculture" into "Agriculture" and "Aquaculture"; and 4) "Light pollution" from "Pollution" (see Appendix 2 for a more detailed explanation). We assumed the same impact score of "bycatch" and "overfishing" for species affected by both (n = 34), as it was not possible to distinguish their relative impacts (see above). The final list of threats considered in the analyses (Table A2.1, Appendix 2) was thus a combination of the original IUCN Red List classification of Level 1 threats (IUCN, 2012), modified as indicated above (see also Table A2.2, Appendix 2).

2.3. Data analysis

All the analyses (except where noted otherwise) considered only threats that were coded to the timing category "ongoing", with a known and non-zero scope and severity. We also analysed the threats separately for "pelagic" and "coastal" species, and for specific groups of seabirds (see Section 2.1 above). For these latter analyses, we distinguished terrestrial threats (e.g. invasive alien species, disturbance at colonies) from marine threats (e.g. overfishing, bycatch). Climate change/severe weather was considered in a separate category (see Table A2.2, Appendix 2 for more details on threats classified as marine or terrestrial). We estimated the impact of each ongoing threat on each species by multiplying the mean scope (the proportion of the population affected; see Table 1) by the mean severity (Table 1; Garnett et al., 2018), and categorised these into four levels, from "low" to "very high" (Table 1). For threats with multiple coding per species (see above), we used the highest value of impact. We also calculated the overall impact of each threat by summing the impact scores across all species.

Finally, we estimated the total number of birds (T) exposed to each threat (i) by summing the product of the global abundance of each species affected by the threat, and the scope of the threat,

$$T_i = \sum_{sp=1}^{n} A_{sp} * S_{i,sp}$$

where A = abundance of species sp, S = scope of the threat i to species sp. The global abundance of each species was extracted from the IUCN Red List database (BirdLife International, 2019) by multiplying the number of mature individuals (available for 95% of the species) by 1.5, to account for the number of non-breeders in the population (Brooke, 2004). In order to address the uncertainty associated with this estimate (given the large range of most estimates of abundance and of the values of scope – see Table 1), we applied a bootstrap approach (1000 repetitions), by selecting random values within the intervals of abundance (i.e. between the minimum and maximum abundance) and scope (i.e. a random value between the minimum and maximum scope for each category – see Table 1) of each species, from which we derived a 95% confidence interval. These analyses were carried out separately for species classified as: 1) globally threatened; 2) Near Threatened and Least Concern with a declining trend and 3) Least Concern with a non-declining trend.

3. Results

3.1. Threats to all seabird species

Invasive alien species, bycatch, hunting/trapping, climate change/severe weather and disturbance are the ongoing threats affecting most seabird species, with each affecting more than a fifth of all species (Fig. 1 and Table 2). Pollution, overfishing and problematic native species also affect many seabird species (> 50 each; Fig. 1). Bycatch, invasive alien species, overfishing and climate change/severe weather are the threats causing highest impacts on average (Fig. 1 and Table 2). The impacts of hunting/trapping and disturbance are relatively low by comparison (Fig. 1 and Table 2); in contrast, diseases and natural system modifications have high impacts on the few species affected (15 and 10 species, respectively; Fig. 1). Invasive alien species have the highest overall impact (i.e. the sum of all impacts on all species affected by this threat), followed by bycatch and climate change/severe weather (Table 2).

We estimate that > 170 million individual birds (> 20% of all seabirds) are currently exposed to the individual impacts of bycatch, invasive alien species and climate change/severe weather (Fig. 2), and that together > 380 million (c. 45% of all seabirds) are exposed to at least one of these three threats.

Overall, 301 (84%) of the 359 seabird species are impacted by at least one ongoing threat. About 70% of these are affected by at least two threats and 46% by at least three threats (n = 301). On average, each seabird species is affected by three ongoing threats (2.85 ± 0.12, range = 1–11, n = 301).

3.2. Threats to globally threatened species

The 110 globally threatened seabird species are largely affected by the same threats highlighted above – invasive alien species, bycatch, climate change/severe weather, hunting/trapping and overfishing (Fig. 3 and Table 2; see also Fig. A2 in Appendix 3). Problematic native species are also a major threat for globally threatened species, both pelagic and coastal. Disturbance is the threat affecting most coastal species, along with hunting/trapping, although mainly with medium or low impact (Fig. 3).

3.3. Threats to Near Threatened and declining Least Concern species

Invasive alien species, climate change/severe weather, bycatch and hunting/trapping are also the threats affecting the highest number of Near Threatened (NT) and Least Concern (LC) species with a declining trend; each affects > 30% of the species in this group (Fig. A3 in Appendix 3).

The populations of these species comprise nearly half of all individual seabirds in the world (45%–47%); about half of birds exposed

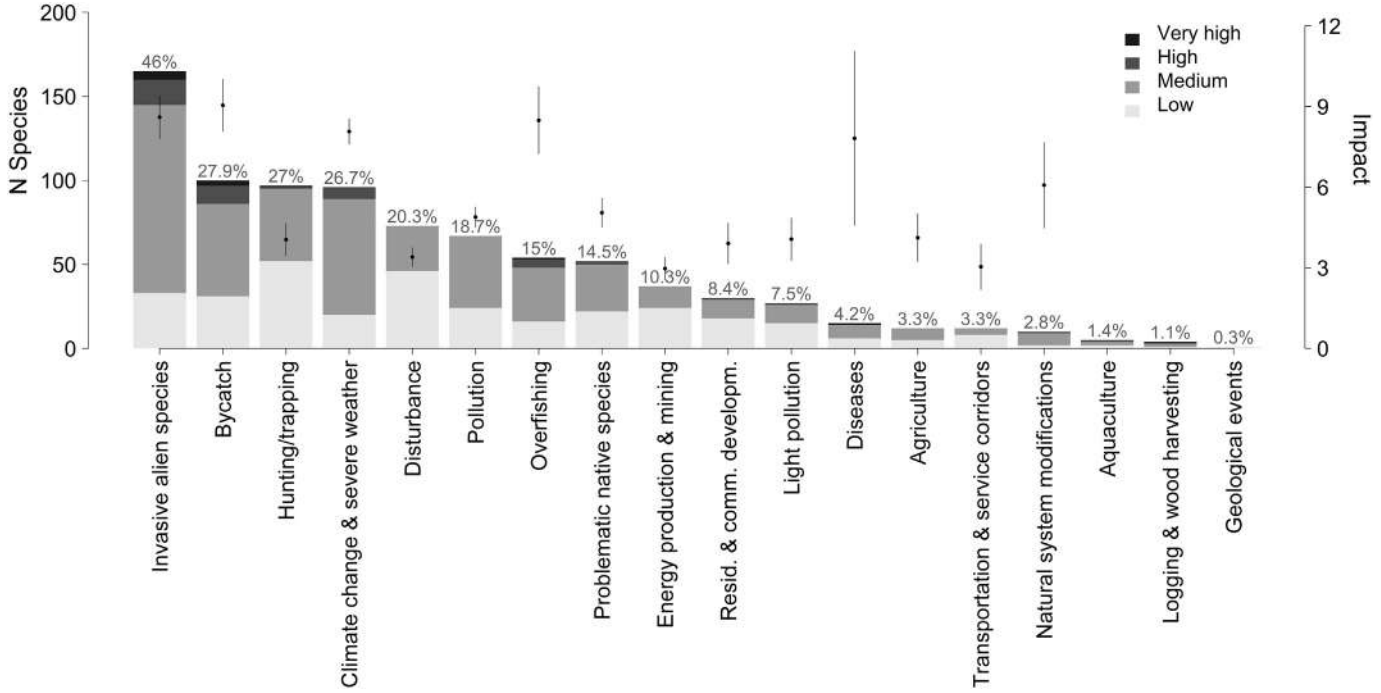

Fig. 1. Ongoing threats to all seabird species (ordered by the number of species affected). Left y axis: total number of species affected; Right y axis: average impact ± SE. Values atop bars indicate the percentage of species affected (n = 359).

to some of the major threats (especially bycatch, climate change/severe weather and invasive alien species) are NT and declining LC (Fig. 2); 81% of the species currently NT or LC with declining trends are impacted by at least one of these three threats.

3.4. Threats to groups of seabirds

The major threats to particular groups of highly threatened species are indicated in Fig. 4 (see also Fig. A4, Appendix 3, for the percentage of threatened species per group). Albatrosses are particularly affected by bycatch (90% of species). In addition, around half of albatross species (13 of 22) are affected by at least one terrestrial threat, mostly invasive alien species but also diseases, which have a high impact (Table 3), and over one-third are affected by climate change/severe weather.

More than 80% of penguin species are affected by climate change/severe weather (a higher proportion than any other seabird group). Marine threats such as overfishing, bycatch and pollution also have large impacts on several species of penguins (Fig. 4). The main threats at colonies are invasive alien species, problematic native species and disturbance, albeit with lower estimated impacts on average. Around half of the penguin species suffer medium, high or very high impacts from both marine and terrestrial threats (marine – pollution or overfishing; terrestrial – usually problematic native species; Table 3).

Invasive alien species and bycatch are also important threats for large petrels and shearwaters (eight species are affected by both these threats; Table 3), as is light pollution (Fig. 4). Cormorants and pelicans are also impacted by a combination of several terrestrial (including invasive alien species and problematic native species) and marine threats (bycatch, overfishing and pollution; Fig. 4 and Table 3). In contrast, gadfly petrels and storm-petrels are almost exclusively impacted by terrestrial threats, particularly by invasive alien species (and light pollution in the case of gadfly petrels; Fig. 4).

3.5. Invasive alien species

Rats *Rattus* spp. and cats *Felis catus* are the invasive alien species impacting the highest number of seabird species (> 100 and 90,

Table 2
Summary of the top threats (impacting > 20% of the species or having an high overall impact) affecting: all seabird species; only globally threatened species; only Near Threatened (NT) species and Least Concern (LC) species with declining trends. N species: number of species affected; N species main threat: number of species for which the threat is the main cause of decline (i.e. highest impact); Mean impact (± SE): mean impact on the species affected by the threat; Overall impact: sum of the impact scores across all species. Threats are listed in descending order of the overall impact on all species.

Threats	All species (n = 359)				Globally threatened species (n = 110)			NT and LC species (declining) (n = 119)		
	N species	N species main threat[a]	Mean impact	Overall impact	N species	Mean impact	Overall impact	N species	Mean impact	Overall impact
Invasive alien species	165	107	8.6 ± 0.8	1419.29	73	12.14 ± 1.64	885.89	62	6.12 ± 0.61	379.36
Bycatch	100	70	9.05 ± 0.97	904.66	50	11.78 ± 1.77	589.00	36	6.68 ± 0.75	240.62
Climate change/severe weather	96	63	8.07 ± 0.47	774.92	37	9.88 ± 0.80	365.53	43	7.44 ± 0.67	319.89
Overfishing	54	24[b]	8.49 ± 1.25	458.25	22	11.89 ± 2.81	261.49	19	6.79 ± 0.83	129.09
Hunting/trapping	97	38	4.05 ± 0.6	392.71	27	6.05 ± 1.98	163.37	35	4.03 ± 0.50	141.18
Disturbance	73	25	3.40 ± 0.36	248.31	26	4.23 ± 0.63	110.09	28	3.37 ± 0.57	94.36

[a] Some species can have more than one threat as the main cause of decline.
[b] Excluding species for which overfishing and bycatch are both indicated as the major threat.

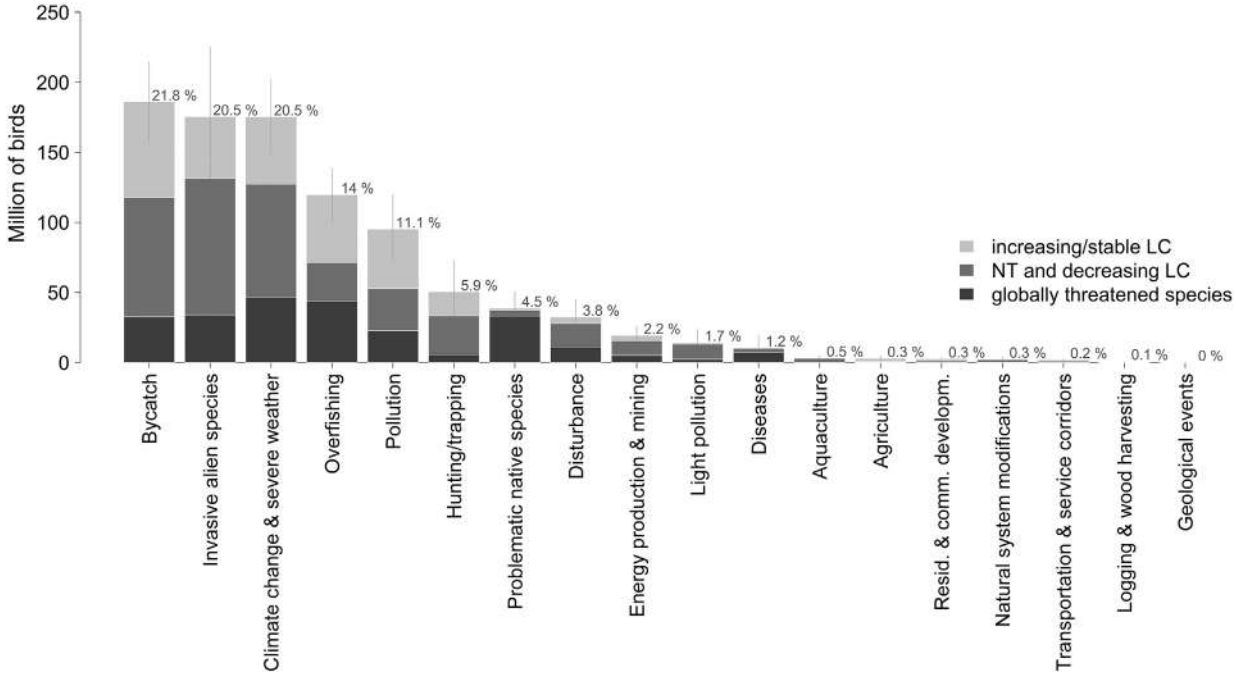

Fig. 2. Estimated total number of seabirds exposed to each threat. Error bars represent the 95% confidence intervals (see methods). Values atop bars indicate percentage of total number of seabirds affected.

respectively; Fig. 5). Sixty-three seabird species (38% of those affected by invasive alien species) are impacted by both rats and cats. Mice (*Mus* spp. and *Peromyscus maniculatus*) affect a smaller number of species (22, of which 20 are Procellariiformes - albatrosses, large petrels & shearwaters, gadfly petrels, prions and storm-petrels), but often with high severity.

3.6. Bycatch

Large-scale fisheries are causing declines of most species affected by bycatch (> 80), whereas < 40 species are affected by small-scale fisheries (Fig. 6). The average impacts (scope and severity) of large and small-scale fisheries are, however, similar (Fig. 6). Gillnet fisheries affect more species than longlining and trawl fisheries; these last two gear types have, however, a greater impact in terms of both average severity and scope (Fig. 6).

3.7. Climate change and severe weather

Climate change/severe weather impacts seabirds mostly due to habitat shifting and alteration, and temperature extremes (almost 40 species are affected by each of these threats, and with relatively high

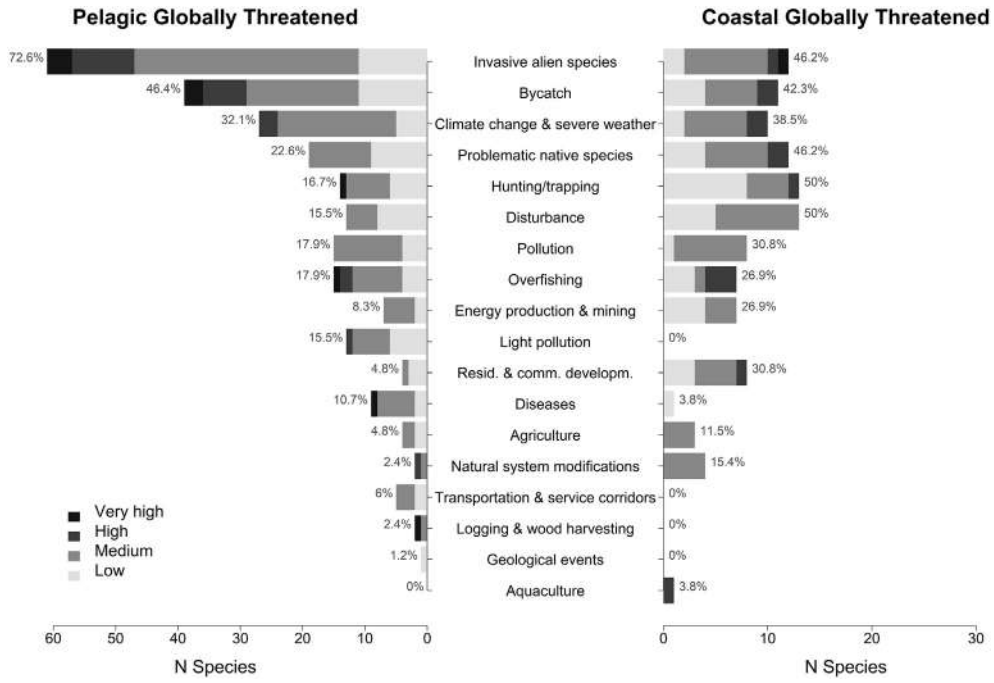

Fig. 3. Ongoing threats to pelagic (n = 84) and coastal (n = 26) globally threatened seabirds; values atop bars indicate percentage of species affected.

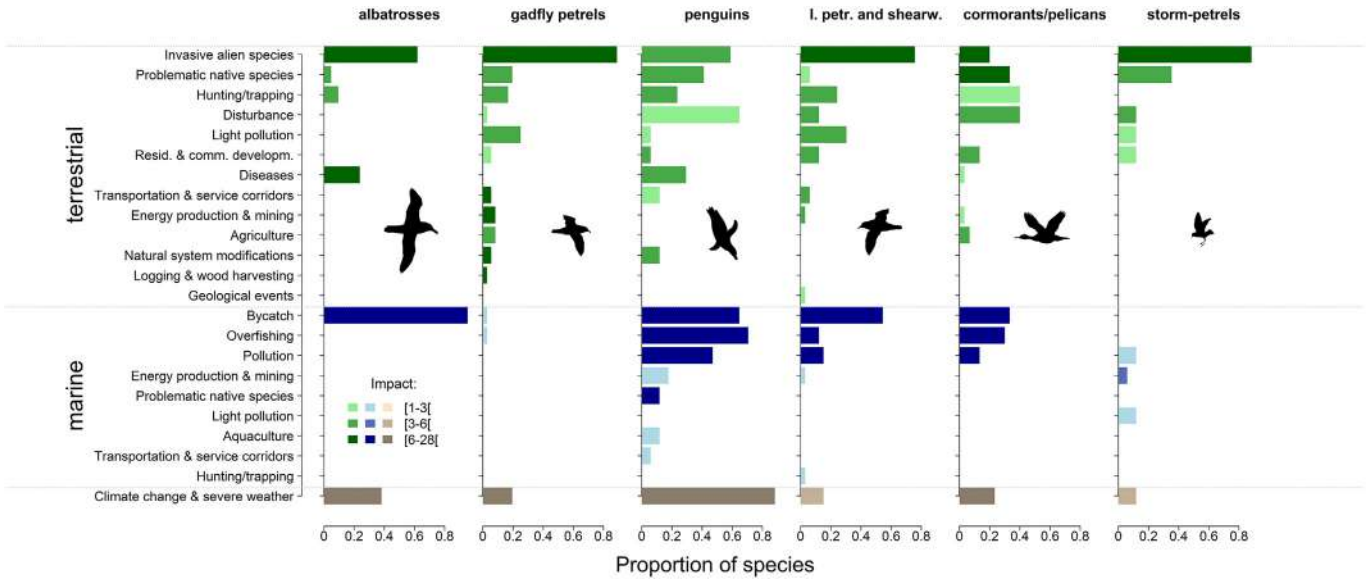

Fig. 4. Main threats (split into marine and terrestrial) by group of seabird species (only groups with > 30% of species classified as globally threatened are shown; see also Fig. A4 in Appendix 3). In column headings, *l. petr. and shearw.* = large petrels and shearwaters.

scope; Fig. 7). Storms/flooding impact > 20 species of seabirds, and with lower scope (Fig. 7).

Species impacted by climate change are also affected by three other threats on average (2.99 ± 0.2; mean ± SE), including invasive alien species (52%), bycatch (43%), and c. 30% by each of overfishing, hunting/trapping and/or pollution (Fig. A5, Appendix 3). For only 11% of seabird species is climate change/severe weather the only threat.

4. Discussion

This is the first comprehensive analysis to use consistent, objective criteria to assess the threats to all 359 seabird species worldwide. We found that invasive alien species, bycatch and climate change/severe weather are the top three threats to seabirds in terms of the number of species affected (165, 100 and 96, respectively; Fig. 1), overall impacts (Table 2), and the estimated total number of individual birds potentially affected (Fig. 2). Hunting/trapping and disturbance also affect many species (97 and 73, i.e. 27% and 20%, respectively), but with a low impact on average; conversely, overfishing has a relatively high impact on fewer species (54, i.e. 15%).

A comparison with the threat assessment made in 2010 is possible for globally threatened species (Croxall et al., 2012), despite minor changes in the list of species, and some differences in methods (e.g. checks for consistency in scoring threats across groups were not made in the previous study). Our results confirm the persistence of top threats such as invasive alien species and climate change/severe weather, which still affect a similar number of species (Fig. 8). Threats related to fishing have increased since the previous assessment, with bycatch now impacting 50 rather than 40, and overfishing 22 rather than 10 globally threatened species (Fig. 8). This is partly due to better understanding of the impacts of gillnet fisheries on seabirds (Žydelis et al., 2009; Crawford et al., 2017), especially coastal species such as sea ducks (Žydelis et al., 2009), including some species which have recently been uplisted to globally threatened (e.g. Long-tailed Duck *Clangula hyemalis*, Horned Grebe *Podiceps auritus*). The relevance of overfishing has also increased, both in pelagic and coastal species (e.g. penguins and cormorants; e.g. Crawford et al., 2015; Trathan et al., 2015). In contrast, the threat from marine pollution has decreased, now affecting 23 rather than 30 globally threatened species. The threat from pollution is largely related to oil spills, a well-known and conspicuous threat to seabirds during the 1970s and 1980s. Oil spill events has decreased greatly in recent decades (Roser, 2018), with a consequent predictable reduction

Table 3
Seabird groups affected by both terrestrial and marine threats (excluding the ones related to the climate change; see methods) with medium, high or very high impact[a], and most frequent interactions (only shown are those affecting > 2 species).

Group	N species with terrestrial and marine threats	Most frequent interactions terrestrial - marine	Number of species affected
Albatrosses	13 (59%)	Invasive alien species - bycatch	10
		Diseases - bycatch	3
Penguins	9 (50%)	Problematic native species - pollution	4
		Invasive alien species - pollution	3
		Problematic native species - overfishing	3
		Hunting/trapping - pollution	3
Auks	11 (46%)	Invasive alien species - pollution	6
		Invasive alien species - bycatch	3
		Disturbance - pollution	3
Large petrels and shearwaters	13 (34%)	Invasive alien species - bycatch	8
		Invasive alien species - overfishing	3
Sea ducks and allies	10 (33%)	Hunting/trapping - pollution	7
		Hunting/trapping - bycatch	4
		Invasive alien species - bycatch	3
Cormorants and pelicans	6 (18%)	Problematic native species - overfishing	3

[a] Only included groups with at least five species with at least one terrestrial and one marine threat.

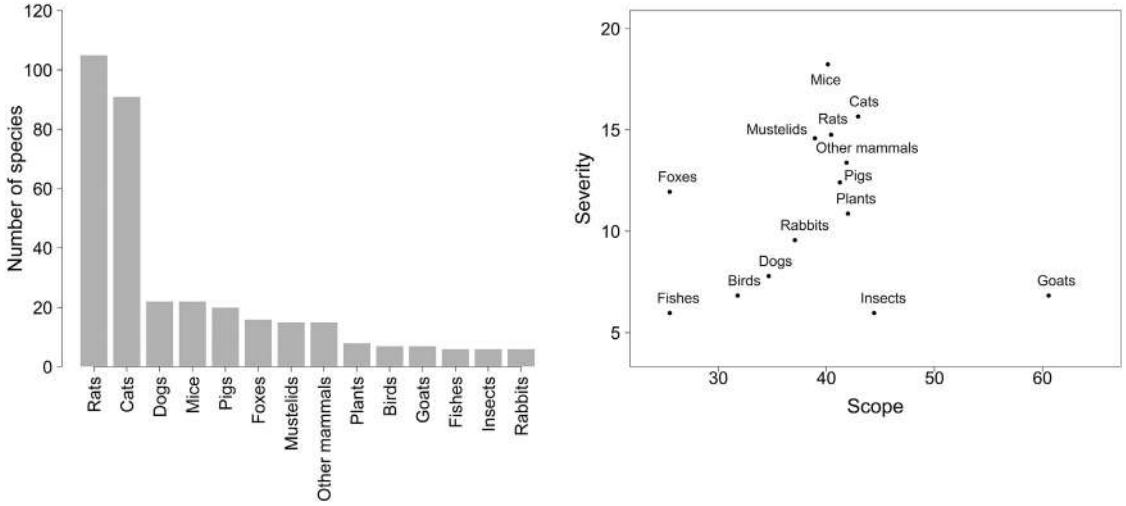

Fig. 5. Left panel: number of seabird species affected by different invasive alien species. Right panel: mean scope and severity of different invasive alien species. Only invasive alien species affecting > 5 seabird species are represented.

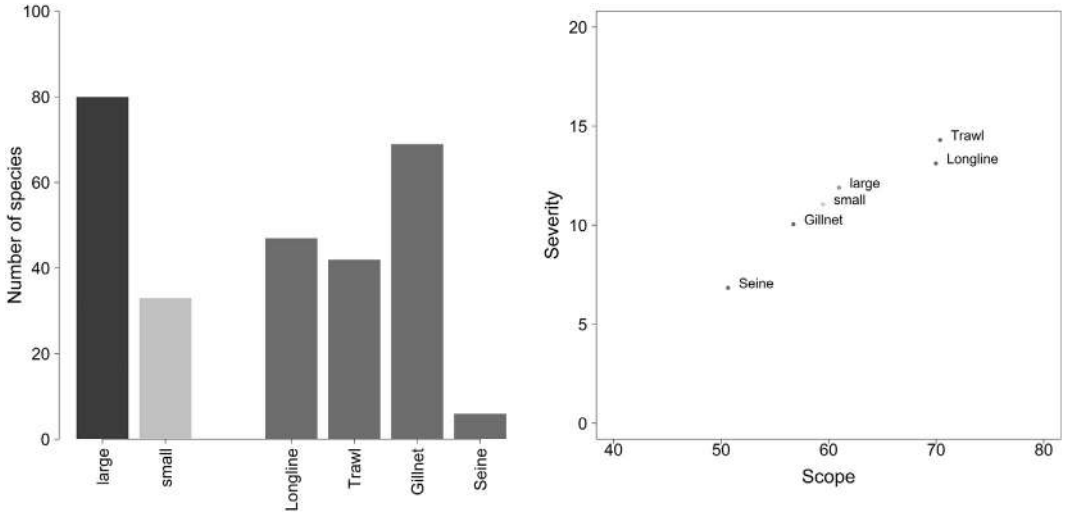

Fig. 6. Left panel: Number of seabird species affected by fisheries (large vs small and different gear types). Right panel: mean scope and severity of large- and small-scale fisheries and of different fishing gear types.

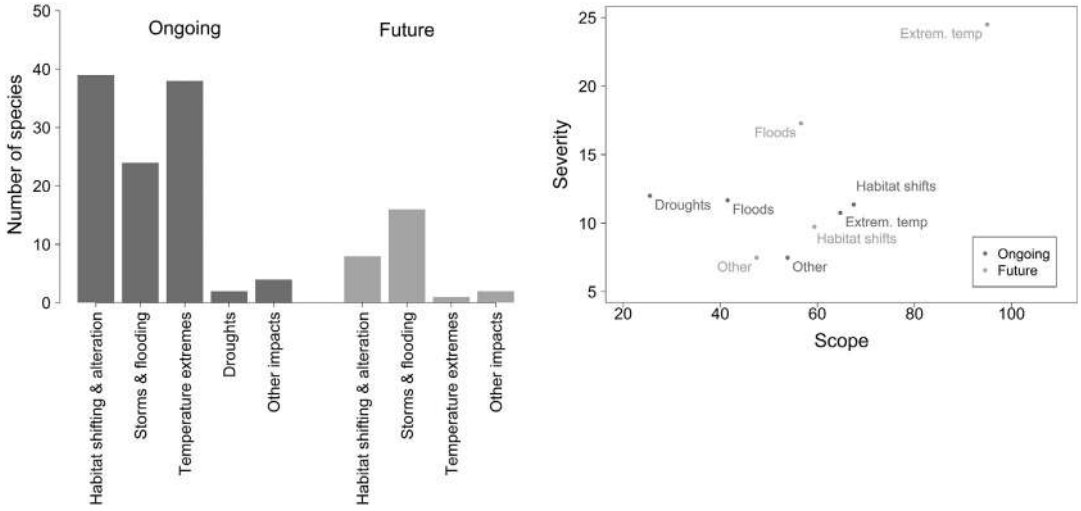

Fig. 7. Left panel: Number of seabird species affected by different "level-2 threats" coded for the threat "climate change/severe weather" (see Table A2.2, Appendix 2). Right panel: mean scope and severity of level 2 threats classified under climate change/severe weather.

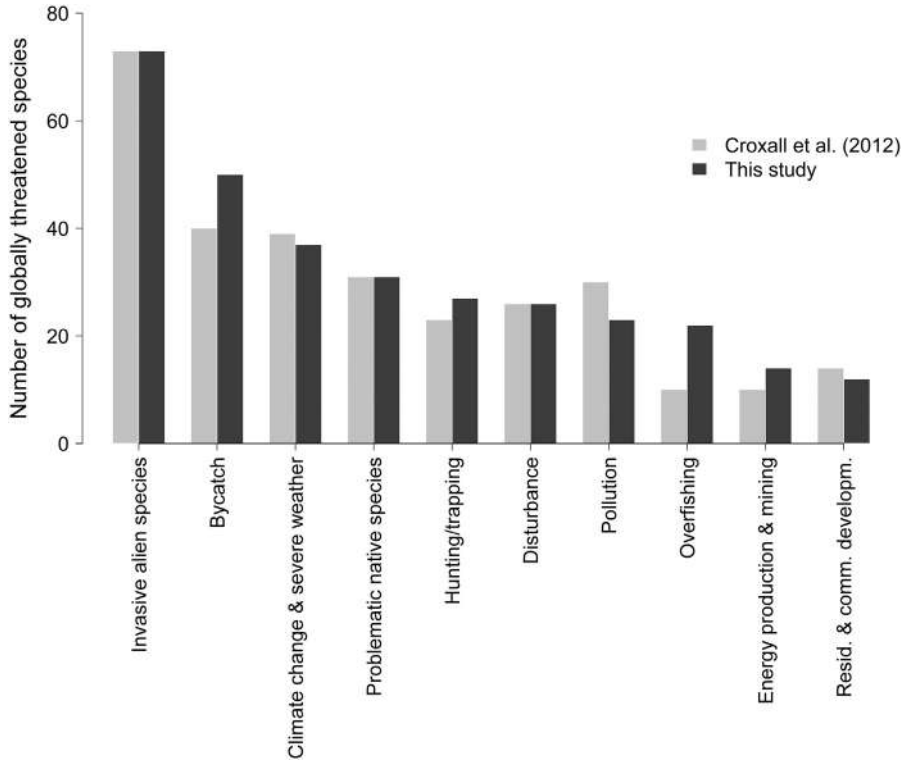

Fig. 8. Comparison between the number of globally threatened seabird species affected by each threat as reported by Croxall et al. (2012) and found in this study. Only threats mentioned in both studies are shown.

of its impact on seabirds when compared with the situation at the end of the last century (Camphuysen, 1998; Clark, 1984).

The top threats currently affecting globally threatened species largely coincide with those affecting NT and LC species with declining trends (Table 2 and Figs. A2 and A3, Appendix 3), which represent one third of all species, and half the total number of individual seabirds. Therefore, tackling the current major problems faced by globally threatened species will also reduce the exposure of hundreds of millions of other (currently non-threatened) seabirds to these threats (Fig. 2).

4.1. Major threats on land

Our study highlighted that invasive alien species, particularly rats and cats, are the major threat to seabird species globally. Therefore, eradication or control of rodents and cats is the major priority in terms of conservation of seabirds at their colonies (Phillips et al., 2016; Spatz et al., 2017; Holmes et al., 2019; Rodríguez et al., 2019) along with enhanced biosecurity measures to prevent re-invasion or new introductions (particularly for sites in proximity to human habitation) and, if necessary and where feasible, post-eradication restoration to provide habitat suitable for recruiting additional seabirds to now-safe sites (Borrelle et al., 2018). The frequent co-occurrence of rats and cats poses an additional challenge in requiring simultaneous eradication (Zavaleta et al., 2001; Rayner et al., 2007).

Hunting/trapping at colonies is the second major threat on land in terms of number of species affected, and the top threat to coastal globally threatened species. This is a well-known issue (Chen et al., 2009; Gaston and Robertson, 2010; Merkel et al., 2014, 2016; Phillips et al., 2016), and needs to be addressed in close collaboration with local communities and authorities. Hunting/trapping can also occur at sea (Bugoni et al., 2008; Alfaro-Shigueto et al., 2016; Frederiksen et al., 2016), although impacts are poorly known (Phillips et al., 2016). Disturbance is also a relevant threat in terms of number of species affected globally, and coastal globally threatened species are particularly affected (Fig. 3). Disturbance of seabirds at their colonies can lead to reduced breeding success (Giese, 1996; Bolduc and Guillemette, 2003; Watson et al., 2014) or even to permanent abandonment of the site (Carney and Sydeman, 1999). The increase of ecotourism activities can pose an additional challenge (Palacios et al., 2018), which is nonetheless solvable by implementing the necessary regulations to control the access to important seabird colonies (Ellenberg et al., 2006).

Other relevant threats on land are light pollution (affecting mostly gadfly petrels, large petrels/shearwaters and storm-petrels; Rodríguez et al., 2017, 2019), problematic native species (especially for cormorants/pelicans, storm-petrels and penguins) and diseases (affecting mostly albatrosses and penguins). These threats also have some known and implementable solutions, such as avoidance or minimization of light sources (especially during fledging periods in high-risk areas; Gineste et al., 2017; Rodríguez et al., 2017), artificial nests for problematic native species competing for nesting burrows (Bolton et al., 2004), and vaccination against diseases in critical cases (Bourret et al., 2018).

4.2. Major threats at sea

We confirmed that bycatch is still a major threat to albatrosses, large petrels/shearwaters and penguins (Trathan et al., 2015; Phillips et al., 2016), and found that large-scale fisheries are driving declines in more than twice as many species as small-scale fisheries (Fig. 6). Although the average impacts (scope and severity) of large and small-scale fisheries seem to be similar, impacts from small-scale fisheries are generally less well-known (Lewison et al., 2004; Chuenpagdee et al., 2006; Soykan et al., 2008). Longline and trawl fisheries involve the gear types with greatest impact in terms of both average severity and scope (especially for albatrosses and large petrels/shearwaters; Tuck et al., 2001; Barbraud et al., 2009).

Many studies have shown that bycatch in longlining and trawl fisheries can be mitigated effectively with the implementation of operational and technical measures. Depending on the characteristics of the fishery, location, season and associated at-risk seabird species,

single measures can be effective, such as discard management or bird-scaring lines on trawl vessels (Bull, 2007; Pierre et al., 2012; Maree et al., 2014; Tamini et al., 2015) and hook-shielding devices in pelagic longline vessels (Sullivan et al., 2018). However, measures used in combination are most effective, such as night setting, bird-scaring lines and weighted branch lines for longline vessels (Brothers et al., 1999; ACAP, 2017a, 2017b, 2017c; Domingo et al., 2017; Paterson et al., 2017).

Many Regional Fisheries Management Organisations (RFMOs), and some national fisheries bodies in areas with high bycatch rates, have adopted regulations that seek to minimize bycatch (Anderson et al., 2011; Gilman, 2011; Phillips et al., 2016). The challenge, however, is ensuring practical implementation of the measures and compliance with the regulations, which requires industry-specific solutions and support to ensure validity of the measures and avoid cross-taxa effects (Gilman et al., 2005; Melvin et al., 2019). Gillnet fisheries are thought to affect more species (Fig. 6), especially diving seabirds such as sea ducks and auks (Žydelis et al., 2009). However, the magnitude of the impact of gillnet fisheries on seabird species is still poorly understood (Žydelis et al., 2013; Bærum et al., 2019). Furthermore, and in contrast with the situation for the fishing gears mentioned above, solutions for gillnet bycatch remain elusive and should be regarded as research priorities (but see Mangel et al., 2018; Melvin et al., 1999).

Overfishing is also a top marine threat. It affects fewer species than other top threats, but with considerably greater impact (Figs. 1, 2, Table 2). Overfishing is the main cause of decline of 24 species (e.g. African Penguin *Spheniscus demersus,* Cape Gannet *Morus capensis* and Cape Cormorant *Phalacrocorax capensis*; Pichegru et al., 2010; Crawford et al., 2015; Grémillet et al., 2016) and is often associated with bycatch (> 60% of the species impacted by overfishing are also affected by bycatch). Tackling the problem of overfishing may involve the creation of Marine Protected Areas (Hyrenbach et al., 2000; Lascelles et al., 2012), including no-take zones (seasonal or permanent) in some critical cases (Daunt et al., 2008; Pichegru et al., 2010). However, it chiefly requires the effective implementation of ecosystem-based management of forage-fisheries within the context of wider, multi-stakeholder Marine Spatial Planning (Ardron et al., 2008). Nevertheless, results from recent analysis suggest that we are still failing to implement such management measures and mitigate the impacts of overfishing, as the global catch of fisheries competing with seabirds synoptically increased during the last four decades (Grémillet et al., 2018). We might be also underestimating the magnitude of this problem, given that it has received considerably less attention than other global issues such as climate change (the effects of which are often difficult to disentangle from overfishing; Grémillet and Boulinier, 2009; Sydeman et al., 2012).

4.3. Scope and scale of management approaches

Many seabird species are impacted by multiple threats (three, on average), which can have cumulative impacts on the populations. Several species (72, including 38 globally threatened species and 20 NT) have at least one marine and one terrestrial threat of medium or higher impact. For example, 27 species are impacted by both invasive alien species and bycatch (particularly albatrosses and large petrels/shearwaters, but also some auks and sea ducks; Table 3). Half of the penguin and auk species face a terrestrial and a marine threat with a medium to very high impact (usually invasive alien species or problematic native species and pollution; Table 3). Gadfly petrels and storm-petrels are two notable exceptions to this pattern, as all major threats are land-based (Fig. 4), although this may also reflect the difficulty in assessing at-sea threats to these highly pelagic and nocturnal species (Ramírez et al., 2016; Ramos et al., 2017), whose foraging areas are only now being revealed (e.g. Ramírez et al., 2013; Ramos et al., 2017; Rayner et al., 2008, 2012).

The co-occurrence of medium or high impact terrestrial and marine threats emphasises the need for “ridge to reef” approaches (Rude et al., 2016; IUCN, 2018), whereby management plans aiming to protect seabird species and their habitats should necessarily include measures to address threats both on land and at sea. The appropriate measures at sea depend on the species and the relevant spatial scales of their foraging ranges: whereas short-ranging species such as cormorants and some penguin species benefit most from site-based forms of protection (e.g. well-managed Marine Protected Areas), wide-ranging species such as albatrosses, petrels and shearwaters will also require measures at the larger scale (even Large Marine Ecosystem; Sherman et al., 2003), particularly in relation to effective fisheries management, notably bycatch regulations (Oppel et al., 2018).

4.4. Climate change

Most of the top threats already mentioned (invasive alien species, bycatch, hunting/trapping, disturbance and overfishing) have known and tested solutions, at least in principle and in part. Climate change/severe weather are different in that there is limited prospect of direct mitigation of most of the main known or potential impacts. These include changes in oceanographic processes (resulting in declining in food availability around colonies), increased frequency of extreme weather events, inundation of colonies due to sea level rise or severe rainfall storms, or increased occurrence and virulence of avian pathogens (reviewed by Grémillet and Boulinier, 2009; Barbraud et al., 2012; Sydeman et al., 2012; Phillips et al., 2016). Translocations (and managed retreat) are a possibility in some cases (Deguchi et al., 2014; Miskelly et al., 2009), but challenging to execute for many species, due to the high costs and logistical difficulties.

Nevertheless, we show that most species (89%) affected by climate change/severe weather are also affected by other threats (3.37 ± 0.2 threats on average ± SE), whose impacts are of the same order of magnitude. The most frequent threats co-occurring with climate change/severe weather are invasive alien species, bycatch, overfishing and hunting/trapping (Fig. A5, Appendix 3). This emphasises the crucial importance of addressing effectively these other major threats in order to compensate for the negative impacts of climate change.

4.5. Emerging threats

The problem of marine plastics, which is global and increasing (Ryan et al., 2009; Kühn et al., 2015), and expected to affect virtually all seabird species in a few decades (Wilcox et al., 2015), is not yet identified as a cause of seabird population declines, with only one report so far of plastics causing a significant impact at this level (Fleshfooted Shearwater *Ardenna carneipes*; Lavers et al., 2014). This threat is predicted to have a higher impact on small, highly pelagic species (such as gadfly petrels, storm-petrels, prions and auklets) (Wilcox et al., 2015; Lavers and Bond, 2016; Roman et al., 2019), whose population sizes, demography and at-sea movements are poorly known in many cases, indicating the difficulty in understanding the real impact of plastics at population levels. However, this problem is recent and so a delay would be expected before population impacts become evident for long-lived species, such as most seabirds.

The occurrence and virulence of avian pathogens is also likely to increase, especially at high latitudes, due to the enhanced spread of ectoparasites as a consequence of a warmer climate and to increasing human presence at seabird colonies (Grémillet and Boulinier, 2009; Uhart et al., 2018).

Offshore wind farming (classified here as “Energy production & mining”) is another fast-growing issue with potential high impacts on seabirds (Furness et al., 2013), but still with limited information regarding the consequences for seabirds at the population level (Green et al., 2016). This threat is expected to affect mostly coastal species such as divers, scoters, terns and shags (Garthe and Hüppop, 2004), especially via displacement (Furness et al., 2013; Cook et al., 2018). However, highly mobile species can also be at particular risk, due to the

cumulative impact resultant from multiple windfarms located across the species distributional range (Busch and Garthe, 2018).

Finally, we anticipate that in a few decades overfishing may become an even more widespread and serious problem for seabirds, including even the more pelagic species. The number of globally threatened species affected by overfishing has more than doubled since the previous assessment based on data collected up to 2010 (Croxall et al., 2012). Depletion of food resources is already regarded as the major cause of decline of 24 species (Table 2), and pressures on stocks of currently exploited coastal forage-fish species are certain to intensify, to the likely detriment of seabirds (Grémillet et al., 2018). In addition, this problem has the potential to increase with the transition of more fisheries to lower trophic levels (Pauly et al., 1998) especially those targeting mesopelagic species (St. John et al., 2016). Mesopelagic fishes, an important part of the diet of many pelagic seabirds, particularly many small nocturnal petrels (Dias et al., 2015; Waap et al., 2017; Watanuki and Thiebot, 2018), are the most abundant marine vertebrates (Irigoien et al., 2014) and remain largely unexploited commercially due to the currently low profitability of fishing deep-water species, especially on the high-seas (St. John et al., 2016; Webb et al., 2010). This situation may soon change due to investment in new fishing technologies, along with the increasing demand for these resources from the aquaculture industry (St. John et al., 2016), with potentially serious implications for their current natural consumers (including seabirds). Despite the difficulty in understanding the complex interactions between seabirds, prey abundance, oceanographic conditions and fisheries, recent studies have shown the detrimental impacts of prey removal on the demography of some species, and highlight the need for defining management objectives for the marine environment that ensure sufficient biomass of forage fish to support seabird communities (Barbraud et al., 2018).

5. Conclusions

Our analysis shows that invasive alien species, bycatch and climate change are the top three threats affecting seabirds globally. Together these threats affect two-thirds of seabird species and hundreds of millions of individuals. Other top threats include overfishing, which affects comparatively few species but with higher impacts, and hunting/trapping and disturbance that affect many species, but with lower impacts. The relative importance of these top threats was largely consistent across different taxonomic groups of seabirds, and when considering only globally threatened species or only NT and declining LC species. These results are also in line with conclusions of some recent threat assessments of well-studied groups of seabirds, such as penguins, albatrosses and petrels (Trathan et al., 2015; Phillips et al., 2016; Rodríguez et al., 2019).

Multiple threats often affect the same species; consequently: a) management approaches tackling simultaneously both marine and terrestrial threats are essential to reverse the declining trend of numerous threatened seabird species; b) the negative effects of climate change can be greatly alleviated by addressing other top threats, for which implementable proven solutions are largely available. However, even for threats with well-known solutions, such as invasive alien species, bycatch and overfishing, there are substantial challenges to overcome. For invasive alien species, many of the priority eradications (Holmes et al., 2019) for islands uninhabited by humans have been completed. Therefore, the focus will increasingly shift to islands with human populations and to mainland areas, both posing substantial problems in relation to mortality of non-target species and control of invasive alien species (as opposed to rapid eradication), which likely require complex, long-term and costly initiatives, even where inherently feasible (Phillips, 2010; Oppel et al., 2011).

Technical solutions to gillnet bycatch have proven hard to develop; in most longline and trawl fisheries compliance with recommended mitigation regulations remains limited (Phillips et al., 2016). Use of remote-recording electronic devices to monitor compliance may be essential to making progress. Seabirds are increasingly impacted by overfishing, especially coastal species. Theoretically, effective ecosystem-based management of marine resources around important seabird colonies can mitigate problems for these particular species, but also requires that effective plans and processes are in place to monitor and enforce compliance. Such potentially effective management systems remain elusive (except at very small scales) anywhere in the world, even within the Economic Exclusive Zones of most developed countries. They are conspicuously absent from the high seas, and radical reform of the RFMOs responsible for such management as does exist is long overdue. Furthermore, the potential detrimental impacts of illegal fishing (e.g. Ortuño Crespo and Dunn, 2017; Petrossian et al., 2018) and of the risk to seabirds of changes in traditional fisheries practices to new models (e.g. reduction fisheries; Smith et al., 2011; balanced harvest; Burgess et al., 2016; Kolding et al., 2016) are practically unknown, and should be a priority area for future research.

Given the continuing deterioration in the conservation status of seabirds, and the increased number and severity of threats confronting them, there is an urgent need to identify and implement practical action to tackle threats to species and sites where feasibility and priority coincide. As seabirds are amongst the best indicators of the status of marine systems, the outlook for the global oceans is not encouraging. However, progress in addressing pollution (Roser, 2018), invasive alien species (Jones et al., 2016) and bycatch (Maree et al., 2014) shows what can be done; effective management of threats in key areas on land and at sea is now the great challenge.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2019.06.033.

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THE MOVEMENT FOR BLACK LIVES

Fossil Fuel Racism

How Phasing Out Oil, Gas, and Coal Can Protect Communities

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Executive Summary

Fossil fuels — coal, oil, and gas — lie at the heart of the crises we face, including public health, racial injustice, and climate change. This report synthesizes existing research and provides new analysis that finds that the fossil fuel industry contributes to public health harms that kill hundreds of thousands of people in the U.S. each year and disproportionately endanger Black, Brown, Indigenous, and poor communities. President Joe Biden and the 117th Congress have a historic opportunity to improve public health, tackle the climate crisis, and confront systemic racism at the same time by phasing out fossil fuel production and use.

Each stage of the life cycles of oil, gas, and coal — extraction, processing, transport, and combustion — generates toxic air and water pollution, as well as greenhouse gas emissions that drive the global climate crisis. Exposure to fossil fuel pollution is linked to negative health impacts for people living near these pollution sources. The impacts of climate change are also strongly linked with rising health risks and threaten the ability of humans to live in various regions of the planet. The public health hazards from air and water pollution, and risks associated with climate change, fall disproportionately on Black, Latinx, Indigenous, Asian, and poor communities.

In addition to the accumulated negative impact on human health and the environment, fossil fuels depend on and contribute to the legacy of systemic racism in the United States. Oil, gas, and coal activity in the United States takes place on the ancestral lands of Indigenous peoples, making the fossil fuel industry complicit beneficiaries of the forced removal and genocide of Indigenous peoples. Racist practices such as redlining and housing discrimination, longstanding social and racial inequalities, colonization, Indigenous genocide and removal, and elected officials who are beholden to corporate power all combine to create a system in which the most dangerous impacts of pollution fall most heavily on the most disadvantaged, particularly Black, Brown, Indigenous, and poor communities.

More recently, the COVID-19 pandemic has exacerbated the disproportionate burden shouldered by these ***historically targeted communities*** across the United States, including by magnifying the health risks of air pollution from fossil fuels.

Tackling this ***fossil fuel racism*** would go a long way to address our present overlapping crises and correct the injustices that historically targeted communities have faced. A ***fossil fuel phaseout*** — an immediate halt to new extraction and infrastructure build-out and managed wind-down of existing production that prioritizes the needs of affected workers and communities — is necessary to end fossil fuel racism and fully address the public health, racial injustice, and climate crises.

Research shows that phasing out fossil fuels will deliver immediate public health improvements to historically targeted communities. Furthermore, any serious policy to address the climate crisis will inevitably require rapid reductions in fossil fuel consumption and production. But, policies that narrowly focus on carbon reductions without considering the full effects of greenhouse gas-emitting sources could fail to reduce local air and water pollution and end up perpetuating the racially inequitable impacts of the fossil fuel economy. Poorly designed climate policies could exacerbate pollution disparities and concentrate pollution in community "hotspots" even as overall carbon emissions decline. A fossil fuel phaseout would ensure air and water pollution as well as greenhouse gas emissions are reduced as part of a comprehensive response to the climate crisis.

President Joe Biden, the 117th Congress, and the nation have a mandate to transform our economy to repair past injustices and build a better future in which all communities can thrive. This decade will be crucial for putting our economy on a climate-safe trajectory, and the next year is a critical window for action to put us on the right track. President Biden can fulfill his promises to

confront systemic racism, tackle the climate crisis, and Build Back Better by initiating a fossil fuel phaseout.

This report gives an overview of recent scientific studies and analyses of the harms due to fossil fuels and their disproportionate impact on Black, Brown, Indigenous, and poor communities in the United States. The report also contributes new analysis of the disproportionate impacts of toxic pollution from petroleum refineries and petrochemical facilities on Black, Brown, and poor communities. The report reviews impacts from each phase of the fossil fuel life cycle — extraction, processing, transport, combustion — and climate change impacts.

Key findings include:

Burning fossil fuels harms public health, disproportionately impacting Black, Brown, Indigenous, and poor communities:

- Harmful air pollution from the burning of fossil fuels (especially particulate matter and ground-level ozone) is a well-established health threat associated with respiratory and cardiovascular risks and premature mortality.[1]
- In 2018 in the United States, there were roughly 355,000 premature deaths due to fossil fuel-linked air pollution,[2] carrying an economic cost of hundreds of billions of dollars.
- People of color, especially Black/African-American people, and poor people are found to bear a disproportionately high burden of fossil fuel pollution across the United States. Black/African-American people have 1.54 times the exposure to particulate matter compared to the overall population.[3]
- Key sources for particulate pollution include coal-fired power plants[4] and vehicle transportation,[5] each of which disproportionately impacts Black, Brown, Indigenous, and poor communities.

Oil and gas extraction releases hazardous air pollutants that disproportionately burden Black, Brown, Indigenous, and poor communities:

- The extraction and processing of oil and gas is associated with emissions of a wide range of hazardous air pollutants, including carcinogens such as benzene and endocrine disruptors.[6]
- Nationally, 17.6 million people live within one mile of an active oil or gas well[7] — more than the populations of New York City, Los Angeles, Chicago, and Houston combined. More than 6.1 million people live within three miles of an oil and gas refinery.[8]
- Emerging scientific studies have linked exposure to oil and gas activity with negative health impacts including elevated cancer risk,[9] pregnancy complications, and respiratory and cardiovascular disease.[10]
- New analysis of the Environmental Protection Agency's (EPA) Toxics Release Inventory finds that oil refineries and petrochemical facilities (which provide key inputs for plastics production) are among the worst polluting sectors of the economy, and that the toxic burden of those sectors falls disproportionately on Black, Brown, Indigenous, and poor communities.[11]
- Although findings vary by region, oil and gas extraction is also found to have disproportionate impacts on people of color, especially Black/African-American people.[12]

Marshall Steam Station. *Smoke billowing from the chimney at the Marshall Steam Station in Catawba County, North Carolina. Operating since 1965, this coal-fired power station has a 2,090-megawatt generating capacity and emitted 11.5 million tons of carbon dioxide in 2011.*

Phasing out fossil fuels would immediately improve public health, tackle the climate crisis, and address historical injustices.

- Many of the same communities dealing with the toxic legacy of fossil fuels are also on the front lines of rising climate impacts. Studies have shown that extreme heat in urban areas puts Black, Brown, Indigenous, and poor communities at disproportionate risk of premature deaths and birth complications.[13]
- Recent studies reveal a relationship between racist policies of the past, such as redlining and housing discrimination, and pollution and climate impacts such as extreme heat,[14] asthma,[15] and flooding.
- The COVID-19 pandemic has exacerbated the disproportionate impacts of fossil fuel pollution. Black/African-American and other people of color have been more likely to die from the disease. Preliminary science indicates that long-standing inequalities in exposure to air pollution could be an especially deadly risk factor for COVID19.
- Some studies show that restoration — undoing the public health harms of fossil fuel pollution — is possible. By taking advantage of "natural experiments" when refineries and power plants are retired, researchers have found measurable improvements in the health of nearby communities.
- Policies to address climate change by phasing out fossil fuels can have immediate local benefits to public health and lower the upfront costs of climate action.

Local and regional case studies of fossil fuel production in highly impacted areas shed greater light on the dangers to nearby communities:

- In south Texas, fracking wastewater wells[16] and oil and gas flares[17] disproportionately harm Latinx communities. Latina women in the Eagle Ford shale — the site of a major fracking boom — face significantly higher risk of giving birth prematurely.[18]
- If completed, the proposed Formosa petrochemical plant in St. James, Louisiana, would likely double toxic air pollution (including carcinogenic benzene and ethylene oxide) in the region, which already bears the nickname "Cancer Alley" following decades of industrial pollution.
- Closing an oil refinery in the Toronto, Canada, suburb of Oakville eliminated 6,000 tons of sulfur dioxide pollution per year and helped reduce hospitalizations for respiratory illnesses in the area.[19]
- In Richmond, California — a predominantly Black and Latinx city in the San Francisco Bay Area — emergency room visits increased sevenfold after a major fire at Chevron's Richmond Refinery in 2012.[20]
- After a series of coal and oil power plants were closed across California in the early 2000s, researchers found a significant decline in preterm births for women living in nearby communities.[21]

President Biden and Congress must confront the overlapping crises we face with a holistic approach to improve public

Explosive Water from Fracking in Pennsylvania. *Sherrie Vargson ignites the water coming out of her kitchen faucet in Bradford County, Pennsylvania. Methane in her well has caused her health problems. The well is just 100 feet from her house.*

health, tackle the climate crisis, and rectify our legacy of systemic racism by phasing out fossil fuel production. Our policy recommendations are rooted in Greenpeace USA's *Just Recovery Agenda* and the Movement for Black Lives' *Red, Black, and Green New Deal* platform. A full list of policy recommendations can be found in Chapter 8. In brief, to truly Build Back Better, President Biden and Congress must:

1. **End fossil fuel racism and reverse the legacies of historical injustices.** Require air and water pollution reductions in environmental justice communities with a "No Hotspots" policy and policies to mitigate cumulative pollution impacts. Pass the *Environmental Justice For All Act* and *Climate Equity Act*, and institutionalize Free, Prior, and Informed Consent regarding federal actions affecting the lands, livelihoods, culture, and spirituality of Indigenous peoples.
2. **Phase out fossil fuel production.** Pass the *End Polluter Welfare Act* to eliminate federal fossil fuel subsidies, halt new fossil fuel leases and permits on public lands and waters, and reject new federal fossil fuel infrastructure permits. Pass the *Break Free from Plastic Pollution Act*. Reinstate the crude oil export ban and prohibit exports of liquified natural gas and coal.
3. **Ensure no worker or community is left behind.** Pass the *Protecting the Right to Organize (PRO) Act* to protect and expand the right of workers to organize. Mandate the use of project labor agreements, Davis-Bacon prevailing wage requirements, community benefit agreements, and high-road labor standards in any federally funded project. Create 250,00 high-quality job-years under a federal oil and gas well remediation program. Establish a federal Energy Worker and Community Protection Fund to guarantee wage, benefit, and pension protections for workers affected by the transition away from fossil fuels, replace lost tax and community revenue, and promote community revitalization and economic development.
4. **Enact a green and just economic recovery.** Pass the *THRIVE Act* to invest at least $1 trillion per year for the next decade to create 15 million good jobs, cut climate pollution in half by 2030, ensure at least 50% of new investments directly benefit frontline and disadvantaged communities, ensure federal investments pass a rigorous environmental justice and equity screen to avoid exacerbating "pollution hotspots," and uphold rigorous labor, climate, and equity standards.
5. **Protect and expand our democracy to make it work for all people.** Pass the *For the People Act* (H.R.1 / S.1) and *John Lewis Voting Rights Act* (H.R. 4) to give more electoral power to the people and protect against racist voter suppression tactics. End the Jim Crow filibuster. Enact the *BREATHE Act* to protect communities from police brutality and racial injustice by investing in Black communities and re-imagining community safety.

Systemic racism and white supremacy have plagued the United States since colonization and have influenced the development of all aspects of society and law, including education, health, work, housing, policing, and more. Our energy systems are no different. The urgency of the interlocking crises we face today gives us an opening to build a better system based on science and justice.

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Playground and Chemical Plant in Louisiana. *Playground built by Shell. Chemical plant is visible in the background.*

Introduction

President Joe Biden and the 117th Congress are confronting multiple overlapping crises, which offer an opportunity and duty to significantly re-orient our economy to improve public health, repair past injustices, combat the climate crisis, and build a better future for all. According to the Intergovernmental Panel on Climate Change (IPCC), the world must cut its carbon dioxide (CO_2) emissions in half this decade in order to have a chance at limiting warming to 1.5°C.[22] The COVID-19 pandemic and unprecedented economic crisis have thrust relief and recovery to the forefront of governmental agendas. The pandemic has further exposed the myriad ways in which the ongoing climate, public health, air pollution, housing, economic, and systemic racism crises intersect and bring harm to historically targeted Black, Brown, Indigenous, and poor communities.

Fossil fuels — oil, gas, and coal — lie at the center of these overlapping crises. Each stage of the life cycles of fossil fuels — extraction, processing, transport, and combustion — generates toxic air and water pollution, as well as greenhouse gas (GHG) emissions that drive the global climate crisis.[23] Decades of research have established that the air pollution and climate change impacts of fossil fuels harm public health and fall disproportionately on Black, Latinx, Indigenous, Asian, and other communities of color and poor communities.

Fossil fuel corporations also depend on and contribute to the legacy of systemic racism in the United States.[24] Racist practices such as redlining and housing discrimination, long-standing social and racial inequalities, colonization, Indigenous genocide and removal, and elected officials who are beholden to corporate power all combine to create a system where the most dangerous impacts of fossil fuel pollution fall most heavily on the most historically targeted communities.

This is ***fossil fuel racism*** in action.

Understanding the fossil fuel industry's deep role in perpetuating racial injustice, public health harms, and the climate crisis is essential to advancing the most effective approach to mitigating the defining challenges of our time. Any comprehensive plan to address our present overlapping crises must include a ***fossil fuel phaseout***: a swift halt to new extraction and a managed wind-down of existing production in a way that prioritizes the needs of workers and communities.

Effective action to combat the global climate crisis will necessarily require sharp reductions in fossil fuel production and consumption in the coming decades. Policies to mitigate climate change should not be judged solely by the metric of carbon emissions, but also by their reductions in air and water pollution and their restoration of communities harmed by decades of industrial pollution. A fossil fuel phaseout would help ensure that efforts to tackle the climate crisis also maximize public health benefits at all stages of our energy systems, and begin to rectify historical injustices.

This report provides a new comprehensive synthesis of the fossil fuel industry's embedded contributions to our present crises, with a focus on the harmful and unequal impacts of deadly air pollution.

Chapter 1 opens with a brief overview of the environmental justice (EJ) movement — the beating heart of this nation's struggle to correct a legacy of environmental racism. *Chapter 2* lays out the primary air pollutants associated with fossil fuels and discusses the data and types of scientific analyses that investigate their prevalence and effects. *Chapter 3* further discusses the concept of *fossil fuel phaseout*, and argues that this is a necessary approach to tackling the climate crisis and racial injustice in tandem.

In *Chapters 4-7*, we review recent scientific studies and analyses of the harms fossil fuels pose to public health, and their disproportionate impacts on Black, Brown, Indigenous, and poor communities in the United States. We also contribute new analysis of the disproportionate impacts of toxic pollution from petroleum refineries and petrochemical facilities on Black, Brown, and poor communities. These chapters are organized to review impacts at each stage of the fossil fuel lifecycle:

- **Extraction**, including oil and gas drilling and fracking, and coal mining;
- **Processing & Transport**, including oil refining, natural gas processing, petrochemical manufacturing, pipelines, and terminals;
- **Combustion**, including point sources such as power plants, industrial facilities, and mobile sources; and
- **Climate Impacts**, due specifically to greenhouse gas emissions associated with all parts of the fossil fuel lifecycle.

Finally, *Chapter 8* provides detailed policy recommendations for President Joe Biden and the 117th Congress. To truly Build Back Better and address public health, systemic racism, and the climate crisis together, President Biden and Congress must:

1. End fossil fuel racism and reverse the legacies of historical injustices.
2. Phase out fossil fuel production.
3. Ensure no worker or community is left behind.
4. Enact a green and just economic recovery.
5. Protect and expand our democracy to make it work for all people.

Shell Refinery in Louisiana. *Clara Smith, 96, stands at the window of her home looking out at Shell refinery just a couple of yards (meters) away.*

1. Environmental Justice

The unequal distribution of pollution has long been recognized by Black, Indigenous, Latinx, Asian, and other communities of color and poor communities across the United States, many of which organized to protect themselves and stop corporate dumping.[25] Community protests in 1982 opposing plans to create a landfill for PCB-contaminated soil in majority-Black Warren County, NC, are often cited as the birth of the Environmental Justice movement. A landmark 1987 study released by the United Church of Christ Commission for Racial Justice was the first to correlate hazardous waste sites with race.[26] Its publication brought the terms *environmental racism* and *environmental justice* (EJ) into the national conversation.

Today, after deep thought leadership from Dr. Robert Bullard, Dr. Beverly Wright, and other scholars, environmental justice is well established as a key framework for understanding environmental goals and policy.[27] Environmental Justice also comprises a social movement directly led by people living on the front lines of industrial activities who face the consequences of racism, consolidated corporate power, and pollution everyday.[28] These ***historically targeted communities*** are predominantly Black/African-American, Latinx, Indigenous, Asian, and/or poor. The EJ movement has forced national leaders and the public at large to place greater focus on the ways in which race and class intersect with our capitalist economy and political systems, and has challenged the structure, funding, and priorities of largely white-led environmental organizations.

During the 2020 campaign, combating environmental racism became a required component of many Democratic presidential candidates' climate platforms. Joe Biden's campaign pledged to make it a priority to "engage in community-driven approaches to develop solutions for environmental injustices affecting communities of color, low-income, and indigenous communities."[29] Yet, despite the growing power of the EJ movement, local pollution risks remain a persistent problem and communities all across the United States and around the world are still fighting to end pollution in their neighborhoods.[30]

Sophisticated analyses of environmental racism have not always translated into concrete policy action, often because EJ communities are denied access to the political and economic power needed to rein in corporate polluters and enact people-first solutions. At the same time, policymakers often become beholden to corporate influence instead of the will of the voters who elect them. Indeed, gerrymandering and laws designed to suppress voter turnout systematically reduce the political influence of many of these communities.[31]

Fossil fuel corporations are key contributors to environmental injustice, as this report demonstrates, but EJ issues go beyond fossil fuels. Many early EJ fights and analyses were focused on incinerators, landfills, and hazardous waste sites. Industrial agriculture and heavy industries such as metal manufacturing have also been the focus of community action. More recently, the Flint, Michigan, water crisis has underscored the toxic legacy of lead in the environment, whether it is found in water pipes, contaminated soil, or paint in old buildings. The EJ concept extends as broadly as the definition of the environment itself, encompassing disparities in access to nature, access to housing and transit, noise and light pollution, and more.

Amidst these myriad environmental justice issues, fossil fuels remain a toxic presence in modern life. Our current intersecting crises arise out of a legacy of systemic racism from which fossil fuel corporations benefit and to which they contribute. The geography of housing and economic activity in the United States has been profoundly shaped

Toxic Patrol in Baton Rouge, Louisiana. *A Greenpeace "toxic patrol," led by Damu Smith, protests against the toxics industry with a banner reading "Environmental Justice Now," which is displayed along with other banners as the activists protest along the street.*

by past practices of redlining (the practice, technically outlawed in 1968, of concentrating Black/African-American and other minority homeowners in certain areas by refusing housing loans)[32], housing discrimination, and job discrimination. The U.S. health system is also plagued by many of the same concerns about systemic racism and inequality.[33] Nationally, Black/African-American people are 40 percent more likely to have asthma and are almost three times more likely to die of "asthma-related causes" than the non-Hispanic white population.[34]

Oil, gas, and coal activity in the United States takes place on the ancestral lands of Indigenous peoples, making the fossil fuel industry complicit beneficiaries of the forced removal and genocide of Indigenous peoples. The first U.S. oil well, the Drake Well, was drilled in 1859 on ancestral Haudenosaunee (Iroquois) territory[35] near Titusville, Pennsylvania, by a company ironically named the Seneca Oil Company.[36]

By its nature, this summary is a reductive look at the public health impacts of fossil fuel-related air pollution and, by itself, does not deeply explore the ways in which these issues overlap and intersect with each other and other systems such as housing, employment, health care, immigration, and more. For example, the report does not fully explore ways in which the geography of fossil fuel extraction intersects with the history of colonization and Indigenous genocide and removal, including means by which those injustices have led to disparate impacts, harm to sacred places, and cultural and spiritual health harm.

Analyses to date have tended to take a piecemeal approach to examining the role of the coal, oil, and gas industries in perpetuating pollution, climate change, and environmental injustice. This report seeks to contribute to the literature by providing a comprehensive overview of the health impacts and disproportionate burdens of air pollution and climate impacts of fossil fuels at each stage of the coal, oil, and gas lifecycles.

There is robust evidence, across a wide variety of contexts and scales, that polluting industries impose health burdens on their neighbors and that these risks are generally higher for Black, Brown, Indigenous, and poor communities.[37,38,39,40,41] Many of these studies have examined cumulative or aggregate impacts; some studies have also summarized health and distributive justice information along fossil fuel supply chains.[42,43,44] This report synthesizes a wide range of recent scientific studies to paint an integrated picture of the fossil fuel industry's role in modern society and its legacy of racial, environmental, and economic injustice.

This report is focused on the U.S. context, but many of the same issues arise across the globe.[45] Indeed, the history of the oil industry is closely intertwined with the history of colonialism, war, and global geopolitics.[46,47,48] Energy and industrial systems are global in nature, and as fossil fuel supply chains stretch across national borders, so too do the harms that result from that activity.[49] With the increasing focus on climate change, the concept of climate justice, along both global and generational axes, has also come to the forefront.

The COVID-19 pandemic has also exposed the stakes of inequality, as Black/African American people and other people of color have been more likely to die from the disease.[50] Although scientific studies have only begun to probe connections between oil and gas development, air pollution, systemic racism, and COVID-19, there are preliminary indications that the mix of factors could be especially deadly.[51,52] One preliminary study found that even small increases in long-term air pollution exposure were associated with higher COVID-19 death rates.[53] As we discuss in detail in this report, Black, Brown, Indigenous, and poor communities have been exposed to higher levels of pollution for decades, in many cases reflecting historical discrimination and redlining.[54]

The Environmental Justice movement has elevated long-overlooked disparities and injustices to the forefront of a national reckoning with the legacy of creating "sacrifice zones" that trade the health of Black, Brown, Indigenous, and poor communities for corporate profits. We hope this report echoes and strengthens the demands from historically targeted communities to move beyond this sordid legacy and create a future in which all communities can thrive.

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Rise for Climate, Jobs & Justice March in San Francisco. *Activists participate in the Rise for Climate, Jobs & Justice march as part of a global day of action to demand our elected leaders commit to no new fossil fuels and a just and fair transition to 100% renewable energy.*

2. Fossil Fuels and Air Pollutants

Although we touch upon water pollution, land use, and other harms due to the fossil fuel industry, the bulk of this report focuses on air pollution. The Clean Air Act draws a distinction between *criteria air pollutants* (widespread pollutants with regional impacts) and *hazardous air pollutants* (typically considered to be more local in their impacts). Above and beyond their greenhouse gas emissions fueling the climate crisis, fossil fuel production and use generates both criteria and hazardous air pollutants.

There are six criteria air pollutants[55] regulated under the National Ambient Air Quality Standards (NAAQS):

- **Particulate matter**, subdivided into $PM_{2.5}$ (fine particulates) and PM_{10}, and sometimes commonly referred to as "soot." Particulates can be generated by combustion and from other sources such as unpaved roads and agricultural fields. PM can also be formed from precursor pollutants such as nitrogen oxide (NOx) and sulfur dioxide (SO_2).[56]
- **Nitrogen dioxide** (NO_2) is commonly grouped with other nitrogen oxides and termed NOx. Nitrogen dioxide is primarily generated by combustion of fossil fuels.[57]
- **Sulfur dioxide** (SO_2) is also primarily generated by combustion of fossil fuels.[58]
- **Ground-level ozone** (O_3) is distinguished from stratospheric ozone, which makes up Earth's "ozone layer." Ground-level ozone is not directly emitted to the atmosphere, but is formed by chemical reactions between NOx and volatile organic compounds (VOCs) in the presence of heat and sunlight.[59] Because ozone formation is affected by temperature, evidence suggests that climate change will itself impose a "climate penalty" and lead to higher ozone levels.[60]
- **Carbon monoxide** (CO) is formed when something is burned, and the largest source of outdoor CO is fossil fuel combustion.[61]
- **Lead** (Pb) air emissions were successfully reduced by 98% from 1980 to 2014 thanks to U.S. Environmental Protection Agency (EPA) regulations that eliminated lead in gasoline.[62]

All of these criteria pollutants are potentially generated as a consequence of the *combustion* of fossil fuels, with $PM_{2.5}$, ozone, and NOx being of particular concern. Because criteria pollutants are associated with mobile sources and a wide range of stationary sources, their pollution is generally more widespread and can degrade regional and national air quality.

The Clean Air Act also regulates hazardous air pollutants (also known as "air toxics" or HAPs), of which there are 187 substances under scrutiny, from a wide range of sources including fossil fuels.[63] These pollutants are often associated with specific facilities, and are regulated differently than the criteria pollutants. Oil and gas produced at the well can contain varying mixtures of many of these substances, and fluids used during the fracking process can introduce numerous other chemicals. Researchers have documented 61 hazardous air pollutants detected near drilling operations.[64] HAP emissions can arise from drilling activities, leaks from infrastructure, during refining and processing, and from end-use combustion.

Notable air toxics that we touch on in this report include:

- The **BTEX** chemicals (**benzene, toluene, ethylbenzene, and xylene**) are naturally occurring substances found in oil and gas deposits, and are present in gasoline and other refined petroleum products. Benzene in particular is a carcinogen, and exposure to these substances can have both acute and chronic health effects.
- Many components of oil and gas mixtures are grouped under the term **volatile organic compounds** (VOCs). These can include the BTEX chemicals, formaldehyde, ethane, propane,[65] butane, pentane, hexane, and many others.[66] These VOCs are also a key ingredient in the formation of ground-level ozone. Another related class are termed **polycyclic aromatic hydrocarbons** (PAHs), which are also associated with fossil fuels.
- **Hydrogen sulfide** (H_2S) is a highly toxic and dangerous gas that occurs naturally in oil and gas deposits. It has long been recognized as a serious hazard for workers

and communities living near oil and gas infrastructure.[67] Oil and gas with high sulfur concentrations are termed "sour" while lower concentrations are called "sweet."

- Heavy metals such as **mercury, chromium, cadmium, lead** and others are byproducts of coal and oil combustion. For example, emissions of inorganic mercury from coal plants can be converted into highly toxic methylmercury by aquatic microbes, which can then bioaccumulate in fish and lead to human exposure.

The EPA maintains the **Toxics Release Inventory** (TRI), which tracks many types of emissions by facility.[68] Using this national dataset, the EPA has also developed a risk screening tool, the **Risk-Screening Environmental Indicators** (RSEI), which combines emissions, toxicity, and impacted population into a single toxic score for each reporting facility.[69] Periodically, the EPA undertakes its **National Air Toxics Assessment** (NATA), which aims to estimate health risks from air toxics emissions.[70] The EPA also maintains **EJScreen**, a tool to map and assess EJ indicators.[71]

Types of Studies

Health effects studies vary significantly by study design, which can provide different information about a potential health hazard and have different limitations on what conclusions can be drawn from them.[72] Studies also vary by geographic scale (from individual facilities to regional to national-scale) and time scale (from single events to cumulative exposure over years). Different pollutants can be associated with a range of different health endpoints, from respiratory issues to birth complications to cancer. Human exposure to a hazard can vary by population patterns, weather patterns, regulation, enforcement, variation among facilities,[73] and other factors.

The EPA uses a risk assessment paradigm to synthesize these various types of scientific information in a manner that can inform policy making. Key stages of this process involve hazard identification, assessing dose-response, assessing exposure, and characterizing the risk.[74]

This chain of causation is most fully developed for some of the widespread criteria air pollutants — notably, PM2.5 and ozone. Through its scientific review processes, the EPA has developed full scientific assessments for those pollutants and used them to set national standards and assess the health impacts of its rulemakings under the Clean Air Act.[75] The picture is more uncertain for many of the numerous air toxics of concern. Challenges to a full risk assessment for HAPs include: uncertainties in emissions inventories and background levels, lack of air monitoring data, small sample sizes, lack of dose-response data, difficulty extrapolating to low doses, difficulty in tracking long-term health impacts (such as cancer), and other factors.[76]

Overlaying demographic patterns to assess disproportionate impact adds yet more complexity. Studies of disproportionate impact can also vary by geographic scale, and comparing across states can run up against data limitations, as well as regional differences in human geography.

Traditional *distributive justice* analyses look at patterns of how environmental risks are distributed among populations, while *benefits sharing* studies analyze how profits or other economic benefits related to polluting industries are shared among stakeholders.[77] *Procedural justice* analyses look at the fairness and inclusiveness of policy and decision-making. Other studies have looked to investigate the dynamics of why these patterns exist, to better understand solutions.[78]

Complicating all of this is that **the scientific process itself has become a target for political interference over recent decades**. Polluting industries have taken a page from the tobacco industry and have sought to protect their products and investments from liability, regulation, and public outrage by "manufacturing doubt" about environmental and health threats.[79] The "capture" of regulatory agencies has also led to significant political interference in the work of government scientists, who might otherwise be expected to work in the general public interest.[80] Under the Trump administration, air pollution science at the EPA became a particular target for political interference.[81] The Biden administration must prioritize both rolling back harmful policies and rebuilding agencies' scientific capacity.

3. Fossil Fuel Phaseout

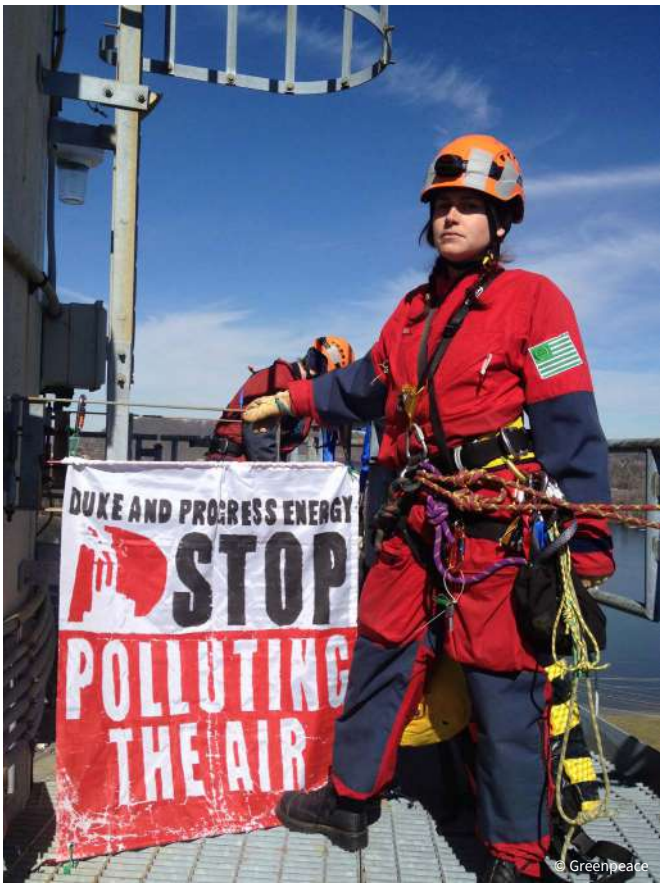
Limiting global warming to the 1.5°C goal to which nations agreed to strive in the Paris Agreement will require cutting carbon dioxide emissions in half in the next decade and reaching net-zero GHG emissions globally by 2050.[82] For scenarios that do not rely on unproven levels of carbon capture or carbon removal, these goals will inevitably require rapid reductions in fossil fuel consumption and production.[83]

Unfortunately, fossil fuel reserves that are already under development contain enough carbon to push us past the 1.5°C carbon budget and exhaust the 2°C budget.[84] For this and other reasons, **any comprehensive climate policy platform must focus not just on building out renewable solutions, but also on policies to phase out fossil fuel production[85] and prevent new investments that lock in future fossil fuel pollution.[86] A "managed decline of the fossil fuel industry[87] could also facilitate plans and programs to ensure that all workers and communities are left better off through the transition**.

A mountain of public health research has convincingly linked air pollution exposure to adverse human health impacts, and has identified air pollution as among the most significant health burdens, both globally[88,89] and in the United States.[90,91] Exposure to air pollution has been linked to higher incidences of heart disease, strokes, asthma, chronic obstructive pulmonary disease, lung cancer, diabetes, and more. Vulnerable populations include the elderly, children,[92] and people with underlying health conditions. The most significant sources of air pollution are the combustion of fossil fuels (and biomass),[93] and as a result, policies to address climate change and phase out fossil fuels are also shown to have significant health co-benefits due to reduced air pollution.[94,95,96] These co-benefits are both immediate and local, and can offset climate mitigation costs.[97,98,99]

For these reasons, strong policies to limit warming to 1.5°C also hold the potential to provide immediate local health benefits to communities that have long battled environmental racism. But these benefits will only be maximized if policymakers explicitly mitigate air and water pollution, advance environmental justice, and meaningfully include historically targeted communities in climate policy-making and implementation.

Environmental justice groups have long voiced skepticism about "carbon-only" and market-focused climate policies.[100,101] Climate policy approaches such as cap-and-trade or carbon taxes have little evidence of achieving the scale of CO_2 emissions reductions needed on their own, and in some cases market-based carbon reduction mechanisms have actually increased local CO_2 emissions and other toxic air pollutants in pollution "hotspots" (i.e., localized areas with elevated pollution levels)." Climate



Action on Asheville Coal Plant. *A Greenpeace activist protesting destruction and pollution caused by coal at the Progress Energy Asheville Power Station in North Carolina looks out from the chimney at the plant with a sign reading, "Duke and Progress Energy Stop Polluting the Air."*

policy-making questions about the scale of pollution co-benefits (*"have we maximized public health gains as we reduce carbon emissions?"*) and their distribution (*"have we reduced long-standing pollution disparities, or do hotspots remain even as overall pollution levels decline?"*) are important to address. "Carbon-only" approaches may miss an opportunity to maximize public health and distributive justice benefits — or even exacerbate existing pollution impacts and inequities.

One recent study investigated the impact of California's cap-and-trade system and found that in the early years of the program, "Neighborhoods that experienced increases in annual average GHG and co-pollutant emissions from regulated facilities nearby after trading began had higher proportions of people of color and poor, less educated, and linguistically isolated residents, compared to neighborhoods that experienced decreases in GHGs."[102] There are many open questions about the ultimate effects of California's system, but these initial results illustrate some of the risks to EJ communities.[103]

Another recent study compared pathways to reduce emissions in the power sector and found that a "carbon-only" policy risks increasing relative pollution disparities, and in some cases, absolute pollution levels in EJ communities.[104] This is because under certain conditions, a "carbon-only" policy favors switching from coal to natural gas-fired power, instead of driving a build-out of renewables. By contrast, a policy to pair CO_2 reduction goals with air pollution and environmental justice goals can achieve greater overall pollution co-benefits with only minimal cost increases.

Similarly, climate action plans that rely on offsets, natural gas fuel switching, or a massive build-out of carbon capture and sequestration (CCS) infrastructure also run the risk of aggravating local air and water pollution impacts and propping up a racist, fossil-fuel powered energy system. Even as CO_2 emissions are "offset" or "captured," these technologies and policies can allow local CO_2 emissions or other pollution to continue, perpetuating harmful health impacts for communities. The United States currently subsidizes the use of captured CO_2 to extract oil from depleted fields, a process known as enhanced oil recovery (EOR). Oil companies are now using EOR and offsets to market "carbon neutral oil."[105] However, such oil will still contribute to air and water pollution as it is extracted and burned — with similar health and justice concerns.

CCS technology captures CO_2 but does not capture other air pollutants from combustion sites, and increased energy requirements of the process itself can lead to greater overall fuel use and increased emissions for some air pollutants.[106] If no additional air pollution control investments are made, widespread adoption of CCS could lead to increases in air pollution related mortality and higher social costs.[107]

Ship Channel and Oil Facilities in Texas. *ExxonMobil Baytown refinery and petrochemical complex in Baytown, Texas, near the Houston Ship Channel.*

California's reliance on carbon offsets as a component of its cap-and-trade system has also drawn opposition from EJ advocates, including the recent resignation of two advisory board members to protest an expansion of the program.[108] Carbon offsets allow in-state facilities to continue emitting and pay to finance projects that purport to reduce emissions elsewhere. Such offsets do nothing to reduce local co-pollutants, and have been plagued by questions about effectiveness and verification of emissions reductions.[109]

Many of the proposed technology-neutral, "carbon-only" plans are explicitly framed as a way of maintaining fossil fuel jobs.[110] This is a misleading frame and will likely have the consequence of allowing a dangerous industry to continue polluting for longer, while delaying and denying justice for polluted communities and fossil fuel workers themselves. There is no doubt that workers and fossil-fuel dependent communities will need support during the energy transition. The Green New Deal has sparked a reorientation of climate policy thinking around a "standards, investments, and justice" framework that opens space to ensure that both fossil fuel workers and polluted communities can get justice.[111]

Fossil fuel corporations have not acted in good faith on climate change nor air pollution, and their stance has been to maintain their economic position for as long as possible while deploying messaging tactics that greenwash their business model. Fossil fuel corporations have known about the scale of the climate threat for decades,[112] but instead of warning the public, their public communications tended to downplay climate risks[113] and they spent millions on a campaign to sow doubt and misinformation.[114] Just as fossil fuel companies were shown to have known about the realities of climate change as far back as the 1960s and '70s, so too did they know about the human health impacts of air pollution during that time. And, as with climate change, the industry responded with denial and delay in the decades that followed, and sought to block or undermine air quality regulations.[115]

In short, "carbon-only" approaches to mitigating climate change that do not explicitly integrate air and water pollution and EJ considerations could fail to alleviate the public health harms and disproportionate burdens of fossil fuel production and use — or even exacerbate them. Instead, holistic approaches are necessary. One ingredient of a holistic climate policy approach could be to include rigorous environmental justice and equity screens in all carbon emission mitigation measures.

Another, perhaps more direct and effective approach, is to pursue a managed phaseout of fossil fuels. A fossil fuel phaseout ensures that the transition away from toxic energy sources is as complete and rapid as possible, neither delaying the necessary transition nor leaving behind pockets of pollution. A fossil fuel phaseout also allows policymakers to plan for the transition needs of workers and communities, rather than leaving it up to unpredictable, market-driven forces.

The next four sections detail the public health harms and disproportionate impacts of fossil fuels at each stage of their supply chain.

Keep It In the Ground Rally at the White House. *Princess Lucaj-Johnson of Alaska speaks at a Keep It In the Ground rally in Washington, D.C.*

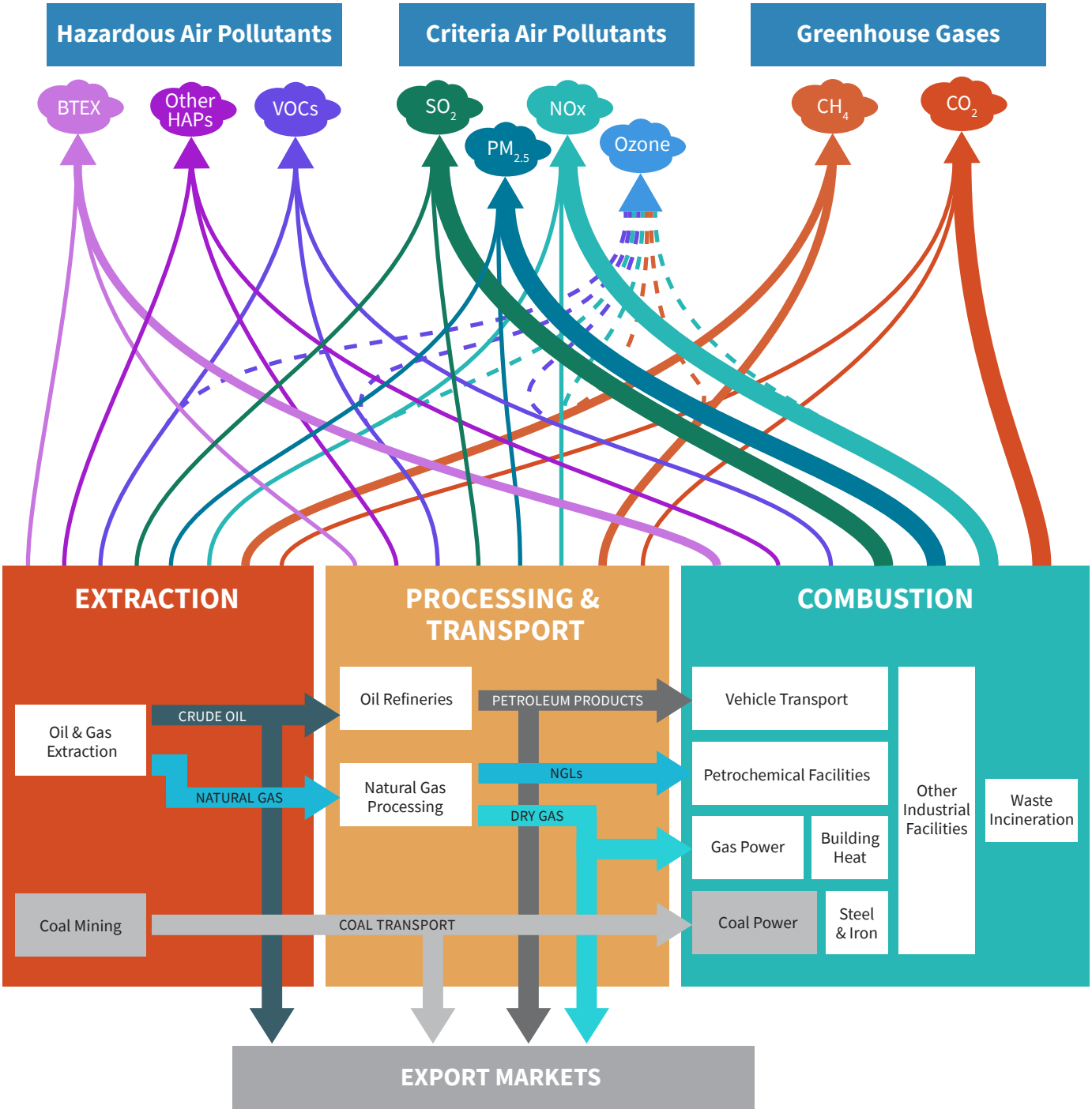


Figure 1: The stages of the oil, gas, and coal lifecycles, and the air pollutants and greenhouse gases associated with them. Note: the graphic only provides a simplified overview of the fossil fuel lifecycle, and does not attempt to account for all uses of fossil fuels in the economy, or all sources or types of emissions associated with fossil fuels. Dashed lines indicate that ground-level ozone is not directly emitted but is formed by chemical reactions involving other precursor pollutants.

4. Extraction

Harm to people and the environment from fossil fuels begins with extraction. Oil and gas deposits contain toxic chemicals, and the processes of drilling, development, and production can lead to human exposure to both hazardous and criteria air pollutants. Coal mining, too, is associated with significant health risks for both workers and nearby communities.

Oil and Gas Extraction

Over the past decade, the shale boom has brought increased scrutiny on oil and gas production in the United States, with a particular focus on air and water pollution related to hydraulic fracturing ("fracking") and other drilling techniques. Although fracking itself is only a specific part of the drilling process for certain wells, the term has become a synecdoche for the entire industry and its negative consequences for health and the environment.

Nationally, 17.6 million people live within one mile of at least one active oil and gas well.[116] Shale now accounts for 65% of oil production and 78% of gas production in the United States, although a significant portion of U.S. production still comes from conventional wells.[117] Tar sands extraction in Alberta has also been a big topic in the United States due to the build-out of pipelines to transport oil south to the Gulf Coast. Many of the health risks discussed below are applicable to conventional and unconventional production because exposure can occur not just at the wellhead or due to a particular well-stimulation event, but at other times and locations as well.

PUBLIC HEALTH HARM

A large volume of studies have investigated the impacts of unconventional oil and gas extraction. Groups such as PSE Healthy Energy,[118,119] Concerned Health Professionals of New York (CHPNY) and Physicians for Social Responsibility (PSR),[120] and other researchers[121,122,123] have published comprehensive reviews of the literature. In this section, we focus primarily on the air pollution risks of fracking,[124] but studies have also detailed risks from water pollution

Shale Fracking in Texas. *Gas flares at a fracking installation in Karnes County, in the Eagle Ford shale region.*

(due to spills, waste discharge, and underground migration of chemicals), earthquakes, naturally occurring radioactive substances, lost ecosystem services,[125] noise pollution, and a variety of economic impacts.[126]

Natural gas is primarily composed of methane (CH_4), which is a greenhouse gas with a warming potential 84-87 times greater than CO_2 over a twenty-year timeframe.[127] One key axis of the fracking debate, which we don't discuss here, has revolved around the climate impacts of methane emissions (either vented or fugitive) and the size of any climate "benefit" relative to coal.[128] However, methane is also a potential ozone precursor and can therefore play a role in increasing ozone concentration in regions with high levels of drilling.[129,130]

In addition to methane, over 60 hazardous air pollutants have been detected near oil and gas extraction sites,[131] including carcinogens, volatile organic compounds (VOCs), endocrine disrupting chemicals,[132] and more. VOC emissions from fracking can lead to ground-level ozone formation and contribute to regional air pollution. In addition to direct emissions, widespread gas flaring in drilling regions[133] and the presence of related infrastructure, such as compressor stations and increased truck traffic, can lead to particulate matter and NOx pollution.[134] Monitoring studies near drilling sites have begun to estimate human exposure levels to various pollutants as a function of distance, although they note that exposure can vary significantly over time.[135,136,137]

Three oil and gas basins — the Permian (located in Texas and New Mexico), Eagle Ford (Texas), and Bakken (North Dakota and Montana) — account for 83% of gas flaring activity in the United States. Half a million people living in those basins reside within three miles (five kilometers) of a flare, with 39% living close to more than 100 flares.[138]

A forthcoming study quantified the health impact of oil and gas production due to criteria pollutants alone, and found that in 2016 the sector's emissions resulted in 7,500 excess deaths, along with other health impacts.[139] A variety of studies have associated proximity to oil and gas activity with health problems such as respiratory impacts (*e.g.*, asthma), cancer, poor birth outcomes, cardiovascular impacts, sleep disturbance, and mental health issues. Although not a comprehensive review, the following paragraphs provide a selection of recent health findings.

An emerging body of evidence associates close proximity to oil and gas activity with elevated cancer risk, although researchers note further study is warranted.[140,141,142] Most notably, **a study in Colorado's oil and gas region used *in situ* monitoring of benzene and other HAPs and found that within 500 feet of oil and gas well sites, cancer risk was eight times higher than EPA's level of concern.**[143] This and earlier studies found that both cancer and non-cancer health risks were greatest within one-half mile of wells, with benzene exposure being the largest risk factor.[144]

© Les Stone / Greenpeace

Boy with Respiratory Problems in Louisiana. *Gregory Jackson, Jr., wearing a breathing mask and standing in front of his home in Norco. Gregory has had respiratory problems since birth. In the background, a hand-painted sign that reads: "Move us Away."*

In recent years, studies have strengthened the association between oil and gas development and adverse birth outcomes. A study in California's San Joaquin Valley found that "exposure to oil and gas well sites is associated with increased risk of spontaneous preterm birth."[145] Another California-based study found proximity to higher oil and gas development "was associated with adverse birth outcomes among mothers residing in rural areas."[146] A study set in the Eagle Ford region of Texas found evidence that exposure to flaring is "associated with an increased risk of preterm birth."[147] Other studies have found similar results in Colorado,[148] Pennsylvania,[149] and Oklahoma.[150]

Oil and gas development is a source of ozone precursors (notably VOCs and NOx) and has been associated with elevated regional ozone levels.[151,152] Elevated ozone levels are in turn associated with asthma exacerbation.[153] Some recent studies have observed associations between oil and gas activity and asthma diagnoses, emergency room visits, and hospitalizations.[154,155,156] Studies have also found associations between oil and gas activity and cardiovascular disease.[157,158]

A recent study in Oklahoma found that an increase in the number of oil and gas wells are associated with increases in "mortality rates, and incidences of cancer, cardiac, and respiratory diseases in communities in close spatial proximity" and decreases in life expectancy.[159] The authors of the most recent CHPNY and PSR compendium of scientific studies concluded, **"Our examination uncovered no evidence that fracking can be practiced in a manner that does *not* threaten human health directly and without imperiling climate stability upon which public health depends."**[160]

DISPROPORTIONATE IMPACT

Unlike industrial facilities, which are sited according to economic, political, and social factors, oil and gas extraction takes place in areas with specific geologic characteristics, although other factors can play a role in how extraction occurs. These drilling areas can vary widely in their demographics and environmental justice impacts, but emerging evidence shows that in general, "pregnant women, children, communities of color, Indigenous people, and impoverished communities are disproportionately harmed by fracking."[161]

Studies of the distributive environmental justice of fracking show that impacted populations can vary by geography.[162,163] In south Texas, there is evidence that fracking wastewater disposal wells are disproportionately located in Black, Brown, Indigenous, and poor communities.[164,165] Similarly, south Texas oil and gas flaring is found to disproportionately occur in Hispanic communities,[166] and the risk of birth complications is higher among Hispanic women.[167] Flaring in the Bakken is found to disproportionately impact the Fort Berthold Indian Reservation in particular, while in the Permian and Eagle Ford basins, a majority of the population living near flaring are people of color.[168]

On the other hand, studies of the location of fracking wells in the Marcellus shale region of Pennsylvania, Ohio, and West Virginia found more mixed evidence on distributive environmental justice metrics. One study found no evidence of racial or income disparities,[169] while another found evidence for income disparities only in Pennsylvania.[170] Notably, the region is largely white with relatively high poverty levels.

In California, people living close to oil and gas wells are disproportionately from low-income households with non-white and Latinx demographics.[171,172] Concerns about drilling and vulnerable populations are found in both rural Kern County[173] and urban Los Angeles.[174] Notably, the history of redlining and oil drilling in Los Angeles stretches back to the earliest days of the oil industry. In the 1930s, the location of oil development and resources were factors used in defining redlined areas.[175]

A more recent study combining data from four states found "robust evidence that minorities, especially African Americans, disproportionately live near fracking wells, but less consistent evidence for environmental injustice by income or educational attainment."[176] The study noted significant variation from state to state, including disparities impacting Black/African-American people in Oklahoma and Texas, and Hispanic people in Texas and urban Colorado, but no racial disparities in Pennsylvania.

Other studies have analyzed the problem from benefit sharing and procedural justice perspectives. One study of Denton, Texas, found that the town's residents (who must deal with pollution risks from drilling) only own 1% of the total mineral wealth value, and the largest share of the town's mineral rights owners live outside of Texas. Denton residents receive some indirect benefits from extraction through taxes and other revenue that is collected by the city.[177] Another study looked at property values near oil and gas wells in Colorado and described complex dynamics in how populations respond to oil and gas development. The authors observed "economic, rural, participatory, and/or distributive injustices that could contribute to health risk vulnerabilities in populations near O&G [oil and gas] wells."[178]

Indigenous communities have long faced high levels of violence, which are being further exacerbated by extractive industries. In recent years, the United States and Canada

have seen an epidemic of murdered and missing Indigenous women (MMIW) at a rate "10 times higher than other ethnicities," and which represents the third-leading cause of death for Indigenous women in the United States.[179] Indigenous communities have identified industrial camps, sometimes called "man-camps," associated with large resource development projects (such as pipelines) as a contributor to violence.[180] A recent U.S.-based study analyzed 23 MMIW "hotspots" and found fracking operations as a "likely contributing factor" in many of them.[181]

Coal Mining

PUBLIC HEALTH HARM

Coal mining in the United States has changed dramatically in recent decades, even as the industry is in structural decline. Industry focus has shifted from Appalachia to Western states, and from underground mining to surface mining (including mountaintop removal mining, or MTR). Coal mining has long generated significant health and safety risks for its workers, and for nearby coal communities. Strong transition policies will be needed to ensure all impacted workers and communities are made better during the transition away from coal.

Coal mining has long been a dangerous profession, and tragic accidents still occur from gas leaks, explosions, cave-ins, and more. Since 1900, over 100,000 miners have been killed in the United States. Black lung disease (pneumoconiosis) has also made a return among Appalachian coal miners.[182] Mine safety regulations seemed to put the disease on a path to eradication in the 1990s, but the trend reversed after 2000 and now as many as one in five miners in Central Appalachia shows evidence of black lung.[183]

Mining creates significant health risks for local communities as well. MTR mining radically alters the landscape, removing mountain ridges and vegetation, and filling valleys with mining "spoil." This process can increase the risks of mudslides and floods, contaminate drinking water, and increase air and noise pollution. Impoundments of coal slurry also create local health hazards due to improper storage, and can lead to significant spills.

A series of health studies in Appalachia found associations between mining intensity and mortality, lung cancer, poor birth outcomes, and other diseases. The excess mortality rate in the region was estimated to be over 2,300 deaths every year.[184]

DISPROPORTIONATE IMPACT

The Appalachian coal-mining region is majority white (although with a long Black/African-American heritage[185]), and has long seen elevated poverty levels and a high proportion of economically distressed counties.[186] Some studies have found that MTR regions have "significantly higher mortality rates, total poverty rates, and child poverty rates" compared to other comparable counties.[187] Other studies have noted that the impact of coal employment on poverty and well-being has been mixed and has changed over time.[188]

The largest coal-producing region in the United States is now the Powder River Basin in Montana and Wyoming, where 16 mines produce 43% of U.S. coal.[189] The region is sparsely populated, and the population is roughly 85% White and 11% Native American.[190] The Basin overlaps with both the Crow Reservation and the Northern Cheyenne Reservation. Decisions in the 1970s to open up the region to coal production led the Crow and Northern Cheyenne Nations to revolt against federal management of Tribal resources and demand greater control over development of their energy resources.[191] Since that time, the Crow and the Northern Cheyenne Nations have pursued different strategies to manage energy extraction on their lands.[192]

Mountain Top Removal in West Virginia. *Aerial photograph documenting mountain top removal mining atop Cherry Pond Mountain.*

5. Processing & Transport

Oil, gas, and coal extraction occurs in specific, although widespread, geographic areas. Extensive networks of pipelines, terminals, storage facilities, tankers, trains, and trucks are needed to transport fuels either to their end markets, or to processing hubs where they are transformed into commercial products. Risks of human exposure to hazardous or criteria air pollutants can occur all along these networks, but certain regions have particularly high concentrations of emitters.

Oil Refining, Natural Gas Processing & Petrochemical Manufacturing

After extraction, crude oil is processed in oil refineries to create a variety of petroleum products, including gasoline, diesel, jet fuel, heavy fuel oil, and others. Natural gas from the wellhead is processed to separate out dry gas (used for power and heating) from natural gas liquids (NGLs), which are common inputs in petrochemical manufacturing, including plastics production.

PUBLIC HEALTH HARM

Refineries, natural gas processing, and petrochemical facilities are associated with both criteria and hazardous air emissions, which impose health risks on nearby communities.[193,194] The Toxic Release Inventory (TRI) tracks the emissions of toxic air and water releases from some of these facilities, although not all industries are covered. Because TRI tracks such a wide diversity of toxic emissions, the EPA has developed Risk-Screening Environmental Indicators (RSEI) to synthesize TRI data into simpler indicators of the overall toxic burden from a facility. RSEI data combines overall emissions with the toxicity of each pollutant and the size of the exposed population.[195]

A 2009 study using RSEI data found that the petroleum refining sector was the 9th most polluting economic sector among industries reporting to TRI.[196,197] **New Greenpeace analysis of the 2018 RSEI data shows that petroleum refineries and petrochemical manufacturing remain the #8 and #9 most polluting industries**, representing 2.7% and 2.6%, respectively, of the total national toxic burden reported to TRI.[198] The only sectors with greater toxic risks are certain types of chemical and metal manufacturing. **One recent study noted that 6.1 million Americans live within three miles of a refinery.**[199]

A number of studies have investigated health impacts associated with specific refineries or petrochemical facilities,[200,201,202] although individuals sites will naturally vary with respect to local populations, pollutants, emissions levels, regulation, enforcement, etc. Meta-analyses have also uncovered evidence associating refineries and petrochemical facilities with both cancer[203,204] and non-cancer health risks.[205,206] **One notable study is a "natural experiment" regarding a refinery closure in Oakville, Ontario, which found that a reduction in SO_2 emissions led to measurable improvements in local health**.[207]

Other studies have looked at the impact of higher emissions levels resulting from breakdowns, accidents, explosions, spills, and other operational failures.[208,209] Studies of residents exposed to benzene following a prolonged flaring disaster at a BP refinery in Texas City, Texas, showed a range of adverse health symptoms and indications of risks of "serious future health complications."[210,211] Another detailed the surge of emergency room visits from nearby neighborhoods following chemical releases in 2007 and 2012 at Chevron's refinery in Richmond, California.[212]

BP's Texas City refinery was also the site of a massive 2005 explosion that killed 15 refinery workers and injured 180 people. Although comprehensive data on refinery accidents is hard to find, a 2015 investigative piece found that at least 58 workers had died in the decade after the BP explosion, only slightly fewer than in the decade prior.[213] Both the Texas City and Richmond refineries release toxic pollution that disproportionately burdens Black, Brown, Indigenous, and poor communities.

Ship Channel and Oil Facilities in Texas. *Ineos and Braskem oil facilities in La Porte, Texas. The Houston area has long been known as a major hub of the petrochemical and fossil fuel industry. As upstream profits in oil and gas production have declined, the fossil fuel industry has increasingly invested in petrochemical divisions as a potential bright spot, citing low price ethane feedstocks generated from the US fracking boom and increased demand for single-use plastics in the consumer goods sector.*

One hazard associated with refineries that has received recent scrutiny is the use of hydrofluoric acid (HF) alkylation to increase the octane of gasoline. HF is a highly toxic chemical that can form an aerosol cloud and potentially travel for miles when released. In 2013, the United Steelworkers and the Tony Mazzochi Center conducted a survey of workers at refineries that use HF and found "131 HF-related incidents or near misses" over three years, and raised concerns about ineffective safety systems.[214] A 2015 explosion at ExxonMobil's Torrance, California refinery reportedly just narrowly missed causing an HF disaster.[215]

Another chemical that has prompted local concern is hydrogen cyanide (HCN), a refinery byproduct that has also been used as a chemical weapon. Houston, Texas activists have mobilized to stop Valero's refinery from receiving a permit for HCN emissions.[216] A review of EPA monitoring data by the Environmental Integrity Project (EIP) found that in 2019, 10 refineries were releasing "cancer-causing benzene into nearby communities at concentrations above federal action levels."[217] Despite this, a recent investigation found that EPA air pollution monitors routinely miss "major toxic releases and day-to-day pollution dangers" — notably including a massive explosion at the Philadelphia Energy Solutions refinery in 2019.[218]

A 2017 report by the NAACP and the Clean Air Task Force (CATF) provided case studies of health impacts on several communities living near oil refineries, but did not attempt to quantify overall health impacts.[219] A recent expert evaluation concluded, "there is ample evidence of the health hazards of refineries during catastrophic failures and sufficient evidence of the health risks of air pollution from refineries during times of normal operation."[220]

A series of reports from CATF analyzed the natural gas supply chain, looking at air toxics,[221] ozone formation,[222] and state-level risks.[223] Making use of the EPA's National Air Toxics Analysis (NATA), the studies concluded that **238 counties in 21 states**[224] **with a total population of nine million "face cancer risk above EPA's one-in-a-million level of concern due to toxic emissions from oil and gas operations."** These reports focus on the entire natural gas supply chain, including drilling sites (see previous section), but exclude oil infrastructure such as refineries.

DISPROPORTIONATE IMPACT

Studies have found that oil and natural gas facilities are disproportionately sited in vulnerable communities and lead to health disparities. Much of this analysis is focused on the Gulf South region, where much of the nation's oil, gas, and petrochemical infrastructure is located.

Studies of nationwide and cross-industry RSEI data have concluded that reporting industries generally have higher risks for Black, Brown, Indigenous, and

poor communities.[225] Researchers at the Political Economy Research Institute (PERI) have used finer grained geographic microdata (RSEI-GM) to estimate the percentage of each facility's toxic score that is borne by minority[226] and poor populations. PERI then combines facilities under the same parent company and publishes annual Top 100 Polluter Indexes.[227]

Greenpeace analysis of 2018 PERI data shows that TRI-reporting facilities overall — and most of the top-polluting sectors individually — impose a disproportionate toxic burden on minorities and poor people.[228]

In particular, petroleum refineries and petrochemical manufacturing are among the sectors which impose the most disproportionate toxic burden on minorities and poor people. The 2009 PERI study found that **the petroleum refining sector was the 2nd worst industrial sector in terms of both minority and poor shares of its aggregate toxic score**.[229] Greenpeace analysis using PERI's 2018 data shows that 56% of refinery toxic burden is borne by minorities (who make up 39% of the population) and 19% by poor people (who make up 14% of the population).

Among the top 10 worst polluting sectors in the 2018 TRI data, the petroleum sector is 2nd worst for minority share, and 4th worst for poor share. In the petrochemical sector, 66% of the toxic burden is borne by minorities and 18% by poor people.

Figure 2 shows 133 U.S. refineries (left) and 48 petrochemical facilities (right) and the share of their pollution burden carried by minorities (x-axis) and poor people (y-axis).

In particular, it is notable that **a majority of toxic burden for both refineries and petrochemicals is in regions that are *both* less-white and lower-income than average** (upper right quadrant). This means that the most polluting facilities in these sectors are located in some of the most vulnerable communities, and in many cases have been located in these regions for decades. Table 1 shows that oil and gas corporations are well-represented in PERI's latest Top 100 Air Polluters list, and that their operations generally disproportionately impact minorities and poor people.

U.S. Refinery and Petrochemical Toxic Scores (2018 data)

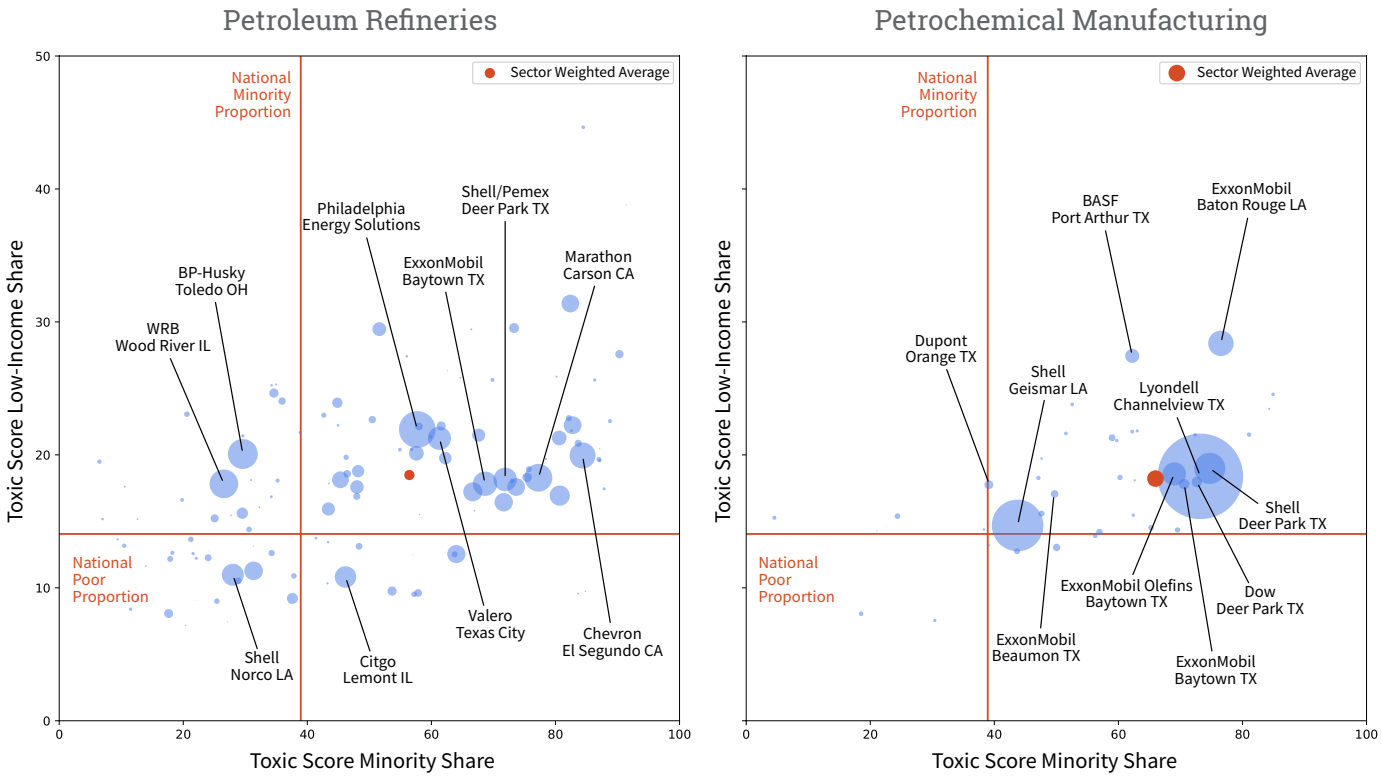


Figure 2: RSEI Toxic Scores for 133 petroleum refineries (left) and 48 petrochemical manufacturing facilities (right), showing the minority share (x-axis) vs. the poor share (y-axis) of the toxic score for each facility. Top 10 polluting facilities are labeled. (Source: Greenpeace analysis of 2018 PERI dataset)

Oil and Gas Corporations in PERI's Toxic 100 Air Polluters

Rank	Corporation	Toxic Score	Poor Share	Minority Share
10	Royal Dutch Shell	3,407,149	16%	52%
12	National Oilwell Varco	2,920,067	15%	56%
21	Exxon Mobil	1,798,756	22%	70%
24	Koch Industries	1,550,053	18%	52%
28	Occidental Petroleum	1,385,291	18%	70%
32	Marathon Petroleum	1,132,246	20%	63%
33	Valero Energy	933,866	21%	64%
36	Phillips 66	812,314	17%	57%
39	Chevron	759,988	17%	74%
52	PBF Energy	516,711	19%	63%
56	BP	461,943	20%	40%
67	Targa Resources	332,436	23%	86%
69	Husky Energy	309,570	20%	29%
95	Total S.A.	208,738	18%	44%

Table 1: Corporations with significant oil and gas business appearing in PERI's most recent Toxic 100 Air Polluters Index.[230] Data taken from the 2020 report, which is based on 2018 TRI data. Table shows ranking and total toxic score across all corporate facilities. Cells colored red show corporations with an aggregate minority or poor share that is larger than the proportion of minority or poor individuals in the U.S. population (39% and 14%, respectively).

The NAACP/CATF report also concluded that the majority of U.S. refineries "are sited in low-income areas and communities of color" and, in particular, quantified significant health impacts imposed on Black/African-American communities from natural gas infrastructure.[231] In particular, emissions attributed to natural gas infrastructure led to elevated cancer risk impacting one million Black/African-Americans, as well as 138,000 asthma attacks and 101,000 lost school days for Black/African-American children.

A 2016 study by the Union of Concerned Scientists and Texas Environmental Justice Advocacy Services (t.e.j.a.s.) compared health risks from chronic and acute exposure among four communities in Houston, Texas: "two predominantly Hispanic and low socio-economic east Houston communities [...] with two primarily white and wealthier west Houston communities." The study "found large disparities between the east and west Houston communities in terms of overall toxicity levels from chemical exposures," with oil refineries and chemical manufacturing ranking among the top polluting sources in the east Houston areas.[232]

The petrochemical industry continues to expand along the Gulf Coast and elsewhere, partly driven by plastics production, which is widely seen as a lifeline for oil and gas companies in a time of increasing ambition on climate.[233]

In 2020, Formosa Plastics (currently #63 on PERI's list) received permits to construct a massive petrochemical complex in the "Cancer Alley'' region of Louisiana. Local Black environmental justice leaders opposing the complex, including RISE Saint James, assert that Formosa's permits would double air toxics emissions (including benzene and ethylene oxide) in the region,[234] which is already home to census tracts with some of the highest cancer risks in the United States.[235]

A recent study of the U.S. refinery industry found that refinery capacity is correlated with higher benzene emissions, and such emissions are associated with lower county per-capita income.[236] However, an earlier study of enforcement penalties levied against oil refineries found "mixed evidence" of inequality in enforcement.[237]

Other studies have looked at disparities in exposure to air toxics, without reference to the source. A 2012 study using the 2005 NATA found **significant cancer risk disparities in "Cancer Alley," with poor areas experiencing 16% more cumulative risk and 12% more risk for areas that are greater than 75% Black/African-American**.[238] A 2018 study found that nationally, public schools with the highest risk for exposure to air neurotoxicants were "significantly more likely to be eligible for free/reduced price meals, and to be Hispanic, [B]lack, or Asian/Pacific Islander (API)."[239]

Pipelines & Terminals

The network of pipelines is regulated by the Pipeline and Hazardous Materials Safety Administration (PHMSA), which differentiates between crude oil, refined petroleum products, highly volatile liquids (often NGLs), and natural gas. Gathering pipelines, which are smaller and less regulated, take oil and gas from the wellhead to a separation facility and then to the larger transmission pipeline, which transports the separated product to a refinery or other destination. At various places along the network are found tank farms, bulk storage facilities, terminals, and other locations where oil and gas is stored, loaded, unloaded, bought, sold, and traded.[240]

PUBLIC HEALTH HARM

Similar to processing facilities, fossil fuel transportation infrastructure is also prone to leaks, spills, explosions, and other disasters.

There is no safe way to transport oil and gas.[241] The oil and gas pipeline network experiences a steady drumbeat of small- to medium-sized spills and leaks every year, as well as occasional catastrophic spills such as the 20,000 barrel tar sands spilled into the Kalamazoo River in 2010. Stronger regulations have led to meaningful declines in spills from oil tankers,[242] but risks of another *Exxon Valdez*-like spill still remain. Transporting oil or gas by truck or train also brings significant safety risks, as illustrated by a 2013 derailment and explosion in Lac-Mégantic, Quebec that killed 47 people.[243] Transporting coal can lead to coal dust pollution.[244]

DISPROPORTIONATE IMPACT

With the shale boom, the United States has seen a rapid build-out of oil and gas pipelines, as well as liquified natural gas (LNG) and crude export terminals. The vast majority of this infrastructure build-out is funneling oil and gas from places like the Bakken, Niobrara (located primarily in Colorado and Wyoming), and Permian regions south to the Gulf Coast, where it is processed or (increasingly) exported. This new terminal and pipeline build-out has the effect of converging significant volumes of oil and gas into regions that are already experiencing environmental justice burdens. These include Corpus Christi, the Houston Shipping Channel,[245] Beaumont, Port Arthur, the "Cancer Alley" region of Louisiana, and other Gulf South communities.

Greenpeace analysis of the 2018 RSEI data finds that the more than 400 Petroleum Bulk Stations and Terminals reporting to TRI are much smaller emitters than refineries or petrochemical plants, but are similarly disproportionately located in poor (19% of toxic score) and minority communities (71% of toxic score).[246]

Pipeline construction has become a particular environmental justice flashpoint in recent years. The construction of the Dakota Access pipeline in 2016 led to massive resistance by Water Protectors at Standing Rock. One issue in that controversy was the apparent rerouting of the pipeline away from a majority White community in favor of a route that put Tribal water resources at risk.[247]

Other major pipeline projects — including the now-canceled Keystone XL, the Transmountain Expansion, and the Line 3 Expansion — have also attracted strong opposition from Indigenous and First Nations activists, as well as from other communities along the routes. A 2018 study found evidence that disproportionately large numbers of Black/African-American, Hispanic, and Indigenous residents live within one mile of the proposed Atlantic Coast Pipeline (ACP) route in North Carolina.[248] The ACP project was canceled in 2020.

The routing of pipelines from extraction regions to processing or export locations is subject to different pressures and processes than the siting of point source facilities like refineries. In particular, the origin and destination of a pipeline are usually fixed by geography and existing infrastructure. While there are choices that are made about the intervening route, many pipelines are routed through low-population density rural areas for much of their length. Pipeline build-out often facilitates an expansion of oil and gas drilling and processing, and can lock in dependence on oil and gas.

However, a 2021 study looking at 84 new, proposed natural gas pipelines found only limited support for the hypothesis that the proposed routes disproportionately impacted environmental justice communities. The authors noted the importance of further research to see if this remains the case for completed pipelines.[249]

Gas Pipeline Warning Sign in Colorado. *A gas pipeline warning sign at the edge of a cornfield near a hydraulic fracturing drilling rig creating a new well on the Niobara shale formation, one of the most intensively fracked areas in the United States.*

6. Combustion

The end use of most (but not all) fossil fuels is combustion for energy, which can occur in both stationary sources (*e.g.*, power plants and industrial facilities) and mobile sources (*e.g.*, cars and trucks). As discussed above, criteria air pollution from the combustion of fossil fuels is one of the most significant global health burdens, and is linked to millions of premature deaths every year globally.

General Exposure to Criteria Air Pollution

PUBLIC HEALTH HARM

A 2020 study by Greenpeace Southeast Asia and the Centre for Research on Energy and Clean Air (CREA) estimated that in 2018, 4.5 million premature deaths worldwide could be attributed to $PM_{2.5}$, ozone, and NOx air pollution from burning fossil fuels. It was estimated that health impacts arising from these pollutants cost roughly US$8 billion per day, or 3.3% of the world's GDP.[250] (In calculating economic costs of air pollution, researchers often include the economic value of lost years of life, lost days of work, and healthcare costs.) In the United States alone, the report found that air pollution due to fossil fuel combustion causes 230,000 premature deaths each year, with an economic cost of US$600 billion.

A 2021 study found even higher numbers: 8.7 million premature deaths globally in 2018 due to $PM_{2.5}$ from fossil fuels, and 355,000 premature deaths in the United States.[251] The elevated numbers were the result of new information on the concentration-response function of $PM_{2.5}$, which relates the size of health effects to the level of $PM_{2.5}$ in the environment to which humans are exposed.

A small portion of the carbon associated with fossil fuels ends up stored in commercial products (*e.g.*, plastics, lubricants, asphalt) instead of being combusted as fuel. Some of those products (notably plastics) are later incinerated at the end of their commercial life, which can lead to both carbon emissions and air pollution. Plastic incineration can result in emissions of dioxin and other air toxics.[252]

DISPROPORTIONATE IMPACT

In the context of the United States, studies have shown that exposure to air pollution is not shared equally. A 2018 study found that for $PM_{2.5}$, **"those in poverty had 1.35 times higher burden than did the overall population, and non-Whites had 1.28 times higher burden." The burden for Black/African-Americans was even higher at 1.54**.[253] Similarly, a 2019 study looked at both $PM_{2.5}$ exposure and consumption patterns that lead to air pollution, and again concluded that "Blacks and Hispanics on average bear a 'pollution burden' of 56% and 63% excess exposure, respectively, relative to the exposure caused by their consumption."[254] Ozone pollution is also found to have a disproportionate impact on Black, Brown, Indigenous, and poor communities.[255]

Over the last several decades, clean air regulation in the United States has significantly reduced pollution levels,[256] although from 2016 to 2018 pollution levels partially rebounded.[257] However, a 2020 study found that **$PM_{2.5}$ *disparities* have persisted even as overall levels have declined** — *i.e.*, "the most polluted census tracts in 1981 remained the most polluted in 2016."[258]

People of color are also more likely to live in areas that violate air quality standards. For example, a 2011 study found non-Hispanic Black/African-American people are "consistently overrepresented in communities with the poorest air quality,"[259] and nearly 50% of Hispanic-Americans live in areas that frequently violate ozone standards.[260]

These broad, nation-wide studies give high-level statistical evidence that Black, Brown, Indigenous, and poor communities suffer disproportionately from fossil fuel combustion. Other studies have looked more closely at particular sources, including coal and natural gas power plants, as well as emissions from transportation.

Coal and Natural Gas Power Plants

Coal- and natural gas-fired power plants are among the largest sources of air pollution, both globally and in the United States.

Although not a main focus of this report, other types of industrial facilities use fossil fuels for on-site energy generation and industrial processes that require high heat — in particular, iron and steel production and various types of manufacturing. Both criteria and air toxics emissions from these industrial sites can be significant, and bring health risks for local communities.[261] Decarbonization strategies for industrial sector emissions must also focus on eliminating criteria and hazardous pollutant emissions and alleviating environmental justice concerns.[262]

PUBLIC HEALTH HARM

Numerous studies have linked coal combustion to significant human health, economic, and environmental harms.[263,264] One study found that the full lifecycle costs of coal to the U.S. public was between $175 and $523 billion every year, with a significant portion of that total ($65 to $187 billion) from monetized health impacts from coal combustion in power plants.[265] As described above, these health impacts are primarily due to exposure to criteria air pollutants $PM_{2.5}$, NOx, and SO_2, but coal plants are also a significant source of mercury as well as various hazardous and carcinogenic air pollutants.[266] Coal plants also generate coal ash as a waste product, which can be harmful as it is often improperly stored and can lead to disasters such as the 2008 Kingston coal ash spill.[267]

In 2015, the Obama administration finalized its Clean Power Plan (CPP) to reduce CO_2 emissions from the nation's fleet of power plants. EPA analysis of the rule projected it would lead to a 32% decline in CO_2 emissions and a 22% decline in coal generation by 2030.[268] These reductions would come with co-beneficial reductions in $PM_{2.5}$ and ozone levels that would avoid 1,500 to 3,600 premature deaths and 90,000 childhood asthma attacks in the year 2030.[269] In 2019, the Trump administration finalized a significantly weaker rule replacing the CPP. The Trump administration's rule, which would have led to as many as 1,400 additional deaths per year compared to the CPP, was struck down by a federal appeals court in January 2021.[270]

Recent studies have measured the health benefits of phasing out fossil fuel power plants. By taking advantage of a "natural experiment" when eight coal and oil power plants were retired in California, researchers were able to measure improvements in the health of nearby populations. The study found plant retirement was associated with a "decrease in the proportion of preterm birth" for mothers living within six miles (10 kilometers).[271] A similar study found improved asthma outcomes following the conversion and retrofitting of coal plants in Kentucky.[272]

Duke Energy Ash Spill Protest in North Carolina. *Protesters outside Duke Energy offices display a variety of dangerous toxic chemicals leaking into the Dan River from an unlined coal ash containment pond at a retired Duke coal plant. The group was demanding Duke clean up its toxic waste.*

Another study analyzed the impacts of closing coal plants in Texas and concluded that "air pollution health impacts often exceed the value of the electricity generated and are of similar magnitude to climate impacts."[273] In the U.S. Northeast, the Regional Greenhouse Gas Initiative (RGGI) — a carbon market designed to reduce power sector emissions — was also found to reduce NOx and SO_2 emissions, and related regional $PM_{2.5}$ concentrations. These reductions were estimated to bring "substantial child health benefits," including avoided asthma cases and avoided preterm births. The study did not attempt to investigate disparities in health impacts by race or income.[274]

Natural gas-fired power plants do not directly generate as much particulate, mercury, or SO_2 pollution, although they do generate NOx emissions, which can lead to ozone and $PM_{2.5}$ via secondary processes. What's more, natural gas power plants are more likely than coal to be located in denser areas, thereby increasing exposure to pollution.[275] So, while natural gas power generation can have lower health impacts than coal, it is not "clean" in comparison to renewable power sources.[276]

DISPROPORTIONATE IMPACT

A 2012 study led by the NAACP found that "coal power plants tend to be disproportionately located in low-income communities and communities of color."[277] The study found that **the six million people who lived within three miles of a coal plant had a lower average income ($18,400) and were more likely to be people of color (39%)** than the population at large ($21,587 and 36%, respectively). The report also gave 75 coal plants a failing grade on environmental justice due to their outsized pollution impacts and disproportionate siting in Black, Brown, Indigenous, and poor communities.

Subsequent government studies have confirmed this finding. A 2015 EPA analysis carried out in support of the Clean Power Plan found "a higher percentage of minority and low-income communities living near power plants than national averages," although the analysis noted wide variability in impacted populations.[278] A 2017 study by Oak Ridge National Laboratory similarly found that the share of minorities living within three miles (five kilometers) of a coal- or oil-fired power plant is 12% larger than the national average.[279]

Other state-level studies have also found disproportionate impacts from the power sector. **In California, the state's CalEnviroScreen 3.0 tool shows that half of all natural gas plants are "located in communities that rank among the 25% most disadvantaged."**[280] In Pennsylvania, one study found that the state's 88 "existing power plants were disproportionately located near disadvantaged communities," and that new proposed plants would exacerbate these disparities.[281]

The power sector has shifted rapidly in recent years, with cheap renewables and natural gas pushing many coal plants into retirement. A 2020 study found that recent coal retirements had decreased overall $PM_{2.5}$ impacts, but "disparities between White and non-White subgroups increased" and burdens remains highest for "below-poverty and non-White subgroups."[282]

A recent study by PERI found that the proportion of Black and Hispanic residents living within three miles (five kilometers) of a power plant was higher for natural gas (13.4% Black, 19.8% Hispanic) than for coal (8.1% Black, 6.1% Hispanic). While overall co-pollutant damages from coal are roughly eight times higher than for natural gas, the coal impact on Hispanic and Black populations is only three to four times greater than natural gas. This data indicates that a climate policy that prioritizes switching from coal to natural gas as a "bridge fuel" risks worsening relative pollution disparities, and in some cases, increasing absolute pollution levels in minority communities.[283]

Mobile Sources and Traffic Exposure

Mobile sources of air pollution, such as tailpipe emissions from gasoline or diesel engines in cars and trucks, also have significant health impacts.[284]

PUBLIC HEALTH HARM

Mobile sources are a key factor in exposure to both $PM_{2.5}$ and ozone. The EPA notes that people who live, work, or attend school near high-traffic roadways are exposed to higher levels of air pollution and suffer adverse health impacts as a result.

Studies have found evidence that exposure to traffic is linked to worsened childhood asthma, cardiovascular mortality,[285] and other adverse effects.[286] The EPA conducted air quality modeling in connection with its 2012 rulemaking on emissions standards for cars and trucks.[287] The agency's regulatory impact analysis (RIA) concluded that improved standards would have "substantial" health benefits both in terms of reduced mortality and net economic benefits.

In March 2020, the Trump administration finalized a much weaker rule that rolled back the Obama-era standards and revoked California's unique authority to set stronger vehicle standards.[288] Taken together, these two rollbacks would increase cumulative greenhouse gas emissions by over a gigaton from 2020 to 2035.[289] Higher levels of

air pollution due to the rollback could also lead to up to 32,000 additional premature deaths from 2017 to 2050.[290]

DISPROPORTIONATE IMPACT

Demographic patterns of major U.S. urban areas have been profoundly shaped by the history of racial discrimination, including policies of redlining and housing discrimination.[291] These patterns were also compounded, in certain U.S. cities, by decisions in the 1940s and '50s to construct major highways through so-called "blighted" areas, erasing some neighborhoods and hemming others in with traffic corridors.[292]

Studies are beginning to explore how these historical patterns have persisted and have uncovered correlations between past redlining practices and present day health burdens. One study of eight California cities found that neighborhoods described as high foreclosure risk in the 1930s were currently associated with higher diesel exhaust pollution levels, higher proportions of minority and poor residents, and a 2.4 times higher rate of asthma-related emergency room visits.[293] Another study that looked at 108 cities nationwide found that previously redlined neighborhoods are disproportionately exposed to extreme heat, and are an average of 2.6°C hotter than non-redlined areas.[294]

Although the sale of leaded gasoline was phased out by the EPA beginning in the 1970s, lead persists in the environment and remains a health hazard. Exposure risks from lead-contaminated soil are often concentrated in urban centers and reflect historical traffic patterns — and thus historical fossil fuel usage.[295]

The EPA's 2012 clean car standards RIA presented evidence that people "living near roads are often socioeconomically disadvantaged," noting that rental properties are twice as likely to be located near large highways, railroads or airports than owner-occupied properties. One U.S.-wide study found "greater traffic volume and density are associated with larger shares of non-white residents and lower median household incomes."[296] Note that air pollution exposure also occurs during daily travel and at work, and mobility should be taken into account in estimating exposure.[297,298]

A number of studies of specific urban areas have also found "higher levels of traffic-related air pollutants in areas with high minority or poor populations."[299,300,301] The EPA's 2012 clean car standards RIA also summarized studies showing that students attending schools with high traffic exposure are "more likely [...] to be of non-white race or Hispanic ethnicity, and more often live in low socioeconomic status locations."

Billboard Messages During Exxon's Shareholder Meeting in Texas. *Billboards addressing climate change are visible in Dallas, Texas, as part of a Greenpeace campaign to confront Exxon before its shareholder meeting in May 2017.*

7. Climate Impacts

Greenhouse gas emissions from fossil fuel usage are the principal factor driving the climate crisis. Climate change is already manifesting itself in a myriad of different forms — from sea-level rise to increased flood risk, from melting permafrost to heat waves, from stronger hurricanes to longer wildfire seasons — with significant consequences for human health and communities.

PUBLIC HEALTH HARM

The impacts of climate change on human health have been well-studied on both a global scale[302] and in the United States.[303] Globally, climate change "threatens to undermine the past 50 years of gains in public health, disrupting the well-being of communities and the foundations on which health systems are built."[304] Should the planet remain on a high emissions trajectory, changes in rainfall patterns[305] and losses of biodiversity[306] will threaten food security for people living in many regions.

In the United States, health hazards related to climate change include "altering exposures to heat waves, floods, droughts, and other extreme events; vector-, food-, and waterborne infectious diseases; changes in the quality and safety of air, food, and water; and stresses to mental health and well-being."[307] Policies to limit emissions could save thousands of lives and hundreds of billions in health-related costs in the United States by the end of the century.[308]

This report has focused on air pollution and its health impacts. Climate change has the potential to increase ozone concentrations, and by amplifying wildfire impacts, can increase $PM_{2.5}$ concentrations during wildfire season. The 2020 California wildfires are estimated to have contributed to over 1,000 premature deaths due to air pollution alone.[309]

Climate change is predicted to increase the frequency and intensity of heat waves, hurricanes and tropical storms, floods, droughts, wildfires, and other weather events. These events can directly harm human health, and can also disrupt public health and health care systems. It is not always possible to directly attribute an individual extreme weather event to climate change, although scientists have made rapid advances in this area.[310] In 2020, the National Oceanic and Atmospheric Administration (NOAA) identified a record-breaking 22 weather/climate disasters that cost over $1 billion in damages each, including seven tropical storms, drought and heatwave in Western states, and the West Coast wildfires.[311] Those 22 events led to 262 deaths and caused a total of $95 billion in damages.

Extreme heat is one of the most direct human health threats arising from climate change. Places such as the Persian Gulf and South Asia may see heat-wave temperatures that approach or exceed the "limit of human survivability" by the end of the century under a high emissions scenario.[312] In the United States, the National Climate Assessment finds that heat-related deaths from warming will outweigh reductions in winter mortality. Limiting climate change could reduce excess heat mortality by over 5,000 deaths per year by the end of the century (in comparison to the high emissions scenario, and without considering adaptation).[313]

Break Free Action in Washington D.C. *Break Free supporters gather in front of the White House in Washington, D.C., before marching to the Lincoln Memorial to call on the Obama Administration to stop offshore drilling, including in the Arctic, Atlantic, and Gulf Coast regions.*

DISPROPORTIONATE IMPACT

Much like air pollution risk, climate change impacts will be unevenly and inequitably shared across the United States[314] and the world. Globally, the United Nations has concluded that "Climate change will have devastating consequences for people in poverty. Even under the best-case scenario, hundreds of millions will face food insecurity, forced migration, disease and death." Climate inaction would be "disastrous for the global economy and pull vast numbers into poverty."[315]

Lower-income regions in the Southeast, with the largest Black/African-American populations in the United States, are among the highest-risk,[316] including many communities already experiencing high pollution burdens and a legacy of environmental racism. The 2018 National Climate Assessment concluded, "People and communities are differentially exposed to hazards and disproportionately affected by climate-related health risks," and noted elevated risks for children, older adults, low-income communities, communities of color, outdoor workers, and "communities disproportionately burdened by poor environmental quality." Lower-income households generally have lower GHG emissions and do not contribute to global climate change at the same scale as high-income households.[317]

The U.S. health system is already plagued by many of the same concerns about systemic racism and inequality, as well as a financial structure that leaves many millions without reliable health insurance. The American Public Health Association (APHA) has warned that the "same physical, social, economic, and services environments that are associated with poor health outcomes for low-income communities and communities of color also increase exposure and vulnerability to the health impacts of climate change."[318]

Alaska Native and Indigenous communities are among the first to experience some of these climate impacts — and are among the United States' first internal climate migrants. Retreating sea ice, increased erosion, and winter storms are forcing several Alaskan villages, such as Kivalina, to relocate to more secure ground.[319] Along Louisiana's coast, rising sea levels and rapid erosion are also forcing Native communities to consider relocation.[320]

Research has shown that the response to climate-driven natural disasters is shaped by and can amplify existing inequalities.[321] Studies looking at the aftermath of Hurricanes Maria and Harvey have found increases in air pollution,[322] threats to water quality,[323] and even the redistribution of existing toxic pollution by floodwaters.[324] In August 2020, Hurricane Laura hit the coast of Louisiana and was immediately followed by a heat wave, impacting a region already suffering from the Covid-19 pandemic, health inequalities, and environmental racism. The hurricane forced emergency shutdowns of numerous refineries and petrochemical plants along the coast, leading to elevated toxic emissions. Hurricane damage triggered a catastrophic chemical plant fire near Lake Charles, releasing chlorine gas and other pollutants, and forcing nearby residents to shelter-in-place and close windows to avoid exposure.[325]

Urban Heat Islands (UHI) can amplify the impact of warming by increasing heat exposure during the day and preventing cooling off at night. Research has found that in 25 cities around the world, 72% of poorer neighborhoods experienced elevated heat exposure — a factor that was potentially mitigated by increasing green space in urban areas.[326] The same pattern holds for most cities across the United States, with studies showing that poor people and people of color live in hotter neighborhoods.[327,328] As noted earlier, exposure to extreme heat is associated with past patterns of redlining.[329] A recent study by the real estate company Redfin also found that formerly redlined areas faced 25% higher flood risks than non-redlined areas, and that those risks were disproportionately borne by people of color, who make up 58% of residents in the formerly redlined neighborhoods studied.[330]

A broad review of health studies found that Black mothers were most at risk from overlapping exposure to air pollution and heat stress, putting them at greater risk of adverse pregnancy outcomes such as preterm birth, low birth weight, and stillbirth.[331] Other groups that face elevated risk of extreme heat include children, the elderly, and outdoor workers, such as agricultural workers and others.

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People's Climate March in New York City. *A participant in the People's Climate March shows off her message as she makes her way through the streets of New York City. The march, two days before the United Nations Climate Summit in 2014, was billed as the largest climate march in history.*

8. Policy Recommendations

As the nation emerges from the COVID-19 pandemic, President Joe Biden and the 117th Congress have a historic opportunity to kick off an ambitious national policy program that puts the United States on a path to rapid decarbonization while ensuring justice for workers and communities. Our national response to the overlapping crises we face must explicitly aim to end fossil fuel racism.

Fossil fuels generate dangerous pollutants at each stage of their lifecycle, from hazardous air toxics emitted from fracking wells to deadly criteria air pollutants and greenhouse gases emitted when they are burned. This pollution harms public health and is driving civilization into existential crisis — and these harms are disproportionately carried by Black, Brown, Indigenous, and poor communities. The time has come to phase out fossil fuel production, build a better economy to take its place, and ensure that workers and communities are made better off through the transition.

The current political opportunity to enact policies to address climate change is also a chance to reduce public health harms and partially alleviate the history of environmental racism — but only if those goals and stakeholders are included in policy design from the start. Pursuing "carbon-only" policies with an eye to extending the life of the fossil fuel industry will only delay and deny justice for communities suffering from toxic legacies and ongoing pollution burdens.

President Biden and Congress must confront the overlapping crises we face with a holistic approach to improve public health, tackle the climate crisis, and rectify our legacy of systemic racism by phasing out fossil fuel production. Our policy recommendations are rooted in Greenpeace USA's *Just Recovery Agenda*[332] and the Movement for Black Lives' *Red, Black, and Green New Deal* platform. To truly Build Back Better, President Biden and Congress must:

1. End fossil fuel racism and reverse the legacies of historical injustices.
2. Phase out fossil fuel production.
3. Ensure no worker or community is left behind.
4. Enact a green and just economic recovery.
5. Protect and expand our democracy to make it work for all people.

1. End fossil fuel racism and reverse the legacies of historical injustices

We must end fossil fuel racism and reverse the legacies of past injustices by placing both resources and power in the hands of impacted communities. Policies to accomplish this vision include:

- Require air and water pollution reductions in environmental justice communities to reduce pollution and pollution disparities by implementing a comprehensive "No Pollution Hotspots" policy.[333]
- Study, measure, and mitigate cumulative impacts of pollution from multiple sources, including legacy pollution.
- Strengthen equity and environmental justice mapping tools to provide more information to the public about health harms and disparities.[334]
- Institutionalize Free, Prior, and Informed Consent to require consent of American Indian and Alaska Native tribes and Indigenous peoples regarding federal actions affecting their lands, livelihoods, culture, and spirituality.
- Pass the *Environmental Justice for All Act* to provide legal remedies to citizens, improve equity mapping tools, expand grant programs, and strengthen consultation with impacted communities.[335]
- Elevate environmental justice to the highest levels of governance, including by passing the *Climate Equity Act*.[336]
- Strengthen the National Ambient Air Quality Standards to align with the best available science and end the politicization of air pollution at the EPA.
- Enact stronger standards, monitoring, and enforcement for hazardous air pollutants under the Clean Air Act.

2. Phase out fossil fuel production

We must halt the expansion of fossil fuel production and infrastructure, and put the industry on track for a just, equitable, and well-managed phaseout of fossil fuel production consistent with a pathway to limit global warming to below 1.5°C. This phaseout must go hand-in-hand with investments in economic security for workers and communities affected during the transition. Policies to accomplish a fossil fuel phaseout include:

- Pass the *End Polluter Welfare Act* to eliminate federal fossil fuel subsidies, including tax breaks, bailouts, loan guarantees, below-market royalties, and public finance.[337]
- Reinstate the crude oil export ban[338] and prohibit exports of liquified natural gas (LNG) and coal.
- Ban new fossil fuel leasing and permitting on public lands and waters, and phase out existing leases.
- Evaluate lifecycle greenhouse gas emissions for all federal infrastructure projects and reject federal permits for projects that would exacerbate the climate crisis.
- Strengthen "polluter pays" requirements, including bonding levels and fees, on oil and gas wells and coal mines to ensure fossil fuel corporations pay the full cost of remediation.
- Pass the *Break Free from Plastic Pollution Act*.
- Establish a national plan and targets to wind down existing fossil fuel production and infrastructure.
- Supporting the establishment of one-mile (minimum 2,500-foot) public health and safety buffer zones to ensure that no oil and gas production occurs near where people live, work, learn, and play.[339]

3. Ensure no worker or community is left behind

We must ensure that workers and communities will be better off during the transition to a more just and regenerative economy. We must invest in economic security and opportunities for workers and communities affected by the transition. Policies to realize this support include:

- Enact the *Protecting the Right to Organize (PRO) Act* to better enable workers to act in solidarity with one another, punish corporations that break the law, limit tactics that intimidate workers, help secure timely collective bargaining agreements, and make it easier for workers to form and join unions.
- Mandate the use of project labor agreements, Davis-Bacon prevailing wage requirements, community benefit agreements, and high-road labor standards in any federally funded project.
- Establish a federal oil well remediation program to create 250,000 high-quality jobs cleaning up abandoned oil and gas wells.[340]
- Provide good jobs cleaning up abandoned mines and fostering economic development in coal communities.
- Create a federal "Energy Worker and Community Protection Fund"[341] to guarantee wage, benefit, and pension protections for workers affected by the transition away from fossil fuels, replace lost tax and community revenue, and promote community revitalization and economic development for affected communities.

4. Enact a green and just economic recovery

We must enact a green and just economic recovery that includes rigorous climate standards to keep temperature increases below 1.5°C. Trillions of dollars in public investment are needed to secure a clean energy economy and guarantee essential public goods for all people. Strong policies are essential to ensure justice and equity for Black, Brown, Indigenous, poor people, and other disadvantaged workers and communities. Policies to accomplish this goal include:

- Pass the *THRIVE Act* to invest at least $1 trillion per year for the next decade to create 15 million good jobs, cut climate pollution in half by 2030, and confront systemic racism and gender, economic, and environmental injustice.[342]
- Honor frontline leadership with a representative governing board to guide federal investments.
- Ensure at least 50% of new investments directly benefit frontline and disadvantaged communities.
- Ensure federal investments pass a rigorous environmental justice and equity screen to avoid exacerbating "pollution hotspots."
- Uphold strong labor, climate, and equity standards to protect and expand workers' rights, mitigate the climate crisis, and ensure investments repair, rather than exacerbate, historic injustices.

5. Protect and expand our democracy to make it work for all people

Our democracy is in peril. We must protect people's rights to have a voice in the democratic process, get corporate money out of politics, defend the fundamental right to protest, and transform the current system to a people-powered democracy that works for all. Policies to achieve this vision include:

- Pass the *For the People Act* (H.R.1 / S.1) to give more electoral power to the people by getting big money out of politics, restoring voting rights, offering new protections for voters, ending gerrymandering, and holding government officials accountable.[343]
- End the Jim Crow filibuster to preserve the will of the majority to enact laws that will address the crises we face and ensure all communities can thrive.
- Pass the *John Lewis Voting Rights Act* (H.R.4) to protect against racist voter suppression tactics and strengthen our electoral system.[344]
- Grant statehood to Washington, D.C., by passing H.R.51, to ensure more than 700,000 thousand residents, a plurality of whom are Black, are afforded the same protections, access, and rights in our democracy that residents of every other state enjoy.[345]
- Passing a constitutional amendment to overturn the *Citizens United* U.S. Supreme Court ruling, which gives corporations and fossil fuel industry leaders unprecedented access to influence elected officials.[346]
- Enact the *BREATHE Act* to protect communities from police brutality and racial injustice by investing in Black communities and re-imagining community safety.

Green New Deal Press Conference in Washington, D.C. *Senator Ed Markey and other Members of Congress host a press conference calling for bold climate action in Congress in 2019.*

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This report is a project of Greenpeace, Inc., a registered Internal Revenue Service 501(c)(4) non-profit entity.

Global Climate Strike in San Francisco. *People across the U.S. left their homes, workplaces, and schools for a youth-led Global Climate Strike. They marched and rallied to demand transformative action to address the climate crisis, and called on leaders to choose to side with young people, not fossil fuel executives polluting the planet for profit.*

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2018 Leatherback sea turtle estimated bycatch reporting requirements as set out in the NMFS Biological Opinion for the continuing authorization of the Pacific Coast groundfish fisheries

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Table of Contents

List of acronyms and abbreviations

A-SHOP: At-Sea Hake Observer Program
BiOp: Biological Opinion
CCL: Curved Carapace Length
CI: Confidence Interval
EEZ: Exclusive Economic Zone
ESA: Endangered Species Act
FMP: Fishery Management Plan
FR: Federal Register
IFQ: Individual Fishing Quota
ITS: Incidental Take Statement
LE: Limited Entry
mt: metric ton
NMFS: National Marine Fisheries Service
NOAA: National Oceanic and Atmospheric Administration
NOI: Northern Oscillation Index
NWFSC: Northwest Fisheries Science Center
OA: Open Access
PacFIN: Pacific Fisheries Information Network
PFMC: Pacific Fishery Management Council
RPM: Reasonable and Prudent Measure
US: United States
USFWS: United States Fish and Wildlife Service
WCGOP: West Coast Groundfish Observer Program

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Introduction

ESA Section 7(a)(2) consultation and the Biological Opinion (BiOp) process

Section 7(a)(2) of the Endangered Species Act of 1973 (ESA), as amended (16 U.S.C. § 1531, *et seq*.), and implementing regulations at 50 Code of Federal Regulations (CFR) Part 400 requires Federal agencies to ensure that any action authorized, funded or carried out by them is not likely to jeopardize the continued existence of listed species or result in adverse modification of their critical habitat. Section 7(a)(2) requires Federal agencies to engage in consultations with either or both National Marine Fisheries Service (NMFS) and the US Fish and Wildlife Service (USFWS) when a federal agency determines that a proposed action may affect a listed species or designated critical habitat. Under Section 7(b)(3) of the ESA, if the activity "adversely affects" ESA-listed species, the agencies undergo formal consultation, which requires a Biological Opinion (BiOp). If incidental take is expected, section 7(b)(4) requires the consulting agency to provide an incidental take statement (ITS) that specifies the impact of any incidental taking and includes non-discretionary reasonable and prudent measures (RPMs) to minimize such impacts and recommendations for further conservation measures.

The 2012 BiOp by NMFS determined that the components of the groundfish fishery were likely to adversely affect eulachon, green sturgeon, humpback whales, Steller sea lions, leatherback turtles, and were likely to destroy or adversely modify critical habitat of green sturgeon. With respect to leatherback turtles interacting with US West Coast groundfish fisheries, the BiOp identified a set of non-discretionary terms and conditions as necessary to implement the RPMs identified in the BiOp. These terms and conditions relate to two main areas: a) management planning and take reporting; and b) take monitoring. The BiOp identified the take limit of leatherback turtles to be 0.38 turtles/year over a 5-year average not exceeding 1 turtle/yr.

Specific Terms and Conditions with respect to leatherback sea turtles that we will address in this report include:

- Preparation of biennial fleet-wide take estimates
- Updating reporting of take considered in the BiOp
- Identifying minimum coverage levels and monitoring goals for those fisheries with anticipated observable take

U.S. West Coast Groundfish Fishery

The U.S. west coast groundfish fishery is a multi-species fishery that utilizes a variety of gear types. The fishery harvests species designated in the Pacific Coast Groundfish Fishery Management Plan (Groundfish FMP; PFMC 2011) and is managed by the Pacific Fishery Management Council (PFMC). Over 90 species are listed in the Groundfish FMP, including a variety of rockfish, flatfish, roundfish, skates, and sharks. These species are found in both federal (> 5.6 km from the coastline) and state waters (0-5.6 km from the coastline). Groundfish are both targeted and caught incidentally by trawl nets, hook-and-line, and fish pots/traps.

Under the FMP, the groundfish fishery consists of four management groups: limited entry, open access, recreational, and tribal fisheries. The Limited Entry (LE) fishery encompasses all commercial fishers who hold a federal limited entry permit. The total number of limited entry permits is restricted. Vessels with an LE permit are allocated a larger portion of the total allowable catch for commercially desirable species, such as sablefish, than vessels without an LE permit. The Open Access (OA) fishery encompasses commercial fishers who do not hold a federal LE permit. Some states require fishers to carry a state-issued permit for certain OA sectors (i.e., a subgroup of a fishery characterized by gear type, target species, and regulation). The Recreational fishery includes recreational anglers who target or incidentally catch groundfish species. Recreational fishery is not included in this report. The Tribal fishery includes native tribal commercial fishers in Washington State that have treaty rights to fish groundfish. Tribal fishery also is not included in this report, with the exception of the observed tribal at-sea Pacific hake sector. These four groups can be further subdivided into multiple sectors. This report includes data from the following sectors.

Limited Entry (LE) sectors

- Individual fishing quota (a.k.a., catch shares) fishery (known as LE bottom trawl and at-sea hake from 2002 to 2010): this sector is subdivided into the following components due to differences in gear type and target species:
    - Bottom trawl: Bottom trawl nets are used to catch a variety of non-hake groundfish species. Catch is delivered to shore-based processors.
    - Midwater non-hake trawl/Midwater Rockfish: Midwater trawl nets are used to target mid-water non-hake species, mainly mid-water rockfish such as yellowtail and widow rockfish. Catch is delivered to shore-based processors.
    - Pot/traps: Pot/traps are used to target groundfish species, primarily sablefish. Catch is delivered to shore-based processors.
    - Hook-and-line: Longlines are primarily used to target groundfish species, mainly sablefish. Catch is delivered to shore-based processors.
    - Electronic Monitoring (EM) Exempted Fishing Permit (EFP): Some IFQ vessels fishing with bottom trawl, midwater trawl, or pots participate in the IFQ EM EFP. EM systems use video recordings to estimate weights of certain IFQ species that are allowed to be discarded at sea. Targets are similar to those targets described above for similar gear types.
    - Vessels fishing using pot or bottom trawl gear followed guidelines for optimized retention and could discard only certain allowed species; on those vessels, observer coverage was targeted at a random sample of 30% of trips to result in 25 to 30% of landings.
    - California halibut trawl: Bottom trawl nets are used to target California halibut by fishers holding a California state halibut permit and an LE federal trawl groundfish permit. Catch is delivered to shore-based processors.

  - Shoreside hake/Midwater hake: Midwater trawl nets are used to target Pacific hake. Catch is delivered to shore-based processors.
  - At-sea motherships and catcher-processors: Midwater trawl nets are used to catch Pacific hake. Catcher vessels deliver unsorted catch to a mothership. The catch is sorted and processed aboard the mothership. Catcher-processors catch and process fish at-sea and may receive unsorted catch from catcher vessels to process. This component also includes the at-sea processing component of the tribal sector. The tribal sector must operate within defined boundaries in waters off northwest Washington State.

- LE fixed gear (non-nearshore): This component is subdivided into two sub-components due to differences in permitting and management:
  - LE sablefish endorsed season: Longlines and pots are used to target sablefish. Catch is generally delivered to shore-based processors.
  - LE sablefish non-endorsed: Longlines and pots are used to target groundfish, primarily sablefish and thornyheads. Catch is delivered to shore-based processors or sold live dockside.

Open Access (OA) federal sector

- OA fixed gear (non-nearshore): Fixed gear, including longlines, pots, fishing poles, stick gear, etc. is used to target non-nearshore groundfish. Catch is delivered to shore-based processors.

Open Access (OA) state sectors

- OA pink shrimp trawl: Trawl nets are used to target pink shrimp. Catch is delivered to shore-based processors.
- OA California halibut trawl: Trawl nets are used to target California halibut by fishers holding a California state halibut permit. Catch is delivered to shore-based processors.

- Nearshore fixed gear: a variety of fixed gear, including longlines, pots, fishing poles, stick gear, etc. are used to target nearshore rockfish and other nearshore species managed by state permits in Oregon and California. Catch is delivered to shore-based processors or sold live at the dock.
- California ridgeback prawn trawl: Trawl nets are used to target prawn in California waters. Observers were first deployed in this fishery in 2017.
- **Pacific halibut hook-and-line derby:** Longline and hook-and-line vessels target Pacific halibut during single day openers as determined by the International Pacific Halibut Commission (IPHC). Observers were first deployed in this fishery in 2017.

Northwest Fisheries Science Center (NWFSC) Groundfish Observer Program

The NWFSC Groundfish Observer Program observes commercial sectors that target or take groundfish as bycatch. The observer program has two units: the West Coast Groundfish Observer Program (WCGOP) and the At-Sea Hake Observer Program (A-SHOP).

The WCGOP was established in May 2001 by NOAA Fisheries (a.k.a., National Marine Fisheries Service, NMFS) in accordance with the Groundfish FMP (50 CFR Part 660,50 FR 20609). This regulation requires all vessels that catch groundfish in the US Exclusive Economic Zone (EEZ) from 3 to 200 miles from shore carry an observer when notified to do so by NMFS or its designated agent. Subsequent state rule-making has extended NMFS' ability to require vessels fishing in the state territorial zone (up to 3 miles from shore) to carry observers.

The NWFSC Groundfish Observer Program's goal is to improve estimates of total catch and discard by observing groundfish fisheries along the U.S. west coast. The WCGOP and A-SHOP observe distinct sectors of the groundfish fishery. The WCGOP observes multiple sectors of the groundfish fisheries, including Individual Fishing Quota (IFQ) shore-side delivery of groundfish and Pacific hake, at-sea mothership catcher-vessels fishing for Pacific hake, LE and OA fixed gear, and state-permitted

nearshore fixed gear sectors. The WCGOP also observes several state-managed fisheries that incidentally catch groundfish, including the California halibut trawl and pink shrimp trawl fisheries. The A-SHOP observes the IFQ fishery that targets Pacific hake at-sea including: catcher-processor, mothership, and tribal vessels.

Leatherback turtles in the US west coast EEZ

The leatherback turtle (*Dermochelys coriacea*) is the sole remaining member of the taxonomic family Dermochelyidae. All other extant sea turtles belong to the family Cheloniidae. Leatherbacks are the largest marine turtle, with a curved carapace length (CCL) of adults often exceeding 150 cm and front flippers that can span 270 cm (NMFS and USFWS, 1998). The leatherback's slightly flexible, rubber-like carapace is distinguishable from other sea turtles that have carapaces with bony plates covered with horny scutes. In adults, the carapace consists mainly of tough, oil-saturated connective tissue raised into seven prominent ridges and tapered to a blunt point posteriorly. The carapace and plastron are barrel-shaped and streamlined.

In the Pacific Ocean, genetic studies have identified three distinct populations (referred to also as genetic stocks or Management Units; see Wallace et al., 2010) of leatherback turtles: (1) Mexico and Costa Rica, which are genetically homogenous but distinct from the western populations; (2) Papua Barat in Indonesia, Papua New Guinea, Solomon Islands, and Vanuatu, which comprise a metapopulation representing a single genetic stock; and (3) Malaysia. The genetically distinct Malaysia nesting population is functionally extinct (Chan & Liew, 1996, Dutton et al., 1999).

Leatherbacks display several unique physiological and behavioral traits that enable this species to inhabit cold water, unlike other sea turtle species. These include a countercurrent circulatory system (Greer et al., 1973), a thick layer of insulating fat (Goff & Lien, 1988, Davenport et al., 1990), large body size that promotes thermal inertia limiting heat loss (i.e., gigantothermy; Paladino et al., 1990), and the ability to elevate body temperature through increased metabolic activity (Southwood et al., 2005,

Bostrom & Jones, 2007, Bostrom et al., 2010). These adaptations also enable leatherbacks to have a larger geographic range than other sea turtle species.

Leatherback turtles have the most extensive range of any living reptile and have been reported circumglobally. Leatherback turtles can forage in the cold temperate regions of the oceans and have been reported at latitudes as high as 71° N and 47° S. Nesting, however, is confined to tropical and subtropical latitudes (reviewed in Eckert et al., 2012). In the Pacific Ocean, nesting aggregations occur primarily in Mexico, Costa Rica, Indonesia, the Solomon Islands, and Papua New Guinea.

Migratory routes of leatherback turtles have been studied in recent years via satellite telemetry. These studies documented transoceanic migrations of adult leatherback turtles between nesting beaches and foraging areas in the Atlantic and Pacific Ocean basins, where they may migrate more than 10,000 km in a year (Ferraroli et al., 2004, Hays et al., 2004, James et al., 2005, Eckert, 2006, Eckert et al., 2006, Benson et al., 2007a, Benson et al., 2011). Leatherback turtles nesting in Central America and Mexico migrate thousands of kilometers into tropical and temperate waters of the South Pacific (Eckert and Sarti, 1997, Shillinger et al., 2008). Females from the western Pacific make long-distance migrations into the central and eastern North Pacific, westward to the Sulawasi and Sulu and South China Seas, northward to the North Pacific Transition Zone and the Sea of Japan, and south to the western South Pacific Ocean and Tasman Sea (Benson et al*.,* 2007a; Benson et al*.,* 2011).

Leatherback turtles mainly eat gelatinous organisms, particularly of the class Scyphozoa, but other taxa including crustaceans, vertebrates, and plants are ingested (reviewed by Eckert *et al.* 2012, Dodge et al., 2011, Jones and Seminoff 2013). Because leatherbacks must consume large amounts of food to meet their energetic demands (Heaslip et al., 2012, Jones et al., 2012), it is important that they have access to areas of high productivity. Leatherback turtles tagged after nesting in July at Jamursba-Medi, Indonesia, arrived in waters off California and Oregon during July- August of the following year (Benson et al., 2007a, 2011) coinciding with the development of seasonal aggregations of jellyfish

(Shenker, 1985, Larson, 1990, Suchman et al., 2008, Suchman & Brodeur, 2005). Other studies similarly have documented leatherback sightings along the Pacific coast of North America during the summer and autumn months, when large aggregations of jellyfish form (Starbird et al., 1993, Bowlby et al., 1994, Benson et al., 2007b). Leatherbacks primarily forage on cnidarians (jellyfish and siphonophores) and, to a lesser extent, tunicates (pyrosomas and salps; NMFS and USFWS, 2013). Within these ecosystems, various oceanic features such as water temperature, down-welling, Ekman upwelling, sea surface height, chlorophyll-a concentration, and mesoscale eddies influence the behavior of leatherbacks (Bailey et al., 2012, Benson et al. 2011, Eguchi et al. 2016).

Genetic evidence presented by Dutton et al. (2000) and telemetry data from both nesting beaches and foraging areas (Benson et al. 2011) indicated that leatherback turtles found along the west coast of the US are actually part of a distinct population originating in the western Pacific. These turtles nest on beaches in Indonesia whose population size has been declining at approximately 6% per annum (Tapilatu et al., 2013). A recent study using stable isotopes indicated that up to two thirds of boreal summer nesting females in Indonesia travel across the Pacific to reach the US west coast (Seminoff et al., 2012). Annual abundance of leatherback turtles in the California Current Ecosystem is affected by local oceanographic events. A positive linear relationship between the Northern Oscillation Index (NOI) and the estimated abundance in a foraging area has been reported (Benson et al., 2007b). Further, a recent study found that the arrival and departure of leatherbacks from the California Current Ecosystem can be predicted using upwelling indices at various latitudes with time lags, indicating effects of physical oceanography on leatherback turtles' presence in a foraging area presumably through affecting abundance of their diet (Eguchi et al. 2016). However, because the vast majority of survey effort has been focused over the continental shelf and up to 32 km from the coast, little information is available on the distribution and abundance of leatherback turtles in offshore waters. Foraging habitat of leatherback turtles along the west coast of the US appears to exist in shallow nearshore waters when

the environmental condition is favorable but it is possible that the offshore areas may be used as their foraging areas in some years (Eguchi et al. 2016).

On January 26, 2012, NMFS designated critical habitat for leatherback turtles within the Pacific Ocean. This designation includes approximately 16,200 square miles (43,798 square kilometers) stretching along the California coast from Point Arena to Point Arguello east of the 3,000 meter depth contour. In addition, approximately 25,004 square miles (64,760 square kilometers) stretching from Cape Flattery, Washington to Cape Blanco, Oregon east of the 2,000-m depth contour was designated. The primary constituent elements essential to the conservation of leatherback turtles include prey, primarily scyphomedusae, of sufficient condition, distribution, diversity, abundance and density necessary to support individual as well as population growth, reproduction, and development of the species.

BiOp Terms and conditions

Data

Fisheries

Data sources for this analysis include onboard observer data (from the WCGOP and A-SHOP), and landing receipt data (referred to as fish tickets, obtained from the Pacific Fisheries Information Network; PacFIN). Observer and electronic monitoring data are the sole source for discard estimation in the U.S. west coast groundfish fisheries. A list of fisheries, coverage priorities and data collection methods employed by WCGOP in each observed fishery can be found in the IFQ and Non-IFQ WCGOP manuals (NWFSC 2013b). A-SHOP program information and documentation on data collection methods can be found in the A-SHOP observer manual (NWFSC 2013b).

The sampling protocol employed by the WCGOP is primarily focused on the discarded portion of catch. To ensure that the recorded weights for the landed portion of the observed catch are accurate,

haul-level retained catch weights recorded by observers are adjusted based on trip-level landing receipts (a.k.a., fish tickets). Fish tickets are issued to fish-buyers by a state agency and must be returned to that state agency for processing. When a vessel delivers fish to a port, fish tickets are completed by fish-buyers and represent single or multiple species. Fish ticket and species-composition data are submitted by state agencies to the PacFIN regional database. This process is described in further detail on the WCGOP Data Processing webpage (NWFSC 2013a). Annual fish ticket landings data were retrieved from the PacFIN database and subsequently divided into various sectors of the groundfish fishery as indicated in further detail online (NWFSC 2013c). For data processing purposes, species and species groups were defined based on management (NWFSC 2013c). A complete listing of groundfish species is defined in the Pacific Coast Groundfish Fishery Management Plan (PFMC 2011). Fishing effort and observer coverages for OA fixed gear, LE Sablefish, LE bottom trawl, and catch share are provided in Tables 1, 2, 3, and 4, respectively.

Leatherback turtles

For leatherback turtles, we provide observed bycatch, sightings of leatherback turtles by observers aboard groundfish fishing vessels, and records of strandings. In the available datasets (2002-2017), one leatherback turtle has been reported entangled and killed in a groundfish fishing gear, whereas five leatherback turtles have been sighted. The death occurred in 2008 (OA pot fishery), whereas the sightings occurred in 2005 (LE sablefish endorsed hook and line fishery), 2007 (LE bottom trawl fishery), 2008 (OA fixed gear), 2011 (catch shares pot fishery), and 2014 (OA fixed gear, hook and line fishery). In this report, we use the single lethal bycatch datum to determine the fleet-wide bycatch estimate of leatherback turtles for the OA pot fishery. All other fishery sectors and gears mentioned in this report and observed by the NWFSC Observer Program have never had an observed bycatch incident with a leatherback sea turtle.

Stranding data were obtained from the Marine Turtle Stranding Network database housed at the NMFS West Coast Regional Office, Long Beach, CA, and Seattle WA. Stranding records are based on discoveries of turtles; therefore, there is a greater probability of encountering turtles ashore when and where there are higher concentrations of people along the coastline. In addition, turtles may have drifted considerably following deaths and subsequent report to the stranding networks. Consequently, the time-series of the number of stranded turtles and spatial distribution alone cannot be directly used to infer the change in strandings over time and space.

From 1963 to 2017, there have been 135 reported leatherback sea turtle strandings along the west coast of the US, including AK, WA, OR, and CA (Hodge & Wing, 2000, R. LeRoux et al. in prep). The quality of data has improved over time, where complete information is available for more recent records than old records. Not all stranded turtles were dead and some stranded leatherback turtles were revived and released back to the sea (n = 7). The number of annual strandings, as defined above, fluctuated from 0 to 12 (Figure 1). Twelve indicated evidence of fishery interactions, where 11 were found in central and southern California and one in Oregon. The majority were in the state of California (118) and no strandings in AK have been reported since 1993 (Figure 1). The annual numbers of stranded leatherback turtles without evidence of human interaction were greatest in the early 1990s and declined thereafter. The stranding locations of leatherback turtles along the west coast of the US were concentrated along central and southern California, including the Channel Islands (Figure 2).

Bycatch Estimation

Statistical Model

We applied two statistical models to characterize uncertainty in the leatherback turtle bycatch in the OA pot fishery. Because only one turtle was encountered as bycatch in this fishery, we were restricted to using simple statistical models while estimating variances of total bycatch. The first approach we used was the Poisson process model, where the total number of entanglements or bycatch

events were assumed to follow a Poisson distribution, $n_{bycatch} \sim Poisson(\lambda_1 \cdot N_y)$. In this approach, the Poisson rate or intensity parameter (λ_1, where $0 \leq \lambda_1 \leq 1$) was fixed at the annual bycatch point estimate (e.g., 1 bycatch event out of 1000 sets would lead to λ_1 = 0.001), and the effort for a particular year (N_y) was used to estimate the total bycatch. A caveat of this first approach was that by fixing λ_1, we were ignoring the uncertainty in the bycatch rate, making the 95% confidence intervals (CIs) overly narrow. For example, two fishery sectors might have the same bycatch point estimate, but if one sector fished with 10x as much effort, that second estimate would be more precise. To incorporate this uncertainty due to variable sample sizes, our second approach was to treat the rate parameter as a random variable (λ_2, where $0 \leq \lambda_2 \leq 1$). We did not use a common approach to model uncertainty in the proportion p of a Binomial distribution using the Normal approximation, $p \sim Normal(\hat{p}, \sqrt{\frac{\hat{p}(1-\hat{p})}{n}})$, where $\hat{p}$ is the estimated proportion and n is the sample size, because the 95% CIs can include negative values due to the small estimated proportion. To keep this parameter (*p*) positive, we instead simulated the number of bycatch events that would have occurred given a certain level of effort, and divided that result by effort. Using our previous numbers as an example, $\lambda_2 \sim Binomial(p = 0.001, N = 1000)/1000$. Both approaches require at least one bycatch event. For each model, we generated 100,000 random draws from the distributions of potential bycatch and calculated summary statistics (mean, median, and variance) as well as measures of uncertainty (95% CIs).

Groundfish landings from OA fixed gear vessels fishing with pots were summarized in 5-year running averages for two periods; 2008-2012 and 2013-2017 (Table 5). Therefore, we present the annual average and five-year total landings. The observed annual average groundfish landings for the first period was 11.59 mt, whereas the fleet-wide total landing over the 5-yr period was 1313.31 mt. For the second period, they were 14.54 mt (annual average) and 871.52 mt (5-yr sum of fleet-wide landing).

Biennial fleet-wide take estimates

We estimated the total bycatch in the most recent two 5-year periods (2008-2012, 2013-2017,Table 5). No leatherback sea turtles were observed as bycatch in the most recent 5-year period (2013-2017) and thus, all U.S. west coast groundfish fisheries are below the BiOp take limit of 0.38 leatherbacks per year for the most recent 5-year period. Since 2003, there has only been one observed leatherback sea turtle caught in U.S. west coast groundfish fishing gear, which occurred in 2008 in the OA fixed gear fishery on a vessel fishing pot gear. The bycatch rate during the 2008-2012 period was 0.017. Given the observer coverage rate of approximately 3%, this produces an estimate of 23 individuals caught by the OA pot fleet during the 2008-12 period (Table 5). It is important to note that extrapolating the bycatch rate computed from only 3% observer coverage to the entire fleet results in large uncertainty.

Estimation of the fleet-wide bycatch is challenging because only one leatherback turtle has been observed taken in the U.S. west coast groundfish fisheries and observer coverage has been low in the OA fixed gear sector (<10%; Table 1). Therefore, to determine the risk of leatherback turtle entanglement in the OA fixed gear fishery, we provide probabilistic estimates of the number of entanglements for the period 2008-2012 (Figure 3) based on the two versions of the Poisson statistical model described above. We also provide summary statistics for each of the model outputs (Table 6). We could only apply these models for the period 2008-2012 because that was the only period in which a leatherback sea turtle was entangled in gear in a U.S. west coast groundfish fishery. Extrapolating these models beyond the 2008-2012 period or to other gear types or fishery sectors would not be appropriate.

For the 2008-2012 period, the model with a fixed bycatch rate (solid line, Figure 3) indicated that the probability of exceeding 1.9 leatherback entanglements in this 5-year period (grey dotted vertical line intersects with solid black line Figure 3) was >90%. Exceeding 1.9 leatherbacks is equivalent to exceeding an average of 0.38 leatherbacks per year for a 5-year period. If we model the bycatch rate

with uncertainty, the probability of exceeding 1.9 entanglements drops to approximately 60% (grey dotted vertical line intersects with dashed black line, Figure 3). The probability of more than 1.9 entanglements decreased more rapidly when uncertainty was included in the model, compared to the model without uncertainty. The large variation in entanglement represented in both models (Figure 3 & Table 6) was a result of only a single bycatch incident in all U.S. west coast groundfish fisheries in the 15 years of observation (2003-2017).

Minimum Observer Coverage

Reasonable and prudent non-discretionary measures for the ESA Section7(a)(2) 2012 BiOp includes "…identify[ing] goals for minimum [observer] coverage levels to achieve fleet-wide take estimates for leatherback sea turtles…and a plan for implementation." (p. 124). Unfortunately, the BiOp provides no guidance on the metrics needed to identify minimum goals for appropriate observer coverage. Interactions between leatherback sea turtles and U.S. west coast groundfish vessels are extremely rare. To date only one leatherback sea turtle has been observed interacting with a groundfish vessel in the 14 years of observation. The lack of data makes any formal investigation into necessary observer coverage rates very challenging. The WCGOP stated target coverage rate (i.e., pre-observation) for the OA fixed gear fishery is to observe 5% of the groundfish landings (excluding Pacific hake). Realized coverage rates (i.e., post-observation) vary around the target coverage rate for a variety of reasons including (but not limited to), resource availability, logistics, safety and fishing effort. The WCGOP plans to maintain historic coverage rates (3-6%) in the OA fixed gear fishery where the single leatherback interaction occurred (Table 1). Target coverage rates for other sectors are as follows: Catch Shares sectors 100%; Catch Shares EM vessels 16-36%; LE sablefish 25-30%; LE Daily Trip Limits 10%; OA ocean shrimp trawl 15%; state nearshore fisheries 7-10%; and California halibut fishery 3-5%; California ridgeback prawn fishery 11%; Pacific halibut derby fishery 7%. Historic coverage rates by year can be found on the NWFSC Observer Program webpage (Somers & Jannot 2014). The WCGOP will assess the

feasibility of a formal investigation of observer coverage for leatherback sea turtle interactions as new data are collected.

Leatherback-specific terms and conditions

The NWFSC Groundfish Observer program maintains a database of both sea turtle interactions with fishing vessels and sightings of sea turtles by at-sea observers. The few records of sightings by the observers indicate that leatherback turtles occur in the fishing grounds. It is also possible that the observed bycatch was an extreme rare event because of the gear configurations and behavior of leatherback turtles where the fishery and turtles co-occur. These possibilities cannot be ruled out without collecting more data through increased observer coverage of all sectors or obtaining more information from fishers.

Because only one leatherback turtle has been observed to be killed by this fishery between 2003 and 2017, it is possible that the likelihood of the fishery affecting the leatherback turtle population is low. However, the population of leatherback turtles that are interacting with this fishery is declining rapidly (Tapilatu et al., 2013). Consequently, every turtle counts for sustaining and hopefully recovering the population. Therefore, for rare but not negligible instances of interactions, some measures are necessary to reduce deaths of bycaught turtles.

The BiOp requires NMFS to provide information and training to observers regarding regulations requiring fishermen to properly handle, release, and resuscitate sea turtles, per 50 CFR 223.206(d)(1), and demonstrate these methods during observer training. In addition, the BiOp requires NMFS to educate observers on handling methods that will reduce sea turtle injury or mortality. The NWFSC Observer Program currently provides this information and training to all its observers. Observers are instructed on the safe handling, release, and resuscitation of sea turtles using model sea turtles in the classroom following protocols set by NMFS (e.g., NMFS 2008). Resuscitation procedures include the following steps: retain on a fishing vessel up to 24 hours, place turtle on plastron, elevate hindquarters

using a cushion, tire etc. (minimum 15 - 30 degrees) to permit the lungs to drain off water for a period of 4 up to 24 hours. Rock sea turtle left to right raising edge of carapace 8 cm each time. Keep turtle in the shade, at a temperature similar to the water temperature, and moist by covering with a wet towel and periodically spraying it with water in a freshly cleaned enclosed area. Periodically test turtle for positive response to resuscitation by gently touching the corner of the eye or eyelid and pinching the tail near the vent to monitor consciousness. Sea turtles may take some time to revive. Turtles that are successfully resuscitated benefit from being held on deck as long as possible (up to 24 hours) to fully recover from the stress of accidental forced submergence. Observers are trained to release sea turtles from the stern or side of the vessel (or the trawl ramp if available) while the vessel's engines are in neutral position with all fishing gear out of the water. Further, NMFS shall provide information on sea turtle biology during groundfish observer training. Observers are instructed on the identifying characteristics of sea turtles using a dichotomous key. Each dichotomy is explained and shown using model sea turtles in the classroom.

Conservation Recommendations

The BiOp requires NMFS to assess the feasibility of collecting data to determine bycatch of jellyfish in the groundfish trawl fisheries. Identifying jellyfish in groundfish trawl fisheries is not feasible. The large trawl nets used in these fisheries result in jellyfish that are not whole specimens. The U.S. west coast bottom trawl survey run by the NWFSC has had little success identifying jellyfish. Jellyfish captured in trawl nets in both the survey and on commercial trawlers are most often recorded as jellyfish unidentified. Incorporating a protocol for identifying jellyfish to species would result in most jellyfish being recorded as unidentified due to poor condition, and any resulting data would be of low quality and value.

Concluding statement

Leatherback turtle entanglements in the groundfish fishery appear to be rare events. Only one bycatch event was recorded in one fishery over the last 15-year period. Because of the low observer coverage of the fishery, however, conclusive statements about leatherback turtle bycatch in the groundfish fishery in general cannot be made without more data on fishery (bycatch or no bycatch) and on overlap between the fishery and leatherback turtles. The large uncertainty resulted from the extrapolation of observed bycatch rate to the entire fleet of the OA fixed-gear fishery. If the bycatch rate was derived from a larger proportion of the total fleet, uncertainty around the estimate would have been smaller.

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Tables and Figures

Table 1. Fishing effort and observer coverage in the OA fixed gear from 2003 to 2017. Leatherback turtle entanglement occurred in 2008 using pot gear. Dark and light gray are used to indicate the two 5-yr periods that were used to compute moving averages in Table 5.

Year	Gear	# vessels	# trips	# hauls	# fixed gear units	Observed Retained (mt)	Landed (mt)	Coverage (%)
2003	Hook and Line	13	41	49	86518	16.54	544.89	3.0
2004	Hook and Line	14	42	52	85895	16.21	473.79	3.4
2005	Hook and Line	10	34	37	58384	9.78	623.95	1.6
2006	Hook and Line	7	10	11	29296	4.50	485.93	0.9
2007	Hook and Line	25	51	67	55215	10.40	263.41	3.9
2008	Hook and Line	33	58	68	73885	16.15	401.17	4.0
2009	Hook and Line	34	69	104	119849	21.81	646.27	3.4
2010	Hook and Line	37	70	105	160570	23.05	756.51	3.0
2011	Hook and Line	40	69	101	162419	20.13	434.34	4.6
2012	Hook and Line	24	34	53	82597	11.48	323.09	3.6
2013	Hook and Line	14	23	30	51870	4.71	193.43	2.4
2014	Hook and Line	21	28	39	71459	11.78	219.47	5.4
2015	Hook and Line	20	38	54	124895	17.47	363.90	4.8
2016	Hook and Line	31	57	79	111092	15.65	291.37	5.4
2017	Hook and Line	43	62	80	95811	14.92	348.03	4.3
2003	Pot	7	16	50	345	2.94	190.30	1.5
2004	Pot	17	96	185	1950	16.99	186.03	9.1
2005	Pot	14	43	50	835	10.67	379.29	2.8
2006	Pot	15	38	39	666	7.90	442.93	1.8
2007	Pot	21	46	75	624	8.75	257.86	3.4
2008	Pot	20	55	75	833	10.43	240.83	4.3
2009	Pot	18	30	45	540	8.82	372.58	2.4

2010	Pot	26	40	71	648	10.66	318.29	3.3
2011	Pot	29	61	85	831	18.94	255.80	7.4
2012	Pot	19	35	70	610	9.13	125.82	7.3
2013	Pot	17	25	48	590	6.30	72.18	8.7
2014	Pot	21	41	63	686	11.66	147.70	7.9
2015	Pot	17	49	64	604	14.60	234.25	6.2
2016	Pot	27	55	73	687	15.27	206.76	7.4
2017	Pot	44	87	127	1249	24.89	210.64	11.8

Table 2. Fishing effort and observer coverage in LE sablefish fishery from 2003 to 2017.

Year	Gear	# vessels	# trips	# hauls	# fixed gear units	Observed Retained (mt)	Landed (mt)	Coverage (%)
2003	Hook and Line	15	48	351	733602	222.85	1051.55	21.2
2004	Hook and Line	17	45	326	492009	180.02	1318.09	13.7
2005	Hook and Line	26	101	678	1456102	481.45	1341.56	35.9
2006	Hook and Line	19	68	471	939951	295.93	1401.23	21.1
2007	Hook and Line	22	75	517	1034046	298.49	1103.93	27.0
2008	Hook and Line	18	77	540	1244141	338.15	1103.35	30.6
2009	Hook and Line	8	45	287	648980	97.81	1441.51	6.8
2010	Hook and Line	21	143	762	1761173	345.77	1304.16	26.5
2011	Hook and Line	23	98	673	1405444	240.74	1153.50	20.9
2012	Hook and Line	17	88	532	1580075	239.32	1079.16	22.2
2013	Hook and Line	18	58	353	1047526	166.42	747.96	22.2
2014	Hook and Line	17	85	495	1200615	203.23	747.12	27.2
2015	Hook and Line	26	97	632	1536820	391.96	939.77	41.7
2016	Hook and Line	21	94	671	1743233	338.12	1031.73	32.8
2017	Hook and Line	25	109	701	2107656	396.86	1060.37	37.4
2003	Pot	6	35	362	9017	148.31	603.97	24.6
2004	Pot	3	13	139	5378	82.68	619.60	13.3
2005	Pot	7	39	492	13822	281.18	615.00	45.7
2006	Pot	7	39	289	10708	200.47	581.80	34.5
2007	Pot	4	30	154	5816	89.97	428.37	21.0
2008	Pot	6	24	329	13638	244.87	432.98	56.6
2009	Pot	3	27	67	3883	66.48	489.07	13.6
2010	Pot	7	43	314	11294	140.39	503.54	27.9
2011	Pot	3	22	227	9029	137.42	371.93	36.9
2012	Pot	5	19	351	14218	101.10	285.98	35.4
2013	Pot	3	14	47	1934	40.52	283.13	14.3
2014	Pot	4	16	195	7561	104.01	338.09	30.8
2015	Pot	9	36	308	11634	223.20	358.21	62.3
2016	Pot	7	55	596	21219	254.27	359.00	70.8
2017	Pot	3	14	186	7852	115.46	375.48	30.8

Table 3. Fishing effort and observer coverage for LE bottom trawl from 2002 to 2010.

Year	# vessels	# trips	# hauls	Tow duration or Soak time (hrs)	Observed Retained (mt)	Landed (mt)	Coverage (%)
2002	132	573	3163	13471.83	2679.97	18005.29	15
2003	125	462	2289	11496.14	2590.36	18388.80	14
2004	103	615	3441	13837.75	4309.78	17673.92	24
2005	105	522	3460	12621.02	4241.57	19286.20	22
2006	87	477	2977	11455.97	3443.33	17794.94	19
2007	88	371	2515	11380.64	3442.33	20442.49	17
2008	100	438	3185	15004.17	4905.22	24188.27	20
2009	101	590	4394	19581.08	6053.39	26055.50	23
2010	83	347	2614	13039.74	4019.52	22320.84	18

Table 4. Fishing effort and observer coverage for catch shares from 2011 to 2017. In Sectors, CS is catch shares and EM are fisheries that are partially monitored for fishing compliance by electronic monitoring systems. In EM fisheries, the coverage is the percentage of observer coverage and exclude electronic monitoring. In gear types (Gear), BT is bottom trawl, MT is midwater trawl, and HL is hook and line.

Year	Sector	Gear	# Vessels	# Trips	# Hauls	# Units	Observed Retained (mt)	Total Landed (mt)	Coverage (%)
2011	CS	BT, MT	72	1134	9195	0	16969.94	16989.16	100
2012	CS	BT	67	1089	8968	0	16949.36	16970.92	100
2013	CS	BT	68	1193	10017	0	18537.64	18512.07	100
2014	CS	BT	64	1033	8322	0	15759.07	15825.47	100
2015	CS	BT	60	904	7480	0	15589.55	15641.96	100
2016	CS	BT	53	802	6623	0	14957.23	14982.93	100
2017	CS	BT	54	839	6398	0	15377.59	15414.12	100
2011	CS	HL	11	94	630	2265264	335.47	364.22	100
2012	CS	HL	8	32	506	1472865	241.27	271.44	100
2013	CS	HL	8	29	215	587238	79.45	81.96	100
2014	CS	HL	8	31	227	601654	88.55	179.47	100
2015	CS	HL	5	16	185	592919	137.84	137.84	100
2016	CS	HL	5	30	351	1110926	192.73	205.39	100
2017	CS	HL	4	13	148	476944	115.94	115.92	100
2011	CS	Pot	17	233	1536	41310	813.82	789.50	100
2012	CS	Pot	19	278	1709	52248	740.69	711.22	100
2013	CS	Pot	10	100	1086	30097	470.84	501.77	100
2014	CS	Pot	14	118	1288	31876	681.15	618.97	100
2015	CS	Pot	8	62	584	18808	405.29	405.29	100
2016	CS	Pot	8	61	584	15785	387.05	394.44	100
2017	CS	Pot	6	44	574	16288	367.35	364.30	100
2015	CS EM	BT	4	9	57	0	134.57	404.20	33
2016	CS EM	BT	7	29	182	0	487.17	1730.93	28
2017	CS EM	BT	8	25	152	0	330.34	2052.25	16
2015	CS EM	Pot	7	18	184	4272	102.37	339.38	30
2016	CS EM	Pot	6	19	249	6275	151.96	470.47	32
2017	CS EM	Pot	7	22	270	7147	184.12	504.53	36

Table 5. The observed number of leatherback turtle (LBT) entanglements, the observed groundfish landings, the bycatch ratio, and the total (fleet-wide) groundfish landings from OA fixed gear vessels fishing pot gear during the last two 5-year periods. Landings and LBT values are summed across years within each 5-year period; bycatch ratio is calculated as the number of LBT / observed landings for each 5-year period. The 2008-2012 values were used in the probability models.

Five Year Period	Observed LBT (# indiv.) 5 yr. sum	Observed groundfish (mt yr^{-1})	Observed Groundfish (mt) 5 yr. sum	Bycatch Ratio	Fleet-wide Groundfish (mt) 5 yr. sum	Estimated number of individuals caught by the fleet
2003-2007	0	9.45	47.25	0.000	1567.49	0
2008-2012	1	11.59	57.97	0.017	1313.31	23
2013-2017	0	14.54	72.72	0.000	871.52	0

Table 6. Summary statistics of the number of entanglements based on 100,000 random draws from the fixed bycatch rate model (no uncertainty) and the model with uncertainty in bycatch rate. See text for model descriptions.

Statistic	Fixed rate	Rate with uncertainty
Minimum	0	0
Mean	4.53	4.53
Median	4	4
Maximum	17	48
Variance	4.54	24.72
Standard Deviation	2.13	4.97

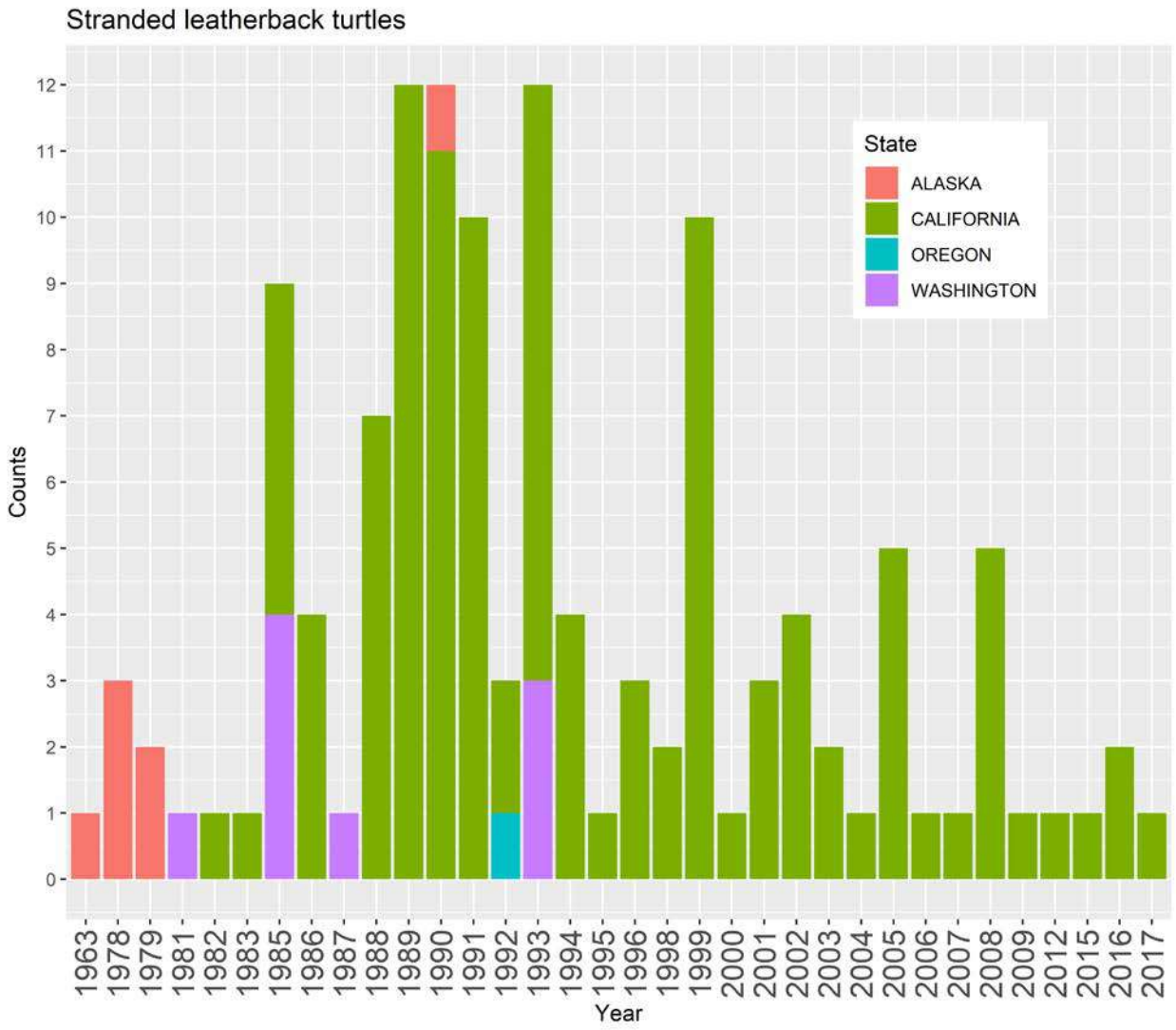

Figure 1. The number of stranded leatherback turtles along the west coast of the US in the stranding database that were not released alive (assumed dead). Data were available up to 2017. Note the time axis (years) is not continuous. Years without stranding records were omitted from the plot to make it concise.

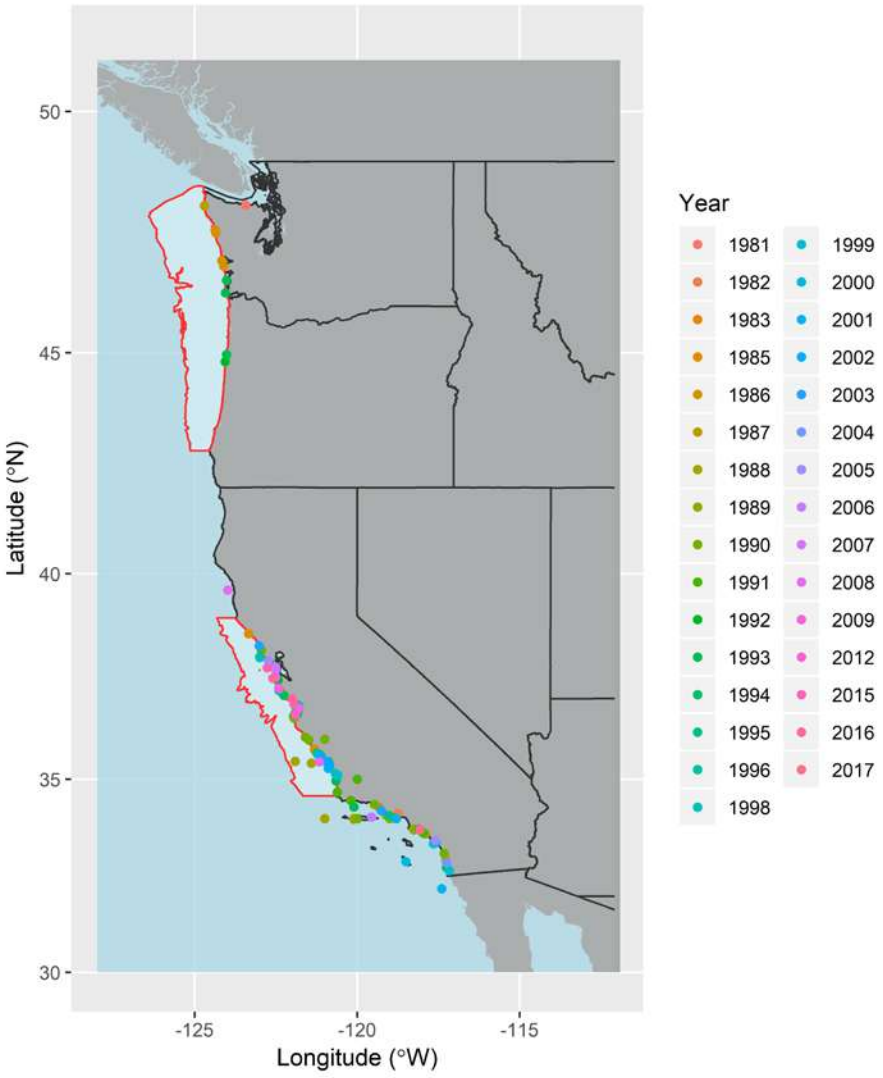

Figure 2. Approximate stranding locations of leatherback turtles along the west coast of the US (those from AK were omitted; n=7) with latitude/longitude data (n = 127). Some strandings are shown on land because of errors in recorded longitude/latitude. The two light-colored polygons with red borders along the coast denote Pacific leatherback critical habitat, which was designated on January 26, 2012 (77 FR 4170).

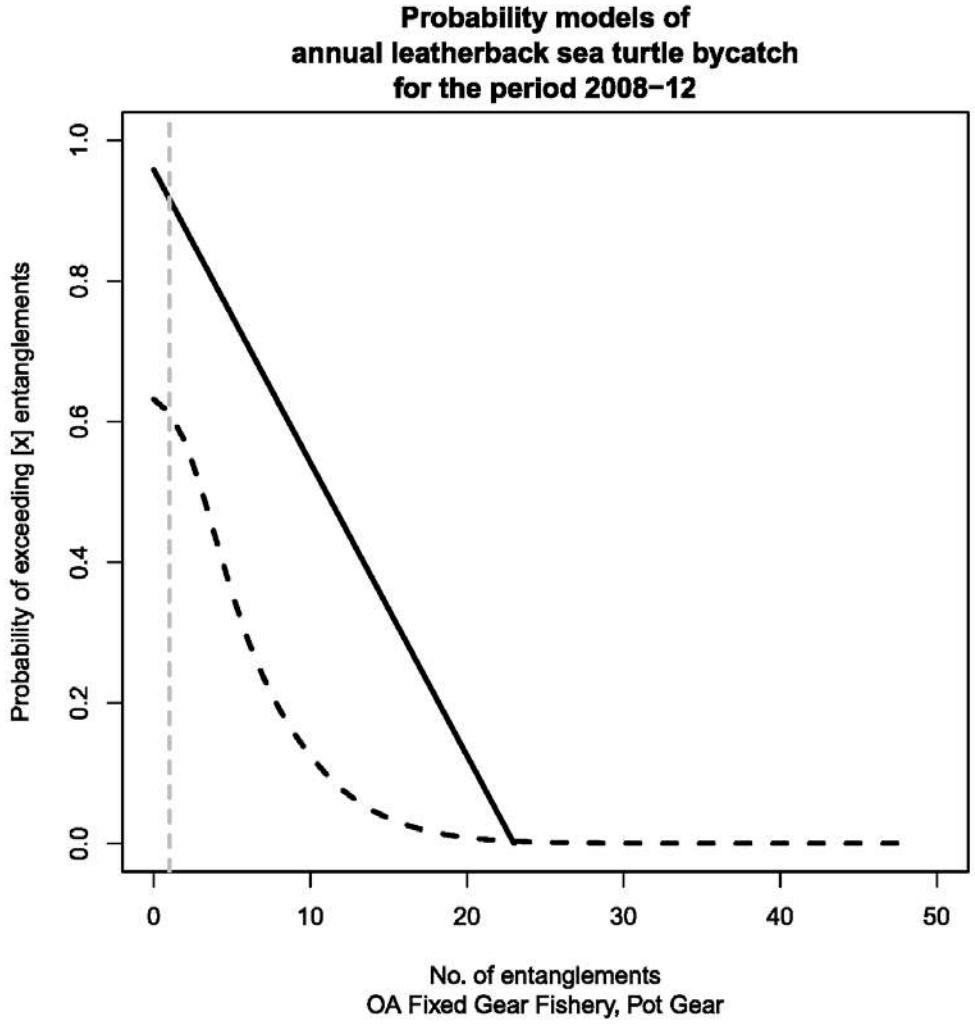

Figure 3. Probability of exceeding the number of entanglements of leatherback turtles in OA fixed gear fishery estimated using a statistical model with fixed bycatch rate (solid line) and another model including uncertainty in the bycatch rate (dashed line). The dotted grey vertical line represents the maximum allowable entanglements across 5 years (1.9) under the 2012 Biological Opinion for leatherback sea turtles in the U.S. west coast groundfish fisheries.

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Contaminant Hazard Reviews
Report No. 11

POLYCYCLIC AROMATIC HYDROCARBON HAZARDS TO FISH, WILDLIFE, AND INVERTEBRATES: A SYNOPTIC REVIEW

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SUMMARY

This account synthesizes available technical literature on ecological and toxicological aspects of polycyclic aromatic hydrocarbons (PAHs) in the environment, with special reference to natural resources. Subtopics include: chemical properties, sources, and fate; background concentrations in biological and nonbiological samples; toxic and sublethal effects to flora and fauna; and proposed criteria and research needs for the protection of sensitive species.

PAHs consist of hydrogen and carbon arranged in the form of two or more fused benzene rings. There are thousands of PAH compounds, each differing in the number and position of aromatic rings, and in the position of substituents on the basic ring system. Environmental concern has focused on PAHs that range in molecular weight from 128.16 (naphthalene, 2-ring structure) to 300.36 (coronene, 7-ring structure). Unsubstituted lower molecular weight PAH compounds, containing 2 or 3 rings, exhibit significant acute toxicity and other adverse effects to some organisms, but are noncarcinogenic; the higher molecular weight PAHs, containing 4 to 7 rings, are significantly less toxic, but many of the 4- to 7-ring compounds are demonstrably carcinogenic, mutagenic, or teratogenic to a wide variety of organisms, including fish and other aquatic life, amphibians, birds, and mammals. In general, PAHs show little tendency to biomagnify in food chains, despite their high lipid solubility, probably because most PAHs are rapidly metabolized. Inter- and intraspecies responses to individual PAHs are quite variable, and are significantly modified by many inorganic and organic compounds, including other PAHs. Until these interaction effects are clarified, the results of single substance laboratory tests may be extremely difficult to apply to field situations of suspected PAH contamination.

PAHs are ubiquitous in nature--as evidenced by their detection in sediments, soils, air, surface waters, and plant and animal tissues--primarily as a result of natural processes such as forest fires, microbial synthesis, and volcanic activities. Anthropogenic activities associated with significant production of PAHs--leading, in some cases, to localized areas of high contamination--include high-temperature (>700 ^{o}C) pyrolysis of organic materials typical of some processes used in the iron and steel industry, heating and power generation, and petroleum refining. Aquatic environments may receive PAHs from accidental releases of petroleum and its products, from sewage effluents, and from other sources. Sediments heavily contaminated

with industrial PAH wastes have directly caused elevated PAH body burdens and increased frequency of liver neoplasia in fishes.

At present, no criteria or standards have been promulgated for PAHs by any regulatory agency for the protection of sensitive species of aquatic organisms or wildlife. This observation was not unexpected in view of the paucity of data on PAH background concentrations in wildlife and other natural resources, the absence of information on results of chronic oral feeding studies of PAH mixtures, the lack of a representative PAH mixture for test purposes, and the demonstrable--and, as yet, poorly understood--effects of biological and nonbiological modifiers on PAH toxicity and metabolism. By contrast, criteria for human health protection and total PAHs, carcinogenic PAHs, and benzo(a)pyrene have been proposed for drinking water and air, and for total PAHs and benzo(a)pyrene in food: drinking water, 0.01 to <0.2 ug total PAHs/l, <0.002 ug carcinogenic PAHs/l, and <0.0006 ug benzo(a)pyrene/l; air, <0.01 ug total PAHs/m^3, <0.002 ug carcinogenic PAHs/m^3, and <0.0005 ug benzo(a)pyrene/m^3; food, 1.6 to <16.0 ug total PAHs daily, and 0.16 to <1.6 ug benzo(a)pyrene daily. In view of the carcinogenic characteristics of many PAH compounds and their increasing concentrations in the environment, it now seems prudent to reduce or eliminate them wherever possible, pending acquisition of more definitive ecotoxicological data.

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ACKNOWLEDGMENTS

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INTRODUCTION

Several polycyclic aromatic hydrocarbons (PAHs) are among the most potent carcinogens known to exist, producing tumors in some organisms through single exposures to microgram quantities. PAHs act at both the site of application and at organs distant to the site of absorption; their effects have been demonstrated in nearly every tissue and species tested, regardless of the route of administration (Lee and Grant 1981). The evidence implicating PAHs as an inducer of cancerous and precancerous lesions is becoming overwhelming, and this class of substances is probably a major contributor to the recent increase in cancer rates reported for industrialized nations (Cooke and Dennis 1984). PAHs were the first compounds known to be associated with carcinogenesis (Lee and Grant 1981). Occupational skin cancer was first documented in London chimney sweeps in 1775 and in German coal tar workers in the late 1800's. By the early 1900's, soot, coal tar, and pitch were all found to be carcinogenic to humans. By 1918, it was shown that topical applications of coal tar produced skin tumors in mice and rabbits; benzo(a)pyrene, a PAH, was identified as one of the most carcinogenic compounds in coal tar (Dipple 1985). The carcinogenic activity to man of soots, tars, and oils is beyond dispute. In addition to the skin cancers noted initially, higher incidences of respiratory tract and upper gastrointestinal tract tumors were associated with occupational exposures to these carcinogens (Dipple 1985). PAH-induced cancers in laboratory animals is well documented. Benzo(a)pyrene, for example, has produced tumors in mice, rats, hamsters, guinea pigs, rabbits, ducks, and monkeys following administration by oral, dermal, and intraperitoneal routes (Pucknat 1981). Teratogenic or carcinogenic responses have been induced in sponges, planarians, echinoderm larvae, teleosts, amphibians, and plants by exposure to carcinogenic PAHs (Neff 1979, 1982b). An unusually high prevalence of oral, dermal, and hepatic neoplasms have been observed in bottom-dwelling fish from polluted sediments containing grossly-elevated PAH levels (Couch and Harshbarger 1985). PAH compounds have damaged chromosomes in cytogenetic tests, have produced mutations in mammalian cell culture systems, and have induced DNA repair synthesis in human fibroblast cultures (EPA 1980). While some PAHs are potent mutagens and carcinogens, others are less active or suspected carcinogens. Some, especially those of biological origin, are probably not carcinogens (Jackim and Lake 1978). Certain lower molecular weight, noncarcinogenic PAHs, at environmentally realistic levels, were acutely toxic to aquatic organisms, or produced deleterious sublethal

responses (Neff 1985). However, few generalizations can be made about the class of PAH compounds because of the extreme variability in toxicity and physicochemical properties of PAHs and their various effects on individual species (Lee and Grant 1981).

PAHs are widely-distributed in the environment, almost ubiquitous, and have been detected in animal and plant tissues, sediments, soils, air, surface water, drinking water, industrial effluents, ambient river water, well water, and groundwater (EPA 1980). Man probably has always been exposed to PAHs from the natural background level in soils and plants (Harrison et al. 1975); avoiding exposure to nanogram quantities of these substances on a daily basis is now considered essentially impossible for all living resources (Dipple 1985). Ever since benzo(a)pyrene was recognized as a carcinogen at the beginning of this century, the presence of it and of other PAHs in the environment has received continuous attention. As one consequence, many reviews have been published on ecological and toxicological aspects of PAH in the environment, with special reference to their carcinogenic properties.[1]

In this report, I summarize selected data on environmental aspects of PAHs, emphasizing PAH effects to aquatic and wildlife resources. This brief review is part of a continuing series prepared in response to informational requests from environmental specialists of the U. S. Fish and Wildlife Service.

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[1] Harrison et al. (1975); Barnett (1976); Suess (1976); Gelboin and Ts'o (1978a, 1978b, 1981); Jackim and Lake (1978); Jones and Freudenthal (1978); Lo and Sandi (1978); Jones and Leber (1979); Neff (1979, 1982a, 1982b, 1985); Tsang and Griffin (1979); Bjorseth and Dennis (1980); EPA (1980); Cooke and Dennis (1981, 1983, 1984); Futoma et al. (1981); Lee and Grant (1981); Pucknat (1981); Sims and Grover (1981); Stegemen (1981); Cooke et al. (1982); Richards and Jackson (1982); Couch et al. (1983); Edwards (1983); Grimmer (1983); Quaghebeur et al. (1983); Sims and Overcash (1983); Couch and Harshbarger (1985); Harvey (1985); Johnson et al. (1985); Sugimura (1986).

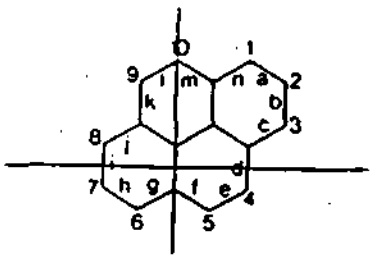
ENVIRONMENTAL CHEMISTRY, SOURCES, AND FATE

PROPERTIES

Polycyclic aromatic hydrocarbons (PAHs), also known as polynuclear aromatic hydrocarbons (PNAs) and polycyclic organic matter (POM), are composed of hydrogen and carbon arranged in the form of two or more fused benzene rings in linear, angular, or cluster arrangements, which may or may not have substituted groups attached to one or more rings (Sims and Overcash 1983). In some cases, the newly defined substituted PAH has strikingly greater toxicological effects than does the parent compound (Cooke and Dennis 1984). The nomenclature of PAH compounds has been ambiguous in the past due to different peripheral numbering systems. The currently accepted nomenclature is shown in Figure 1.

Of major environmental concern are mobile PAHs that vary in molecular weight from 128.16 (naphthalene, $C_{10}H_8$) to 300.36 (coronene, $C_{24}H_{12}$). Higher molecular weight PAHs are relatively immobile because of their large molecular volumes and their extremely low volatility and solubility. Among the mobile forms are thousands of compounds that differ in the number and position of aromatic rings, and in the position of substituents on the basic ring system. The lower molecular weight unsubstituted PAH compounds, containing 2 to 3 rings, such as naphthalenes, fluorenes, phenanthrenes, and anthracenes (Figure 2), have significant acute toxicity to some organisms, whereas the higher molecular weight 4- to 7-ring aromatics do not. However, all known PAH carcinogens, cocarcinogens, and tumor producers are in the high molecular weight PAH group (Figure 3).

Physical and chemical characteristics of PAHs generally vary with molecular weight. With increasing molecular weight, aqueous solubility decreases, and melting point, boiling point, and the log Kow (octanol/water partition coefficient) increases (Table 1), suggesting increased solubility in fats, a decrease in resistance to oxidation and reduction, and a decrease in vapor pressure. Accordingly, PAHs of different molecular weight vary substantially in their behavior and distribution in the environment and in



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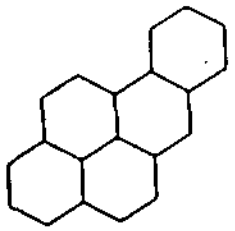

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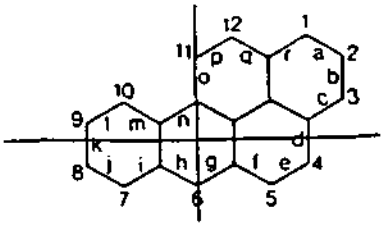

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Figure I. Nomenclature of PAHs (modified from Lee and Grant 1981, and Grimmer 1983). The PAH formula is oriented so that the greatest number of rings are in a horizontal row and a maximum number of rings are above and to the right of the horizontal row. The first carbon atom that belongs to the uppermost ring and is not engaged in ring fusion with another ring is given the number C-1; numbering continues in a clockwise direction omitting those carbon atoms which do not carry a hydrogen atom. The bond between C-1 and C-2 is designated as side "a"; other peripheral sides continue in clockwise direction in alphabetical order. Examples are: (1) pyrene (correctly oriented, numbered, and lettered), (2) benzo(a)pyrene (not oriented correctly), (3) benzo(a)pyrene (correctly oriented, numbered, and lettered).

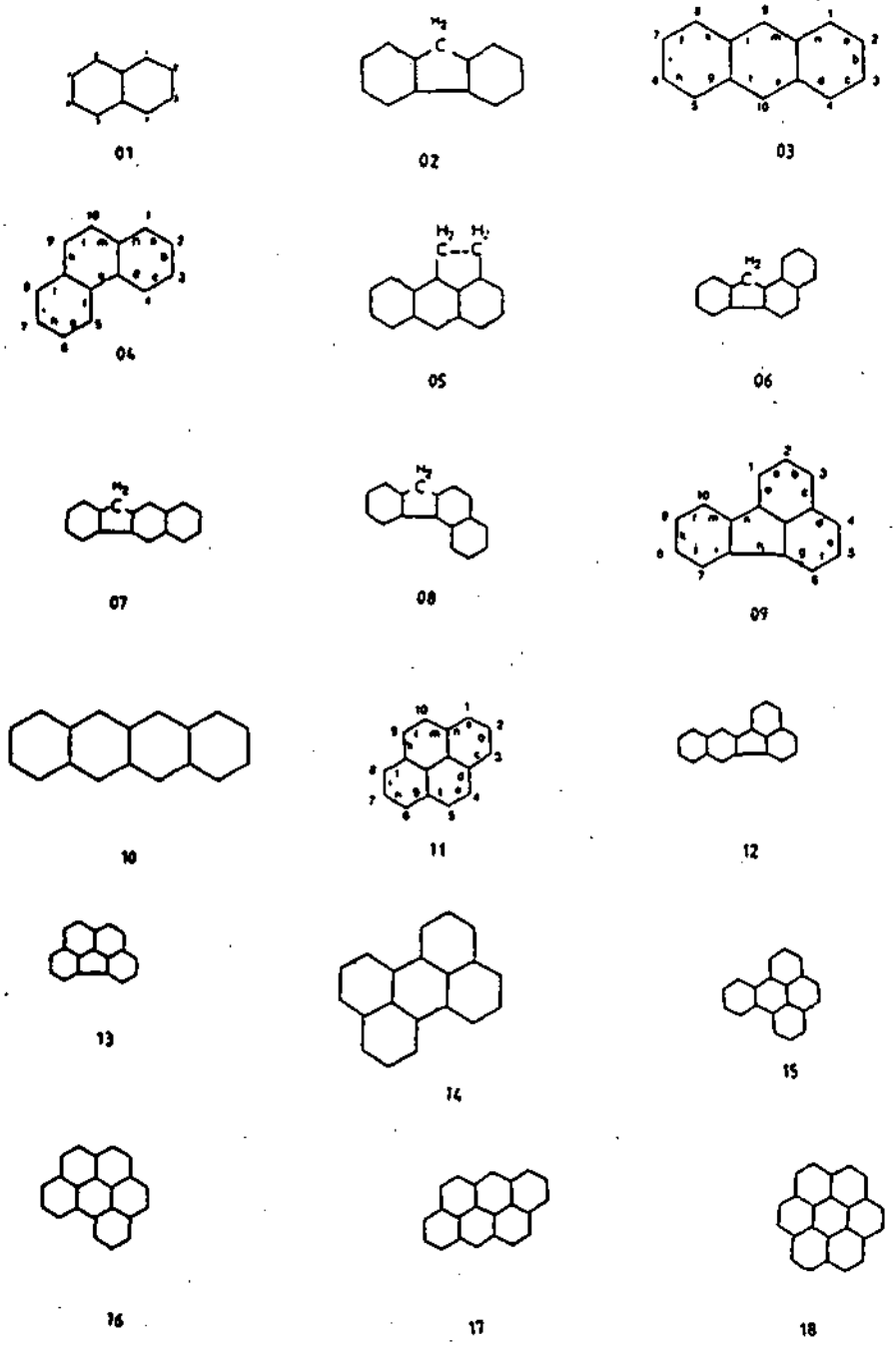

Figure 2. Ring structures of representative noncarcinogenic PAHs (modified from Lee and Grant 1981, and Neff 1985). The numbering and lettering system for several PAHs is also given. Compounds are: (1) naphthalene, (2) fluorene, (3) anthracene, (4) phenanthrene, (5) aceanthrylene, (6) benzo(a)fluorene, (7) benzo(b)fluorene, (8) benzo(c)fluorene, (9) fluoranthene, (10) naphthacene, (11) pyrene, (12) benzo(k)fluoranthene, (13) benzo(g,h,i)fluoranthene, (14) perylene, (15) benzo(e)pyrene, (16) benzo(g,h,i)perylene, (17) anthanthrene, (18) coronene.

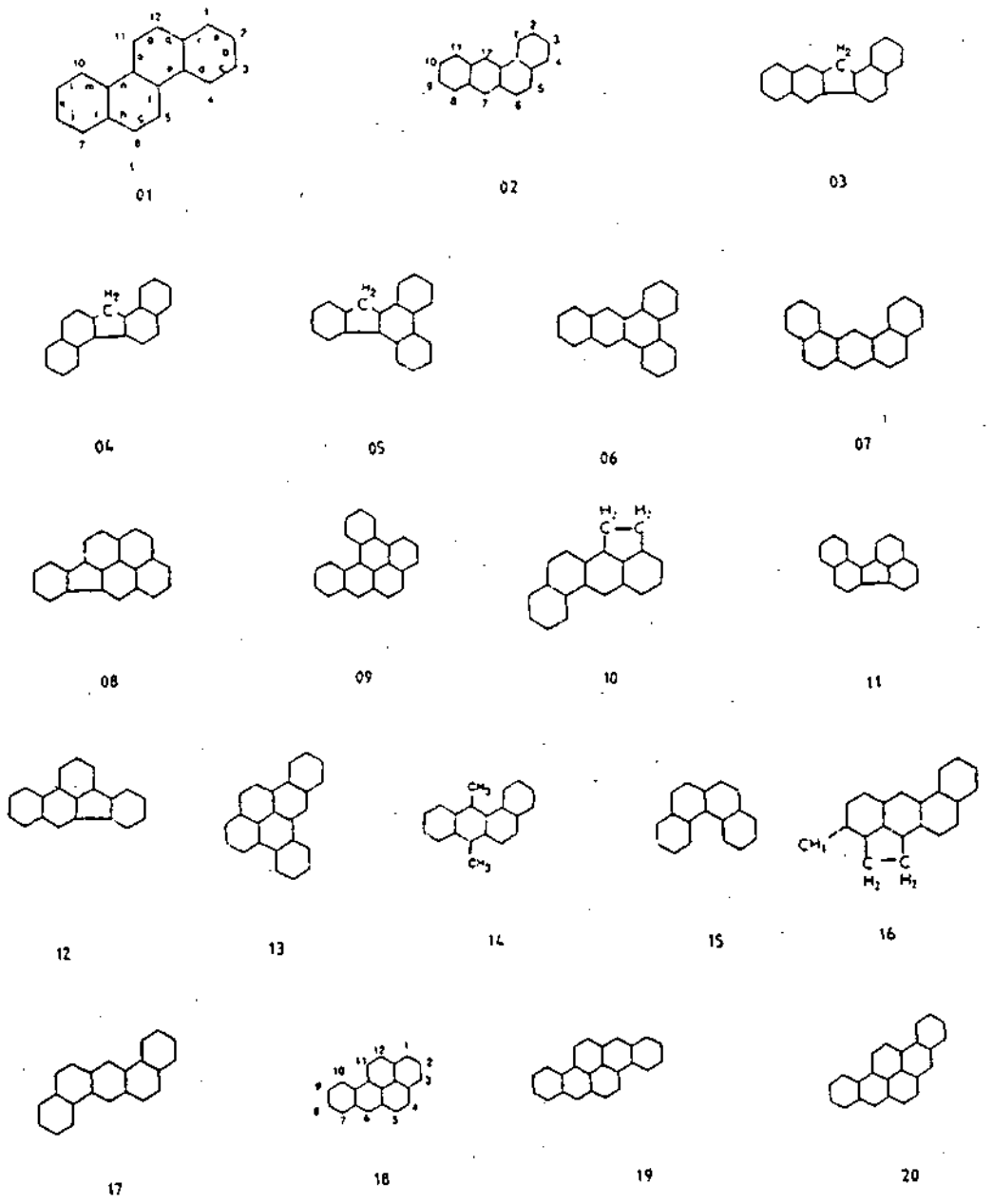

Figure 3. Ring structures of representative tumorogenic, cocarcinogenic, and carcinogenic PAHs (modified from Lee and Grant 1981). The numbering and lettering system for several PAHs is also given. Compounds are: (1) chrysene, (2) benz(a)anthracene, (3) dibenzo(a,h)fluorene, (4) dibenzo(a,g)fluorene, (5) dibenzo(a,c)fluorene, (6) dibenz(a,c)anthracene, (7) dibenz(a,j)anthracene, (8) indeno (1,2,3-cd) pyrene, (9) dibenzo(a,l)pyrene, (10) cholanthrene, (11) benzo(j)fluoranthene, (12) benzo(b)fluoranthene, (13) dibenzo(a,e)pyrene, (14) dimethylbenz(a)anthracene, (15) benzo(c)phenanthrene, (16) 3-methylcholanthrene, (17) dibenz(a,h)anthracene, (18) benzo(a)pyrene, (19) dibenzo(a,h)-pyrene, (20) dibenzo(a,i)pyrene. Compounds 1 to 9 are weakly carcinogenic, cocarcinogenic, or tumorogenic; compounds 10 to 13 are carcinogenic; compounds 14 to 20 are strongly carcinogenic.

Table 1. Some physical and chemical properties of selected PAHs.

Compound	Number of rings	Approximate molecular weight	Melting point (°C)	Solubility in water (mg/l)	log Kow
Naphthalene	2	128	80	30.0	3.37
Anthracene	3	178	216	0.07	4.45
Benz(a)anthracene	4	228	158	0.014	5.61
Benzo(a)pyrene	5	252	179	0.0038	6.04
Benzo(g,h,i)perylene	6	276	222	0.00026	7.23

their biological effects. Additional and more comprehensive data on the physical and chemical properties of PAHs are given in Barnett (1976), Lo and Sandi (1978), Neff (1979, 1985), EPA (1980), Futoma et al. (1981), Lee and Grant (1981), Pucknat (1981), Edwards (1983), Grimmer (1983), Sims and Overcash (1983), and Whitehouse (1985).

SOURCES

About 43,000 metric tons of PAHs are discharged into the atmosphere each year, and another 230,000 tons enter aquatic environments (Table 2). PAHs are ubiquitous in nature as a consequence of synthesis in terrestrial vegetation, microbial synthesis, and volcanic activity, but quantities formed by these natural processes are small in comparison with those produced from forest and prairie fires and anthropogenic sources (Barnett 1976; Suess 1976; Lo and Sandi 1978; Neff 1979, 1985; EPA 1980; Lee and Grant 1981; Pucknat 1981; Edwards 1983; Grimmer 1983; Sims and Overcash 1983). Anthropogenic activities associated with significant production of PAHs include: coke production in the iron and steel industry; catalytic cracking in the petroleum industry; the manufacture of carbon black, coal tar pitch, and asphalt; heating and power generation; controlled refuse incineration; open burning; and emissions from internal combustion engines used in transportation. Thus, the formation of PAHs in the environment is due to an endogenous synthesis by microorganisms,

Table 2. Major sources of PAHs in atmospheric and aquatic environments (modified from Lo and Sandi 1978; Neff 1979; Edwards 1983; Sims and Overcash 1983).

Ecosystem and sources	Annual input, in metric tons
ATMOSPHERE	
Total PAHs	
Forest and prairie fires	19,513
Agricultural burning	13,009
Refuse burning	4,769
Enclosed incineration	3,902
Heating and power	2,168
Benzo(a)pyrene	
Heating and power	
Worldwide	2,604
USA only	475
Industrial processes (mostly coke production)	
Worldwide	1,045
USA only	198
Refuse and open burning	
Worldwide	1,350
USA only	588
Motor vehicles	
Worldwide	45
USA only	22
AQUATIC ENVIRONMENTS	
Total PAHs	
Petroleum spillage	170,000
Atmospheric deposition	50,000
Wastewaters	4,400
Surface land runoff	2,940
Biosynthesis	2,700
Total benzo(a)pyrene	700

algae, and macrophytes which provide natural background, and to a second process which is connected to man-controlled high-temperature (>700 °C) pyrolysis of organic materials, to open burning, and to natural volcanic activities. The discovery in fossil fuels of complex mixtures of PAHs spanning a wide range of molecular weights has led to the conclusion that, given sufficient time (i.e., millions of years), pyrolysis of organic materials at temperatures as low as 100 to 150 °C can also lead to production of PAHs (Neff 1985).

Forest and prairie fires release much greater amounts of PAHs to the atmosphere than does fossil fuel burning. Nearly all of the airborne PAHs produced by flame pyrolysis are associated with the particulate fraction produced during combustion, and these are significantly modified by the chemical composition of the fuel, the pyrolysis temperature, the duration of exposure to elevated temperatures, and to other factors (Neff 1979; Edwards 1983). In one study, a PAH profile was established for a series of laboratory fires simulating the prescribed burning of pine needle litter (McMahon and Tsoukalas 1978). Heading fires (moving with wind) produced more total particulate matter than backing fires (moving against wind), but backing fires produced significantly higher amounts of PAHs, with the actual amounts formed dependent on fuel loading and the residence time of combustible gases in the burning zone. Emission factors for benzo(a)pyrene varied from 238 to 3,454 ug/kg in backing fires and 38 to 97 ug/kg in heading fires.

PAHs present in the atmosphere enter rain as a result of in-cloud and below-cloud scavenging (van Noort and Wondergem 1985). Total PAHs deposited on land and water are almost equivalent to PAH content in rainfall; significant quantities of PAHs are found in presumed pollution-free areas, indicating the importance of rain in transport and distribution of PAHs (Quaghebeur et al. 1983).

PAHs may reach aquatic environments in domestic and industrial sewage effluents, in surface runoff from land, from deposition of airborne particulates, and especially from spillage of petroleum and petroleum products into water bodies (Jackim and Lake 1978; Lake et al. 1979; Neff 1979; EPA 1980; Martens 1982; Boehm and Farrington 1984; Hoffman et al. 1984; Prahl et al. 1984). The majority of PAHs entering aquatic environments remains close to sites of deposition, suggesting that lakes, rivers, estuaries, and coastal marine environments near centers of human populations are the primary repositories of aquatic PAHs (Neff 1979). Large variations in aquatic PAH contents were evident due to localized source inputs and physicochemical conditions. For example, urban runoff from stormwater and highways to Narragansett Bay, Rhode Island, accounted for 71% of the total inputs for higher molecular weight PAHs, and 36% of the total PAHs (Hoffman et al. 1984). More than 30% of all combustion-derived PAHs in coastal sediments of Washington State is supplied by riverine transport of suspended particulate materials, while direct atmospheric input accounts for a maximum of 10% (Prahl et al. 1984). In contrast, concentrations of PAHs in sediments from the

vicinity of Georges Bank, off the US northeastern coast, varied from 1 to 100 ug/kg dry weight, and were directly related to total organic carbon, silt, and clay contents in sediments; combustion-derived PAHs dominated at the higher concentrations, while lower levels were often associated with a fossil fuel origin (Boehm and Farrington 1984).

Discharge water from hydrostatic testing of natural gas pipelines is a significant source of PAH loading into aquatic environments, contributing as much as 32,000 ug PAHs/l of discharge water, mostly as naphthalenes (Eiceman et al. 1984). More than 25 PAHs, primarily anthracenes and pyrenes, were detected in pipeline residues on inner walls of natural gas pipelines at concentrations up to 2,400 ug/m^2 of inner surface; the same compounds may be reasonably expected in aqueous wastes from pipeline maintenance (Eiceman et al. 1985). Release of these, or similar, discharge waters directly into aquatic environments will result in contamination similar to that caused by oil spills; however, these sites for pollution may occur in locations far distant from oil production and refinery activities (Eiceman et al. 1984). PAHs are also present in tap water at concentrations of 0.1 to 1.0 ng/l, primarily as mono- and dichlorinated derivitives of naphthalene, phenanthrene, fluorene, and fluoranthene (Shiraishi et al. 1985). The presence of PAHs and chlorinated PAHs in tap water indicates the reaction of PAHs with chlorine; however, their significance to human health and to aquatic biota is unknown.

FATE

Concern about PAHs in the environment is due to their persistence and to the fact that some are known to be potent mammalian carcinogens, although environmental effects of most noncarcinogenic PAHs are poorly understood (Neff 1985). Prior to 1900, a natural balance existed between the production and the degradation of PAHs. Synthesis of PAHs by microorganisms and volcanic activity and production by man-made high temperature pyrolytic reactions and open burning seemed to be balanced by PAH destruction via photodegradation and microbial transformation. With increased industrial development and increased emphasis of fossil fuels as energy sources, the balance has been disturbed to the extent that PAH production and introduction into the environment greatly exceeds known PAH removal processes (Suess 1976; Sims and Overcash 1983).

When released into the atmosphere, PAH compounds will become associated with particulate materials. Their residence time in the atmosphere and transport to different geographic locations are governed by particle size, meteorological conditions, and atmospheric physics. The highly reactive PAHs photodecompose readily in the atmosphere by reaction with ozone and various oxidants; degradation times range from several days to six weeks for PAHs adsorbed onto particulates <1 um in diameter (in the absence of rainfall) to <1 day to several days for those adsorbed to larger particles (Suess 1976).

Smaller atmospheric particulates containing PAHs are easily inhaled (Lee and Grant 1981), and may pose special problems, as yet unevaluated, for airborne organisms such as birds, insects, and bats. Photooxidation, one of the most important processes in the removal of PAHs from the atmosphere, can also produce reaction products that are carcinogenic or mutagenic, although little is known of their persistence (Edwards 1983). One of the more common photooxidation reactions of PAHs is the formation of endoperoxides that ultimately undergo a series of reactions to form quinones (Edwards 1983). Various parameters may modify chemical and photochemical transformation of PAHs in the atmosphere, including light intensity, concentration of gaseous pollutants (O_3, NO_x, SO_x), and chemicophysical characteristics of particulates or substrates into which the PAHs are adsorbed; depending on these variables, the half-life of benzo(a)pyrene in the atmosphere varies from 10 minutes to 72 days (Valerio et al. 1984). Atmospheric PAHs are transported over relatively long distances from industrial areas and from natural forest and prairie fires (Edwards 1983); however, sites nearer urban centers have much higher PAH deposition rates than more rural areas (Hites and Gschwend 1982).

Much of the PAHs released into the atmosphere eventually reaches the soil by direct deposition or by deposition on vegetation. The PAHs may be adsorbed or assimilated by plant leaves before entering the animal food chain, although some adsorbed PAHs may be washed off by rain, chemically oxidized to other products, or returned to the soil as the plants decay. PAHs assimilated by vegetation may be translocated, metabolized, and possibly photodegraded within the plant. In some plants growing in highly contaminated areas, assimilation may exceed metabolism and degradation, resulting in an accumulation in plant tissues (Edwards 1983).

In water, PAHs may either evaporate, disperse into the water column, become incorporated into bottom sediments, concentrate in aquatic biota, or experience chemical oxidation and biodegradation (Suess 1976). The most important degradative processes for PAHs in aquatic systems are photooxidation, chemical oxidation, and biological transformation by bacteria and animals (Neff 1979). Most PAHs in aquatic environments are associated with particulate materials; only about 33% are present in dissolved form (Lee and Grant 1981). PAHs dissolved in the water column will probably degrade rapidly through photooxidation (EPA 1980), and degrade most rapidly at higher concentrations, at elevated temperatures, at elevated oxygen levels, and at higher incidences of solar radiation (McGinnis and Snoeyink 1974; Suess 1976; Bauer and Capone 1985). The ultimate fate of those PAHs that accumulate in sediments is believed to be biotransformation and biodegradation by benthic organisms (EPA 1980). PAHs in aquatic sediments, however, degrade very slowly in the absence of penetrating radiation and oxygen (Suess 1976), and may persist indefinitely in oxygen-poor basins or in anoxic sediments (Neff 1979). PAH degradation in aquatic environments occurs at a slower rate than that in the atmosphere (Suess 1976), and the cycling of PAHs in aquatic environments, as is true for other ecological systems, is poorly understood (Neff 1979).

Animals and microorganisms can metabolize PAHs to products that may ultimately experience complete degradation. The degradation of most PAHs is not completely understood. Those in the soil may be assimilated by plants, degraded by soil microorganisms, or accumulated to relatively high levels in the soil. High PAH concentrations in soil can lead to increased populations of microorganisms capable of degrading the compounds. Of equal importance to PAH cycling dynamics is the physical state of the PAH, i.e., whether in vapor phase or associated with particles such as flyash. Particles may increase or decrease the susceptibility of PAHs to degradation, depending on the PAH and particles involved (Edwards 1983).

PAHs can be taken into the mammalian body by inhalation, skin contact, or ingestion, although they are poorly absorbed from the gastrointestinal tract. The main routes of elimination of PAHs and their metabolites include the hepatobiliary system and the gastrointestinal tract (Sims and Overcash 1983). In mammals, an enzyme system variously known as the cytochrome P-450-dependent mixed-function oxidase, mixed-function oxidase, mixed-function oxygenase, aryl hydrocarbon hydroxylase, or drug metabolizing system, is responsible for initiating the metabolism of various lipophilic organic compounds, including PAHs. The primary function of this system is to render poorly water soluble lipophilic materials more water soluble, and therefore more available for excretion. Some PAHs are transformed to intermediates, which are highly toxic, mutagenic, or carcinogenic to the host. Oxidative metabolism of PAHs in this system proceeds via high electrophilic intermediate arene oxides, some of which bind covalently to cellular macromolecules such as DNA, RNA, and protein. Most authorities agree that metabolic activation by the mixed-function oxidase system is a necessary prerequisite for PAH-induced carcinogenesis and mutagenesis (Neff 1979). This enzyme system is known to be present in rodent tissues, and human liver, skin, placenta, fetal liver, macrophages, lymphocytes, and monocytes (Lo and Sandi 1978). Studies with rodents have shown that the mixed-function oxidase system can convert PAHs to various hydroxylated derivatives including phenols, quinones, and epoxides, and can also activate PAHs to produce carcinogenic metabolites (Lo and Sandi 1978). Fish and most crustaceans tested to date possess the enzymes necessary for activation (Statham et al. 1976; Varanasi et al. 1980; Fabacher and Baumann 1985), but some molluscs and other invertebrates are unable to efficiently metabolize PAHs (Jackim and Lake 1978; Varanasi et al. 1985). Although many aquatic organisms possess the requisite enzyme systems for metabolic activation of PAHs, it is not certain in most cases whether these enzymes produce the same metabolites as those produced by mammalian enzymes (Neff 1979).

PAHs are metabolized by liver mixed-function oxidases to epoxides, dihydrodiols, phenols, and quinones. The intermediate metabolites have been identified as the mutagenic, carcinogenic, and teratogenic agents (Sims and Overcash 1983). The activation mechanisms occur by hydroxylation or

production of unstable epoxides of PAHs which damage DNA, initiating the carcinogenic process (Jackim and Lake 1978). Metabolic formation of bay region diol epoxides represents an important pathway by which PAHs are activated to carcinogens (Figure 4). Such metabolic activation proceeds via initial formation of the dihydrodiol with the bay region double bond, followed by subsequent oxidation of the dihydrodiol to the bay region diol epoxide (Sims and Overcash 1983). Active epoxides may be converted to less toxic products by various enzymatic and other reactions (Neff 1979). In the case of benzo(a)pyrene, the "ultimate carcinogen" (7 beta, 8 alpha-dihydroxy-,7,8,9,10 tetrahydrobenzo(a)pyrene- 9 alpha, 10 alpha-epoxide) reacts with the guanine of RNA and DNA, the linkage taking place between the C-10 atom of benzo(a)pyrene and the C-2 amino group of guanine (Grimmer 1983; Dipple 1985; Figure 4). Additional information on actual and theoretical mechanisms involved in the metabolic activation of PAHs are given in Cavalieri et al. (1978, 1980), Bjorseth and Dennis (1980), Herd and Greene (1980), Cooke and Dennis (1981), Sims and Grover (1981), Grimmer (1983), Szentpaly (1984), Harvey (1985), and Yan (1985).

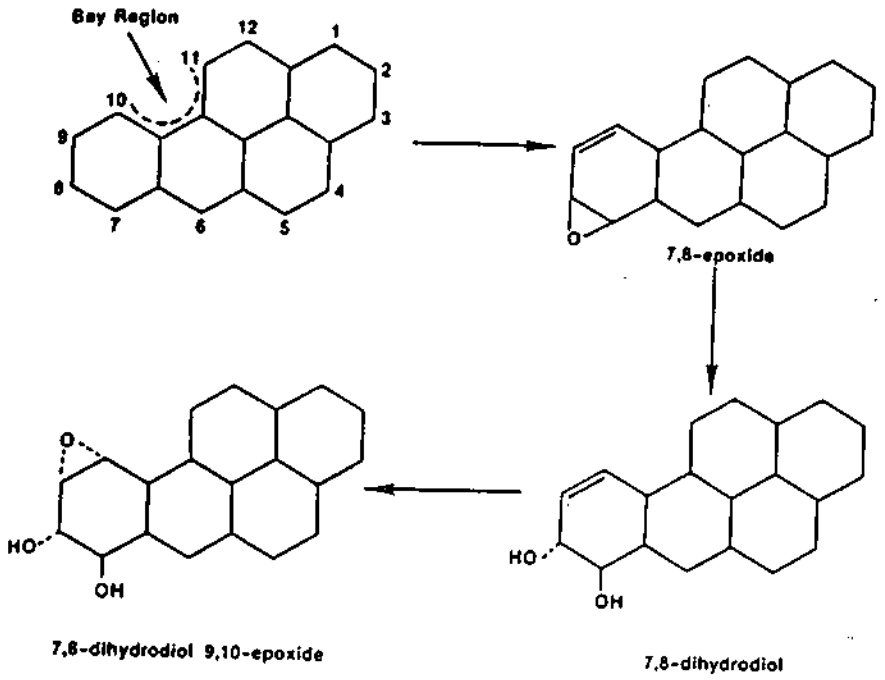

Figure 4. The bay region dihydrodiol epoxide route of benzo(a)pyrene (modified from Dipple 1985).

BACKGROUND CONCENTRATIONS

GENERAL

PAHs are ubiquitous in the environment. In nonbiological materials, concentrations are elevated in the vicinity of urban industrialized locales, and from areas of significant wood burning activities such as forest fires and residential home heating. Terrestrial vegetation and aquatic invertebrates can accumulate significant concentrations of PAHs, possibly due to inefficient or missing mixed-function oxidase systems. Fish do not appear to contain grossly elevated PAH residues; this may be related to their efficient degradation system. At present, data are lacking or unavailable on PAH background concentrations in natural populations of birds and other wildlife --although it seems unlikely that significant accumulations will occur. Some investigators have shown that aquatic invertebrates, fish, and amphibians collected from areas of high sediment PAH content show elevated frequencies of hyperplasia and neoplasia (Rose 1977; Mix 1982; Black 1983; Malins et al. 1984, 1985a, 1985b; Black et al. 1985; Baumann et al., in press), and, recently, that hepatic carcinoma has been induced in rainbow trout (Salmo gairdneri) by benzo(a)pyrene through dietary and intraperitoneal injection routes (Hendricks et al. 1985).

More comprehensive information on PAH background levels in various biological and nonbiological compartments is given in Lo and Sandi (1978), Neff (1979, 1985), Pucknat (1981), Edwards (1983), Grimmer (1983), and Sims and Overcash (1983).

NONBIOLOGICAL SAMPLES

Total PAH levels in air are usually much higher in winter than in summer, higher in urban communities than in rural areas (Table 3; Grimmer 1983), and appear to be related primarily to the weight of total suspended particulates in the atmosphere (Hites and Gschwend 1982; Greenberg et al. 1985; Srivastava et al. 1985; Ang et al. 1986). PAH levels in precipitation are significantly higher in winter than in summer, primarily due to emissions from household heating (Quaghebeur et al. 1983; van Noort and Wondergem 1985). Among

Table 3. PAH concentrations in selected nonbiological materials.

Material (units), and other variables	Concentration	Reference[a]
AIR (ng/m^3)		
USA cities, 1959, total PAHs		
Detroit	95.1	EPA 1980
Birmingham	63.4	
Nashville	60.6	
New Orleans	33.6	
Los Angeles	31.8	
Atlanta	26.3	
San Francisco	13.7	
Sydney, Australia		
Winter	8.2	Barnett 1976
Summer	0.6	
USA cities, 1971-1977		
Benzo(a)perylene = BaPER	0.2-9.2	EPA 1980
Benzo(e)pyrene = BeP	0.9-4.6	
Benzo(k)fluoranthene = BkFL	0.03-1.3	
Pyrene = PYR	0.18-5.2	
Coronene = COR	0.2-6.4	
Perylene = PER	0.01-1.2	
Anthracene = A	0.07-0.3	
Naphthalene = NA	Max. 0.4	
Benz(a)anthracene = BaA	Max. 4.6	
Indeno(1,2,3-cd)pyrene = IP	Max. 1.3	
Steel mill, Ontario, Canada, 1971-1979		
Station 0.8 km distant		
Benzo(a)pyrene = BaP	9.4 (Max. 110.0)	Potvin
BkFL	8.9 (Max. 142.0)	et al. 1981
Fluoranthene = FL	7.0 (Max. 43.3)	
PER	9.1 (Max. 106.0)	
Benzo(g,h,i)perylene = BghiPER	13.7 (Max. 90.0)	
Station 2.8 km distant		
BaP	0.4 (Max. 7.9)	
BkFL	0.7 (Max. 5.1)	
FL	1.1 (Max. 4.8)	
PER	0.7 (Max. 9.1)	
BghiPER	1.4 (Max. 8.5)	
Benzo(a)pyrene = BaP		
Urban areas	0.1-61.0	Edwards 1983
Downwind from coal gasification plant, Yugoslavia	Max. 80.0	

Table 3. (Continued)

Material (units), and other variables	Concentration	Reference[a]
Urban areas		
1966	3.2	EPA 1980
1970	2.1	
1976	0.5	
Rural areas	0.01-1.9	Edwards 1983
Rural areas		
1966	0.4	EPA 1980
1976	0.1	
SOILS		
Near M6 Motorway, Lancaster, UK (maximum deposition rate, ng/m^2/week)		
Distance from roadway		
3.8 meters		
A	2,300	Johnston and
FL	15,200	Harrison 1984
BaA	5,800	
Benzo(b)fluoranthene = BbFL	7,300	
BkFL	2,800	
BaP	4,900	
9.0 - 47 meters		
A	420	
FL	1,700	
BaA	260	
BbFL	690	
BkFL	470	
BaP	290	
Vicinity slash burn site, Oregon (g/ha)		
0-2 cm depth		
Preburn		
Phenanthrene = PHEN	0.5	Sullivan
FL	0.6	and Mix 1985
103 days postburn		
PHEN	9.8	
FL	3.6	
365 days postburn		
PHEN	ND	
FL	0.8	
2-5 cm depth		

Table 3. (Continued)

Material (units), and other variables	Concentration	Reference[a]
105 days postburn		
PHEN	1.3	
FL	0.3	
365 days postburn		
PHEN	ND	
FL	ND	
BaP (ug/kg)		
Rural areas	0.4	Barnett 1976
Industrial areas	400.0	
Nonpolluted areas	up to 1,000	Edwards 1983
Near known sources	>100,000	
Near coal-tar pitch disposal site, Germany	650,000	Lee and Grant 1981
Near recreation area, USSR	0.4	Harrison et al. 1975
Forest soil	1.5-4.0	
LITTER		
Forest, Oregon (g/ha)		
3 days postburn		
PHEN	603	Sullivan and Mix 1985
FL	245	
32 days postburn		
PHEN	ND	
FL	ND	
Coniferous trees (ug/kg)		
BghiPER	42	Thomas et al. 1984
BaP	51	
IP	47	
FL	164	
SEDIMENTS (ug/kg)		
Buffalo River, near Buffalo, NY		
Sediments		
BaA	7,300	Black 1983
Chrysene = CHRY	4,300	
BbFL	3,500	

Table 3. (Continued)

Material (units), and other variables	Concentration	Reference[a]
BaP	4,500	
Dibenz(a,h)anthracene = DBA	1,000	
IP	4,400	
Sediment extracts		
BaA	16,000	
CHRY	14,000	
BbFL	13,900	
BaP	15,400	
DBA	3,300	
IP	12,300	
Cayuga Lake, Ithaca, NY, 1978		
Total PAHs		
Within marinas	4,600-13,900	Heit 1985
Deepwater	1,260-2,500	
Near power plant	104-6,800	
FL		
Within marinas	1,700	
Deepwater	285	
Near power plant	8-1,000	
Penobscot Bay, Maine		
Total PAHs	286-8,794	Johnson
PHEN	17-252	et al. 1985
A	ND-49	
FL	156-3,700	
Pyrene = PYR	16-539	
BaA	14-540	
CHRY	9-578	
BbFL	17-1,000	
BkFL	14-696	
BaP	10-540	
DBA	2-120	
BghiPER	23-641	
IP	9-228	
Casco Bay, Maine, total PAHs	215-14,425	
Charles River, Mass., total PAHs	87,000-120,000	
Boston Harbor, Mass., total PAHs	8,500	
New Bedford Harbor, Mass., total PAHs	63,000	
Lake Erie, total PAHs	530-3,750	
Adirondack Lakes, total PAHs	4,070-12,807	

Table 3. (Continued)

Material (units), and other variables	Concentration	Reference[a]
Alaska, total PAHs	5-113	
Tamar estuary, UK, total PAHs	4,900	
Southampton estuary, UK, total PAHs	91,000-1,791,000	
Severn estuary, UK, total PAHs	1,600-25,700	
Monaco, total PAHs	5,200-12,100	
Gulf of Finland, total PAHs	437	
Norway, total PAHs	284-99,452	
Walvis Bay, Africa, total PAHs	68	
Amazon River system, total PAHs	ND-544	
SEWAGE		
Waters, worldwide, total PAHs(ug/l)	100-500	Lee and Grant 1981
Sludge, total PAHs		
United Kingdom, 12 sites, (ug/kg)		
Fresh weight	80-1,760	McIntyre
Dry weight	200-50,300	et al. 1981
Texas, Reese Air Force Base		
Effluent lagoon (ug/kg fresh weight)		
PER	300.0	Rose 1977
PYR	5.8	
FL	5.7	
BaA	1.4	
CHRY	1.3	
BaP	0.5	
BeP	0.2	
A	0.2	
MOTOR OILS (ug/l)		
Unused		
BaP	115	Pasquini and
CHRY	56	Monarca 1983
PER	11	
Used		
BaP	1,382	
CHRY	10,170	
PER	1,024	

Table 3. (Continued)

Material (units), and other variables	Concentration	Reference[a]
GROUNDWATER (ug/l)		
Worldwide		
Total PAHs	0.01-0.05	Lee and Grant 1981
Total PAHs	0.045-0.51	Harrison et al. 1975
Carcinogenic PAHs	0.00-0.081	
Germany		
Total PAHs	0.04	EPA 1980
Carcinogenic PAHs	0.003	
Champaign, Illinois		
Total PAHs	0.007	
Carcinogenic PAHs	0.003	
Elkhart, Indiana		
Total PAHs	0.02	
Carcinogenic PAHs	0.004	
DRINKING WATER (ug/l)		
USA, total PAHs	0.015	Lee and Grant 1981
Europe, total PAHs	0.04-0.06	
Monongehala River, Pittsburgh, PA		
Untreated		
Total PAHs	0.6	EPA 1980
Carcinogenic PAHs	0.14	
Treated		
Total PAHs	0.003	
Carcinogenic PAHs	0.002	
Ohio River, Wheeling, WV		
Untreated		
Total PAHs	1.59	
Carcinogenic PAHs	0.57	
Treated		
Total PAHs	0.14	
Carcinogenic PAHs	0.011	
Lake Winnebago, Appleton, WI		
Untreated		
Total PAHs	0.007	
Carcinogenic PAHs	0.002	

Table 3. (Concluded)

Material (units), and other variables	Concentration	Reference[a]
Treated		
Total PAHs	0.006	
Carcinogenic PAHs	0.002	
SURFACE WATER (ug/l)		
Worldwide		
Low level contamination	0.05-0.25	Lee and
Medium polluted	0.2-1.0	Grant 1981
Germany, Rhine River		
Total PAHs	1.12	EPA 1980
Carcinogenic PAHs	0.49	
Thames River, UK		
Total PAHs	0.5-1.33	
Carcinogenic PAHs	0.18-0.56	

[a]Each reference applies to data in the same row and in the rows that immediately follow for which no reference is indicated.

industrial sources, the production of metallurgical coke is the single most significant source of atmospheric PAHs in Ontario, Canada. Coke production in 1977 represented about 52% of all PAH emissions from Ontario sources versus about 46% formed as a result of forest fires (Potvin et al. 1981). Beyond 2 km distant from the coke point source, PAH concentrations in air were typical of those measured in major urban nonindustrialized areas (Table 3; Potvin et al. 1981). A variety of PAHs have been detected in ambient air in the USA and elsewhere. Benzo(a)pyrene, because of its carcinogenic properties, has been monitored extensively, and has frequently been used as an indicator of PAHs (EPA 1980). In general, total PAHs in air is about 10X higher than benzo(a)pyrene levels, although this relation is extremely variable (Lee and Grant 1981). Benzo(a)pyrene levels, like total PAHs, were higher in winter than summer, probably due to residential and industrial heating; air levels in urban areas with coke ovens were 40% to 70% higher than in cities without coke ovens, but this may be related to higher industrial emissions in those cities (Lee and Grant 1981). In one case, benzo(a)pyrene levels in air from the center of a remote mountain community in Colorado were several times higher than what is usually found in U.S. metropolitan areas, and was attributed to extensive residential wood burning (Murphy et al. 1982). Average concentrations of benzo(a)pyrene in urban air Nationwide declined from 3.2 ng/m^3 in 1966 to 0.5 ng/m^3 in 1978, an 80% decrease (Lee and Grant 1981). These decreases are believed to be due primarily to decreases in coal consumption for commercial and residential heating, improved disposal of solid wastes, and restrictions on open burning (EPA 1980).

A major source of PAHs in soils and soil litter is from emissions and deposition from forest fires. In a controlled burn study, Sullivan and Mix (1985) showed that lower molecular weight PAHs, such as phenanthrene and fluorene, which had been deposited in soil litter, degraded to nondetectable levels within 2 years after burning. Higher molecular weight PAHs such as benzo(k)fluorene, benzo(a)pyrene, benzo(g,h,i)perylene, perylene, and indeno(1,2,3-cd)pyrene, were more persistent in litter, decreasing after 5 years to about 20% of initial deposition. Although movement into the top 2 cm of the soil profile was initially more pronounced for lower molecular weight PAHs, all compounds appeared to reach equilibrium between litter and soil on the basis of organic content within one year postburn. Differential persistence and fate of PAHs on slash burn sites is explained by solubility, Kow, and other physicochemical properties (Sullivan and Mix 1985). PAHs from vehicle emissions constitute a minor, but measurable, source of soil PAHs (Table 3). The majority of highway-derived PAHs appears to be deposited within 3.8 m of the road, but the influence of the highway may extend to nearly 70 m (Johnston and Harrison 1984). The use of composted municipal wastes for conditioning of agricultural soils is not recommended, as these contain at least nine identified carcinogenic PAHs (Martens 1982).

Some sediments were found to be highly contaminated with PAHs. Sediments and sediment extracts from the Buffalo River, New York, contained elevated levels of carcinogenic PAHs (1,000-16,000 ug/kg). Brown bullheads (Ictalurus

nebulosus), in response to repeated applications of Buffalo River sediment extracts, showed epidermal hyperplasia and neoplasia when compared to controls (Black 1983). PAH concentrations in sediments from the Great Barrier Reef, Australia, were always <0.8 ug/kg dry weight, except in small areas close to sites frequently visited by power boats; in those instances, total PAH levels exceeded 13,400 ug/kg (Smith et al. 1985). Highest PAH levels measured in sediments of Cayuga Lake, New York, were found in marinas or areas of the lake receiving urban runoff, and were apparently not related to stack emissions from a nearby coal-fired power plant; Heit (1985) believed that stack emissions were either masked by other sources or were atmospherically transported and deposited elsewhere. Coastal and offshore sediments are subject to highly elevated PAH levels from a variety of sources, mostly unknown, relative to preindustrial times (Johnson et al. 1985). For example, PAH levels in sediments of Penobscot Bay, Maine, fell within the range found in sediments near industrialized regions, and were significantly higher than expected for an area previously considered to be uncontaminated (Table 3; Johnson et al. 1985).

Sewage effluents usually contained measurable levels of PAHs, although extreme variability between and among sites is common. For example, during a heavy storm, individual PAH levels in a sewage works may increase more than 100X over a dry weather period (Harrison et al. 1975). Conventional sewage treatment plant processes remove up to 90% of carcinogenic PAHs, and this may be increased to 99% using percolating filters and activated sludge processes (Harrison et al. 1975). Tiger salamanders (Ambystoma tigrinum), collected in 1975 from a 13-ha sewage effluent lagoon at Reese Air Force Base, Texas, showed a remarkably high incidence (53%) of neoplastic and other lesions (Rose 1977). Analysis of sludge composites showed elevated PAH levels, especially perylene; levels of organochlorine and organophosphorus pesticides, nitrosamines, and heavy metals were judged to be nonelevated (Rose 1977).

Careful disposal of used motor oils is warranted, as these contain high quantities of mutagenic and carcinogenic PAHs (Table 3; Pasquini and Monarca 1983).

All but the most heavily contaminated fresh and marine waters contain total PAH concentrations in the part-per-trillion or low part-per-billion range (Table 3; Neff 1982b). A large proportion of the PAH content in water is probably adsorbed onto suspended solids (Harrison et al. 1975). In Lake Michigan, concentrations of total PAHs in the surface microlayer varied from 0.15 to 0.45 ug/l, representing on a relative scale 10^6 times the concentration in air, suggesting that aerosols are a major source of these compounds and that the microlayer is a repository until the PAHs are removed by adsorption and sedimentation (Strand and Andren 1980).

BIOLOGICAL SAMPLES

Carcinogenic PAHs have been extracted from a large variety of fresh plants, including root and leaf vegetables, fruits, grains, and edible mushrooms, as well as from various marine bacteria and phytoplankton under circumstances suggesting that PAHs were present due to local biosynthesis (Suess 1976). Vegetation and soil near known PAH sources are more highly contaminated with PAHs than those collected at greater distances (Edwards 1983). PAH levels in lettuce (Lactuca sativa) grown in Sweden seemed to be directly related to its proximity to local recognized point sources of PAH emitters (Table 4; Larsson and Sahlberg 1982). Washing lettuce with water had little effect on phenanthrene levels, but significantly reduced other PAHs, such as benzo(a)pyrene, benz(a)anthracene, and benzo(g,h,i)perylene by 68% to 87% (Larsson and Sahlberg 1982). Fruits and vegetables grown in polluted atmospheres may contain up to 100X higher levels of total PAHs than those grown in unpolluted environments (EPA 1980; Lee and Grant 1981). PAH concentrations for plants are generally greater on plant surfaces than internal tissues, greater in above ground plant parts than those below ground, and greater in plants with broad leaves (greater surface area) than those with narrow leaves (Edwards 1983). Plants can become contaminated with PAHs through environmental pollution, particularly through deposition from the atmosphere, and also through food processing. For example, the bran portion of milled wheat, as well as finished bran cereal, had a considerably higher PAH content than other fractions or finished products (Lawrence and Weber 1984b). Enrichment of PAHs in plants is associated with deposition of atmospheric particulate matter with relatively small particle sizes; thus, PAH content is usually in the order of humus > mosses > lichens (Thomas et al. 1984). Mosses appear to be good indicators of regional PAH air pollution and have been recommended for this purpose (Herrmann and Hubner 1984). Concentrations of total PAHs in soils, usually the sum of 5 to 20 PAHs, typically exceeded benzo(a)pyrene levels by at least one order of magnitude; however, concentrations of benzo(a)pyrene in vegetation were generally less than those in soil where plants were growing (Edwards 1983).

PAH accumulations in marine molluscs have been reported (Table 4); however, some of these data may be misleadingly low. For example, lengthy cold storage of 10 months can result in loss of volatile PAHs, such as anthracene, in tissues of mussels (Smith et al. 1984); accordingly, background concentrations in these organisms may be underreported. Bivalve molluscs tend to accumulate high PAH levels due to their inability to metabolize and excrete them (Lawrence and Weber 1984a), presumably due to inefficient or missing mixed-function oxidase systems (Sirota and Uthe 1981). Cellular proliferative disorders, resembling neoplastic conditions in vertebrates, were found in mussels with the greatest PAH concentrations: 9.5% vs. 0.7% in control site (Mix 1982). Baseline levels of PAHs in indigenous bivalve molluscs reflected the degree of human onshore activity at the various sample sites, and presumably the level of water contamination; however, little relation was

Table 4. PAH concentrations in field collections of selected biota. Values are shown in ug/kg (ppb) fresh weight (FW), or dry weight (DW).

Taxonomic group, compound, and other variables	Concentration	Reference[a]
ALGAE AND OTHER PLANTS		
Marine algae, Greenland		
Total PAHs	60 FW	Harrison et al. 1975
Marine algae, Benzo(a)pyrene=BaP	Up to 60 DW	Lee and Grant 1981
Freshwater alga,		
Chlorella vulgaris, BaP	10-50 DW	Suess 1976
Bacteria, BaP	2-6 DW	
Moss, Hypnum cupressiforme		
Southern Finland, 1982		
Near center of industrial town		
BaP	110 DW	Herrmann and Hubner 1984
Fluoranthene=FL	250 DW	
Benzo(g,h,i)perylene=BghiPER	90 DW	
Indeno(1,2,3 cd)pyrene=IP	41 DW	
Vegetation		
Total PAHs		
Nonpolluted areas	20-1,000 DW	Edwards 1983
Near known source	25,000 DW	
BaP	0.1-150.0 DW	
Lettuce,		
Lactuca sativa, total PAHs		
Sweden, summer 1980		
Grown near highway		
8-15 m distant	50 FW	Larsson and Sahlberg 1982
15-50 m distant	26 FW	
Near airport, 150-800 m	24 FW	
Aluminum smelter		
0.5-1.5 km distant	654 FW	
2.0-6.5 km	128 FW	
Industrial areas	13 FW	
Residential areas		
Urban	13 FW	
Rural	12 FW	
Seedlings, wheat and rye, BaP	10-20 DW	Suess 1976

Table 4. (Continued)

Taxonomic group, compound, and other variables	Concentration	Reference[a]
INVERTEBRATES		
Rock crab, Cancer irroratus		
Edible portions, 1980		
New York Bight		
Total PAHs	1,600 FW	Humason and
BaP	1 FW	Gadbois 1982
Long Island Sound		
Total PAHs	1,290 FW	
BaP	ND	
American oyster, Crassostrea virginica, soft parts		
South Carolina, 1983, residential resorts		
Total PAHs		
Spring months		
Palmetto Bay	520 FW	Marcus and Stokes 1985
Outdoor Resorts	247 FW	
Fripp Island	55 FW	
Summer months		
Palmetto Bay	269 FW	
Outdoor Resorts	134 FW	
Fripp Island	21 FW	
American lobster, Homarus americanus		
Edible portions, 1980		
New York Bight		
Total PAHs	367 FW	Humason and
BaP	15 FW	Gadbois 1982
Long Island Sound		
Total PAHs	328 FW	
BaP	15 FW	
Softshell clam, Mya arenaria		
Coos Bay, Oregon, 1978-1979		
Soft parts		
Contaminated site		
Total PAHs	555 FW	Mix 1982
Phenanthrene = PHEN	155 FW	
FL	111 FW	
Pyrene = PYR	62 FW	
BaP	55 FW	
Benz(a)anthracene = BaA	42 FW	

Table 4. (Continued)

Taxonomic group, compound, and other variables	Concentration	Reference[a]
Chrysene = CHRY	27 FW	
Benzo(b)fluoranthene = BbFL	12 FW	
Others	<10 FW	
Uncontaminated site		
Total PAHs	76 FW	
PHEN	12 FW	
FL	10 FW	
Others	<10 FW	
Bay mussel, Mytilus edulis		
Oregon, 1979-1980		
Soft parts, total PAHs		
Near industrialized area	106-986 FW	Mix and Schaffer 1983b
Remote site	27-274 FW	
Sea scallop, Placopectin magellanicus		
Baltimore Canyon, east coast USA		
Muscle		
BaA	1 FW	Brown and
BaP	<1 FW	Pancirov 1979
PYR	4 FW	
New York Bight, 1980		
Edible portions		
Total PAHs	127 FW	Humason and
BaP	3 FW	Gadbois 1982
Clam, Tridacna maxima		
Australia, 1980-1982, Great Barrier Reef		
Soft parts, total PAHs		
Pristine areas	<0.07 FW	Smith et al. 1984
Power boat areas	Up to 5 FW	
BaP		
Marine plankton		
Greenland	5 FW	Harrison et al. 1975
Italy	6-21 FW	
France	400 FW	
Worldwide	Up to 400 DW	Lee and Grant 1981
Mussel, Mytilus sp.		
Greenland		
Shell	60 FW	Harrison et al. 1975
Soft parts	18 FW	

Table 4. (Continued)

Taxonomic group, compound, and other variables	Concentration	Reference[a]
Italy		
Shell	11 FW	
Soft parts	130-540 FW	
Bivalve molluscs, 5 spp.		
Edible portions	6 (Max. 36) FW	Stegeman 1981
Decapod crustaceans, 4 spp.		
Edible portions	2 (Max. 8) FW	
Softshell clam, Mya arenaria, soft parts		
Coos Bay, Oregon		
1976-1978		
Near industrialized areas	6-20 FW	Mix and Schaffer 1983a
Remote areas	1-2 FW	
1978-1979		
Near industrialized areas	9 FW	
Remote areas	4 FW	
VERTEBRATES		
Fish, muscle		
Lake Ontario, 6 spp., total PAH	3-8 FW	Lawrence and Weber 1984a
Baltimore Canyon, east coast, USA, 5 spp.		
BaA	Max. 0.3 FW	Brown and Pancirov 1979
BaP	Max. <5 FW	
PYR	Max. <5 FW	
Smoked		
FL	3 FW	EPA 1980
PYR	2 FW	
Nonsmoked		
FL	Max. 1.8 FW	
PYR	Max. 1.4 FW	
Winter flounder, Pseudopleuronectes americanus		
Edible portions, 1980		
New York Bight		
Total PAHs	315 FW	Humason and Gadbois 1982
BaP	21 FW	
Long Island Sound		
Total PAHs	103 FW	
BaP	ND	

Table 4. (Continued)

Taxonomic group, compound, and other variables	Concentration	Reference[a]
Windowpane, Scopthalmus aquosus		
Edible portions, 1980		
New York Bight		
Total PAHs	536 FW	
BaP	4 FW	
Long Island Sound		
Total PAHs	86 FW	
BaP	ND	
Red hake, Urophycus chuss		
Edible portions, 1980		
New York Bight		
Total PAHs	412 FW	
BaP	22 FW	
Long Island Sound		
Total PAHs	124 FW	
BaP	5 FW	
BaP		
Fish		
Marine, edible portions		
9 spp.	Max. 3 FW	Stegeman 1981
Greenland	15 FW	Harrison et al. 1975
Italy	65 FW	
Steak, charcoal broiled	5-8 DW	Barnett 1976
Ribs, barbecued	11 DW	
INTEGRATED STUDIES		
Michigan, 1978, Hersey River		
Near wastewater treatment plant		
PHEN		
Sediments	4,097 FW	Black et al. 1981
Insects, whole	5,488 FW	
Crustaceans, muscle	447 FW	
Fish, muscle	28-15,313 FW	
BaA		
Sediments	3,504 FW	
Insects	2,893 FW	
Crustaceans	40 FW	
Fish	0.2-19 FW	

Table 4. (Concluded)

Taxonomic group, compound, and other variables	Concentration	Reference[a]
BaP		
Sediments	1,194 FW	
Insects	725 FW	
Crustaceans	8 FW	
Fish	0.07-1 FW	
Control location		
Sediments and biota		
PHEN	2-42 FW	
BaA	ND-6.7 FW	
BaP	0.04-1.2 FW	
Nova Scotia, 1980, total PAHs		
Near coking facility		
Sediments	2,830,000 DW	Sirota et al. 1983
American lobster, *Homarus americanus*		
Hepatopancreas	57,300-88,100 FW	
Tail muscle	1,910-2,670 FW	
Control area		
Sediments	<8,220 DW	
American lobster		
Hepatopancreas	1,185 FW	
Tail muscle	216 FW	
Black River, Ohio, contaminated area, total PAHs		
Sediments	6,700 DW	West et al. 1984
Brown bullhead, *Ictalurus nebulosus*	660 FW	
Water	153 FW	

[a]Each reference applies to data in the same row and in the rows that immediately follow for which no reference is indicated.

evident between accumulated levels of individual PAHs and total PAHs (Mix 1982). Elevated PAH concentrations, especially benz(a)anthracene, chrysene, fluorene, phenanthraene, and pyrene in oyster tissues and sediments were measured in samples from the vicinity of marinas, and were higher in oysters in cooler months, when lipids and glycogen were being stored preparatory to spawning (Marcus and Stokes 1985). In general, PAH concentrations in marine clams were highest in areas adjacent to industrialized bayfronts and lowest in clams inhabiting more remote areas; concentrations were lowest in autumn-winter, and highest during spring-summer (Mix and Schaffer 1983a). A similar pattern was observed in mussels, Mytilus edulis, with the more water soluble, lower molecular weight, PAHs bioconcentrated 10X to 100X above that of the higher molecular weight, less water soluble PAHs (Mix and Schaffer 1983b); PAH levels in mussels seemed to be independent of water salinity (Mix and Schaffer 1979). Clams contaminated with PAHs and removed to clean seawater for 24 hours showed significant depuration of unsubstituted 3- and 4-ring PAHs; in contrast, concentrations of all 5-, 6-, and 7-ring compounds, which includes most of the carcinogenic PAHs, were not significantly depurated (Mix 1982). A positive relation exists between PAH isomers in sediments, soft tissues of the mussel Mytilus edulis, and a seaweed (Fucus sp.) collected at Vancouver, British Columbia (Dunn 1980). For mussels, the general trend towards lower levels of higher molecular weight PAHs relative to levels in sediments suggests an uptake mechanism which involves the solution of PAHs in water; superimposed on this pattern is the more rapid turnover and shorter half-life of lower molecular weight PAHs in mussels (Dunn 1980).

PAH residues were higher than expected in American lobsters (Homarus americanus) collected offshore (mean weight 3.6 kg) when compared to smaller (0.6 kg) lobsters collected inshore (Sirota and Uthe 1981), suggesting that age or body size are important modifiers in PAH accumulation dynamics. PAH concentrations in sediments collected near a coking facility in Nova Scotia in 1980 contained up to 2,830 mg/kg dry weight, or more than 20X the levels recorded in Boston (Mass.) Harbor; concentrations in excess of 100 mg/kg dry weight sediment were recorded for phenanthrene, fluorene, pyrene, benz(a)anthracene, chrysene, benzo(e)pyrene, benzo(b)fluoranthene, and benzo(a)pyrene, and these seemed to reflect the elevated tissue levels in American lobsters collected from that locale (Sirota et al. 1983). PAH residues in digestive glands of American lobsters collected in 1979 in Nova Scotia from the vicinity of a major oil spill were higher than those from coastal control sites; however, PAH contents of edible muscle from control and oiled lobsters were similar (Sirota and Uthe 1981).

PAH levels in fish are usually low because this group rapidly metabolizes PAHs (Lawrence and Weber 1984a); furthermore, higher molecular weight PAHs, which include the largest class of chemical carcinogens, do not seem to accumulate in fish (West et al. 1984). Raw fish from unpolluted waters usually do not contain detectable amounts of PAHs, but smoked or cooked fish contain varying levels. The concentration of benzo(a)pyrene in skin of cooked

fish was much higher than in other tissues, suggesting that skin may serve as a barrier to the migration of PAHs in body tissues (EPA 1980).

Sediments and biota collected from the Hersey River, Michigan, in 1978, were heavily contaminated with phenanthrene, benz(a)anthracene, and benzo(a)pyrene when compared to a control site. Elevated PAH concentrations were recorded in sediments, whole insect larvae, crayfish muscle, and flesh of lampreys (family Petromyzontidae), brown trout (Salmo trutta), and white suckers (Catostomus commersoni), in that general order (Black et al. 1981). The polluted collection locale was the former site of a creosote wood preservation facility between 1902 and 1949, and, at the time of the study, received Reed City wastewater treatment plant effluent, described as an oily material with a naphthalene-like odor (Black et al. 1981). In many cases, aquatic organisms from PAH-contaminated environments have a higher incidence of tumors and hyperplastic diseases than those from nonpolluted environments. Carcinogenic PAHs have not been unequivocally identified as the causative agent for an increased incidence of cancer in any natural population of aquatic organisms, according to Neff (1982b). However, a growing body of evidence, mostly circumstantial, links PAHs to cancer in feral fish populations, especially bottom dwelling fish from areas with sediments heavily contaminated with PAHs (Baumann and Lech, in press).

TOXIC AND SUBLETHAL EFFECTS

GENERAL

A wide variety of PAH-caused adverse biological effects have been reported in numerous species of organisms under laboratory conditions, including effects on survival, growth, metabolism, and especially tumor formation. Inter- and intraspecies responses to carcinogenic PAHs were quite variable, and were significantly modified by many chemicals including other PAHs that are weakly carcinogenic or noncarcinogenic. Until these interaction effects are clarified, the results of single substance laboratory tests may be extremely difficult to apply to field situations of suspected PAH contamination.

FUNGI

Fungal degradation of PAHs may be important in the detoxification and elimination of PAHs in the environment. The fungus Cunninghamella elegans, for example, inhibited the mutagenic activity of benzo(a)pyrene, 3-methyl cholanthrene, benz(a)anthracene, and 7,12-dimethylbenz(a)anthracene, as judged by results of the Ames test using Salmonella typhimurium (Cerniglia et al. 1985). The rate of decrease in mutagenic activity in bacterial cultures incubated with PAHs was coincident with the rate of increase in fungal metabolism. C.elegans metabolized PAHs to dihydrodiols, phenols, quinones, and dihydrodiol epoxides, and to sulfate, glucuronide, and glucoside conjugates of these primary metabolites in a manner similar to that reported for mammalian enzyme systems, suggesting that this organism (and perhaps other fungi) is important in PAH metabolism and inactivation (Cerniglia et al. 1985).

TERRESTRIAL PLANTS

Biological effects of PAHs on terrestrial vegetation have been reviewed by EPA (1980), Lee and Grant (1981), Wang and Meresz (1982), Edwards (1983), and Sims and Overcash (1983). In general, these authorities agreed on several points. First, plants and vegetables can absorb PAHs from soils through their roots, and translocate them to other plant parts such as developing shoots. Uptake rates were governed, in part, by PAH concentration, PAH water solubility, soil type, and PAH physicochemical state (vapor or particulate). Lower molecular weight PAHs were absorbed by plants more readily than higher molecular weight PAHs. Under laboratory conditions, some plants concentrated selected PAHs above that of their immediate geophysical surroundings, but this has not been conclusively demonstrated in field-grown cultivated crops or other vegetation. Second, above-ground parts of vegetables, especially the outer shell or skin, contained more PAHs than underground parts, and this was attributed to airborne deposition and subsequent adsorption. Externally deposited PAHs in vegetables were difficult to remove with cold water washings; not more than 25% were removed from lettuce, kale, spinach, leeks, and tomatoes using these procedures. Third, PAH-induced phytotoxic effects were rare; however, the data base on this subject is small. Fourth, most higher plants can catabolize benzo(a)pyrene, and possibly other PAHs, but metabolic pathways have not been clearly defined. Finally, the biomagnification potential of vegetation in terrestrial and aquatic food chains needs to be measured; this work should be conducted with a variety of PAHs in both field and laboratory experiments.

Some plants contain chemicals known to protect against PAH effects. Certain green plants contain ellagic acid, a substance that can destroy the diol epoxide form of benzo(a)pyrene, inactivating its carcinogenic and mutagenic potential (Edwards 1983). PAHs synthesized by plants may act as plant growth hormones (Edwards 1983). Some vegetables, such as cabbage, brussel sprouts, and cauliflower, contain naturally occurring antineoplastic compounds including benzyl isothiocyanate and phenethyl isothiocyanate; these compounds are known to inhibit mammary cancers, stomach tumors, and pulmonary edemas induced in rats by benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene (EPA 1980). Decreased activation of carcinogens has also been demonstrated in animals fed diets that were high in protein, low in carbohydrate, and containing adequate choline; the reverse was observed in diets high in carbohydrate, low in protein, or containing certain organophosphorus insecticides, piperonyl butoxide, carbon tetrachloride, nickel carbonyl, or tin (EPA 1980). In cases where dietary constituents can alter the metabolism of foreign agents, such as PAHs, the anticarcinogenic effect may result from an alteration of steady state levels of activated versus detoxified metabolites (EPA 1980). The implications of these observations to herbivorous wildlife are unknown at present.

AQUATIC BIOTA

PAHs vary substantially in their toxicity to aquatic organisms (Table 5). In general, toxicity increases as molecular weight increases (although high molecular weight PAHs have low acute toxicity, perhaps due to their low solubility in water) and with increasing alkyl substitution on the aromatic ring. Toxicity is most pronounced among crustaceans and least among teleosts (Neff 1979; Table 5). In all but a few cases, PAH concentrations that are acutely toxic to aquatic organisms are several orders of magnitude higher than concentrations found in even the most heavily polluted waters (Neff 1979). Sediments from polluted regions, however, may contain PAH concentrations similar to those which are acutely toxic, but their limited bioavailability would probably render them substantially less toxic than PAHs in solution (Neff 1979).

A growing literature exists on uptake, retention, and translocation of PAHs by aquatic plants and animals. Authorities generally agree that: most species of aquatic organisms studied to date rapidly accumulate (i.e., bioconcentrate) PAHs from low concentrations in the ambient medium; uptake of PAHs is highly species specific, being higher in algae, molluscs, and other species which are incapable of metabolizing PAHs; bioconcentration factors (BCF) tend to increase as the molecular weight of the PAH increases, with increasing octanol/water partition coefficient values, with time until approaching an apparent equilibrium level (sometimes within 24 hours), and with increases in dissolved organic matter in the medium, lipid content of organism, and a variety of endogenous and exogenous factors (Jackim and Lake 1978; Southworth et al. 1978; Lee and Grant 1981; Neff 1982a). BCF values have been determined for selected PAHs and aquatic organisms (Table 6); additional BCF data for aquatic biota are available for plants (Dobroski and Epifanio 1980; Boyle et al. 1984), crustaceans (Southworth 1979; Sirota and Uthe 1981; Fox and Rao 1982; Neff 1982a; Williams et al. 1985), tunicates (Baird et al. 1982), molluscs (Jackim and Wilson 1979; Dobroski and Epifanio 1980; Neff 1982a), and fishes (Southworth 1979; Neff 1982a; Stoker et al. 1984). Algal accumulation of benzo(a)pyrene increased linearly in a 24-hour exposure period, and correlated positively with surface area (Leversee et al. 1981), suggesting adsorption rather than absorption. Algae readily transform benzo(a)pyrene to oxides, peroxides (Kirso et al. 1983), and dihydrodiols (Warshawsky et al. 1983). Photosynthetic rates of algae, and presumably PAH accumulations, were significantly modified by light regimens. For reasons still unexplained, algae grown in "white" light (major energy in blue-green portion of the spectrum) were more sensitive to benzo(a)pyrene than were cultures grown in "gold" light (Warshawsky et al. 1983; Schoeny et al. 1984). Accumulation by oysters (Crassostrea virginica) and clams (Rangia cuneata) of naphthalene, phenanthrene, fluorene, and their methylated derivatives increased with increasing methylation and PAH molecular weight; uptake was more rapid under conditions of aontinuous flow than in static tests (Neff et al. 1976). When returned to PAH-free seawater, molluscs released PAHs to non-

Table 5. Toxicities of selected PAHs to aquatic organisms.

PAH compound, organism, and other variables	Concentration in medium (ug/l)	Effect[a]	Reference[b]
BENZ(a)ANTHRACENE			
Bluegill, *Lepomis macrochirus*	1,000	LC-87 (6 m)	EPA 1980
BENZO(a)PYRENE			
Sandworm, *Neanthes arenceodentata*	>1,000	LC-50 (96 h)	Neff 1979
CHRYSENE			
Sandworm	>1,000	LC-50 (96 h)	
7,12-DIMETHYLBENZ(a)ANTHRACENE			
Minnows, *Poeiciliopsis* spp.			
Juveniles	250	LC-0 (20 h)	Schultz
Juveniles	500	LC-100 (20 h)	and Schultz 1982
DIBENZ(a,h)ANTHRACENE			
Sandworm	>1,000	LC-50 (96 h)	Neff 1979
FLUORANTHENE			
Sandworm	500	LC-50 (96 h)	
FLUORENE			
Grass shrimp, *Palaemonetes pugio*	320	LC-50 (96 h)	
Bluegill	500	LC-12 (30 d)	Finger et al. 1985
Amphipod, *Gammarus pseudoliminaeus*	600	LC-50 (96 h)	
Rainbow trout, *Salmo gairdneri*	820	LC-50 (96 h)	
Bluegill	910	LC-50 (96 h)	
Sandworm	1,000	LC-50 (96 h)	Neff 1979

Table 5. (Continued)

PAH compound, organism, and other variables	Concentration in medium (ug/l)	Effect[a]	Reference[b]
Sheepshead minnow, Cyprinodon variegatus	1,680	LC-50 (96 h)	
Snail, Mudalia potosensis	5,600	LC-50 (96 h)	Finger et al. 1985
Mayfly, Hexagenia bilineata	5,800	LC-50 (120 h)	
Fathead minnow, Pimephales promelas	>100,000	LC-0 (96 h)	
NAPHTHALENE			
Copepod, Eurytemora affinis	50	LC-30 (10 d)	Neff 1979
Pink salmon, Oncorhynchus gorbuscha, fry	920	LC-50 (24 h)	
Dungeness crab, Cancer magister	2,000	LC-50 (96 h)	Neff 1985
Grass shrimp	2,400	LC-50 (96 h)	Neff 1979
Sheepshead minnow	2,400	LC-50 (24 h)	
Brown shrimp, Penaeus aztecus	2,500	LC-50 (24 h)	
Amphipod, Elasmopus pectenicrus	2,680	LC-50 (96 h)	
Coho salmon, Oncorhyncus kisutch, fry	3,200	LC-50 (96 h)	Neff 1985
Sandworm	3,800	LC-50 (96 h)	Neff 1979
Mosquitofish, Gambusia affinis	150,000	LC-50 (96 h)	
1-METHYLNAPHTHALENE			
Dungeness crab, Cancer magister	1,900	LC-50 (96 h)	
Sheepshead minnow	3,400	LC-50 (24 h)	
2-METHYLNAPHTHALENE			
Grass shrimp	1,100	LC-50 (96 h)	Neff 1985
Dungeness crab	1,300	LC-50 (96 h)	
Sheepshead minnow	2,000	LC-50 (24 h)	Neff 1979

Table 5. (Concluded)

PAH compound, organism, and other variables	Concentration in medium (ug/l)	Effect[a]	Reference[b]
TRIMETHYLNAPHTHALENES			
Copepod, *Eurytemora affinis*	320	LC-50 (24 h)	
Sandworm	2,000	LC-50 (96 h)	
PHENANTHRENE			
Grass shrimp	370	LC-50 (24 h)	
Sandworm	600	LC-50 (96 h)	EPA 1980
1-METHYLPHENANTHRENE			
Sandworm	300	LC-50 (96 h)	

[a] m = months, d = days, h = hours.

[b] Each reference applies to data in the same row and in the rows that immediately follow for which no reference is indicated.

detectable levels in about 60 days, with high molecular weight PAHs depurated more slowly than low molecular weight compounds; brown shrimp (Penaeus aztecus) and longnose killifish (Fundulus similis), which can metabolize PAHs, lost PAHs more quickly than clams and oysters, which apparently lack the detoxifying enzymes (Neff et al. 1976). Pink shrimp (Penaeus duorarum) exposed to 1.0 ug chrysene/l for 28 days and then transferred to unpolluted seawater for an additional 28 days contained concentrations of chrysene (91 ug/kg fresh weight in abdomen, 48 ug/kg in cephalothorax) that were considered potentially hazardous to human consumers over extended periods (Miller et al. 1982). Eggs of the sand sole (Psettichthys melanostictus) exposed to 0.1 ug benzo(a)pyrene /l for 5 days showed reduced and delayed hatch and, when compared to controls, produced larvae with high accumulations (2.1 mg/kg fresh weight) and gross abnormalities, such as twinning and tissue overgrowths, in 50% of the test larvae (Hose et al. 1982). Naphthalene and benzo(a)pyrene were rapidly accumulated from the medium by three species of California marine teleosts; loss was rapid, being >90% for naphthalene in 24 hours, and 20% (muscle) to 90% (gill) for benzo(a)pyrene in a similar period (Lee et al. 1972). Phenanthrene is metabolized by many species of aquatic organisms, including fish. A marine flounder, Platichthys flesus, given a single oral dose of 0.7 mg phenanthrene/kg body weight, contained elevated phenanthrene concentrations in lipids, melanin-rich tissues (such as skin), and the eye lens; most was eliminated within 2 weeks (Solbakken et al. 1984). Different rates of accumulation and depuration of benzo(a)pyrene and naphthalene in bluegill (Lepomis macrochirus) and Daphnia magna have been documented by McCarthy and Jimenez (1985) and McCarthy et al. (1985). Benzo(a)pyrene accumulations in bluegill, for example, were 10X greater than naphthalene, but benzo(a)pyrene is extensively metabolized, whereas naphthalene is not. Consequently, postexposure accumulations of naphthalene greatly exceeded that of the parent benzo(a)pyrene. Because the more hydrophobic PAHs, such as benzo(a)pyrene, show a high affinity for binding to dissolved humic materials and have comparatively rapid biotransformation rates, these interactions may lessen or negate bioaccumulation and food chain transfer of hydrophobic PAHs (McCarthy and Jimenez 1985; McCarthy et al. 1985).

Time to depurate or biotransform 50% of accumulated PAHs (Tb 1/2) varied widely. Tb 1/2 values for Daphnia pulex and all PAH compounds studied ranged between 0.4 and 0.5 hours (Southworth et al. 1978). For marine copepods and naphthalene, a Tb 1/2 of about 36 hours was recorded (Neff 1982a). For most marine bivalve molluscs, Tb 1/2 values ranged from 2 to 16 days. Some species, such as the hardshell clam (Mercenaria mercenaria), showed little or no depuration, while others, such as oysters, eliminated up to 90% of accumulated PAHs in 2 weeks--although the remaining 10% was released slowly, and traces may remain indefinitely (Jackim and Lake 1978). Percent loss of various PAHs in oysters (Crassostrea virginica), 7 days postexposure, ranged from no loss for benzo(a)pyrene to 98% for methylnaphthalene; intermediate were benz(a)anthracene (32%), fluoranthene (66%), anthracene (79%), dimethylnaphthalene (90%), and naphthalene (97%) (Neff 1982a). Teleosts and

Table 6. PAH bioconcentration factors (BCF) for selected species of aquatic organisms.

PAH compound, organism, and other variables	Exposure period[a]	BCF	Reference[b]
ANTHRACENE			
Cladoceran, Daphnia magna	60 m	200	EPA 1980
Fathead minnow, Pimephales promelas	2 to 3 d	485	Southworth 1979
Cladoceran, Daphnia pulex	24 h	760 to 1200	Southworth et al. 1978; Southworth 1979; EPA 1980; Neff 1985
Mayfly, Hexagenia sp.	28 h	3,500	EPA 1980
Rainbow trout, Salmo gairdneri	72 h	4,400 to 9,200	Linder et al. 1985
9-METHYLANTHRACENE			
Cladoceran, Daphnia pulex	24 h	4,583	Neff 1985
BENZ(a)ANTHRACENE			
Cladoceran, Daphnia pulex	24 h	10,109	Southworth et al. 1978
BENZO(a)PYRENE			
Teleosts, 3 spp., Muscle	1 h to 96 h	0.02 to 0.1	EPA 1980
Clam, Rangia cuneata	24 h	9 to 236	Neff 1979; EPA 1980
Bluegill, Lepomis macrochirus	4 h	12	Leversee et al. 1981
Atlantic salmon, Salmo salar Egg	168 h	71	Kuhnhold and Busch 1978
Midge, Chironomus riparius, larvae	8 h	166	Leversee et al. 1981
Rainbow trout, liver	10 d	182 to 920	Gerhart and Carlson 1978

Table 6. (Continued)

PAH compound, organism, and other variables	Exposure period[a]	BCF	Reference[b]
Oyster, Crassostrea virginica	14 d	242	EPA 1980
Northern pike Esox lucius			
Bile and gallbladder	3.3 h	3,974	Balk et al. 1984
"	19.2 h	36,656	
"	8.5 d	82,916	
"	23 d	53,014	
Liver	3.3 h	259	
"	19.2 h	578	
"	8.5 d	1,376	
"	23 d	619	
Gills	3.3 h	283	
"	19.2 h	382	
"	8.5 d	372	
"	23 d	213	
Kidney	3.3 h	192	
"	19.2 h	872	
"	8.5 d	1,603	
Other tissues	3.3 h to 23 d	<55	
Mosquitofish, Gambusia affinis	3 d	930	Lu et al. 1977
Bluegill			
No dissolved humic material (DHM)	48 h	2,657	McCarthy and Jimenez 1985
20 mg/l DHM	48 h	225	
Cladoceran, Daphnia magna	6 h	2,837	Leversee et al. 1981
Alga, Oedogonium cardiacum	3 d	5,258	Lu et al. 1977
Periphyton, mostly diatoms	24 h	9,600	Leversee et al. 1981
Mosquito, Culex pipiens quinquefasciatus	3 d	11,536	Lu et al. 1977
Sand sole, Psettichthys melanostictus			
Egg	6 d	21,000	Hose et al. 1982
Snail, Physa sp.	3 d	82,231	Lu et al. 1977
Cladoceran, Daphnia pulex	3 d	134,248	

Table 6. (Continued)

PAH compound, organism, and other variables	Exposure period[a]	BCF	Reference[b]
CHRYSENE			
Clam, *Rangia cuneata*	24 h	8	Neff 1979
Mangrove snapper, *Lutjanus griseus*			
Liver	4 d	83 to 104	Miller et al. 1982
Liver	20 d	258 to 367	
Pink shrimp, *Penaeus duorarum*			
Cephalothorax	28 d	248 to 361	
Cephalothorax	28 d + 28 d postexposure	21 to 48	
Abdomen	28 d	84 to 199	
Abdomen	28 d + 28 d postexposure	22 to 91	
FLUORANTHENE			
Rainbow trout, liver	21 d	379	Gerhart and Carlson 1978
FLUORENE			
Bluegill	30 d	200 to 1,800	Finger et al. 1985
NAPHTHALENE			
Clam, *Rangia cuneata*	24 h	6	Neff 1979
Sandworm, *Neanthes arenaceodenta*	3 to 24 h	40	Neff 1982a
Sandworm	24 h + 300 h post-treatment	not detectable	
Atlantic salmon, egg	168 h	44 to 83	Kuhnhold and Busch 1978
Cladoceran, *Daphnia pulex*	24 h	131	Neff 1985
Crustaceans, 3 spp.	72 h	195 to 404	Neff 1979
Bluegill, whole	24 h	310	McCarthy and Jimenez 1985

Table 6. (Concluded)

PAH compound, organism, and other variables	Exposure period[a]	BCF	Reference[b]
DIMETHYLNAPHTHALENES			
Crustaceans, 3 spp.	72 h	967 to 1,625	Neff 1979
PERYLENE			
Cladoceran, Daphnia pulex	24 h	7,191	Neff 1985
PHENANTHRENE			
Clam, Rangia cuneata	24 h	32	Neff 1979
Cladoceran, Daphnia pulex	24 h	325	Neff 1985
PYRENE			
Cladoceran Daphnia pulex	24 h	2,702	
Rainbow trout, liver	21 d	69	Gerhart and Carlson 1978

[a] m = minutes, h = hours, d = days.

[b] Each reference applies to the values in the same row and in the rows that follow for which no other reference is indicated.

arthropods usually had low Tb 1/2 values. In bluegill, 89% loss of benzo(a)pyrene was recorded 4 hours postexposure; for midge larvae it was 72% in 8 hours, and for daphnids it was 21% in 18 hours (Leversee et al. 1981).

The role of sediments in PAH uptake kinetics should not be discounted. Sediment-associated anthracene contributed about 77% of the steady state body burden of this compound in the amphipod Hyalella azteca (Landrum and Scavia 1983). For benzo(a)pyrene and the amphipod Pontoporeia hoyi, the sediment source (including interstitial water) accounted for 53% in amphipods collected at 60 m, but only 9% at 23 to 45 m (Landrum et al. 1984). Benthos from the Great Lakes, such as oligochaete worms (Limnodrilus sp., Stylodrilus sp.) and amphipods (Pontoporeia hoyi), obtain a substantial fraction of their PAH body content from the water when sediment PAH concentrations are low. However, when sediment PAH concentrations are elevated, benthos obtain a majority of their PAHs from that source through their ability to mobilize PAHs from the sediment/pore water matrix; the high concentrations of phenanthrene, fluorene, benzo(a)pyrene, and other PAHs measured in these organisms could provide a significant source of PAHs to predator fish (Eadie et al. 1983). Great Lakes benthos appear to contain as much PAHs as the fine grain fraction of the sediment which serves as their food, although overlying water or pore water appears to contribute a larger proportion of PAHs to the organism's body burden than does sediments (Eadie et al. 1984). Marine mussels (Mytilus edulis) and polychaete annelid worms (Nereis virens) exposed for 28 days to sediments heavily contaminated with various PAH compounds accumulated significant concentrations (up to 1,000X control levels) during the first 14 days of exposure, and little thereafter; during a 5-week postexposure period, depuration was rapid, with the more water soluble PAHs excreted most rapidly; PAH levels usually remained above control values to the end of the postexposure period (Lake et al. 1985). English sole (Parophrys vetulus), during exposure for 11 to 51 days to PAH-contaminated sediments, showed significant accumulations of naphthalenes in liver (up to 3.1 mg/kg dry weight) after 11 days, with concentrations declining markedly thereafter; uptake of phenanthrene, chrysene, and benzo(a)pyrene was negligible during the first 7 days (Neff 1982a).

Fluorene effects in freshwater pond ecosystems have recently been evaluated (Boyle et al. 1984, 1985; Finger et al. 1985). In ponds exposed to initial fluorene concentrations of 0.12 to 2.0 mg/l, Tb 1/2 values in water ranged from 6 to 11 days. Ten weeks after fluorene introduction, little degradation had occurred in the organic bottom sediments; fluorene residues were present in fish, invertebrates, and rooted submerged macrophytes. Studies with fingerling bluegills showed that 0.062 mg fluorene/l adversely affected their ability to capture chironomid prey, 0.12 mg/l reduced growth, and 1.0 mg fluorene/l increased their vulnerability to predation by largemouth bass (Micropterus salmoides). The authors concluded that fluorene, at concentrations well below its solubility and at levels that could realistically occur in the environment, represents a potential hazard to aquatic organisms.

Large interspecies differences in ability to absorb and assimilate PAHs from food have been reported. For example, crustaceans (Neff 1982a) and fish (Maccubbin et al. 1985; Malins et al. 1985a, 1985b) readily assimilated PAHs from contaminated food, whereas molluscs and polychaete annelids were limited (Neff 1982a). In all cases where assimilation of ingested PAHs was demonstrated, metabolism and excretion of PAHs were rapid (Neff 1982a). Thus, little potential exists for food chain biomagnification of PAHs (Southworth 1979; Dobroski and Epifanio 1980; Neff 1982a). In laboratory aquatic ecosystem studies, Lu et al. (1977) found that benzo(a)pyrene can be accumulated to high, and potentially hazardous, levels in fish and invertebrates. In the case of mosquitofish (Gambusia affinis), almost all of the accumulated benzo(a)pyrene was from its diet, with negligible accumulations from the medium. However, mosquitofish degraded benzo(a)pyrene about as rapidly as it was absorbed, in contrast to organisms such as snails (Physa sp.) which retained most (88%) of the accumulated benzo(a)pyrene for at least 3 days postexposure, presumably due to deficiencies in their mixed-function oxidase detoxication system (Lu et al. 1977). Benzo(a)pyrene, when administered to northern pike (Esox lucius) through the diet or the medium, followed similar pathways: entry via the gills or gastrointestinal system, metabolism in the liver, and excretion in the urine and bile (Balk et al. 1984). Benthic marine fishes exposed to naphthalene or benzo(a)pyrene, either in diet or through contaminated sediments, accumulated substantial concentrations in tissues and body fluids (Varanasi and Gmur 1981). The tendency of fish to metabolize PAHs extensively and rapidly may explain why benzo(a)pyrene, for example, is frequently undetected, or only detected in low concentrations in livers of fish from environments heavily contaminated with PAHs (Varanasi and Gmur 1980, 1981). Extensive metabolism of benzo(a)pyrene plus the presence of large proportions of polyhydroxy metabolites in liver of English sole indicates the formation of reactive intermediates such as diol epoxides and phenol epoxides of benzo(a)pyrene, both of which are implicated in mammalian mutagenesis and carcinogenesis (Varanasi and Gmur 1981).

Cytotoxic, mutagenic, and carcinogenic effects of many PAHs are generally believed to be mediated through active epoxides formed by interaction with microsomal monooxygenases. These highly active arene oxides can interact with macromolecular tissue components and can further be metabolized or rearranged to phenols or various conjugates. They can also be affected by epoxide hydrolase to form dihydrodiols, which are precursors of biologically active diol epoxides--a group that has been implicated as ultimate carcinogens. Investigators generally agree that marine and freshwater fishes are as well equipped as mammals with liver PAH-metabolizing enzymes; rapidly metabolize PAHs by liver mixed-function oxidases, with little evidence of accumulation; translocate conjugated PAH metabolites to the gall bladder prior to excretion in feces and urine; and have mixed-function oxidase degradation rates that are significantly modified by sex, age, diet, water temperature, dose-time relationships, and other variables. In addition, many species of fishes can convert PAHs, benzo(a)pyrene for example, to potent mutagenic metabolites, but because detection of the 7,8-dihydrodiol, 9,10-epoxide by analytical

methods is extremely difficult, most investigators must use biological assays, such as the Ames test, to detect mutagenic agents. At present, the interaction effects of PAHs with inorganic and other organic compounds are poorly understood. Specific examples of the above listed phenomena for PAH compounds and teleosts are documented for benzo(a)pyrene (Ahokas et al. 1975; Lu et al. 1977; Gerhart and Carlson 1978; Melius et al. 1980; Varanasi et al. 1980, 1984; Stegeman et al. 1982; Couch et al. 1983; Hendricks 1984; Melius 1984; Schoor 1984; Schoor and Srivastava 1984; Hendricks et al. 1985; Neff 1985; Fair 1986; von Hofe and Puffer 1986), 3-methylcholanthrene (Gerhart and Carlson 1978; Melius et al. 1980; Melius and Elam 1983; Schoor and Srivastava 1984; Neff 1985), benz(a)anthracene, chrysene, and pyrene (Gerhard and Carlson 1978), and 7,12-dimethylbenz(a)anthracene (Stegeman et al. 1982).

Baumann et al. (1982) summarized reports on increasing frequencies of liver tumors in wild populations of fish during the past decade, especially in brown bullhead (Ictalurus nebulosus) from the Fox River, Illinois (12% tumor frequency), in Atlantic hagfish (Myxine glutinosa) from Swedish estuaries (6%), in English sole from the Duwamish estuary, Washington (32%), and in tomcod (Microgadus tomcod) from the Hudson River, New York (25%). In all of these instances, significant levels of contaminants were present in the sediments, including PAHs. PAHs have been identified as genotoxic pollutants in sediments from the Black River, Ohio, where a high incidence of hepatoma and other tumors has been observed in ictalurid fishes (West et al. 1984, 1986). Reports of tumors in Great Lakes fish populations have been increasing. Tumors of thyroid, gonad, skin, and liver are reported, with tumor frequency greatest near areas contaminated by industrial effluents such as PAHs; liver tumors were common among brown bullhead populations at sites with large amounts of PAHs in sediments (Baumann 1984). A positive relationship was finally established between sediment PAH levels and prevalence of liver lesions in English sole in Puget Sound, Washington (Malins et al. 1984; Varanasi et al. 1984), and sediment levels and liver tumor frequency in brown bullheads from the Black River, Ohio (Baumann and Harshbarger 1985; Black et al. 1985). Sediment PAH levels in the Black River, Ohio, from the vicinity of a coke plant outfall, were up to 10,000 times greater than those from a control location: concentrations were greater than 100 mg/kg for pyrene, fluoranthene, and phenanthrene; between 50 and 100 mg/kg for benz(a)anthracene, chrysene, and benzofluoranthenes; and between 10 and 50 mg/kg for individual naphthalenes, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene, and anthanthrene (Baumann et al. 1982). Brown bullheads from this location contained >1.0 mg/kg of acenapthalene (2.4), phenanthrene (5.7), fluoranthene (1.9), and pyrene (1.1), and lower concentrations of heavier molecular weight PAHs; bullheads also exhibited a high (33%) liver tumor frequency, which seemed to correspond to their PAH body burdens. Investigators concluded that the elevated frequency of liver neoplasia in Black River bullheads was chemically induced, and was the result of exposure to PAHs (Baumann et al. 1982; Baumann and Harshbarger 1985).

Neoplasms in several species of fishes have been produced experimentally with 3-methylcholanthrene, acetylaminofluorene, benzo(a)pyrene, and 7,12-dimethylbenz(a)anthracene, with tumors evident 3 to 12 months postexposure (Couch and Harshbarger 1985; Hendricks et al. 1985). Under laboratory conditions, liver neoplasms were induced in two species of minnows (Poeciliopsis spp.) by repeated short-term exposures (6 hours once a week, for 5 weeks) to an aqueous suspension of 5 mg/l of 7,12-dimethyl-benz(a)anthracene. About 44% of the fish surviving this treatment developed hepatocellular neoplasms 6 to 9 months postexposure (Schultz and Schultz 1982). Eastern mudminnows (Umbra pygmaea) kept in water containing up to 700 ug PAHs/l for 11 days showed increased frequencies of chromosomal aberrations in gills: 30% vs. 8% in controls (Prein et al. 1978). High dietary benzo(a)pyrene levels of 500 mg/kg produced significant elevations in hepatic mixed-function oxidase levels in rainbow trout after 9 weeks (Hendricks et al. 1985). Rainbow trout fed diets containing 1,000 mg benzo(a)pyrene/kg for 12 months developed liver tumors (Couch et al. 1983). About 25% of rainbow trout kept on diets containing 1,000 mg benzo(a)pyrene/kg for 18 months had histologically confirmed liver neoplasms as compared to 15% after 12 months, with no evidence of neoplasia in controls (Hendricks et al. 1985). Young English sole may activate and degrade carcinogenic PAHs, such as benzo(a)pyrene, to a greater extent than adults, but additional research is needed to determine if younger fish are at greater risk than older sole to PAH-induced toxicity (Varanasi et al. 1984). In English sole, a high significant positive correlation between PAH metabolites (1-and 3-hydroxy benzo(a)pyrene, hydroxy and dihydrodiol metabolites of pyrene and fluoranthene) in bile, and idiopathic liver lesions, prevalance of neoplasms, megalocytic hepatosis, and total number of hepatic lesions (Krahn et al. 1986) suggests that selected PAH metabolites and key organs or tissues may be the most effective monitors of PAH contamination in aquatic organisms.

In addition to those effects of PAHs emphasizing survival, uptake, depuration, and carcinogenesis previously listed, a wide variety of additional effects have been documented for aquatic organisms. These include: inhibited reproduction of daphnids and delayed emergence of larval midges by fluorene (Finger et al. 1985); decreased respiration and heart rate in mussels (Mytilus californianus) by benzo(a)pyrene (Sabourin and Tullis 1981); increased weight of liver, kidney, gall bladder, and spleen of sea catfish (Arius felis) by 3-methylcholanthrene, which was dose-related (Melius and Elam 1983); photosynthetic inhibition of algae and macrophytes by anthracene, naphthalene, phenanthrene, pyrene (Neff 1985), and fluorene (Finger et al. 1985); immobilization of the protozoan, Paramecium caudatum, by anthracene, with an EC-50 (60 min) of 0.1 ug/l (EPA 1980); perylene accumulation by algae (Stegeman 1981); accumulation without activation of benzo(a)pyrene and benzo(a)anthracene by a marine protozoan (Parauronema acutum), and biotransformation of various fluorenes by P. acutum to mutagenic metabolites (Lindmark 1981); interference by toluene and anthracene with benzo(a)pyrene uptake by freshwater amphipods (Landrum 1983); abnormal blood chemistry in oysters (Crassostrea virginica) exposed for one year to 5 ug 3-methyl-

cholanthrene/l (Couch et al. 1983); and enlarged livers in brown bullheads from a PAH-contaminated river (Fabacher and Baumann 1985).

AMPHIBIANS AND REPTILES

Limited data were available on biological effects of benzo(a)pyrene, 3-methylcholanthrene, and perylene to reptiles and amphibians (Balls 1964; Stegeman 1981; Anderson et al. 1982; Schwen and Mannering 1982a, 1982b; Couch et al. 1983).

Implantation of 1.5 mg of benzo(a)pyrene crystals into the abdominal cavity of adult South African clawed toads (Xenopus laevis) produced lymphosarcomas in 11 of the 13 toads (85%) after 86 to 288 days (Balls 1964). Immature toads were more resistant, with only 45% bearing lymphoid tumors of liver, kidney, spleen, or abdominal muscle 272 to 310 days after implantation of 1.5 mg of benzo(a)pyrene crystals in the dorsal lymph sac or abdominal cavity. Implantation of 3-methylcholanthrene crystals into X. laevis provokes development of lymphoid tumors similar to those occurring naturally in this species; moreover, these tumors are readily transplantable into other Xenopus or into the urodele species Triturus cristatus (Balls 1964). Intraperitoneal injection of perylene into tiger salamanders can result in hepatic tumors (Couch et al. 1983).

A critical point of interaction between PAHs and reptiles/amphibians involves the transformation of these compounds by cytochrome P-450-dependent monooxygenase systems (Stegeman 1981); in general, reaction rates in this group are considerably slower than those observed in hepatic microsomes from mammals (Schwen and Mannering 1982a). Mixed-function oxidation systems can be induced in liver and skin of tiger salamanders by perylene (Couch et al. 1983) and 3-methylcholanthrene (Anderson et al. 1982), and in liver of the leopard frog (Rana pipiens) and garter snake (Thamnophis sp.) by benzo(a)pyrene and 3-methylcholanthrene (Stegeman 1981; Schwen and Mannering 1982a, 1982b). A single dose of 40 mg/kg body weight of 3-methylcholanthrene was sufficient to induce mixed-function oxidase activity for several weeks in the leopard frog (Schwen and Mannering 1982b). Amphibians, including tiger salamanders, are quite resistant to PAH carcinogenesis when compared to mammals, according to Anderson et al. (1982). This conclusion was based on studies with Ambystoma hepatic microsomes and their inability to produce mutagenic metabolites of benzo(a)pyrene and perylene (as measured by bacterial Salmonella typhimurium strains used in the Ames test); however, rat liver preparations did produce mutagenic metabolites under these procedures (Anderson et al. 1982).

BIRDS

Only two articles were available on PAHs and avian wildlife, and both concerned mallards (Anas platyrhynchos). In one study, Patton and Dieter (1980) fed mallards diets that contained 4,000 mg PAHs/kg (mostly as naphthalenes, naphthenes, and phenanthrene) for a period of 7 months. No mortality or visible signs of toxicity were evident during exposure; however, liver weight increased 25% and blood flow to liver increased 30%, when compared to controls. In the second study, Hoffman and Gay (1981) measured embryotoxicity of various PAHs applied externally, in a comparatively innocuous synthetic petroleum mixture, to the surface of mallard eggs. The most embryotoxic PAH tested was 7,12-dimethylbenz(a)anthracene: approximately 0.002 ug/egg (equivalent to about 0.036 ug/kg fresh weight, based on an average weight of 55 g per egg) caused 26% mortality in 18 days, and, among survivors, produced significant reduction in embryonic growth and a significant increase in the percent of anomalies, e.g., incomplete skeletal ossification, defects in eye, brain, liver, feathers, and bill. At 0.01 ug 7,12-dimethylbenz(a)anthracene/egg, only 10% survived to day 18. Similar results were obtained with 0.015 ug (and higher) chrysene/egg. For benzo(a)pyrene, 0.002 ug/egg did not affect mallard survival, but did cause embryonic growth reduction and an increased incidence of abnormal survivors. At 0.01 ug benzo(a)pyrene/egg, 60% died in 18 days; at 0.05 ug/egg, 75% were dead within 3 days of treatment. Embryos may contain microsomal enzymes that can metabolize PAHs to more highly toxic intermediates than can adults, and avian embryos may have a greater capacity to metabolize PAHs in this manner than do mammalian embryos and fetuses (as quoted in Hoffman and Gay 1981); this observation warrants additional research. Several investigators have suggested that the presence of PAHs in petroleum, including benzo(a)pyrene, chrysene, and 7,12-dimethylbenz(a)anthracene, significantly enhances the overall embryotoxicity in avian species, and that the relatively small percent of the aromatic hydrocarbons contributed by PAHs in petroleum may confer much of the adverse biological effects reported after eggs have been exposed to microliter quantities of polluting oils (Hoffman and Gay 1981; Albers 1983).

MAMMALS

Numerous PAH compounds are distinct in their ability to produce tumors in skin and in most epithelial tissues of practically all animal species tested; malignancies were often induced by acute exposures to microgram quantities. In some cases, the latency period can be as short as 4 to 8 weeks, with the tumors resembling human carcinomas (EPA 1980). Certain carcinogenic PAHs are capable of passage across skin, lungs, and intestine, and can enter the rat fetus, for example, following intragastric or intravenous administration to pregnant dams (EPA 1980). In most cases, the process of carcinogenesis occurs

over a period of many months in experimental animals, and many years in man. The tissue affected is determined by the route of administration and species under investigation. Thus, 7,12-dimethylbenz(a)anthracene is a potent carcinogen for the mammary gland of young female rats after oral or intravenous administration; dietary benzo(a)pyrene leads to leukemia, lung adenoma, and stomach tumors in mice; and both PAH compounds can induce hepatomas in skin of male mice when injected shortly after birth (Dipple 1985). Acute and chronic exposure to various carcinogenic PAHs have resulted in destruction of hematopoietic and lymphoid tissues, ovotoxicity, antispermatogonic effects, adrenal necrosis, changes in the intestinal and respiratory epithelia, and other effects (Table 7; EPA 1980; Lee and Grant 1981). For the most part, however, tissue damage occurs at dose levels that would also be expected to induce carcinomas, and thus the threat of malignancy predominates in evaluating PAH toxicity. There is a scarcity of data available on the toxicological properties of PAHs which are not demonstrably carcinogenic to mammals (EPA 1980; Lee and Grant 1981).

Target organs for PAH toxic action are diverse, due partly to extensive distribution in the body and also to selective attack by these chemicals on proliferating cells (EPA 1980). Damage to the hematopoietic and lymphoid system in experimental animals is a particularly common observation (EPA 1980). In rats, the target organs for 7,12-dimethylbenz(a)anthracene are skin, small intestine, kidney, and mammary gland, whereas in fish the primary target organ is liver (Schultz and Schultz 1982). Application of carcinogenic PAHs to mouse skin leads to destruction of sebaceous glands and to hyperplasia, hyperkeratosis, and ulceration (EPA 1980). Tumors are induced in mouse skin by the repeated application of small doses of PAHs, by a single application of a large dose, or by the single application of a subcarcinogenic dose (initiation) followed by repeated application of certain noncarcinogenic agents (promotion) (Dipple 1985). Newborn mice were highly susceptible to 3-methylcholanthrene, with many mice dying from acute or chronic wasting disease following treatment; some strains of mice eventually developed thynomas, but other strains showed no evidence despite serious damage to the thymus (EPA 1980).

In general, PAH carcinogens transform cells through genetic injury involving metabolism of the parent compound to a reactive diol epoxide. This, in turn, can then form adducts with cellular molecules, such as DNA, RNA, and proteins, resulting in cell transformation (Dipple 1985; Ward et al. 1985). In the case of benzo(a)pyrene, one isomer of the 7,8-diol, 9,10-epoxide is an exceptionally potent carcinogen to newborn mice and is believed to be the ultimate carcinogenic metabolite of this PAH (Slaga et al. 1978). One of the most toxicologically significant processes involved in the response to PAH absorption is the interaction with drug metabolizing enzyme systems (Lee and Grant 1981). Increased production of mixed-function oxidase enzymes in various small mammals has been induced by halogenated napthalenes (Campbell et al. 1983), 3-methylcholanthrene (Miranda and Chhabra 1980), and numerous other PAHs (EPA 1980). PAH metabolites produced by microsomal enzymes in mammals

Table 7. Some effects of PAHs on selected laboratory animals.

Effect (units), organism, PAH compound	Concentration	Reference[a]
LD-50, ACUTE ORAL (mg/kg body weight)		
Rodents (*Rattus* spp., *Mus* spp.)		
Benzo(a)pyrene	50	Sims and Overcash 1978
Phenanthrene	700	
Naphthalene	1,780	
Fluoranthene	2,000	
CARCINOGENICITY, CHRONIC ORAL (mg/kg body weight)		
Rodents		
7,12-dimethylbenz(a)anthracene	0.00004-0.00025	Lo and Sandi 1978
Benzo(a)pyrene	0.002	Sims and Overcash 1983
Dibenz(a,h)anthracene	0.006	
Benz(a)anthracene	2.0	
Benzo(b)fluoranthene	40.0	
Benzo(k)fluoranthene	72.0	
Indeno(1,2,3-cd)pyrene	72.0	
Chrysene	99.0	
Anthracene	3,300.0	
CARCINOGENICITY, APPLIED EXTERNALLY AS TOPICAL (mg)		
Mice, *Mus* spp.		
Benzo(a)pyrene	0.001	Lo and Sandi 1978
Dibenz(a,c)anthracene	0.001	
7,12-dimethylbenz(a)anthracene	0.02	
Dibenz(a,j)anthracene	0.039	
Anthracene	0.08	
Benzo(g,h,i)perylene	0.8	
Benz(a)anthracene	1.0	
CARCINOGENICITY, SUBCUTANEOUS (mg)		
Mice		
Dibenz(a,h)anthracene		
Adults	>0.0002	

Table 7. (Continued)

Effect (units), organism, PAH compound	Concentration	Reference[a]
Newborn	>0.00008	
Dibenzo(a,i)pyrene		
In sesame oil	0.05	
In peanut oil	0.6	
Benzo(a)pyrene	0.06	
Dibenzo(a,e)pyrene	>0.6	
Benzo(b)fluoranthene	1.8	
Benz(a)anthracene	5.0	
Dibenzo(a,h)pyrene	6.0	
TESTICULAR DAMAGE (mg)		
Rat, Rattus spp.		
Benzo(a)pyrene, oral	100.0 (no effect)	EPA 1980
7,12-dimethylbenz(a)anthracene		
Intravenous		
Young rats	0.5 - 2.0	
Older rats	5.0	
Oral	20.0	
OOCYTE AND FOLLICLE DESTRUCTION, SINGLE INTRAPERITONEAL INJECTION (mg/kg body weight)		
Mice		
Benzo(a)pyrene	80.0	Mattison 1980
3-methylcholanthrene	80.0	
7,12-dimethylbenz(a)anthracene	80.0	
ALTERED BLOOD SERUM CHEMISTRY AND NEPHROTOXICITY, SINGLE INTRAPERITONEAL INJECTION (mg/kg body weight)		
Rat		
Phenanthrene	150.0	Yoshikawa
Pyrene	150.0	et al. 1985

Table 7. (Concluded)

Effect (units), organism, PAH compound	Concentration	Reference[a]
FOOD CONSUMPTION, DAILY FOR 5 DAYS (mg/kg body weight)		
Deer mice, Peromyscus maniculatus		
2-methoxynaphthalene		
30% reduction	825	Schafer and Bowles 1985
2-ethoxynaphthalene		
3% reduction	1,213	
House mice, Mus musculus		
2-methoxynaphthalene		
50% reduction	825	
2-ethyoxynaphthalene		
50% reduction	1,213	

[a]Each reference applies to the values in the same row, and in the rows that follow for which no other reference is indicated.

can be arbitrarily divided into water soluble groups, and organosoluble groups such as phenols, dihydrodiols, hydroxymethyl derivatives, quinones, and epoxides (EPA 1980). In the case of benzo(a)pyrene, the diol epoxides are usually considered as the ultimate carcinogens. Other microsomal enzymes convert epoxide metabolites to easily excretable water soluble compounds, with excretion primarily through feces and the hepatobiliary system (EPA 1980). Interspecies differences in sensivity to PAH-induced carcinogenesis are due largely to differences in levels of mixed function oxidase activities, and these will directly affect rates at which active metabolites are converted to less active products (Neff 1979).

Investigators agree that unsubstituted aromatic PAHs with less than 4 condensed rings have not shown tumorgenic activity; that many, but not all, 4-, 5-, and 6-ring PAH compounds are carcinogenic; and that only a few unsubstituted hydrocarbons with 7 rings or greater are tumorogenic or carcinogenic (Neff 1979; EPA 1980; Dipple 1985). Many PAH compounds containing 4 and 5 rings, and some containing 6 or more rings, provoke local tumors after repeated application to the dorsal skin of mice; the tumor incidence exhibited a significant dose-reponse relationship (Grimmer et al. 1985). Among unsubstituted PAHs containing a nonaromatic ring, e.g., cholanthrene and acenapthanthracene, all active carcinogens retained an intact phenanthrene segment (EPA 1980). The addition of alkyl substitutents in certain positions in the ring system of a fully aromatic PAH will often confer carcinogenic activity or dramatically enhance existing carcinogenic potency. For example, monomethyl substitution of benz(a)anthracene can lead to strong carcinogenicity in mice, with potency depending on the position of substitution in the order 7 > 6 > 8 = 12 > 9; a further enhancement of carcinogenic activity is produced by appropriate dimethyl substitution, with 7,12-dimethylbenz(a)anthracene among the most potent PAH carcinogens known. Alkyl substitution of partially aromatic condensed ring systems may also add considerable carcinogenic activity, as is the case with 3-methylcholanthrene. With alkyl substitutes longer than methyl, carcinogenicity levels decrease, possibly due to a decrease in transport through cell membranes (EPA 1980).

A good correlation exists between skin tumor initiating activities of various benzo(a)pyrene metabolites and their mutagenic activity in mammalian cell mutagenesis systems (Slaga et al. 1978), although variations in chromosome number and structure may accompany tumors induced by various carcinogenic PAHs in rats, mice, and hamsters (Bayer 1978; EPA 1980). Active PAH metabolites, e.g., dihydrodiols or diol epoxides, can produce sister chromatid exchanges in Chinese hamster ovary cell (Bayer 1978; EPA 1980; Pal 1984). When exchanges were induced by the diol epoxide, a close relationship exists between the frequency of sister chromatid exchanges and the levels of deoxyribnucleoside-diol-epoxide adduct formation (Pal 1984). In general, noncarcinogenic PAHs were not mutagenic (EPA 1980).

Laboratory studies with mice have shown that many carcinogenic PAHs adversely affect the immune system, thus directly impacting an organism's

general health, although noncarcinogenic analogues had no immunosuppressive effect; further, the more carcinogenic the PAH, the greater the immunosuppression (Ward et al. 1985).

Destruction of oocytes and follicles in mice ovary is documented following intraperitoneal injection of benzo(a)pyrene; 3-methylcholanthrene, and 7,12-dimethylbenz(a)anthracene; the rate of destruction was proportional to the activity of the ovarian cytochrome P-450 dependent monooxygenase, as well as the carcinogenicity of the PAH (Mattison 1980). However, no information is presently available to indicate whether PAHs present a hazard to reproductive success. In those cases where teratogenic effects are clearly evident, e.g., 7, 12-dimethylbenz(a)anthracene, the required doses were far in excess of realistic environmental exposures (Lee and Grant 1981).

Numerous studies show that unsubstituted PAHs do not accumulate in mammalian adipose tissues despite their high lipid solubility, probably because they tend to be rapidly and extensively metabolized (EPA 1980; Lee and Grant 1981).

Biological half-life (Tb 1/2) of PAHs is limited, as judged by rodent studies. In the case of benzo(a)pyrene and rat blood and liver, Tb 1/2 values of 5 to 10 minutes were recorded; the initial rapid elimination phase was followed by a slower disappearance phase lasting 6 hours or more (EPA 1980). Tb 1/2 values from the site of subcutaneous injection in mice were 1.75 weeks for benzo(a)pyrene, 3.5 weeks for 3-methylcholanthrene, and 12 weeks for dibenz(a,h)anthracene; the relative carcinogenicity of each compound was directly proportional to the time of retention at the injection site (Pucknat 1981).

Many chemicals are known to modify the action of carcinogenic PAHs in experimental animals, including other PAHs that are weakly carcinogenic or noncarcinogenic. The effects of these modifiers on PAH metabolism appear to fall into three major categories: those which alter the metabolism of the carcinogen, causing decreased activation or increased detoxification; those which scavenge active molecular species of carcinogens to prevent their reaching critical target sites in the cell; and those which exhibit competitive antagonism (DiGiovanni and Slaga 1981b). For example, benz(a)anthracene, a weak carcinogen, when applied simultaneously with dibenz(a,h)anthracene, inhibited the carcinogenic action of the latter in mouse skin; a similar case is made for benzo(e)pyrene or dibenz(a,c)anthracene applied to mouse skin shortly prior to initiation with 7,12-dimethylbenz(a)anthracene, or 3-methylcholanthrene (DiGiovanni and Slaga 1981a). Benzo(a)pyrene, a known carcinogen, interacts synergistically with cyclopenta(cd)pyrene, a moderately strong carcinogen found in automobile exhausts, according to results of mouse skin carcinogenicity studies (Rogan et al. 1983). Other PAH combinations were cocarcinogenic, such as benzo(e)pyrene, pyrene, and fluoranthene applied repeatedly with benzo(a)pyrene to the skins of mice (DiGiovanni and Slaga 1981a). Effective

inhibitors of PAH-induced tumor development include selenium, vitamin E, ascorbic acid, butylated hydroxytoluene, and hydroxyanisole (EPA 1980). In addition, protective effects against PAH-induced tumor formation have been reported for various naturally occurring compounds such as flavones, retenoids, and vitamin A (EPA 1980). Until these interaction effects are clarified, the results of single substance laboratory studies may be extremely difficult to apply to field situations of suspected PAH contamination. Additional work is also needed on PAH dose-response relationships, testing relevant environmental PAHs for carcinogenicity, and elucidating effects of PAH mixtures on tumor formation (Grimmer 1983).

RECOMMENDATIONS

At present, no criteria or standards have been promulgated for PAHs by any regulatory agency for the protection of sensitive species of aquatic organisms or wildlife. This observation is not unexpected in view of several factors: (1) the paucity of data on PAH background concentrations in wildlife and other natural resources; (2) the absence of information on results of chronic oral feeding studies of PAH mixtures and the lack of a representative PAH mixture for test purposes; and (3) the demonstrable--and as yet, poorly understood--effects of biological modifiers, such as sex, age, and diet, and interaction effects of PAHs with inorganic and other organic compounds, including other PAHs.

Nevertheless, the growing data base for aquatic life indicates a number of generalizations: (1) many PAHs are acutely toxic at concentrations between 50 and 1,000 ug/l; (2) deleterious sublethal responses are sometimes observed at concentrations in the range of 0.1 to 5.0 ug/l; (3) uptake can be substantial, but depuration is usually rapid except in some species of invertebrates; and (4) whole body burdens in excess of 300 ug benzo(a)pyrene/kg (and presumably other PAHs) in certain teleosts would be accompanied by a rise in the activity of detoxifying enzymes.

Current aquatic research has focused on PAHs because of their known relationship with carcinogenesis and mutagenesis. Many reports exist of high incidences of cancer-like growths and developmental anomalies in natural populations of aquatic animals and plants, but none conclusively demonstrate the induction of cancer by exposure of aquatic animals to environmentally realistic levels of carcinogenic PAHs in the water column, diet, or sediments (Neff 1982b, 1985). However, recent studies by Baumann, Malins, Black, Varanasi and their coworkers, among others, have now established that sediments heavily contaminated with PAHs from industrial sources were the direct cause of elevated PAH body burdens and elevated frequencies of liver neoplasia in fishes from these locales. At present, only a few sites containing high PAH concentrations in sediments have been identified (Couch and Harshbarger 1985), suggesting an urgent need to identify and to evaluate other PAH-contaminated aquatic sites. Most fishery products consumed by upper trophic levels, including man, contain PAH concentrations similar to those in green vegetables and smoked and charcoal-broiled meats, and would probably represent a minor source of PAH toxicity; however, consumption of aquatic

organisms, especially filter-feeding bivalve molluscs, from regions severely contaminated with petroleum or PAH-containing industrial wastes, should be avoided (Jackim and Lake 1978; Neff 1982b). Neff (1982b) suggested that repeated consumption of PAH-contaminated shellfish may pose a cancer risk to humans. If true, this needs to be evaluated using seabirds, pinnipeds, and other wildlife groups which feed extensively on molluscs that are capable of accumulating high burdens of carcinogenic PAHs, in order to determine if similar risks exist.

For avian wildlife, data are missing on PAH background concentrations and on acute and chronic toxicity; these data should be collected posthaste. Studies with mallard embryos and PAHs applied to the egg surface showed toxic and adverse sublethal effects at concentrations between 0.036 and 0.18 ug PAH/kg whole egg (Hoffman and Gay 1981). Additional research is needed on petroleum-derived PAHs and their effects on developing embryos of seabirds and other waterfowl.

PAH criteria for human health protection (Table 8) were derived from tests with small laboratory mammals, primarily rodents. Accordingly, these proposed criteria should become interim guidelines for protection of nonhuman mammalian resources pending acquisition of more definitive data. The proposed PAH criteria are controversial. Pucknat (1981) states that there is no way at present to quantify the potential human health risks incurred by the interaction of any PAH with other PAHs or with other agents in the environment, including tumor initiators, promotors, and inhibitors. The problem arises primarily from the diversity of test systems and bioassay conditions used for determining carcinogenic potential of individual PAHs in experimental animals, and is confounded by the lack of a representative PAH mixture for test purposes, the absence of data for animal and human chronic oral exposures to PAH mixtures, and the reliance on data derived from studies with benzo(a)pyrene to produce generalizations concerning environmental effects of PAHs--generalizations which may not be scientifically sound--according to Pucknat (1981). EPA (1980) emphasizes that only a small percentage of PAH compounds are known to be carcinogenic, and that measurements of total PAHs (i.e., the sum of all multiple fused-ring hydrocarbons having no heteroatoms) can not be equated with carcinogenic potential; furthermore, when the term "total PAHs" is used, the compounds being considered should be specified for each case. Lee and Grant (1981) state that an analysis of dose-response relationships for PAH-induced tumors in animals shows, in some cases, deviation from linearity in dose-response curves, especially at low doses, suggesting a two-stage model consistent with a linear nonthreshold pattern. Because overt tumor induction follows a dose-response relationship consistent with a multihit promotion process, the multihit component of carcinogenesis may be supplied by environmental stimuli not necessarily linked or related to PAH exposure.

The well-documented existence of carcinogenic and anticarcinogenic agents strongly suggests that a time assessment of carcinogenic risk for a particular

Table 8. Proposed PAH criteria for human health protection (modified from EPA 1980; Lee and Grant 1981; Pucknat 1981).

Criterion, PAH group, and units	Concentration
DRINKING WATER	
Total PAHs	
ug/l[a]	0.0135-0.2
Daily intake, ug[a]	0.027-0.4
Yearly intake, ug	4.0
Benzo(a)pyrene	
ug/l	0.00055
Daily intake, ug	0.0011
Carcinogenic PAHs	
ug/l[b]	0.0021
Daily intake, ug[b]	0.0042
ug/l[c]	
Cancer risk 10^{-5}	0.028
Cancer risk 10^{-6}	0.0028
Cancer risk 10^{-7}	0.00028
Daily intake, ug[c]	
Cancer risk 10^{-5}	0.056
Cancer risk 10^{-6}	0.0056
Cancer risk 10^{-7}	0.00056
FOOD	
Total PAHs	
Daily intake, ug[d]	1.6-16.0
Yearly intake, ug	4,150.0
Benzo(a)pyrene	
Daily intake, ug[e]	0.16-1.6
AIR	
Total PAHs	
ug/m^3	0.0109
Daily intake, ug[f]	0.164-0.251
Cyclohexane extractable fractions	
Coke oven emissions, coal tar products, ug/m^3, 8 to 10 hour-weighted average	100.0-150.0

Table 8. (Concluded)

Criterion, PAH group, and units	Concentration
Benzene soluble fractions	
Coal tar pitch volatiles, ug/m^3, 8-hour, time-weighted average	200.0
Benzo(a)pyrene	
ug/m^3	0.0005
Daily intake, ug[f]	0.005-0.0115
Carcinogenic PAHs	
ug/m^3	0.002
Daily intake, ug[f]	0.03-0.046
ALL SOURCES	
Total PAHs	
Daily, ug	1.79-16.6
Benzo(a)pyrene	
Daily intake, ug	0.166-1.61
Daily allowable limit, ug[g]	0.048
Carcinogenic PAHs (except diet)	
Daily intake, ug[b]	0.086-0.102

[a]Total of 6 PAHs: fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, benzo(b)fluoranthene, benzo(k)fluoranthene, and indeno(1,2,3-cd)pyrene.

[b]Total of 3 PAHs: benzo(a)pyrene, benzo(j)fluoranthene, and indeno(1,2,3-cd)pyrene.

[c]Based on all carcinogenic PAHs.

[d]Assuming 1,600 g food daily, 70 kg adult, 1 to 10 ug total PAHs/diet.

[e]As above, except 0.1 to 1.0 ug benzo(a)pyrene/diet.

[f]Assuming average of 15 to 23 m^3 of air inhaled daily.

[g]From Wang and Meresz (1982).

PAH can be evaluated only through a multifactorial analysis (Lee and Grant 1981). One of the most toxicologically significant processes involved in the response to PAH absorption is the interaction with drug metabolizing enzyme systems. The induction of this enzyme activity in various body tissues by PAHs and other xenobiotics is probably critical to the generation of reactive PAH metabolites at the target site for tumor induction. At present, wide variations occur in human and animal carcinogen-metabolizing capacity. Moreover, it has not yet been possible to definitely correlate enzyme activity with susceptibility to carcinogenesis. The obligatory coupling of metabolic activation with PAH-induced neoplasia in animals indicates that the modulation of drug metabolizing enzymes is central to carcinogenesis (Lee and Grant 1981).

PAHs from drinking water contribute only a small proportion of the average total human intake (Harrison et al. 1975). The drinking water quality criterion for carcinogenic PAH compounds is based on the assumption that each compound is as potent as benzo(a)pyrene, and that the carcinogenic effect of the compounds is proportional to the sum of their concentrations (EPA 1980). Based on an oral feeding study of benzo(a)pyrene in mice, the concentration of this compound estimated to result in additional risk of one additional case for every 100,000 individuals exposed (i.e, 10^{-5}) is 0.028 ug/l. Therefore, with this assumption, the sum of the concentrations of all carcinogenic PAH compounds should be less than 0.028 ug/l in order to keep the lifetime cancer risk below 10^{-5} (EPA 1980). The corresponding recommended criteria which may result in an incremental cancer risk of 10^{-6} and 10^{-7} over the lifetime are 0.0028 and 0.00028 ug/l, respectively (Table 8). If the above estimates are made for consumption of aquatic organisms only, the levels are 0.311 (10^{-5}), 0.031(10^{-6}), and 0.003(10^{-7}) ug/kg, respectively (EPA 1980). The use of contaminated water for irrigation can also spread PAHs into other vegetable foodstuffs (EPA 1980). When vegetables grown in a PAH-polluted area are thoroughly washed and peeled, their contribution to total PAH intake in humans is not significant (Wang and Meresz 1982). Herbivorous wildlife, however, may ingest significant quantities of various PAHs from contaminated vegetables--but no data were available on this subject.

PAHs are widely distributed in the environment as evidenced by their detection in sediments, soils, air, surface waters, and plant and animal tissues. However, the ecological impact of PAHs is uncertain. PAHs show little tendency for bioconcentration, despite their high lipid solubility (Pucknat 1981), probably because most PAHs are rapidly metabolized. Sims and Overcash (1983) list a variety of research needs regarding PAHs in soil-plant systems. Specifically, research is needed to establish: the rates of PAH decomposition in soils; the soil PAH levels above which PAH constituents adversely affects the food chain; and enhancement factors that increase degradation rates of PAHs, especially PAHs with more than 3 rings. Once these factors have been determined, PAH disposal into soils may become feasible at environmentally nonhazardous levels.

Diet is the major source of PAHs to humans. Authorities agree that most foods contain 1 to 10 ug total PAHs/kg fresh weight, that smoking or barbecuing fish and meats increases total PAH content up to 100X, that contaminated molluscs and crustaceans may contribute significantly to PAH intake, and that PAH carcinogenic risk to humans has existed at least since man began to cook his food (Barnett 1976; EPA 1980; Lee and Grant 1981; Lawrence and Weber 1984a). A total of 22 PAHs has been identified in foods, of which 11 have been found to be carcinogenic in experimental animals. Of these, only 5 (benzo(a)pyrene, benz(a)anthracene, 3-methylcholanthrene, dibenz(a,h)anthracene, and 7,12-dimethylbenz(a)anthracene) have been demonstrated to induce tumors following oral administration to rats and mice, and only 3 of the 11 exhibited positive dose-response relationships in chronic studies with mice (Lo and Sandi 1978). At the present time, there is no evidence that any of the 11 known carcinogenic PAHs or their combinations can cause cancer in human beings via the oral route, especially in quantities likely to be present in foods (Lo and Sandi 1978).

In view of the carcinogenic characteristics of many PAH compounds, their increasing concentrations in the environment should be considered alarming, and efforts should be made to reduce or eliminate them wherever possible (Suess 1976).

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This report synthesizes technical literature on ecological and toxicological aspects of polycyclic aromatic hydrocarbons (PAH) in the environment, with special reference to fisheries and wildlife resources. Subtopics include: chemical properties, sources, and fate; background concentrations in biological and nonbiological samples; toxic and sublethal effects of PAH to flora and fauna; and proposed criteria and research needs for the protection of sensitive, nonhuman organisms.

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Impact of the Keystone XL pipeline on global oil markets and greenhouse gas emissions

Peter Erickson* and Michael Lazarus

Climate policy and analysis often focus on energy production and consumption[1,2], but seldom consider how energy transportation infrastructure shapes energy systems[3]. US President Obama has recently brought these issues to the fore, stating that he would only approve the Keystone XL pipeline, connecting Canadian oil sands with US refineries and ports, if it 'does not significantly exacerbate the problem of carbon pollution'[4]. Here, we apply a simple model to understand the implications of the pipeline for greenhouse gas emissions as a function of any resulting increase in oil sands production. We find that for every barrel of increased production, global oil consumption would increase 0.6 barrels owing to the incremental decrease in global oil prices. As a result, and depending on the extent to which the pipeline leads to greater oil sands production, the net annual impact of Keystone XL could range from virtually none to 110 million tons CO_2 equivalent annually. This spread is four times wider than found by the US State Department (1–27 million tons CO_2e), who did not account for global oil market effects[5]. The approach used here, common in lifecycle analysis[6], could also be applied to other pending fossil fuel extraction and supply infrastructure.

Globally, the International Energy Agency projects that nearly $700 billion per year will be invested in the upstream oil and gas sector over the next two decades[7]. The resulting infrastructure could contribute to carbon lock-in and further the problem of 'carbon entanglement'[8]. Accordingly, it is crucial to understand the implications of fuel supply infrastructure for future greenhouse gas (GHG) emissions[9]. Innovations such as extraction-based carbon accounting[10] have helped quantify the emissions associated with fossil fuel supply, not just consumption, as has traditionally been the focus. However, few analyses have quantified the incremental GHG emissions impact of new fossil fuel supply infrastructure.

Broadly speaking, construction of fuel supply infrastructure could result in several categories of GHG impacts, including emissions associated with project construction and operation[5]; 'lifecycle' emissions associated with fuel extraction, processing and transportation[5]; and emissions associated with increased fuel use and combustion, due to price effects[6], if the infrastructure increases global fuel supply. Furthermore, high-profile decisions such as the US government approval of Keystone XL could have indirect, political or structural effects, if they lead other decision-makers to reject new fossil fuel infrastructure on GHG grounds or, conversely, lead to a political backlash that inhibits other efforts to reduce emissions[11]. Although this last category may be the most significant, quantification is difficult and inherently speculative, so we do not further analyse it here.

The three categories of emissions impact can be reflected, sequentially, as:

$$\Delta\text{Emissions} = \text{Emissions}_{\text{const}} + \Delta\text{Production} * (\text{EF}_{\text{proj}} - \text{EF}_{\text{ref}}) + \Delta\text{Consumption} * \text{EF}_{\text{ref}} \quad (1)$$

where: $\text{Emissions}_{\text{const}}$ = Emissions associated with infrastructure construction and operation, in tonnes CO_2 equivalent (CO_2e); ΔProduction = Increase in production of fuel handled by infrastructure project; EF_{proj} = Emissions factor, per unit of fuel handled, lifecycle basis; EF_{ref} = Emissions factor, per unit of displaced, reference fuel, lifecycle basis; ΔConsumption = Increase in fuel consumption resulting from increased production.

Factoring out the increase in production from the second two terms of equation (1) yields:

$$\Delta\text{Emissions} = \text{Emissions}_{\text{const}} + \Delta\text{Production} * \left((\text{EF}_{\text{proj}} - \text{EF}_{\text{ref}}) + \left(\text{EF}_{\text{ref}} * \frac{\Delta\text{Consumption}}{\Delta\text{Production}} \right) \right) \quad (2)$$

For the Keystone XL pipeline, the State Department has estimated all terms in equation (2) except the final one, a ratio that expresses the extent to which expanding oil sands production may increase global oil consumption. This term, and the effect it embodies, has not received significant attention in discussions of Keystone XL (ref. 12), and is therefore the subject of this Letter.

Microeconomic theory provides the tools to examine the price effect of adding new production capacity to an existing market[13]. Our simple model simulates the interaction between global oil demand[14] and supply[15] for the year 2020, as depicted in Fig. 1.

Similar economic models have been used to analyse the oil market impact of other US policies—for example, for the proposed expansion of oil extraction from the Arctic National Wildlife Refuge[13], expanded production of US biofuels[6], or recent proposals for new coal export terminals that may open new markets for Powder River Basin coal that might otherwise be shut in[16].

For small shifts in supply (830,000 barrels per day (bpd) is less than 1% of global oil supply), and for which the supply and demand curves can be represented as linear, the ratio of increased consumption to increased production can be approximated as the elasticity of demand (E_d) divided by the difference between the elasticities of demand and supply (E_s; ref. 13):

$$\frac{\Delta\text{Consumption}}{\Delta\text{Production}} \cong \frac{E_d}{E_d - E_s} \quad (3)$$

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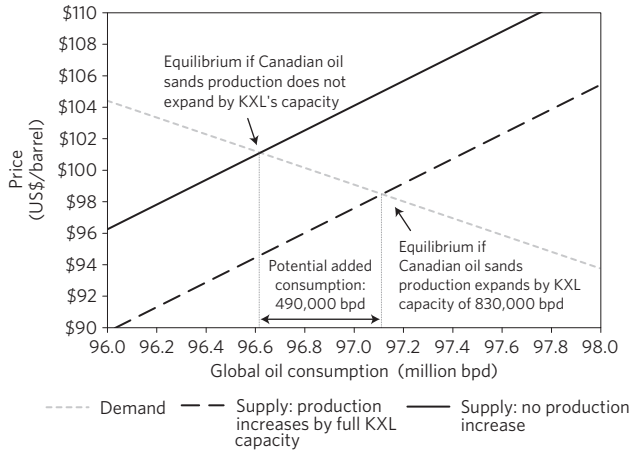

Figure 1 | Simple model of global supply and demand for oil: how increasing global oil supply via Keystone XL would decrease prices and increase consumption. We fix the demand curve, and adjust the supply curve to reflect the extent to which Keystone XL might affect Canadian oil sands production, from no effect to the full 830,000 bpd pipeline capacity.

Table 1 | Increase in annual crude oil consumption per barrel of added Canadian oil sands production under a range of demand and supply elasticities.

	Supply elasticity		
Demand elasticity	0.1	0.13	0.6
−0.054	0.35	0.29	0.08
−0.20	0.66	0.59	0.25
−0.36	0.78	0.73	0.38

Added consumption resulting from each unit of increased production ranges from 0.08 in the case of high supply and low demand elasticities to 0.78 in the case of low supply and high demand elasticities.

Using a long-run elasticity of supply of 0.13, as derived from a global oil supply curve for 2020[15] used by the International Energy Agency[7], and a long-run elasticity of demand of −0.20 from a literature survey[14], equation (3) results in an increase of 0.59 barrels of oil consumed for each barrel (bbl) of increased production. We use this ratio in equation (2); the value is similar to the market adjustment effect [0.5 (±0.2)] found in a recent modelling assessment of the impact of increased biofuel supply on global oil consumption[6].

To characterize uncertainties in the demand and supply relationships around the market-clearing price, we conduct a sensitivity analysis by varying demand elasticities by one standard deviation of values found in a literature survey[17] and supply elasticities across the values found in an OECD review[18], as shown in Table 1, and discussed further in the Methods section. In addition, there are a number of possible effects that our model does not capture—such as the increased availability of highly efficient vehicles, increased switching to non-petroleum transport fuels, or cartel behaviour among a small number of producers—although these effects, as noted in the Methods, are likely to be small.

For all other terms in equation (2), we use the State Department's findings. The GHG emissions impact of pipeline construction is minor, far less than 1 million tons CO_2e per year when spread over the pipeline's 50-year lifetime[5]. The GHG emissions of pipeline operation are similarly small, and slightly less than for alternative transport modes such as rail[19]. Because these net effects are small (less than 1 million tonnes CO_2e per year), we do not consider them further here.

The difference in lifecycle emissions between the oil sands and a reference crude may, however, be substantial. The State Department estimated the lifecycle emissions factor of oil sands ($EF_{proj} = EF_{oil\ sands} = 569$ kg CO_2e/bbl) to be 18% higher than that of the most likely alternative, reference crude, Middle Eastern Sour ($EF_{ref} = 481$ kg CO_2e/bbl). Equation (2) therefore suggests a GHG impact of 373 kg CO_2e $[(569-481)+481*0.59]$ for each barrel of increased production. It remains possible that the reference crude could have a lifecycle GHG emissions intensity more similar to the oil sands. For example, the State Department provides one set of estimates for oil sands ($EF_{oil\ sands} = 557$ kg CO_2e/bbl) and a reference, Venezuelan crude ($EF_{ref} = 552$ kg CO_2e/bbl), that differ by only 1% (ref. 5). In this case, the increase in emissions from the substitution of oil sands for the reference crude would be less, but the emissions associated with increased global consumption would be greater, yielding 331 kg CO_2e $[(557-552)+552*0.59]$ per barrel of increased production; 11% lower than if substituting for Middle Eastern Sour.

The overall GHG emissions impact of Keystone XL is determined, as shown in equation (2), by the extent to which Keystone XL leads to an increase in oil sands production. Here, the State Department concludes that owing to availability of other pipelines (for example, the proposed expansion of the Trans Mountain pipeline to Vancouver, British Columbia., or the proposed Northern Gateway pipeline to Kitimat, British Columbia) or rail for transporting oil sands crude, the rate of Canadian oil sands extraction would most likely be the same with or without Keystone XL (ΔProduction = 0), and therefore there is no GHG emissions impact. Other analysts suggest that the State Department may be overly optimistic, however, and that regulatory, environmental and local community barriers faced by other pipeline and rail options could ultimately restrict expansion of oil sands production[20,21].

The State Department also suggests a case in which the oil sands production could increase by Keystone's full capacity (ΔProduction = 830,000 bpd). If future oil prices are lower than expected, specifically $65–$75 per barrel, 'higher transportation costs (due to pipeline constraints) could have a substantial impact on oil sands production levels, possibly in excess of the capacity of the proposed Project'[5]. Oil prices could be lower than now forecast for a number of reasons. For example, technological progress in extraction and processing or the introduction of new low-cost supplies could increase competition among suppliers, shifting the supply curve to the right and lowering prices. Slower-than-expected growth in vehicle use in developing countries, or faster uptake of vehicle efficiency technologies, could shift the demand curve to the left, lowering prices. A combination of these and other factors could also present themselves, as in the US Energy Information Agency's (EIA) Low Oil Price projection, which falls within this range for nearly all of the next 20 years[22]. Furthermore, widespread implementation of GHG emission reduction policies would reduce demand for oil and, in turn, oil prices seen by producers, even though consumers might see higher prices under a carbon price[23–25].

The State Department calculates the GHG impact under the scenario where the Canadian oil sands production increases by the full amount of Keystone XL's capacity as 1.3–27.4 million tCO_2e per year, corresponding to the estimates of lifecycle emissions associated with oil sands relative to Venezuelan and Middle Eastern Sour reference crudes, respectively, as discussed above, and assuming perfect substitution of one fuel for another[5]. Using those same lifecycle emissions estimates and assumptions about increased oil sands production, our analysis suggests incremental GHG emissions of 100–110 Mt CO_2e, or four times the upper State Department estimate. The sole reason for this difference is that we account for the changes in global oil consumption resulting from increasing oil sands production levels, whereas the State Department does not. (We include results for all supply and demand elasticities

considered, assuming a reference Middle Eastern Sour crude, in the Supplementary Information).

To put the scale of potential emissions increases from Keystone XL in context, consider that projected emission decreases in 2020 due to various US government climate policies under consideration are estimated to range from 20 to 60 Mt CO_2e for performance standards on industrial boilers, cement kilns and petroleum refiners (combined), and from 160 to 575 Mt CO_2e for performance standards on new and existing power plants[26].

Our simple model shows that, to the extent that Keystone XL leads to greater oil sands production, the pipeline's effect on oil prices could substantially increase its total GHG impact. Similar models are common in the lifecycle analysis literature. Methodological reviews have emphasized the importance of considering market-mediated effects in policy assessments, including in oil markets, and warned against the practice employed by the State Department of assuming perfect substitution of one fuel for another with no consideration of price and scale effects[27,28].

We see no indication that the State Department has considered these market effects in its assessment. The proprietary model it uses (EnSys' WORLD model)[5,29] is opaque with respect to key assumptions and features, such as global oil market response to changes in supply. By contrast, advantages of our simple model—using publicly available supply curves and peer-reviewed elasticities—are transparency and the ability to gauge the magnitude of possible price effects. Similar approaches could also be applied to other pending investments in fossil fuel extraction and supply infrastructure, such as deepwater oil rigs, new ports or rail lines to transport coal, or any of a host of investments under consideration that would expand global fossil-fuel supply[9].

The question of whether Keystone XL will 'significantly exacerbate the problem of carbon pollution' hinges on how much the pipeline increases global oil supply and, through price effects, global oil consumption. This Letter offers no new insights on whether Keystone XL will ultimately enable higher oil sands production levels: there are diverse viewpoints on whether alternative transportation options can fully substitute for Keystone XL. Instead, this Letter focuses on price effects and finds that, to the extent that Keystone XL may increase global oil supply, the State Department's assessment has overlooked the pipeline's potentially most significant GHG impact: increasing oil consumption as the result of increasing supplies and lowering prices.

Methods

Our model of global oil supply and demand is based on the standard approach for supply and demand analysis, for example as outlined by Perloff[13].

We draw our global oil supply curve for 2020 from the work of Rystad Energy[15]. Similar to other oil supply curves[30,31], Rystad's curve starts with significant conventional oil production in lower-cost regions (such as the Middle East), followed by a more steeply rising segment of higher-cost, less conventional resources (such as deepwater, enhanced recovery, oil sands) that represent the marginal resource. For example, Rystad's curve shows the cost of oil supply in 2020 rising sharply after 90 million barrels per day (mbpd). At the assumed equilibrium consumption level of 96.62 mbpd in 2020, per the US EIA (ref. 22), the real oil price is $101 US$/barrel and the elasticity of supply is 0.13. (See Appendix 1 in the Supplementary Information for the full cost curve.) For simplicity, we assume that Rystad's cost curve does not already include the oil to be carried by Keystone XL. If it did already include it, we estimate that the elasticity of supply at the equilibrium consumption level would instead be 0.11.

To model a demand response, we use the results of a literature review that estimates a long-run demand elasticity of −0.2 (ref. 14) which we use to approximate a demand curve that intersects the supply curve at the equilibrium consumption level noted above.

Assuming small changes in supply, a change in consumption can be estimated as the shift in the supply curve (change in production) multiplied by the elasticity of demand divided by the difference between the elasticities of demand and supply, $E_d/(E_d - E_s)$ (ref. 13).

Demand elasticities tend to be greater in the longer term than in the shorter term[14], as there is more time to invest capital in alternatives such as biofuels or high-efficiency or electric vehicles. Uncertainties also exist on the supply side. Technological progress in oil extraction and processing could flatten the curve, increasing the price elasticity of supply. (The elasticity of supply could also be lower if overall demand was less, and hence the equilibrium price was lower). Alternatively, if depletion effects (whether in conventional or unconventional sources) are stronger than assumed by industry analysts, the curve could steepen, decreasing the elasticity of supply. To characterize these uncertainties, we also consider a range of supply and demand elasticities. For demand elasticities we use a range from one of the studies cited by the literature review we use for our central estimate[17]. For supply elasticities, we use a range reported by the Organization for Economic Cooperation and Development[18].

We do not consider substitution or market effects with other fuels because most oil is consumed in the transport sector, where few alternatives are currently available and where the literature on elasticities of substitution for the key alternative—biofuel—is sparse[32]. If this method were applied to other fossil fuels, however—for example, the expanded supply of coal, which in most sectors, such as power, competes directly with other fuels and energy sources such as natural gas or renewable energy—such substitution effects would need to be considered.

Last, this simple model may miss more complicated effects, such as cartel behaviour, in which a small number of producers may manipulate the oil supply and prices. However, our literature review and analysis of global oil price behaviour found little compelling evidence of effective cartel influence; in the case of recent price increases, we found that low demand price elasticity, low supply elasticity (or the 'failure of global production to increase'), and growing demand from emerging economies are the main determinants of price[14]. Just as underinvestment has tended to lead to price increases[33], investment in supply infrastructure will tend to lead to price decreases. Our simple model also misses any market, and consequent emissions, impact should increased oil sands production increase the supply and depress the prices of refining co-products such as petroleum coke, LPG, or electricity, increasing their consumption and substituting for lower or higher carbon fuels.

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Author contributions

P.E. and M.L. designed the research. P.E. designed and constructed the spreadsheet model. P.E. and M.L. analysed the results and wrote the paper.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to P.E.

Competing financial interests

The authors declare no competing financial interests.