

# Effects of Pollution on Marine Organisms

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**ABSTRACT:** This review covers selected 2018 articles on the biological effects of pollutants, including human physical disturbances, on marine and estuarine plants, animals, ecosystems and habitats. The review, based largely on journal articles, covers field and laboratory measurement activities (bioaccumulation of contaminants, field assessment surveys, toxicity testing and biomarkers) as well as pollution issues of current interest including endocrine disrupters, emerging contaminants, wastewater discharges, marine debris, dredging and disposal etc. Special emphasis is placed on effects of oil spills and marine debris due largely to the 2010 Deepwater Horizon oil blowout in the Gulf of Mexico and proliferation of data on the assimilation and effects of marine debris. Several topical areas reviewed in the past (e.g. mass mortalities ocean acidification) were dropped this year. The focus of this review is on effects, not on pollutant sources, chemistry, fate or transport. There is considerable overlap

across subject areas (e.g. some bioaccumulation data may be appear in other topical categories such as effects of wastewater discharges, or biomarker studies appearing in oil toxicity literature). Therefore, we strongly urge readers to use keyword searching of the text and references to locate related but distributed information. Although nearly 400 papers are cited, these now represent a fraction of the literature on these subjects. Use this review mainly as a starting point. And please consult the original papers before citing them.

**KEYWORDS:** Tissue residues, toxicity, bioaccumulation, biomagnification, biomarkers, sediment quality, ecological risk assessment, endocrine disrupters, metals, nano particles, POPs, PCBs, PAHs, PBDEs, radionuclides, pharmaceuticals, personal care products, trace metals, pesticides, biomarkers, marine biocides, oil spills, dispersants, field survey methods, sewage, marine debris, entanglement, dredging, eutrophication, fishing impacts, human disturbance, noise and light pollution, Arctic, Antarctic, microbes, plankton, invertebrates, fish, birds, turtles, mammals, marshes, mangroves, inter-tidal.

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## Memoriam: Donald J. Reish

This Water Environment Research annual review of the literature on Effects of Pollution on Marine Organisms began over a half-century ago under the leadership of Dr. Donald J. Reish at California State University at Long Beach (Fulcher, 2016). Beginning in the 1950s Dr. Reish was an early leader of studies on the

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/WER.1218](https://doi.org/10.1002/WER.1218)

effects of, and recovery from, marine pollution and over many decades produced biology graduate students who themselves have led in the field of marine and aquatic pollution. Dr. Reish passed away during summer, 2018, 50 years after he first authored this annual review. He is greatly missed and we are proud to continue this review.

### Field Studies Using Biomarkers

**Algae.** Toniolo *et al.* (2018) examined the effects of the Costa Concordia wreck and recovery operations off of Italy on the seagrass *Posidonia oceanica*. Secondary metabolites, including catechin, chlorogenic acid and isoharmnetin-3-rutinoside, all decreased in leaves and rhizomes in proportion to the shading caused by both the wreck and the recovery platform. Mutagenicity was absent in tissue samples, however, leading the authors to believe that minimal absorption of contaminants occurred at the site. As the effects observed were primarily caused by shading, natural recovery of the seagrass was expected after removal of the wreck.

**Molluscs.** Mollusc studies included Breitwieser *et al.* (2018), who used a multiblock statistical analysis with a path modeling approach (Path ComDim) to determine the relationship between heavy metals and organic contaminants in tissues, biochemical assays in the gills and digestive glands, and genetic data in scallops (*Mimachlamys varia*) sampled at 13 sites off the French Atlantic coast. The strongest links were between organic contaminants and low genetic diversity, and between heavy metals and biochemical assays in the digestive gland. In the second linkage lipid peroxidation (LPO, oxidative lipid damage) and glutathione-S-transferase (GST, detoxification enzyme) activity were positively correlated with Cu, Se, As and Zn. Those four metals, along with pesticides and PCBs 28 and 77, were also negatively correlated with laccase activity (immune response). Biochemical assays in the gills did not have any strong links with other blocks in the analysis, possibly due to high background noise in the data. David *et al.* (2018) examined the potential effects of contamination in two estuaries in Brazil on the ability of an oyster (*Crassostrea rhizophorae*) to regulate water volume in tissues when exposed to varying salinities. Oysters from

three contaminated sites had an impaired ability to regulate water volume compared to those from a reference location, alongside an increase in multi-xenobiotic resistance phenotype (MXR) activity in gill tissue, indicative of exposure to pollutants. Diaz-Garduño *et al.* (2018) used a mobile laboratory to examine the effects of tertiary treatment of wastewater effluent with photobiotreatment (high rate algal pond) on survival and biomarker response in Manila clams (*Ruditapes philippinarum*). Seven-day survival in the highest concentration tested (50%) more than doubled after tertiary treatment, but there was little effect on lysosomal membrane stability (LMS, cellular stress biomarker). A biomarker index applied to other measured biomarkers (total lipid storage [energy reserves], mitochondrial electron transport [MET, energy transport], cyclooxygenase activity [inflammation], acetylcholinesterase activity [AChE, neurotoxicity]) indicated a lower level of response after tertiary treatment, driven mainly by MET. Laranjeiro *et al.* (2018) reported trends in imposex in dog whelks (*Nucella lapillus*) and netted whelks (*Nassarius reticulatus*) from 2000 to 2014 at 45 sites along the coast of Portugal. Tissue concentrations of tributyltin (TBT) compared to its degradation products, mono- and dibutyltin, in netted whelks in 2014 indicated that some tributyltin inputs were still present. However TBT in netted whelks and imposex markers in both species have decreased over time in almost all locations. The steepest decrease was in commercial ports versus the smaller fishing ports and marinas, which could be due to a higher frequency of dredging of contaminated sediment or better water exchange in the larger ports. One small fishing port had an increase in imposex and occurrence of sterile females over time, leading the authors to believe that TBT-based antifouling paints were possibly being used illegally on the vessels in the port.

**Crustaceans.** Cenov *et al.* (2018) measured metallothionein (MT, metal scavenger) content and metals in the digestive gland of Norway lobster (*Nephrops norvegicus*) from two locations off of Croatia. Positive correlations between MT and Cd, As and Hg concentrations were observed in larger lobsters, and negative correlations between MT and Mn, Cd, Cu and Zn

were observed in smaller lobsters. There was a negative correlation between reactive oxygen species concentration and MT in small lobsters, and negative correlations between lipids and metals in both sizes of lobsters. There were no consistent spatial or temporal trends.

**Fish.** Fish studies included an examination of parent polycyclic aromatic hydrocarbons (PAHs) in liver, PAH metabolites in bile, and ethoxyresorufin-o-deethylase (EROD) activity (response to bioavailable PAH exposure) in liver of yellowfin seabream (*Acanthopagrus latus*) collected off the Kuwait coast in the winter and summer of 2005-2006 and 2015 (Beg *et al.* 2018). There was little difference in liver PAH content or EROD activity between sampling sites or years, but the concentration of three and four ring metabolites in bile was higher in 2015. High molecular weight metabolites were more common in winter than summer for both sampling periods, and EROD activity was higher in winter during the 2015 sampling period. Aksem *et al.* (2018) reported the spatial and temporal trends of EROD activity and PAH metabolites in bile of dab (*Limanda limanda*), plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) collected from 61 sites off Great Britain between 2001 and 2015. Samples for AChE activity were also collected in 2014 and 2015. The majority of the sites were below the Background Assessment Criteria (BAC) under OSPAR's Hazardous Substances Strategy. EROD activity was significantly increasing at seven sites in male flatfish (five of which were above the BAC) and at six sites in female flatfish (three of which were above the BAC). Twenty sites had upward trends in PAH metabolites, eight of which were above the BAC but still below the upper-level Environmental Assessment Criteria (above which unacceptable effects may be occurring). Two regions, Clyde and Tees, were flagged as areas of potential concern. AChE activity varied 5-fold between sites, with the highest inhibition found at sites in the Eastern channel area, close to the coast.

**Sea Turtles.** Sea turtle studies included a sampling of blood from 100 nesting olive ridley turtles (*Lepidochelys olivacea*) from Oaxaca, Mexico for metals and biochemical parameters (Cortés-Gómez *et al.* 2018). Metals were found in the following relative concentrations:

Zn>Se>As>Sr>Cu>Mn>Cr>Cd>Ni>Ti>Pb. Zinc was negatively correlated with aspartate aminotransferase (AST), albumin and creatinine and positively correlated with P-nitrophenyl acetate esterase activity (EA). Selenium was correlated negatively with urea and EA and positively with creatinine and glucose. Arsenic was correlated negatively with glucose and cholesterol and positively with AST and urea. Strontium was positively correlated with alanine amino transferase (ALT), AST, urea and albumin. Copper was correlated negatively with ALT and positively with cholesterol. Chromium was negatively correlated with cholesterol. Cadmium was negatively correlated with creatinine and glucose. Titanium was positively correlated with creatinine, urea, cholesterol and cortisol, and lead was correlated negatively with EA and positively with AST and glucose. Casini *et al.* (2018) sampled 75 loggerhead turtles (*Caretta caretta*) in Italian rescue centers and off the Mediterranean coast of Spain. PCBs, DDTs, and carcinogenic and high molecular weight PAHs were higher in older turtles. No significant difference in Hg, Pb and Cd concentrations in the carapace was found between age classes. CYP1A (enzyme that bioactivates some carcinogenic organic compounds) content in skin biopsies was not correlated with organochlorines or grouped PAHs, but there were correlations with fluoranthene and pyrene individually. There was no difference in plasma LPO between age classes, but plasma butylcholinesterase (BChE, neurotoxicity) activity in juveniles and subadults was significantly inhibited compared to adults. Cadmium levels were correlated with gamma glutamyl transferase in plasma, a measure of kidney damage. Comet assay (DNA fragmentation, considered a potentially repairable form of DNA damage) results in erythrocytes were correlated with carcinogenic PAHs, and there was also a correlation between the comet assay and micronucleus formation (another measure of DNA damage, considered irreparable), though comet assay results were highest in juveniles, whereas micronuclei were higher in adults.

#### Laboratory Studies Using Biomarkers

**Metals.** Harayashiki *et al.* (2018) exposed yellowfin bream (*Acanthopagrus australis*) via diet to 0.7 to 6 mg/kg (actual) inorganic mercury for up to 16 days. In the liver, catalase (CAT, antioxidant enzyme) activity was higher in exposed fish after 8 days, and LPO levels increased after 16 days. In the muscle, GST activity was reduced after 4 days, but increased thereafter, while LPO levels were lower than in the control throughout most of the exposure period, especially in the lowest treatment. In the intestine, CAT activity was affected in fish exposed to the highest treatment, and GST activity was correlated with Hg in food. And in the gills, no correlation was observed between biomarkers and Hg exposure. There were no significant long-term effects on feeding behavior. Gallo (2018) was interested in the effects of toxicants on ion currents in tunicate (*Ciona intestinalis*) eggs and possible correlations with fertilization and development success. Sperm and eggs were exposed, both separately (for 30 minutes) and together (for 24 hours), to 0.04 to 1.1  $\mu\text{M}$  Pb, 7 to 550  $\mu\text{M}$  Zn, 1 to 10  $\mu\text{M}$  TBT, or 1 to 10  $\mu\text{M}$  of the herbicide phenylurea (all nominal concentrations). There was no effect on fertilization or development when the eggs and sperm were exposed separately to any of the toxicants, however when the eggs and sperm were exposed together zinc completely inhibited fertilization at all tested concentrations. TBT had a fertilization  $\text{EC}_{50}$  of 1.7  $\mu\text{M}$  and a development  $\text{EC}_{50}$  of 0.90  $\mu\text{M}$ , phenylurea had no effect on fertilization and a development  $\text{EC}_{50}$  of 2.8  $\mu\text{M}$  and Pb had no effect on either endpoint. All of the toxicants reduced the amplitude of  $\text{Na}^+$  currents in the eggs prior to fertilization. The fertilization current (FC, inward ion current occurring at the moment of fertilization) was absent in eggs exposed to Zn while TBT decreased the frequency of the FC in a dose-dependent manner, but Pb and phenylurea had no effect on FC frequency, leading the authors to believe that effects on the FC may be linked to decreased fertilization rates.

**Nanoparticles.** Studies with nanoparticles (NPs) included an examination of the effects of two  $\text{CeO}_2$  NPs with different physicochemical properties of on the hemolymph of mussels (*Mytilus galloprovincialis*, Sendra *et al.* 2018). Hemocytes were incubated *in vitro* to 1 to 50

mg/L (nominal) of the two NPs for up to 4 hours. The NP (CNP2) with a negative zeta potential, rounded shape and protein corona formation caused lower LMS, phagocytosis capacity (immune function biomarker) and extracellular reactive oxygen species (ROS) production than the NP (CNP1) with an almost neutral zeta potential, well-faceted shape and no protein corona. The protein corona formed around CNP2 in hemolymph serum appeared to be composed of a form of Cu,Zn-Superoxide dismutase (SOD, an antioxidant enzyme). Liu *et al.* (2018) exposed the red macroalgae *Gracilaria lemaneiformis* to 5 to 40 mg/L (nominal)  $\text{TiO}_2$  NPs for up to 15 days. The relative growth rate of the algae after 15 days decreased in a dose-dependent manner, along with the soluble protein content. Nitrate reductase activity decreased after 3 days, and at 15 days it was highly inhibited at all concentrations, indicating a negative effect on the algae's ability to absorb nitrogen. Superoxide radical levels increased in algae exposed to all concentrations after 15 days, while the antioxidant capacity, as measured by both DPPH radical and hydroxyl radical scavenging activity, decreased, indicating potential oxidative damage was occurring. Huang *et al.* (2018) examined the effects of salinity on the toxicity of  $\text{TiO}_2$  NPs in juvenile olive flounder (*Paralichthys olivaceus*). The fish were exposed to 1 to 10 mg/L (nominal) NPs in 10 and 30 psu seawater for 4 days, measuring effects on CAT, SOD, and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  (necessary for osmoregulation) activity, LPO, and gill histology. An integrated biomarker response index indicated that at 10 psu treatment with  $\text{TiO}_2$  NPs caused an overall decrease in biomarker levels, while at 30 psu the treatment increased biomarker levels, which may have been due in part to an increase in NP agglomerates observed at 30 psu. Histological abnormalities were observed in the gills of treated fish at both salinities, but were more marked at 30 psu. To determine the combined effects of exposure to endocrine disrupting compounds and NPs, Shi *et al.* (2018) exposed blood clams (*Tegillarca granosa*) for 10 days to 99 to 199 ng/L (actual)  $17\beta$ -estradiol (E2) and 0.05 to 0.1 mg/L (nominal)  $\text{TiO}_2$ , both alone and in combination. Total hemocyte count was reduced and there was a higher percentage of basophil granulocytes when clams were

exposed to 199 ng/L E2 or the coexposures (99 ng/L E2 plus either 0.05 or 0.1 mg/L TiO<sub>2</sub>), but not to 99 ng/L E2 or TiO<sub>2</sub> alone. Phagocytosis was reduced by all exposures, but the reduction was greatest in the coexposures and 199 ng/L E2. Production of Vtg was increased by E2 exposure, and coexposure with 0.1 mg/L TiO<sub>2</sub> caused a higher increase in production, though exposure to TiO<sub>2</sub> alone had no effect. Expression of immune-related genes was repressed in TiO<sub>2</sub> and 199 ng/L E2 exposures, with coexposure causing further repression, especially for expression of *TRAF3* and *NFκβ*. In all, the study demonstrated enhanced toxicity of E2 in the presence of TiO<sub>2</sub>.

**Organic Contaminants.** Abreu *et al.* (2018) exposed crabs (*Carcinus maenas*) to the industrial chemicals acrylonitrile (100 and 1000 µg/L actual) and aniline (5 and 50 µg/L actual) for up to 21 days, measuring a range of biomarkers in several tissues to develop an integrated biomarker response fingerprint for each compound. Acrylonitrile appeared to cause neurotoxic effects in muscle coupled with enhanced biotransformation and significant oxidative damage, particularly in the gills. Aniline appeared to cause neurotoxic effects in the ganglion, affect aerobic and anaerobic metabolism in muscle and also cause low levels of oxidative damage in the gills. The effects of both chemicals were time and dose-dependent. Li *et al.* (2018) developed enzyme-linked immunosorbent assays (ELISAs) to measure Vtg in marbled flounder (*Pseudopleuronectes yokohamae*), black rockfish (*Sebastes schlegelii*) and fat greenling (*Hexagrammos otakii*). There was good cross-reactivity between marbled flounder and black rockfish Vtg, but not fat greenling. The ELISA was good at detecting dose-dependent increases in Vtg levels upon exposure of marbled flounder to 0.1 to 10 mg/L pentadecafluorooctanoic acid (PFOA, nominal) for 14 days. Marigoudar *et al.* (2018) exposed sea bass (*Lates calcarifer*) to 0.04 to 0.56 µg/L (actual) of the insecticide chlorpyrifos for 30 days, resulting in a survival LOEC of 0.08 to 0.09 µg/L. There were varying effects on esterases depending upon the isoform. Isoform 1 was inhibited at all concentrations, indicating neurotoxicity; there was no effect on isoform 2 and isoform 3 was slightly inhibited.

Activities of SOD and malate dehydrogenase (energy production enzyme) were also inhibited. Histology indicated low to moderate damage in the gills and pathological lesions in the eyes.

**Organics and Microplastics.** Magara *et al.* (2018) determined the effects of polyethylene microplastic (MP) beads on the uptake and effects of the PAH fluoranthene (Flu) in the blue mussel (*Mytilus edulis*). Mussels were exposed for 96 hours to 50 or 100 µg/L Flu (nominal) and 100 or 1000 MP/mL in the following treatments: 1) Flu only; 2) MP only; 3) Flu and MP coexposure; 4) Flu allowed to adsorb onto MP prior to testing. Coexposure with MP did not affect Flu concentrations in gill or digestive gland, but pre-test adsorption significantly decreased tissue concentrations of Flu compared to the Flu-only exposure. Biomarker responses indicated the following trends: 1) antioxidant responses were more likely in the gill than in the digestive gland; 2) both contaminants caused effects, including decreased glutathione (ROS scavenger) content and increased glutathione peroxidase activity (GPx, antioxidant enzyme) in the gill and decreased SOD and CAT activity and increased selenium-dependent GPx (SeGPx) activity in the digestive gland for Flu and increased CAT, GPx and SeGPx activity and decreased glutathione content in the gill for MP, but the effects were not additive or synergistic; 3) exposure concentrations were not a consistent predictor of effect; and 4) the internal Flu dose was not a consistent predictor of effect. O'Donovan *et al.* (2018) exposed peppery furrow shell clams (*Scrobicularia plana*) for up to 14 days to 1 mg/L of low density polyethylene MP that was either unaltered, with 16.87 µg/g (actual) benzo(a)pyrene (BaP) adsorbed to it, or with 70.22 µg/g (actual) perfluorooctane sulfonic acid (PFOS) adsorbed to it. Unaltered MP exposure was linked to an increase in CAT and GST activities and LPO in the gill, whereas in the digestive gland it was linked to a decrease in SOD, GPx and CAT activities and LPO, as well as a decrease in olive tail moment (Comet Assay DNA damage endpoint) in hemocytes. A somewhat different set of responses was seen in clams exposed to BaP-adsorbed MP, with an increase in tail length (Comet Assay) in hemocytes, an increase in

SOD, AChE, and GST activities and LPO in the gill, and a decrease in GPx activity and an increase in LPO in the digestive gland. Relative to the other two exposures PFOS-adsorbed MPs were linked to relatively few effects, including an increase in GPx activity and LPO and a decrease in GST activity in the gill only. All of the effects observed were time-dependent.

**Polycyclic Aromatic Hydrocarbons.** Santana *et al.* (2018) performed a literature review and meta-analysis of the effects of PAHs on biomarkers in fish livers to shed light on the following questions: 1) what is the overall effect of PAHs on biotransformation and oxidative stress biomarkers; 2) do the effects of PAHs vary among experimental approaches, environments, habitats and exposure routes; 3) do time and dose dictate PAH effects; 4) which biomarkers are most responsive? Overall, EROD, GST, SOD, GPx, oxidized glutathione and LPO increased in fish exposed to PAHs, while CAT, glutathione reductase (GR, recycles oxidized glutathione into the ROS-scavenger reduced glutathione), and reduced glutathione were not altered. EROD activity was generally higher in laboratory vs. field exposures, in freshwater fish, and increased with PAH concentration and time of exposure. GST activity was higher in field and caged-fish vs. laboratory exposures, in freshwater fish, in sediment exposures vs. other laboratory exposures, and increased with concentration and time. GPx was also higher in sediment exposures and increased with concentration and time. SOD activity and LPO both decreased with time at a given concentration, and oxidized glutathione was higher in laboratory exposures and increased with concentration. The authors indicated that EROD is the best specific biomarker for PAH exposure, and GST, GPx and LPO, though less specific, may also be good biomarkers. Mechanistic Target of Rapamycin (mTOR) is a serine/threonine protein kinase that senses and integrates a variety of cellular physiological and environmental signals to regulate eukaryotic cell growth. The phosphorylated active form of mTOR activates protein translation and inhibits autophagic activity. Sforzini *et al.* (2018) investigated the possible mechanistic role of mTOR in the effects of BaP on lysosomal autophagy and function in digestive gland cells of the mussel *Mytilus*

*galloprovincialis*, exposing them for 3 days to 5 to 100 µg/L BaP (nominal). Immunolabelling indicated that BaP accumulated inside lysosomes, and BaP also stimulated lysosomal fatty acid accumulation. Lysosomal membrane stability decreased in a dose-dependent manner, and the lysosomal/cytoplasmic (L/C) volume ratio increased, indicating that autophagy was highly stimulated and the catabolism occurring was not compensated by new protein synthesis. The level of the phosphorylated form of mTOR decreased even though the total concentration of the enzyme in the cytoplasm increased, indicating an inactivation of mTOR that may have led to the increased autophagy observed. Similar effects on LMS, L/C volume ratio and mTOR phosphorylation were observed in mussels caged for 28 days in an industrial and commercial port in Italy compared to a reference location.

Braga *et al.* (2018) exposed the polychaete *Laeonereis culveri* and the clam *Anomalocardia flexuosa* to the water-soluble fraction of diesel oil (0.26 to 71.0 µg/L ΣPAHs, actual, at start of exposure) for up to 7 days. The frequency of micronuclei and GST activity increased significantly in both species after 7 days, so were determined to be the best biomarkers of diesel oil exposure in the two species. Activity of SOD (both species) and CAT (clams) only increased non-significantly at the beginning of the exposure, and LPO results were inconclusive. Tarrant *et al.* (2018) exposed infaunal starlet sea anemones (*Nematostella vectensis*) for 24 hours to nominal concentrations of 2 ppm Corexit 9500 dispersant (DISP), 20 ppm water-accommodated fraction (WAF) of Macondo sweet crude oil, or a combination of the two (CEWAF), and all of the exposures were performed both with and without exposure to UV light for the first 6 hours of the exposure. Without UV-exposure, DISP upregulated 6 transcripts and downregulated 85, WAF upregulated 9 and downregulated 16, and CEWAF upregulated 2 and downregulated 9. Addition of UV dramatically increased the number of differentially-expressed transcripts, with UV alone upregulating 434 and downregulating 171, DISP + UV upregulating 480 and downregulating 221, WAF + UV upregulating 541 and downregulating 248, and CEWAF + UV upregulating 592 and downregulating 278. Phase I and

II metabolic genes were not upregulated in response to DISP, WAF or CEWAF, however they were induced by the addition of UV exposure, along with genes involved in DNA repair, production of UV-protective compounds and novel antioxidants. To determine the effects of oil and dispersant on mussels in Arctic/Subarctic conditions, Counihan (2018) exposed *Mytilus trossulus* at 4°C for up to 21 days to the WAF of 10 ppm Alaska North Slope crude oil (total petroleum hydrocarbons = 4237 µg/L actual), 1 ppm Corexit 9500 dispersant, or the WAF combined with either 2 ppm, 1 ppm or 0.5 ppm dispersant. Concentrations were allowed to decline over time to simulate real-world oil spill conditions. The majority of the physiological impacts caused by the treatments occurred during the first 7 days, including elevated P450 activity (biotransformation enzymes), SOD activity, heat shock protein levels (HSP, molecular chaperones that help stabilize proteins during stress), and the effects were similar in all of the treatments. However, mussels exposed to WAF also had higher mortality and immune suppression, and DISP and CEWAF had higher RNA:DNA ratios. After 21 days, mussels in all treatments exhibited evidence of genetic damage, tissue loss and continued stress response. Osse *et al.* (2018) also looked into oil effects in Arctic/Subarctic environments, exposing sea stars (*Asterias rubens*) brittle stars (*Ophiopholis aculeata*), sea urchins (*Strongylocentrotus droebachiensis*), and sea cucumbers (*Cucumaria frondosa*) at 6°C for 96 hours to the WAF of used lubricating oil (0.22 to 0.33 mL/L nominal). Exposures were timed to coincide with pre-spawning, spawning, and post-spawning periods, and GPx and EROD activity were measured in various tissues including gonads, intestines, stomach, pyloric caecum and respiratory tree, depending upon the species. Response to the WAF exposure varied by sex, season, species and tissue type. For example, in sea stars stomachs, exposure to the WAF elicited a higher GPx activity in males than in females. The gonads of male sea urchins exposed to WAF displayed a decrease in EROD activity, but the gonads of female urchins were more influenced by season than WAF exposure. Overall it was determined that GPx activity was a better biomarker for

WAF exposure than EROD activity, particularly in the sea star.

#### **Pharmaceuticals and Personal Care Products.**

Pusceddu *et al.* (2018) exposed invertebrates to sediment spiked with either the anti-bacterial triclosan or the anti-inflammatory ibuprofen via elutriate and sediment-water interface (SWI, both the mussel *Perna perna* and the sea urchin *Lytechinus variegatus*) or whole sediment exposure (the mussel *Mytella charruana*). The LOECs for urchin embryo development were 75 ng/g (actual) triclosan and 15 ng/g (actual) ibuprofen, regardless of whether they were exposed via elutriate or SWI, whereas *P. perna* had the same LOECs as the urchins in the SWI exposure, but the elutriate exposure was less toxic, with LOECs of 750.2 ng/g triclosan and 150.8 ng/g ibuprofen. The LOECs for LMS in *M. charruana* exposed to whole sediment for 24 hours were 0.08 ng/g triclosan and 0.15 ng/g ibuprofen, much lower than the LOECs above and below the concentrations that were measured in sediment at an outfall in Santos Bay, Brazil (15.14 ng/g triclosan and 49.0 ng/g ibuprofen). The antihypertensive losartan produced EC<sub>50</sub>s of 219.2 mg/L (nominal) for fertilization and 84.6 mg/L for embryo development in *P. perna* (Cortez *et al.* 2018). A 96-hour exposure of adult mussels to 277 to 2811 ng/L (actual) losartan induced dibenzylfluorescein dealkylase activity in the gills, which is indicative of P450 CYP2 activity, one of the enzyme families responsible for metabolism of losartan in humans. The activities of GST, GPx and ChE along with DNA damage were also induced in the gills, but EROD activity was inhibited in the digestive gland and LMS was reduced in hemocytes. Concentrations of losartan measured in Santos Bay, Brazil ranged from 0.60 to 8.70 ng/L, lower than the observed effects levels. Barreto *et al.* (2018) exposed juvenile gilthead seabream (*Sparus aurata*) for 96 hours to 1.5 to 15,000 µg/L (nominal) gemfibrozil, a lipid regulator. The activities of CAT and GR increased in the gills and GPx and GR activity increased in the liver. Lipid peroxidation in the liver was increased at the lowest dose only, whereas it was reduced in the gill at intermediate concentrations. The ability to swim against water flow was significantly reduced at all tested concentrations, making it the most

sensitive endpoint measured. Araújo *et al.* (2018) exposed the sole *Solea senegalensis* to the sunscreen agent 4-methylbenzylidene camphor from eggs through hatch to 96-hours post fertilization. The LC<sub>50</sub> was 0.439 mg/L (actual), and the EC<sub>50</sub> for malformation was 0.372 mg/L. Effects on growth and swimming time were also observed, with swimming time being the most sensitive endpoint (NOEC <0.068 mg/L). Lactate dehydrogenase activity (measures anaerobic metabolism) was reduced from 0.025 to 0.051 mg/L, and at 0.085 mg/L AChE activity increased, but there was no effect on CAT activity or LPO.

### Biomarkers of Climate Change

Akbarzadeh *et al.* (2018) set out to identify genes that could act as thermal stress biomarkers in the gills of salmonids. By analyzing data from previous microarray studies followed by deep literature mining they identified 39 candidate genes related to 24 proteins. The 32 genes with the best polymerase chain reaction (PCR) efficiencies were tested by analyzing gill tissue from adult sockeye salmon (*Oncorhynchus nerka*) and juvenile Chinook salmon (*O. tshawytscha*) thermally stressed ( $\Delta+3-6^{\circ}\text{C}$ ) for 5 to 7 days. Eight genes were ultimately identified to be used in concert as biomarkers of thermal stress: *SERPINH1* (on chromosome [ch] 9), *SERPINH1* (ch20), *HSP90AA1* (ch6), *HSP90AA1* (ch15), *FKBP10* (ch3&6), *EEF2* (ch14), *MAP3K14* and *SFRS2*. If testing thermal stress in moribund fish, *MAP3K14* and *SFRS2* should be replaced with *HSP70* (ch6) and *NEK4* (ch12). The biomarker genes are involved in chaperoning and protein rescue, protein biosynthesis and oxidative stress. Maulvault *et al.* (2018) examined the possible effects of climate change on the toxicity of the anti-inflammatory drug diclofenac in juvenile seabass (*Dicentrarchus labrax*). The fish were fed an equivalent of 2% body weight of feed spiked with 500 ng/kg (actual, dry weight) diclofenac for 28 days, and were exposed to a temperature increase of 5°C and a pH decrease of 0.4 ( $\Delta\text{pCO}_2 \sim 1000 \mu\text{atm}$ ), all three exposures performed both alone and in combination with one or both of the other factors. Biomarkers of animal condition, hematological parameters, genotoxicity, oxidative stress, heat shock

response, protein degradation, endocrine disruption and neurotoxicity from brain, liver, muscle and blood were combined into an integrated biomarker response index to measure overall effects. Results indicated that diclofenac induced more severe stress when fish were co-exposed with warming, compared to diclofenac alone or combined with acidification. Nardi *et al.* (2018) aimed to examine the effects of climate change on cadmium uptake and toxicity in mussels (*Mytilus galloprovincialis*), and also to determine if seasonality played a role in potential climate change effects. Mussels were exposed for four weeks to 20  $\mu\text{g/L}$  Cd (nominal), a temperature increase of 5°C (from 10 to 15°C in winter, from 20 to 25°C in summer) and a pH decrease of 1.0 ( $\Delta\text{pCO}_2 \sim 2600 \mu\text{atm}$ ), all three exposures performed both alone and in combination with one or both of the other factors. Hazard quotients were calculated from results of multiple biomarkers measuring metal detoxification, oxyradical metabolism and cellular toxicity in the digestive gland, gills and hemocytes. Cadmium accumulation increased with an increase in temperature in the winter but not the summer, and was unaffected by acidification. There were clearer synergistic effects of Cd and warming and/or acidification on biomarkers in the summer, however. Hazard quotients calculated from winter data indicated that the digestive gland was more sensitive to variations in Cd than acidification or warming. Gills had higher overall hazard quotients in all exposures, with warming and Cd + warming producing the highest values. Carbon capture and storage (CCS), in which CO<sub>2</sub> is separated from industrial and energy-related sources and transported to offshore geological formations, is considered by some to be one of the best choices for atmospheric CO<sub>2</sub> reduction. However there is some concern about the effects of CO<sub>2</sub> leakage from these sites on the marine environment. Passarelli *et al.* (2018) examined the potential effects of acidification caused by CO<sub>2</sub> leakage from CCS locations on mussels (*M. galloprovincialis*) exposed to sediments containing metals. Adult mussels were exposed for up to 10 days to whole sediments from a location with moderate metal contamination and a reference location, with the pH of the overlying water adjusted from a pH of 8.0 to a pH range of 7.5 to 5.5 by adding CO<sub>2</sub>. In mussels exposed



contaminated sediment the LC<sub>50</sub> was a pH of 7.22, as opposed an LC<sub>50</sub> of pH 6.4 in the reference sediment. There were also longer-term effects on LMS at a pH of 7.0 in mussels exposed to contaminated sediment. Dissolved Zn in the overlying water was strongly correlated with pCO<sub>2</sub>. Similar results were obtained from mussel embryos exposed to acidified elutriates for 2 days, with significant effects on development observed at a pH of 7.5 in the contaminated elutriate versus a pH of 6.5 in the reference elutriate, though there was little difference observed between the two elutriates at pH 8.0. Dissolved Al and Cu were strongly correlated with pCO<sub>2</sub> in the elutriate.

### Effects of Dredging, Dumping, and Extraction

Donazar-Aramendia *et al.* (2018) reported that the benthic community in a recurrent marine disposal area in southwestern Spain had greater diversity and species richness than nearby control sites, with no significant differences in food web structure between the sites based on isotopic analysis; the authors found that univariate indices (i.e., diversity and richness) better reflected the changes driven by the historical disposal operations over time than the AZTI Marine Biotic Index (AMBI) and Benthic Families Ecological Status Index (BENFES).

Exposure of two soft corals, cauliflower coral (*Duva florida*) and red tree coral (*Primnoa resedaeformis*), to mine tailings in the laboratory resulted in significantly decreased food intake in *D. florida* and significantly increased food intake in *P. resedaeformis*; while histological analysis revealed mine tailing particles <10 µm embedded in the tissues of both species (Liefmann *et al.*, 2018). High concentrations of small sized (9.3 µm) suspended sediment in a low food availability environment was found to have the greatest negative impact on ingestion rate, egg production, and mortality of the copepod (*Acartia tonsa*), though these effects were reduced with increased food availability (Sew *et al.*, 2018).

Mesocosm studies that exposed intact macrobenthic communities to three different types of mine tailings found that all tailings negatively impacted the benthic community at thin layers (< 2 cm) of deposition,

with the strongest response observed for fine-grained tailings combined with flotation chemicals and the weakest response observed for tailings combined with flocculation chemicals (Trannum *et al.*, 2018). Mestdagh *et al.* (2018) reported that increasing thickness of deposited sediment layers produced a shift in intertidal microbenthic communities to lower densities of low motility and surface-dwelling animals, with decreased ecosystem functioning as more motile and deeper-living fauna could not sustain oxygen penetration through increasing thickness of the deposited layer.

### Effects of Wastewater Discharges

Berasategui *et al.* (2018) investigated the effects of different fractions of a sewage plume on the fecundity and survival of the calanoid copepod (*Eurytemora americana*); the authors found the water taken from 0.5 m above the sediment bed at the discharge point was lethal to all tested copepods, while exposure to waters collected from the surface of the sewage plume and 1.5-2 m below the plume's surface caused no copepod mortality but did significantly reduce egg production, number of nauplii, and number of fecal pellets compared to unexposed copepods.

Significant oxidative stress, as measured by a suite of biomarkers, was reported in amphipods (*Gammarus pulex*) following exposure to sewage effluent from Tunceli municipal wastewater treatment plant in Turkey (Tatar *et al.*, 2018). Using a suite of biomarkers of exposure, antioxidant response, and effects, Díaz-Garduño *et al.* (2018a) reported that urban wastewater from Chiclana de la Frontera, province of Cádiz, in Southern Spain produced oxidative stress levels in exposed flat fish (*Solea senegalensis*) as reflected by increases in Phase I metabolism proteins measured by ethoxyresorufin-O-deethylase (EROD) and dibenzylfluorescein (DBF) assays as well as increased glutathione reductase antioxidant enzymes, all of which were generally reduced after photobiotreatment of the effluent. Similarly, sublethal effects, including lysosomal membrane stability alterations, changes in energy status storage, changes in mitochondrial electron transport, inhibition of inflammatory mechanisms, and neurotoxic effects, to Manila clam (*Ruditapes*

*philippinarum*) exposed to urban wastewater from Chiclana de la Frontera were reduced after photobiotreatment of the effluent (Díaz-Garduño *et al.*, 2018b).

### Effects of Fishing and Aquaculture

No significant differences were found in the physico-chemical parameters of Spanish fish farm effluents (with the exception of turbidity) from those of the input or control water, and bioassays with various dilutions of fish farm effluents found little toxicity to three species (*Vibrio fischeri*, *Isochrysis galbana*, and *Paracentrotus lividus*), representing different aquatic trophic levels (Carballeira *et al.*, 2018).

Electric pulses used to catch brown shrimp (*Crangon crangon*) were found to have no significant impact on the survival and development of Sole (*Solea solea*) embryos and larvae in laboratory studies of Sole embryos at 2 days postfertilization and larvae at 11 days posthatching (Desender *et al.*, 2018).

Callier *et al.* (2018) reviewed the literature describing the primary mechanisms and effects of attraction and repulsion of wild animals to or from aquaculture operations, including effects related to the provision of physical structure, effects related to the provision of food, and effects from certain farm activities; the authors found a variety of direct and indirect effects on wild organisms at the level of individuals and populations, with considerable uncertainty regarding the long-term and ecosystem-wide consequences of these interactions. Comparison of AMBI and BENTIX biotic indices computed for a study of two aquaculture farms in a coastal transitional area of Tuscany, Italy, produced generally similar results; however, the health status at one farm was deemed to be better within the farm than at the reference site, while the opposite was found at the other farm (Forchino *et al.*, 2018). Exposure of white sea urchin (*Gracilechinus acutus*) to aquafeed diets in the laboratory produced adverse effects to egg fertilization and larval survival, though white sea urchin were found to be 10 times more abundant at aquaculture sites in western Norway than at control sites (White *et al.*, 2018).

Commercial fish species caught within a major fishing ground in the northeastern Mediterranean Sea were reported to exhibit higher biomass in areas with intermediate and low fishing pressure, as well as increasing maximum and mean total length with decreasing fishing effort (Dimarchopoulou *et al.*, 2018). Investigated the effects of bottom trawling and eutrophication on benthic species, Josefson *et al.* (2018) compared records from the 1880s with more recent data collected from the Kattegat off the coast of Sweden and reported that the depth distribution structure of benthic invertebrates in the eastern Kattegat has changed substantially since the 1880s, with species most affected by intensive trawling having changed maximal depth limits above and away from trawling areas. Another study in the Kattegat found that chronic bottom trawling reduced diversity of soft sediment macrofauna and may boost the abundances of species, such as the brittle star *Amphiura chiajei*, that are resistant to bottom trawling and benefit from reduced predation by target fishery species (Skold *et al.*, 2018).

Applying a functional group analysis to data from field surveys of benthic communities from two large, widely separated areas in New Zealand's EEZ (Chatham Rise and Challenger Plateau), Lundquist *et al.* (2018) found differences in functional responses and reductions in functional groups at different levels of fishing effort, which the authors suggest indicates increased functional homogenization with increased disturbance because less tolerant functional groups are excluded and functional diversity is reduced.

Dureuil *et al.* (2018) found that commercial trawl fishing effort within marine protected areas (MPAs) in the European Union was 38% higher than trawling effort in unprotected areas and occurred in 59% of European MPAs; evaluating the potential impact to a sensitive indicator species group, the authors reported that elasmobranch catch per unit effort from research surveys was 2.3-fold higher outside MPAs than within MPAs, with endangered and critically endangered species  $\geq 5$ -fold more abundant outside MPAs. Investigating the condition of horse mussel (*Modiolus modiolus*) beds historically subjected to intense scallop fishing seven years after the introduction of

legislation banning all forms of fishing in Strangford Lough, Northern Ireland, Farinas-Franco *et al.* (2018) reported lower biodiversity, particularly for sponges, hydroids, and tunicates, and up to an 80% decline in densities of *M. modiolus* across the current distributional range of the species, leading the authors to conclude that protection alone is insufficient to support natural recovery of biogenic reefs that are already substantially degraded.

Using fuzzy correspondence analysis of six biological traits spanning 32 modalities, Howarth *et al.* (2018) evaluated how primary production and trawl fishing affect the functioning of benthic ecosystems by comparing the functional composition, diversity, and evenness of benthic communities in the English Channel and in the Celtic and Irish Seas, across interacting gradients of bottom trawling activity and primary production; the authors found that bottom trawling had negative effects on the functional diversity, evenness, and biomass of 12 of the 32 modalities, with greater effects in areas of high primary production. Kaplan *et al.* (2018) reported that the invasive tunicate *Didemnum vexillum* has a greater impact on biodiversity decline among macroinvertebrates in the epibenthic environment of Georges Bank than disturbance and extraction from bottom-fishing. The density of horseshoe crabs on the southwestern coast of Deep Bay, Hong Kong were found to be significantly reduced in bottom-laid low-density and high-density simulated oyster cultch compared with the adjacent bare flats (Kwan *et al.*, 2018). Quantitative analysis of Remote Operated Vehicle (ROV) video transects compared with the recent patterns of trawling intensity over the soft-bottom margins of the Gioia Canyon (Southeastern Tyrrhenian Sea) revealed that vulnerability of megabenthic species that are indicative of Vulnerable Marine Ecosystems (VMEs) differs among species, as the bamboo coral *Isidella elongate* showed a clear negative relationship with trawling intensity, while results for the sea pen *Funiculina quadrangularis* were less straightforward (Pierdomenico *et al.*, 2018).

## Effects of Light Pollution

Satellite Visible Infrared Imaging Radiometer Suite (VIIRS) nighttime annual average radiance composite image data were used to estimate light pollution along Florida beaches, which was then incorporated into generalized linear models with 2012-2016 nest density data from the Florida Statewide Nesting Beach Survey program for green turtles (*Chelonia mydas*), loggerheads (*C. caretta*), and leatherbacks (*Dermochelys coriacea*) to demonstrate that nest density is significantly negatively correlated with light pollution for each sea turtle species, with the greatest negative association for green turtles, followed by loggerheads and, lastly, leatherbacks (Hu *et al.*, 2018). Price *et al.* (2018) found similar results for loggerhead sea turtles (*C. caretta*) nesting on a Florida barrier island in the northern Gulf of Mexico; increased luminance of landward light was significantly associated with a decreased number of nests and increased disorientation of hatchlings. Sea turtle nesting on barrier islands in North Carolina was also found to be more concentrated in areas with low light pollution (Windle *et al.*, 2018).

Assessing multiple metrics (e.g., fan spread of hatchling tracks; offset angle from a direct line to the sea; and the number and direction of stray tracks), Dimitriadis *et al.* (2018) found a statistically significant relationship between the level of artificial nighttime lighting at a beach and hatchling sea-finding orientation for loggerhead sea turtles (*C. caretta*) in the Mediterranean Sea. Cruz *et al.* (2018) investigated whether exposure to artificial light at different colors and intensities could affect the in-water swimming direction of hatchling olive ridley sea turtles (*Lepidochelys olivacea*) collected from Parque Nacional Marino Las Baulas on the Pacific coast of Costa Rica; the authors found hatchlings were attracted to yellow and green light at low intensities (>9 lx and > 5 lx, respectively) and red light at high intensities (> 39 lx), which caused initial swimming misorientation in experimental exposures to yellow and green light. Using acoustic telemetry to track the movement of flatback turtle (*Natator depressus*) hatchlings dispersing through nearshore waters, Wilson *et*

*al.* (2018) found that hatchlings spent 50% longer in the nearshore area in the presence of high pressure sodium vapour (amber in color) light and 150% longer in the area in the presence of metal halide (white in color) light.

Increased growth of microphytobenthos, stimulated by artificial light at night, was found to be tempered on an Italian rocky coast by subsequent increased grazing by a small littorinid snail (*Melarhaphé neritoides*), which Maggi and Benedetti-Cecchi (2018) suggest demonstrates that trophic interactions can provide a stabilizing mechanism to counteract foodweb effects resulting from artificial light at night. Longcore *et al.* (2018) developed a model to assess predicted responses of four different organisms to lighting of different spectral output; using indices based on action spectra from behavioral or visual characteristics of organisms and lamp spectral irradiance, the authors found filtered yellow-green and amber light-emitting diodes (LEDs) to have lower predicted effects on wildlife than high pressure sodium lamps, while blue-rich lighting would have greater effects.

### Effects of Noise Pollution

The soundscape of plainfin midshipman (*Porichthys notatus*) at two locations around Vancouver Island, Canada was found to be dominated by their hum at night, with possible anthropogenic noise more prevalent during the day and overlapping with plainfin midshipman vocalizations in 28% of recordings (Halliday *et al.*, 2018). Significant effects on acoustic and visual courtship behavior (males) and spawning behavior (females) were reported from continuous noise exposure of the painted goby (*Pomatoschistus pictus*) in aquarium experiments (de Jong *et al.*, 2018). Ferrari *et al.* (2018) conducted laboratory and field experiments to examine the effect of boat noise on the ability of juvenile Ambon damselfish (*Pomacentrus amboinensis*) to learn to recognize a novel predator; the authors found that the presence of boat noise at the time of learning reduced subsequent antipredator response and survival compared to fish that were trained with exposure to reef noise alone. Exposure of staghorn damselfish (*Amblyglyphidodon curacao*) embryos in

shallow reef conditions to noise from either a 2-stroke or 4-stroke power boat engine resulted in statistically significant increases in the heart rate of the embryos, with a greater effect observed for exposure to noise from the 2-stroke engine (Jain-Schlaepfer *et al.*, 2018). Australian snapper (*Pagrus auratus*) demonstrated different behavioral responses to boat noise exposure depending on whether the snapper were exposed within or outside of a marine protected area (Mensing *et al.*, 2018).

McCormick *et al.* (2018) investigated the interactive effects of elevated CO<sub>2</sub> and boat noise on the kinematics of predator-prey interactions between the predator dottyback (*Pseudochromis fuscus*) and prey juvenile damselfish (*Pomacentrus wardi*); the authors found elevated boat noise had a similar effect on the kinematics of the predator-prey response as elevated CO<sub>2</sub> conditions when tested alone, and there was no evidence of an interactive effect between stressors when tested together.

No impacts to commercial catch rates of snow crab (*Chionoecetes opilio*) were detected following seismic explorations in the western north Atlantic Ocean (Morris *et al.*, 2018). Similarly, no evidence of scallop (*Pecten fumatus*) mortality was detected following a 2015 marine seismic survey in the Gippsland Basin, Australia (Przeslawski *et al.*, 2018).

Cholewiak *et al.* (2018) investigated potential communication masking of four species of baleen whales using an agent-based modeling framework to calculate changes in communication space in relation to different types of vessel noise in the Stellwagen Bank National Marine Sanctuary; the authors found that “gunshot” communications from North Atlantic right whales (*Eubalaena glacialis*) experienced the least amount of masking, while fin (*Balaenoptera physalus*), humpback (*Megaptera novaeangliae*) and minke (*B. acutorostrata*) whale signals experienced masking levels of 80% or more. Atlantic bottlenose dolphins (*Tursiops truncatus*) in the northwest Atlantic Ocean were found to produce less complex calls during increased ambient noise in the 2.5 and 20 kHz one-third octave band levels, suggesting that the signaller responded by simplifying the call as opposed to the received call losing components through masking

(Fouda *et al.*, 2018). Combining long-term acoustic monitoring data with AIS vessel-tracking data and acoustic propagation modelling, Putland *et al.* (2018) reported that routine vessel passages in the Hauraki Gulf of north-eastern New Zealand decreased communication space by up to 61.5% for nocturnal bigeye fish (*Pempheris adspersa*) and 87.4% for Bryde's whales (*Balaenoptera edeni*), with communication space being reduced by up to 99% when the vessel passed within 10 km of an experimental listening station.

Branstetter *et al.* (2018) explored the effects of vibratory pile driver noise on the vigilant capabilities of Atlantic bottlenose dolphins (*T. truncatus*) using five trained dolphins in an experimental design that recorded each dolphin's ability to echolocate, detect, and report targets during exposure to different levels of recorded pile driver noise; the authors found large differences between individual dolphins, with two dolphins unaffected by any level of pile driver noise and three initially affected at the highest exposure level (140 dB re 1  $\mu$ Pa) and then rapidly acclimating on subsequent exposures. Acoustic monitoring data around seven large-scale offshore wind farm projects in Germany demonstrated a clear gradient in the decline of harbor porpoise (*Phocoena phocoena*) detections after pile driving operations, with declines found at sound levels exceeding 143 dB re 1  $\mu$ Pa<sup>2</sup>s for up to 17 km from the piling operation with no noise mitigation system in place and up to 14 km at locations with noise mitigation systems in operation (Brandt *et al.*, 2018). While the abundance of harbor seals (*Phoca vitulina*) in a narrow channel on the west coast of Scotland was not found to be affected by acoustic playbacks of tidal turbine noise, Hastie *et al.* (2018) found that the seals significantly increased their distance from the playback location during turbine playbacks. Kastelein *et al.* (2018) investigated whether exposure to broadband pile driving sounds produced temporary hearing threshold shifts (TTS) in two female harbor seals (*Phoca vitulina*) and found that TTS were small, only occurred after 360 minute exposures, and recovered within one hour after exposure ceased.

Conducting an experiment on the effect of broadband noise on foraging behavior of shore crab

(*Carcinus maenas*) and common shrimp (*Crangon crangon*) in a quiet cove in the Oosterschelde estuary in The Netherlands, Hubert *et al.* (2018) found that the number of crabs that gathered around a food source decreased relative to controls in the presence of noise, while the number of shrimp increased in the presence of noise, which was negatively correlated with the number of crabs present. Cox *et al.* (2018) conducted a meta-analysis of 42 field and laboratory studies from 11 countries on the impacts of noise pollution to fish behavior and physiology and found that anthropogenic sound had statistically significant negative effects on both fish behavior and physiology, which are not limited to specific responses or species.

### **Effects of Offshore Wind Energy Development**

Comparing local populations of viviparous eelpout (*Zoarces viviparus*) in the Lillgrund Offshore Wind Farm with natural populations in the Öresund strait in Sweden, Langhamer *et al.* (2018) found that the operating wind farm had no potentially negative effects on the individual health of eelpout or on the reproductive performance of the fish based on measured condition indices, histosomatic indices, and gonadosomatic indices as well as studies of population dynamics, biometrics, reproductive success, and fry development. Exclusion of fishing effort during construction of the Westernmost Rough wind farm, located within the Holderness coast lobster fishery on the northeast coast of the United Kingdom, was found to increase the total number of European lobster (*Homarus gammarus*) as well as the proportion of larger lobsters within the area, though these increases returned to baseline levels within weeks of reopening the exclusion area to fishing (Roach *et al.*, 2018).

### **Assessment Methods and Pollution Indicators**

Keeley *et al.* (2018) developed a multi-trophic Metabarcoding Biotic Index (mt-MBI) to characterize the enrichment level of sediment at fish farms using environmental DNA and RNA (eDNA/eRNA) metabarcoding of three taxonomic groups (foraminifera,

bacteria, and general eukaryotes) and compared its performance of the established Enrichment Stage index; the authors found strong correspondence between the mt-MBI and Enrichment Stage and exclusion of the foraminiferal data did not affect the overall performance of the mt-MBI.

Linder and Horne (2018) developed thirteen regression models from three classes (6 generalized regression models, 4 time series models, and 3 nonparametric) to detect, quantify, and forecast changes in nekton density for a case study of a tidal turbine site in Admiralty Inlet, WA; parametric models that did not include lagged dependent variables were the most capable at detecting change; deterministic time series models in conjunction with semi-parametric generalized regression models provided the best at quantifying change; and nonparametric models most accurately forecasted change.

Using a paired control-impact study design, Fowles *et al.* (2018) investigated the effects of pollution from four different anthropogenic point sources (marinas, storm-water drains, sewage outfalls and fish farms) on the species richness and cover of recruited algae and sessile invertebrates in the Derwent Estuary, Tasmania, Australia; the authors found that marinas had the greatest impact on recruitment of algal dominated species assemblages compared to paired controls, while higher algal cover was observed near sewage outfalls and fish farms compared to controls, including higher coverage of native algae species near sewage outfalls compared to controls.

Menegon *et al.* (2018) developed a cumulative effects assessment (CEA) backsourceing application that supported the spatial identification and quantification of anthropogenic pressures affecting environmental components, from which they were able to identify a clear distinction among local human impacts (trawling, small scale fishery) versus long-range diffusive human impacts (underwater noise and marine litter) at two case study sites in the Northern and Southern Adriatic Sea. A process-based modeling framework for assessing population consequences from anthropogenic disturbances based on simulated individual animals' movements, energetics, and survival demonstrated in a case study that the North Sea harbor porpoise (*Phocoena phocoena*) population was

unaffected by exposure to noise from the construction of 65 wind farms (Nabe-Nielsen *et al.*, 2018).

Qualitative mathematical models were developed and visualized in Bayesian networks to provide a framework to better understand and predict the cumulative impact of increased hard-substrate benthic organisms resulting from the construction of an offshore wind farm, decreased fishing due to safety measures within the offshore wind farm, and changes in sole, cod, and king scallop distributions caused by global warming in the Bay of Seine (Raoux *et al.*, 2018); the combined model predicted that king scallop abundance in the wind farm would decrease concurrent with decreased fishing effort, while the abundance of benthos, benthic feeding fish, and piscivorous fish would increase. Causon and Gill (2018) reviewed the available literature on the interaction of habitat modification, epibenthic colonization, biodiversity, and ecosystem function to create a model of biodiversity mediated linkages between habitat modification, ecosystem processes and functions, and the provision of ecosystem services in relation to offshore wind farm structures; the authors note that whether changes in biodiversity associated with offshore wind farms will have positive, negative, or neutral effects on ecosystem services is unclear.

### **Effects of Marine Debris: Ingestion**

The majority of published papers in 2018 on the effects of marine debris focused on ingestion. In many studies, researchers examined stomach contents and confirmed the chemical composition of debris fragments through spectroscopic methods (e.g., Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy). These studies provide a snapshot of ingestion at certain points in time, and over an increasingly broad geographic scope, but are not always coupled with experiments or investigations into the effects of this ingestion at the organismal level, or an analysis of debris source that would enable development of source prevention and reduction strategies. Studies have also not followed standardized procedures, as discussed by Hermsen *et al.* (2018). Hermsen *et al.* reviewed studies that quantify micro-plastic

ingestion in marine organisms, and proposed a quality assessment using ten criteria, such as sample size, processing and storage, negative and positive controls, and polymer identification. The authors determined that few studies meet their quality criteria and call for stricter quality assurance protocols when conducting studies that quantify micro-plastic ingestion. The sections below outline key papers on microplastic ingestion by resource type.

**Fish.** Many studies examined ingestion of microplastics in commercially-important fish and invertebrates. However, few attempted to describe disparate particles that could have a natural origin. Kroon *et al.* (2018) conducted a literature review on studies reporting ingestion of microplastic by fish. The authors used the review to develop a suggested new classification scheme for future research (e.g., clearer separation of particles that are non-plastic). Kroon *et al.* applied this classification to coral trout caught at the Great Barrier Reef and demonstrated the ingestion of so-called “semi-synthetic” fibers (e.g., rayon created from cellulose) and naturally-derived fibers (e.g., cotton, wool). Other research was more similar to Halstead *et al.* (2018), which quantified ingested microplastics in estuarine fish through gut content analysis in combination with attenuated total reflectance FTIR and Raman microscopy. Fish species caught in Sydney Harbor, Australia ingested plastic at a frequency of 21-64%, though no differences were noted when debris was standardized to gut content weight. In the Gulf of Thailand, Azad *et al.* (2018) described the first record of microplastic ingestion in several commercial fish species, including demersal, pelagic, and reef-associated fishes. Azad *et al.* documented the percent occurrence of ingested microplastics (<5 mm) and meso-plastics (5-25 mm) in 66.7% of fish stomachs (110 of 165 samples) and determined that fishing net fibers were the most abundant type of ingested debris. Cheung *et al.* (2018) examined wild and farmed flathead grey mullet (*Mugil cephalus*) collected near Hong Kong. The authors detected ingested microplastics in 60% of wild-caught fish but only 16.7% of farmed fish, and found a positive correlation between microplastic abundance and fish body size. As in many

other studies, fibers were the most prevalent type of plastic, and 90% of the identified plastics were smaller than 2 mm.

In the Persian Gulf and Musa estuary, Abbasi *et al.* (2018) collected demersal and pelagic fish species as well as the tiger prawn, *Penaeus semisulcatus*, analyzed for microplastics in the gastrointestinal tract, skin, muscle, gills, and liver of each fish species, calculated particle abundance and particles per gram of tissue (ranging from 0.16 particles/g to 1.5 particles/g), and examined microplastics using microscopy to determine color, size, morphology, and elemental composition; which allowed the authors to speculate on the source of the fragments as potentially components of antifouling paint. Baalkhuyur *et al.* (2018) investigated stomach contents of commercial as well as non-commercial fish species in a study conducted in the Saudi Arabian Red Sea, and found that 10 of 13 commercial fish species and 8 of 13 non-commercial species had ingested microplastic particles such as films and fishing net fibers. Anastasopoulou *et al.* (2018) collected fish from the Adriatic and Ionian Seas and conducted an analysis of fish stomach contents. The authors claimed the first record of marine litter ingested by the spotted flounder, *Citharus linguatula*, and documented the percent occurrence of micro-litter in 40% of fish captured from the Ionian Sea and 87% of fish captured in the Adriatic Sea. Also in the Ionian Sea, Digka *et al.* (2018) collected mussels and commercially important fish (*Sardine pilchardus*, *Pagellus erythrinus*, and *Mullus barbatus*) and found a variety of ingested microplastic particles of various color and sizes. Approximately 46% of mussels and 41% of fish had quantifiable microplastics in digestive tract tissues, and examination confirmed the majority of particles were plastic fragments. In another study from the Adriatic Sea, Pellini *et al.* (2018) collected a benthic flatfish, the common sole, and quantified microplastic particles in the digestive tract. This commercial fishing species had a 95% frequency of plastic ingestion (of 533 individuals sampled), with fragments accounting for 72% of particles.

Bernardini *et al.* (2018) published the first record of plastic debris ingestion by blue sharks (*Prionace glauca*) from the northwestern Mediterranean Sea. The authors used

a standard protocol developed by the European Commission's Marine Strategy Framework Directive and documented ingested plastic debris in approximately 25% of sampled stomach contents, with the greatest abundance of polyethylene sheets. Compa *et al.* (2018) investigated the ingestion of micro-plastics in two commercial fish species captured along the Mediterranean coast of Spain, and found approximately 15% of the 210 fish had ingested micro-plastics and natural fibers. In the Mondego estuary in Portugal, Bessa *et al.* (2018) investigated the occurrence of ingested plastics in commercial fish, including sea bass (*Dicentrarchus labrax*), seabream (*Diplodus vulgaris*) and flounder (*Platichthys flesus*). The authors examined 120 stomachs and found that 38% contained micro-plastics, the vast majority of which were fibers (96%).

Silva *et al.* (2018) documented microplastic contamination in grunt (*Haemulidae*) fishes in the Goiana Estuary, Brazil. The authors found trends in ingestion that coincided with peak ingestion of certain prey species, and point to the importance of life histories in understanding sources and impacts of micro-plastic on fish. Ferreira *et al.* (2018) investigated stomach contents of the Brazilian estuarine predator, *Cynoscion acoupa*, and found that more than half of the sampled individuals had ingested micro-plastic particles. Their analysis of particles documented a higher frequency of larger filaments in the upper estuary, which they attribute to proximity of sources as well as hydrodynamics within the sampled estuary. Ferreira *et al.* also found the highest incidence of plastic ingestion during the rainy season, in concert with high fishing pressure, which indicates that the fishing industry may be an important source of ingested microplastics. In addition, Cardozo *et al.* (2018) found that almost half of the Atlantic bigeye (*Priacanthus arenatus*) stomach contents examined contained plastic debris, and that 63% of those fragments were derived from fishing activities (e.g., paint fragments from vessels and synthetic net fibers). Pegado *et al.* (2018) claimed the first recorded evidence of microplastic particles in 26 of 189 fish gastrointestinal tracts, representing 14 species from the Amazon River estuary. Microplastic particles were overwhelmingly (97.4%) categorized as pellets.

Beer *et al.* (2018) investigated decadal trends associated with micro-plastic ingestion by plankton and planktivorous forage fish (Atlantic herring and European sprat) in the Baltic Sea. Time series samples from 1987 to 2015 did not find a significant increase in the concentration of micro-plastic in plankton or fish stomach contents over time, though the authors found a positive correlation between fish size and ingested micro-plastic. Approximately 20% of the 814 fish contained plastic particles; 95% of those particles were smaller than 5 mm and 93% were classified as fibers. Also in the Baltic Sea, Budimir *et al.* (2018) developed a new method for extracting micro-plastic from fish stomach contents based on a sodium hydroxide digestion, and determined an 84% recovery of particles in spiked samples. Budimir *et al.* then used this method to analyze microplastics in planktivorous fish, and found a low percent occurrence in herring (1.8%, n=164) and sprat (0.9%, n=154), and no ingested micro-plastic in three-spined sticklebacks (n=355). Interestingly, the ingestion rates observed by Beer *et al.* and Budimir *et al.* differ for the same species investigated from the Baltic Sea, which may indicate the importance of standardizing collection and analytical methods to control for variability caused by isolation and identification methods.

McGoran *et al.* (2018) collected fish and invertebrates from the Thames Estuary and Firth of Clyde, examined plastic ingestion in 876 individuals, and found that 14 of the 21 estuarine species had ingested plastics and 32% of organisms had ingested plastic. The most common type of plastic was fibers (88% of all identified plastic particles), and the authors did not find a geographic difference in the average amount of ingested plastic. In northeastern Greenland, Morgana *et al.* (2018) conducted a case study that collected sculpin and cod as well as subsurface water samples to investigate microplastic concentration in the Arctic. Frequency of ingestion differed between species, as demersal bigeye sculpin had a 34% ingestion rate (n=71) compared to an 18% ingestion rate (n=85) in pelagic polar cod, which may be explained by feeding behavior and habitat differences. The authors also measured microplastic concentrations in subsurface waters that were detected at higher concentrations than at lower



latitudes, and highlighted the reach of microplastic pollution to the Arctic. However, Liboiron *et al.* (2018) examined the gastrointestinal tracts of 134 silver hake collected from Newfoundland, Canada, and found a 0% incidence of ingested micro-plastic particles. The authors had hypothesized that silver hake, as a predator species that feeds at the surface and in benthic environments, would consume prey that had been exposed to micro-plastics. Liboiron *et al.* discussed these results in the context of other studies, and found that 41% of 100 fish ingestion rates reported in peer-reviewed literature reported a 0% incidence of microplastic ingestion. They recommend other researchers similarly publish findings that include null results to obtain a more complete understanding of the factors affecting debris ingestion.

**Mammals.** Due to the difficulty in surveying marine mammals, few data exist on the abundance of ingested microplastics in mammal species. Hernandez-Gonzalez *et al.* (2018) recorded the presence of microplastics in all digestive tracts (n=35) of common dolphins collected from the Iberian Peninsula. An average of 12 items were identified per sample, with high variation between organisms, and fibers were the most common type of microplastic. Similarly, Perez-Venegas *et al.* (2018) recorded the presence of microplastic fibers in South American fur seals in northern Patagonia. The authors quantified microplastics in female scat, finding no microplastic particles but microplastic fibers in 67% of samples; therefore, this medium could provide a valuable monitoring tool for other pinnipeds. In a trophic transfer experiment, Nelms *et al.* (2018) analyzed scat collected from captive grey seals and digestive tracts from wild-caught Atlantic mackerel for the presence of microplastic particles. The authors found that almost half of the analyzed scat samples (n=15) and one third of fish (n=10) contained microplastic particles, which measured a mean length of 1.5 mm and 2 mm in scat and fish tissue, respectively.

**Birds.** In the same vein as mammal studies that examined scat for microplastics, Alvarez *et al.* (2018) used regurgitated pellets to evaluate the presence of ingested and depurated microplastics in the European shag. The authors

found microplastic particles in 63% of the 41 examined pellets, and identified nylon and polyester fibers as the most abundant type of particle. They also found a correlation between abundance of microplastics and presence of benthic prey, indicating a potential source of microplastics is fibers that settle into sediments. Verlis *et al.* (2018) conducted a study of wedge-tailed shearwaters that nest nearshore and offshore of the eastern coast of Australia, in which birds were lavaged and the regurgitated material was analyzed for microplastic particles. Ingestion rates on nearshore sites reached 20% of surveyed birds, and these beaches were significantly more polluted, while ingestion rates on less polluted offshore sites reached 8% of shearwaters. Ingested plastics differed (in material and color) from offshore beaches surveyed, implying that birds nesting offshore may not feed locally as compared to birds that nest closer to shore. Provencher *et al.* (2018) examined the gastrointestinal tract of northern fulmars to investigate the presence of microplastics in guano. In particular, the authors evaluated the terminal portion of gastrointestinal tract and fecal precursor samples, though the frequency of microplastic occurrence did not correlate between these compartments. However, Provencher *et al.* found a positive relationship between the number of plastic particles in the gut and the quantity of microplastics in guano, and concluded that fulmars may be a vector of microplastic as guano collects around their colonies.

**Sea Turtles.** Caron *et al.* (2018) documented the ingestion of microplastics in two green sea turtles (*Chelonia mydas*) in the Great Barrier Reef, Australia, while also developing an extraction protocol for chyme to better assess the stomach contents as it reaches the small intestine. Using the newly developed analytical technique, the authors identified two microplastic particles and seven microplastics, composed of paint chips and synthetic fabric fibers. In New Zealand, Godoy and Stockin (2018) conducted 35 necropsies of immature and sub-adult green sea turtles that stranded between 2007 and 2013. Twelve individuals had ingested synthetic marine debris, with soft plastics (e.g., plastic bags) and white, clear, or translucent particles the most abundant debris types. Organismal size metrics did not correlate with the volume or number of

ingested debris items, though the authors indicate that anthropogenic factors such as debris ingestion may impact turtle aggregations. Near Hawaii and American Samoa, Jung *et al.* (2018) identified microplastics ingested by multiple species of sea turtles that were caught as bycatch in longline fisheries. The authors identified the majority of ingested particles as low-density, floating polymers such as low-density polyethylene and polypropylene, but did not find a significant correlation between ingested debris type and the feeding strategy employed by the species.

### **Entanglement in Marine Debris.**

Far fewer studies published in 2018 studied the effects of entanglement in or smothering by marine debris. However, multiple studies published research on the abrasion of benthic habitats by fishing gear and other sunken marine debris. For example, Galgani *et al.* (2018) extended this concept by considering the entanglement of habitats composed of sessile suspension feeders (e.g., in tropical coral reef, deep-sea coral, and sponge aggregation habitats) and their use for monitoring benthic entanglement trends. Ballesteros *et al.* (2018) conducted a dive study around the island of Koh Tao in the Gulf of Thailand to document the impact of various derelict fishing gear materials (e.g., nets, ropes, cages, lines) on the surrounding coral reef. The study found that more than 95% of gear items were composed at least partially of plastic, and that nets were the dominant type of lost gear. Ballesteros also documented that fragmentation of coral was less common than expected, perhaps due to characteristics of the dominant species, and that corals located underneath derelict gear showed the most damage and abrasion. Consoli *et al.* (2018) surveyed video footage of 19 transects along the Straits of Sicily, an Ecologically or Biologically Significant Area as designated by the Convention on Biological Diversity. Similarly to Ballesteros *et al.*, the purpose of this study was to quantify the marine litter and its impact of benthic fauna. Consoli *et al.* found a litter density that ranged from 0 to 14.02 items per 100 square meters, with a greater density at depths greater than 100 meters than in shallower waters. The most abundance type of marine debris was lost or abandoned fishing lines,

accounting for 98.07% of the total litter density; effects of litter included entanglement and/or coverage of benthic species and damage to sessile organisms, including species of conservation concern. Continuing this theme, Ferrigno *et al.* (2018) observed video footage from the southern Bay of Naples to characterize coral habitat morphology and assess the presence of lost or abandoned fishing gear. The authors found that derelict fishing gear entangles and covers benthic assemblages, which leads to necrosis and growth of parasitic epibionts on coral habitat.

### **Effects of Oil Spills**

As in recent years, a majority of the oil pollution literature focused on the effects associated with the Deepwater Horizon (DWH) oil spill that occurred in 2010. However, other papers described monitoring studies conducted in the years after the Exxon Valdez oil spill (1989), behavior changes in oiled and rehabilitated brown pelicans oiled during the Refugio oil spill (2015), and community changes associated with hydrocarbon seeps in the Gulf of Mexico, among others. Studies covered impacts in the nearshore to the offshore to the deep-sea benthos, and across species from microorganisms to megafauna.

### **Effects of the 2010 Deepwater Horizon Oil Spill**

**Marsh and Nearshore Impacts.** Bam *et al.* (2018) detailed significant changes both among years (2013 and 2014) and between sites in salt-marsh terrestrial arthropod communities of reference, lightly-DWH oiled, and heavily-DWH oiled sites after DWH oil re-distribution during Hurricane Isaac (2012). In both sampling years, reference sites had significantly higher taxonomic richness (0.8) than any of the oiled sites (0.5-0.6) and distinctly different community structure. For all sites, species richness and diversity increased over time, suggesting on-going community recovery.

Three years after the DWH oil spill, densities of nematodes, copepods, and annelids had recovered to reference site levels in moderately and heavily oiled sites, concurrent with the recovery of *Spartina alterniflora* stem density. However, populations of gastropods, bivalves,

ostracods, and amphipods remained significantly depressed six years after the spill (Fleeger *et al.* 2018)). The reasons for depressed recovery is likely species dependent and related to high sediment concentrations of total petroleum hydrocarbons, reduced underground biomass of *S. alterniflora*, and life history characteristics (e.g. larval dispersal mechanisms).

Five years after the DWH oil spill, Husseneder *et al.* (2018) found that populations of the greenhead horse fly (*Tabanus nigrovittatus*) were recovering in oil impacted marshes. Fly numbers increased, genetic bottlenecks were gone, migration into oiled sites occurred, and parameters of family structure (number of breeding parents, effective population size, number of family clusters) were no different than in non-oiled sites.

Rabalais *et al.* (2018) determined that the size and extent of hypoxia in the Northern Gulf of Mexico in the summer of 2010, concurrent with the DWH oil spill, was not significantly different from documented hypoxic zones in other years (1990-2014).

Six years after the DWH oil spill (2010), there were no significant differences in plant belowground biomass, sediment extractable  $\text{NH}_4^+$ , inorganic nitrogen flux, and bacterial abundance and diversity at sites that had been lightly, moderately, and heavily oiled by the DWH oil spill. Despite the lack of difference among these parameters, denitrifying capacity at moderately and heavily oiled sites was still nearly 50% less than that of sites that had been only lightly oiled ( $27.7 \pm 14.7$  and  $37.2 \pm 24.5$  vs  $71.8 \pm 33.8 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ , respectively). However, denitrifying capacity at all sites was similar to that of other Gulf of Mexico marshes prior to and during the spill (Tatariw *et al.* 2018).

**Deep Sea and Offshore Effects.** DeLeo *et al.* (2018) noted significantly different expression of 1,439 genes associated with five gene clusters that are linked to oxidative stress, immune response, wound repair/inflammation, metabolism of xenobiotics, and tissue regeneration pathways in colonies of the deep-sea octocoral (*Paramuricea biscaya*) collected in November 2010 that were coated by oil contaminated flocculent associated with the DWH oil spill.

After evaluating 5300 offshore water samples collected as part of the DWH oil spill Natural Resource Damage Assessment, Driskell and Payne (2018) reported significantly accelerated lower and intermediate weight PAH (e.g. C2-phenanthrenes) dissolution, and inhibited saturated hydrocarbon (saturated n-alkanes (C9–C40) and selected (C15–C20) acyclic isoprenoids (e.g., pristane and phytane)) biodegradation in water samples containing dispersant components collected from the deep sea plume of oil droplets. The authors were unable to determine whether the seeming inhibition of microbial degradation of the saturated hydrocarbons was the result of an actual depression in biodegradation or whether the inhibition was reflective of such rapid dissolution that the microbial community had not yet had sufficient time to respond.

Seven years after the DWH oil spill, Girard and Fisher (2018) documented on-going impacts to three deep sea octocoral (*Paramuricea biscaya*) communities adversely affected by the deposition of oil contaminated flocculent material associated with the DWH oil spill. Corals with a greater level of initial visible impact had significantly slower rates of recovery and greater visible damage (broken and unhealthy branches, hydroid colonization) than those at reference sites.

Using a structured matrix model populated by data collected with high-resolution imagery, Girard *et al.* (2018) estimated that recovery of *Paramuricea biscaya* coral colonies adversely impacted by the DWH oil spill will take approximately three decades. However, growth rate data suggest recovery to pre-impact biomass levels may actually take hundreds of years.

In 2011 and 2012, Schwing *et al.* (2018a) documented stable carbon isotopic ( $\delta^{13}\text{C}$ ) depletion (0.2-0.4%) in the foraminifera *Cibicides* spp. sampled from the sediment intervals deposited post-DWH at sites impacted by marine oil snow sediment flocculent accumulation (MOSSFA) formed from the DWH oil spill (2010). By 2014,  $\delta^{13}\text{C}$  levels were similar to those in sediment intervals down-core of the DWH event.

Benthic foraminifera species richness ( $27.2\% \pm 3.6\%$ ) and heterogeneity ( $19.8\% \pm 0.2\%$ ) steadily

increased in surface sediment samples between 2011 and 2013 at seven offshore sites (water depths of 401-1577m) in the northern Gulf of Mexico impacted by MOSSFA from the DWH oil spill (2010). By 2014, samples were more consistent, and Schwing *et al.* (2018b) estimated a resilience rate of approximately three years. DWH oil spill as compared to those before the spill.

After comparing concentrations of polycyclic aromatic hydrocarbons (PAH) in mesopelagic fishes caught prior to, during, and after the DWH oil spill, Romero *et al.* (2018) documented a dramatic increase in PAHs in fish muscle tissues in 2011 ( $4972 \pm 1477$  ng/g) as compared to 2007 ( $630 \pm 236$  ng/g). PAH concentrations returned to near background levels by 2015-2016 ( $827 \pm 138$  ng/g), but the composition of the PAHs more closely resembled those in tissues from fish collected in 2011 than those in pre-spill fish tissues.

**Fish Populations.** Using the model Atlantis, Ainsworth *et al.* (2018) predicted shifts in biomass, age structure, and distribution for eight fish species guilds (snappers (Lutjanidae), groupers (Serranidae), Sciaenidae, elasmobranchs, large pelagic fish, small pelagic fish, small demersal and reef fish, and large demersal fish) of the Northern Gulf of Mexico potentially impacted by the DWH oil spill. Modeling resulted in large decreases in biomass occurred across all guilds; small demersal and reef fish experienced the largest decline, decreasing from between 50-75% biomass while large reef and demersal fish biomass declined 25-70%. According to the model, full recovery of some populations may take greater than 30 years.

In contrast to the predictions of Ainsworth *et al.* (2018), Ward *et al.* (2018) found no significant anomalies in fish species occurrence or catch rates after the DWH oil spill. The authors reached their conclusion using a spatiotemporal statistical approach to analyze long-term fisheries monitoring datasets (trawl, bag seine, gill nets for shrimp, crabs, groundfish, finfish in estuarine and open water) collected by the Louisiana Department of Fish and Wildlife. Using population genomic tools, Shaefer *et al.* (2018) found little evidence of population level effects on Gulf killifish (*Fundulus grandis*) sampled in 2015 from

DWH oiled barrier islands (Cat and Horn Islands) in coastal Mississippi. Finally, up to seven years after the DWH oil spill, Murawski *et al.* (2018) determined that fish assemblages in the Northern Gulf of Mexico were no different after the DWH oil spill as compared to those before the spill.

#### **Seafood Contamination and Consumption.**

Despite making extremely conservative assumptions (59+years daily lifetime exposure, 40kg body weight, extremely high consumption rates (45.2 g/day of shrimp; 64.9, 142.4, or 540 g/day of finfish), and complete absorption of any PAHs present), Wyckliffe *et al.* (2018) found no elevated cancer risk from eating finfish and shrimp collected in 2012-2015 from the waters of southeastern Louisiana. PAH levels in finfish and shrimp were generally below the minimum detection levels (1.2 to 2.1 PPB) or were less than 10PPB.

**Recreation Impacts.** English *et al.* (2018) estimated a loss of \$520 million ( $\pm 166$ ) of lost shoreline recreational user days as a result of the DWH oil spill, out of a total of \$661 million (2015\$) of recreational damages.

**Seafood.** Bianchini and Morrissey (2018) related elevated sediment tPAH levels in samples from 2015-2017 with later departure times for both Sanderling (*Calidris alba*) and Red Knot (*C. canutus*), but only Sanderling displayed slower refueling at sites with elevated tPAHs. Bird staging sites in Louisiana (Elmer's Island Wildlife Refuge, Grand Isle, Port Fourchon) that had documented oiling from the DWH oil spill had the highest pooled average tPAH concentrations ( $322 \pm 318$  ng/g wet mass) as compared to sites in Texas (Bolivar Flats (26.4 ng/g wet mass) and North Padre Island ( $28.2 \pm 14.0$  ng/g wet mass)).

One year after the DWH spill, Burger (2018) found that the shoreline oiling category designation (no oil, little oil, moderate to heavy oil) resulted in no significant differences in bird colony phenology or in the mean number or size of chicks in nests of colonies located within 2km of a particular designation.

Perez-Umphrey *et al.* (2018) recorded significantly elevated cytochrome p450 1A (CYP1A) levels in livers of Seaside Sparrows collected from areas of Barataria Bay, Plaquemines Parish, Louisiana that were

heavily oiled by the DWH oil spill as compared to those collected from unoiled areas. CYP1A levels declined by 2012, increased in 2013, following Hurricane Isaac, and then declined again in 2014. Sediment PAH levels in oiled plots followed a similar pattern, decreasing from a mean PAH concentration of  $9525.81 \text{ ng g}^{-1} \pm 4499.13$  in 2011 to less than  $1000 \text{ ng g}^{-1}$  in 2012 pre-hurricane Isaac, then jumping back up to nearly  $5000 \text{ ng g}^{-1}$  in 2012 and 2013 post-hurricane Isaac, before declining again to less than  $1000 \text{ ng g}^{-1}$  in 2014.

Although the number of Seaside Sparrows (*Ammodramus maritimus*) and Red-winged Blackbirds (*Agelaius phoeniceus*) within 10km of areas oiled by the DWH oil spill declined in the first two years after the DWH oil spill (-31% and -28% respectively), their numbers had rebounded by 2014. Clapper Rail (*Rallus spp.*) declined by 46%, and remained depressed through 2014 (Yaukey 2018).

**Marine Mammals.** Balmer *et al.* (2018) ruled out persistent organic pollutants (POP) as the source of the severe health issues observed in Barataria Bay, Louisiana dolphins (*Tursiops truncatus*) in the aftermath of the DWH oil spill. POP concentrations in samples collected in 2011, 2013, and 2014 in Barataria Bay dolphins ( $\Sigma$ POPs 36,100 ng/g lipid (32,000–40,800)) were significantly lower than those of dolphins sampled from the Sarasota Bay, FL reference site ( $\Sigma$ POPs 49,800 ng/g lipid (41,500–59,800)) or another oil impacted area, Mississippi Sound, Mississippi ( $\Sigma$ POPs 85,500 ng/g lipid (68,100–107,000)).

Pitchford *et al.* (2018) suggested that increased search efforts in response to the DWH oil spill had the capacity to increase the frequency of stranding reports, regardless of the cause of death.

Between the years 2010 – 2012, Wise *et al.* (2018) documented significantly declining concentrations of metals (Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Aluminum (Al), Chromium (Cr), and Nickel (Ni)) associated with MC252 (DWH) oil in skin samples taken from sperm whales (*Physeter microcephalus*), Bryde's whales (*Balaenoptera edeni*), and short-finned pilot whales (*Globicephala macrorhynchus*) after the DWH oil spill. As an example, in sperm whales,

Al declined from  $>75 \text{ ug/g}$  in 2010 to less than  $>12 \text{ ug/g}$  in 2012, and Mg declined from  $>300 \text{ ug/g}$  in 2010 to  $<265 \text{ ug/g}$  in 2012.

### Exxon Valdez oil spill (EVOS)(1989)

Aderhold *et al.* (2018) summarized the topics covered in the special issue of Deep-Sea Research Part II that is devoted to the results of monitoring studies conducted since the EVOS. Those studies, funded by the Exxon Valdez Oil Spill Trustee Council (EVOSTC), range from examinations of persistent spill effects including the distribution and effects of lingering oil and wildlife population recoveries to changing physical and biological oceanographic conditions, and how the interplay of these variables impact nearshore ecosystem and population dynamics.

Using gene transcription, Bowen *et al.* (2018) found significant elevation in the expression of two genes (Cytochrome P450, family 3 (Cyp 3) and Caspase 8 (Casp 8)) associated with hydrocarbon exposure in Pacific blue mussels (*Mytilus trossulus*) collected between 2012-2015 from PAH contaminated harbors (Cordova, Seward, and Whittier Harbors). Surprisingly, gene expression in mussels collected from Prince William Sound that had varying levels of oil exposure from the EVOS (Herring Bay, Hogan Bay, Iktua Bay, Johnson Bay, and Whale Bay) did not display the same elevation, indicating no PAH exposure.

After evaluating 24 years (1989-2012) of bird population data collected after the EVOS, Cushing *et al.* (2018) found that the average rate of recovery for eight bird genera injured by the spill was 0.1% per year. Of those eight genera, four (Loon spp. (*Gavia*), Goldeneye spp. (*Bucephala*), Black oystercatcher (*Haematopus*), and Murre spp. (*Uria*)) had no significant change in abundance, two (Pigeon guillemot (*Cepphus*) and Murrelet spp. (*Brachyramphus*)) had significant decreases in abundance, and two (Harlequin duck (*Histrionicus*) and Cormorants (*Phalacrocorax*)) had significant increases. The declines in the populations of pigeon guillemots and murrelets appear to be linked to environmental conditions rather than chronic effects from the EVOS spill.

After reviewing monitoring results reported through 2015, Esler *et al.* (2018) presented a timeline of recovery for a variety of species impacted by the EVOS. Species discussed included Pigeon guillemots (*Cephus columba*), marbled murrelets (*Brachyramphus marmoratus*), glaucous-winged gulls (*Larus glaucescens*), harlequin ducks (*Histrionicus histrionicus*), bald eagles (*Haliaeetus leucocephalus*), river otters (*Lontra canadensis*), sea otters (*Enhydra lutris*), and killer whales (*Orcinus orca*). Recovery timeframes ranged from less than a year for glaucous-winged gulls to 20-25 years for sea otters and harlequin ducks. Killer whales have yet to recover, and one pod (AT1) is likely to become extinct. Species-specific life and natural history traits drive the variation in recovery rates.

Twenty-six years after the EVOS (1989), both Lindeberg *et al.* (2018), and Nixon and Michel (2018), found nearly unweathered subsurface oil primarily in the mid-intertidal zone at various locations in Prince William Sound, Alaska. Comparison to surveys from 2001 indicated that the distribution, mass, and toxicity of the oil is unchanged.

Moran *et al.* (2018) suggested that the presence of Dall's porpoises (*Phocoenoides dalli*) in novel habitats (nearshore, shallow waters, and bays) in Prince William Sound, Alaska may in part result from the decline in Killer whale (*Orcinus orca*) populations in the aftermath of the EVOS. Presence of overwintering and spawning populations of Pacific herring (*Clupea pallasii*) in these habitats, a preferred prey, may also play a role.

Using spatial-temporal models populated with fisheries independent groundfish data, Shelton *et al.* (2018) suggested that overall biomass of groundfish declined 1-2% for year in EVOS impacted areas between 1990-1999. Loss of apex predators (e.g. Pacific cod (*Gadus microcephalus*), Lingcod (*Ophiodon elongates*), Pacific Halibut (*Hippoglossus stenolepis*) and pelagic foragers (e.g. Pacific hake (*Merluccius productus*), rockfish species (*Sebastes sp.*) appears to have driven the decline.

### Effects Studies at Other Oil Spills

Lamb *et al.* (2018) documented altered behavior in California brown pelicans (*Pelecanus occidentalis*

*californicus*) oiled and rehabilitated (OAR) during the Refugio oil spill (2015) as compared to non-oiled, non-rehabilitated brown pelicans. During the monitoring period (mean 271 days), OAR pelicans spent less time at breeding colonies, flew farther more frequently, had a greater home range, and foraged in higher productivity waters. In particular, OAR female pelicans dispersed over significantly longer distances and spent significantly less time in residence.

While PAH levels ( $\Sigma$ 16 EPA PAHs, but specifically, naphthalene, acenaphthene, acenaphthylene, fluorene and phenanthrene) steadily declined from ( $\Sigma$ 16 PAHs) 4465.71  $\mu\text{g}/\text{kg}$  of wet weight five days post spill to 327.60  $\mu\text{g}/\text{kg}$  of wet weight five months later in blue mussel (*Mytilus edulis* spp.) tissues after a 180,000 l diesel spill (December 2013) in Skjervøy harbour, Norway, biomarker enzyme (Glutathione S-Transferase (GST), Superoxide dismutase (SOD), laccase, Malondialdehyde (MDA)) responses remained significantly altered. However, the enzyme expression modulated as compared to what it was immediately after the spill (Breitwieser *et al.* (2018)).

Stimmelmayer *et al.* (2018) documented low tissue PAH levels (<50 ng/g wet weight) and hepatic, pulmonary, and cardiac lesions in three seals (two spotted seals (*Phoca largha*), one ringed seal (*Phoca hispida*) oiled in 2012 by an unknown source in the Bering Sea.

Vad *et al.* (2018) noted that there are few studies of the impacts of oil exposure on deep-sea sponges, but outlined a variety of potential impacts including declines in health, compromised immune system, bioaccumulation, disruption of larval dispersal and settlement, and changes in community abundance, age structure, and diversity among others.

After evaluating samples collected over a number of years (2000–2002, 2009–2013), Washburn *et al.* (2018) reported that community structure and abundance in macrofaunal assemblages associated with hydrocarbon seeps in the Gulf of Mexico are significantly different and higher than in background soft-sediment habitats. These differences are most pronounced in the top 3 cm of the

sediments and at seeps between 1000 and 2000 meters deep.

### Laboratory Studies on Effects of Oil

Oil does not easily mix with water. Therefore researchers use various means to enhance oil concentrations, resulting in what is known as Water Accommodated Fraction, or WAF. These methods include physical mixing resulting in Low Energy Water Accommodated Fraction (LEWAF) High Energy Water Accommodated Fractions (HEWAF); use of dispersants results in Chemically Enhanced Water Accommodated Fraction (CEWAF).

A May 2015 spill of crude oil at Refugio in the Santa Barbara Channel, CA, inspired Ladd *et al* (2018) to conduct a series of laboratory experiments on the effects of oil on two key harmful algal bloom phytoplankton species, the Domoic Acid (DA)-producing *Pseudonitzschia australis* and the coccolithophore *Emiliana huxleyi*. Cultures from field collections were raised in nutrient enhanced sea water and then subjected in triplicate to exposures to 100% or 50% WAF of Refugio oil and also seep oil. Total Petroleum Hydrocarbon (TPH), Polycyclic Aromatic Hydrocarbon (PAH) and Saturated Hydrocarbon (SH) concentrations were measured and were comparable to the upper range of concentrations measured during other oil spills. Over 5 days the exposures reduced or inhibited both species during exponential growth phases. However *P. australis* did not cease production of DA while *E. huxleyi* suffered coccolith shedding. The authors provided a detailed bibliography of the effects of oil on phytoplankton and discussed implications for the effects of oil WAF on bloom dynamics and marine food web dynamics. Bioaccumulation potential of two compounds commonly found in crude oils (dodecane and phenanthrene) in copepods was reported by Agersted *et al.* (2018). 89-hour static exposure to non-toxic concentrations of the two compounds was followed by 75-hour depuration periods in non-tainted seawater. While measurable differences in uptake, elimination, and depuration rates were observed between compounds, and copepod developmental stages, the authors conclude that elimination and depuration were

slow in all cases and short-term exposure to low concentrations of WAF of crude oil can result in bioaccumulation, leading to broader ecological impacts throughout the food web.

Kelly and Taylor (2018) found a significantly higher proportion of blue crab (*Callinectes sapidus*) embryos hatched in the pre-zoeal stage after five to six days of exposure to nominal 500 ppb of water-accommodated fraction (WAF) of south Louisiana crude oil (24 hour renewal cycle of WAF) than in seawater controls. No significant difference in the proportion of zoeal versus pre-zoeal stages was observed in oil-exposed versus control embryos in three to four day incubations.

Morris *et al.* (2018) compared morphological and functional cardiotoxic effects of HEWAF and LEWAF of MC252 crude oil on embryonic red drum (*Sciaenops ocellatus*). After observing defects and abnormalities in pericardial area, atrioventricular angle, atrial contractility, and ventricular contractility, the group did not observe a difference in biological effects between WAF types..

Cave and Kajiura studied the olfactory response of Atlantic Stingray (*Hypanus sabinus*) to water accommodated fractions of Deep Water Horizon crude oil. After 48-hour exposure to WAF (0.09 g/L with partial renewal every 8 hours), the stingrays displayed an average decrease in olfactory function of 45.8%.

Three studies focused on the effects of oil contaminated sediment. Boulais *et al.* (2018) investigated the sub-lethal exposure of three early life stages of the Eastern oyster (*Crassostrea virginica*) to Deepwater Horizon oil-contaminated sediments. Gametes, Embryos, and Veliger larvae were exposed to elutriate from contaminated sediment for 1, 24, and 48 hours respectively. Fertilization inhibition in the gamete stage was the most sensitive endpoint with EC20 and EC50 values of 40.6 and 173.2 micrograms tPAH50 per liter respectively. Embryo abnormalities (EC20 77.7 and EC50 151 microgram tPAH50 per liter) and veliger larvae shell growth (EC20 only, 1180 micrograms tPAH50 per liter) were less sensitive to the unfiltered elutriate. Franco *et al.* (2018) studied the effects of sediment-entrained Sweet Louisiana crude oil on two species of Fidler crab (*Uca longisignalis*

and *Uca panacea*). All bioturbation endpoints (burrow frequency, burrow size, and surface sediment transport) were reduced in the presence of oil; oxidative stress (lipid peroxidation), and histological abnormalities (blister cells in hepatopancreas) were also elevated in oil-exposed crabs.

Marine snow describes organic material suspended in the water column that settles and accumulates on the seafloor Van Eenennaam *et al.* (2018) studied the combined effects of marine snow and spilled, lightly weathered crude oil (MC252 surrogate) on three species of invertebrates, *Corophium volutator* (amphipod), *Macoma balthica* (bivalve), and *Hydrobia ulvae* (gastropod), representing a naturally occurring benthic community. Behavioral observations during 16-day exposure (10g oil/m<sup>2</sup>), and species survival rates after 16 days, led the authors to conclude that oil-contaminated marine snow increases adverse effects of oil; a species' motility, feeding behavior, sensitivity to hypoxic conditions, and sensitivity to oil toxins are influencing factors.

While the salt-tolerant submerged aquatic grass, *Ruppia maritima* is tolerant to oil, Martin and Swenson (2018) found that exposure to oil reduced the carbon:nitrogen (C:N) ratio, thus altering the food value to herbivorous animals including Grass shrimp (*Palaemonetes pugio*) and amphipods (*Gammarus mucronatus*). Both species, showed preference to feeding on oiled extracts of leaves *Ruppia* grown in sediment at four oil concentrations, compared to unoiled sediment. *Ruppia* C:N ratios decreased with increasing oil exposure. Type of oil and weathering status was not reported. The authors speculated on how decreased food value to herbivores of submerged plants surviving direct oiling might affect food web dynamics.

Little work has been done on the effects of multiple stressors, including oil, on developing fish embryos. Perrichon *et al* (2018) exposed 8-hour post-fertilization *Mahi mahi* embryos for 24 hours to six serial dilutions of HEWAF of naturally weathered Deepwater Horizon surface slick oil at two temperatures (26 and 30C), reporting effects on three morphological and three cardiac function endpoints. Measured PAH concentrations across 5 HEWAF dilutions ranged from about 3 to about 48 µg/L.

Within this exposure range, significant effects included increased edema and decreased heart rate, stroke volume and cardiac output. While effects were reduced at 30C, the hypothesis that higher temperature protects developing embryos was not supported. Arnberg *et al.* (2018) studied the individual and cumulative effects of ocean warming, ocean acidification, and mechanically dispersed North Sea crude oil on the green sea urchin (*Strongylocentrotus droebachiensis*), and the northern shrimp (*Pandalus borealis*). Each stressor had a negative impact on larval survival, development, and growth, but compounding interactions were not statistically observed; i.e. cumulative effects were generally equal to the sum of individual effects.

### Toxicity of Dispersants Alone

Three studies focused on the effects of dispersants alone, and their component chemicals. MacInnis *et al.* (2018) studied the toxicity of Corexit EC9500A on freshwater rainbow trout (*Oncorhynchus mykiss*), and saltwater Coho (*Oncorhynchus kisutch*), Chinook (*Oncorhynchus tshawytscha*), and Chum (*Oncorhynchus keta*) salmon. 24-hour static exposures yielded LC50 ranges of 35.3 mg/L to 47.6 mg/L for saltwater species (more sensitive), and 59.8 to 92.1 mg/L for rainbow trout (less sensitive). The group also evaluated the use of dioctyl sulfosuccinate, DOSS, a surfactant component of several common dispersants, as an indicator of residual oil dispersant in the environment. The authors concluded long-term detection is not reliable with this method. Echols *et al.* (2018) reported on the short-term static exposure of 18 marine species (five mollusks, four algae, three fish, three crustaceans, two echinoderms, and a cnidarian) to the chemical dispersant Corexit 9500 in the absence of oil. Exposure periods were 24-, 48-, 72-, or 96-hours depending on species. LC50, EC50, and EC10 values were derived for each species and HC5 was calculated for the dispersant marker dipropylene glycol n-butyl ether (DPnB). While oysters and algae were most sensitive to exposure conditions, all taxa were evenly distributed on the sensitivity index curve. The DPnB HC5 value of 1172 micrograms/L was significantly higher than concentrations



measured in the field during Deepwater Horizon. Dasgupta *et al.* (2018) exposed sheepshead minnow (*Cyprinodon variegatus*) larvae to both Corexit 9500 and its separated surfactant component dioctyl sodium sulfosuccinate (DOSS). The study included both high and environmentally relevant (low) concentrations of each substance over 24-hour and 96-hour exposures. The study suggests DOSS causes significantly higher levels of oxidative stress at both concentrations and time points than with exposure to Corexit alone.

See below for studies involving both dispersant and dispersed oil toxicity.

### Effects of Intentionally Dispersed Oil

Several studies focused on the effects of dispersants and chemically-dispersed oil on fishes. Xiang *et al.* (2018) used enzymatic antioxidant activity and the blue sac disease (BSD) index to assess the sub-acute toxicity of WAF, CEWAF, and biologically enhanced water accommodated fractions (BEWAF) of crude oil to embryos of the marine medaka (*Oryzias melastigma*). The group monitored malformation, mortality, and hatching throughout a 22-day exposure period, solutions renewed daily. Chemical and biological dispersants increased the levels of bioavailable TPHs and PAHs in the water, corresponding to an increase in biological toxicity. Exposure concentration and duration positively correlated with BSD index scores. McConville *et al.* (2018) investigated the toxicity of toluene, 2-methyl naphthalene, phenanthrene, Corexit 9500, and CEWAF (Corexit 9500 and ANS crude) to *Anoplopoma fimbria*, the cold-water, deep-sea Sablefish. Using static exposure tests at 24, 48, 72, and 96-hour periods, with partial water renewal every 24 hours, the group calculated LC50s for each toxin and time point. The authors also provide alternatives and improvements to oil exposure characterization in toxicity testing. Hansen *et al.* (2018) exposed fertilized eggs (24-hour static exposure, 14 day-post fertilization) of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) to both mechanically and chemically dispersed crude oil. Differences in adhesion of oil-droplets to eggs between species was significant and may be due to

differences in egg surface morphology. In both species, the presence of chemical dispersant caused less oil-egg adhesion when compared to mechanically dispersed oil. Beirao *et al.* (2018) examined the effects of crude oil WAF, CEWAF, and the effects of the chemical dispersant Corexit EC9500A alone, on the reproductive ability of capelin (*Mallotus villosus*). Specifically, sperm behavior (motility, curvilinear velocity, and linear velocity) and fertilization rates were analyzed. Sperm behavior was not affected by any of the treatments but egg fertilization rates were reduced by both exposure to CEWAF and treatment solutions using the chemical dispersant alone. The mechanism for reduced fertilization is unclear but the authors provide several possible explanations. In a relevant study involving a freshwater fish, Barron *et al.* (2018) reported on the phototoxicity of South Louisiana Crude (SLC) to Zebra fish larvae (*Danio rerio*). Population survival was measured after 24-hour static exposure to either contaminated sediment, water-accommodated fractions of SLC, chemically enhanced (Corexit 9500A) water-accommodated fractions of SLC, or Corexit 9500A alone, followed by 3- and 3.5-hour (water and sediment groups respectively) exposure to high (100% natural sunlight) and low (10% natural sunlight) levels of UVA. The authors describe a dose-dependent, direct relationship between oil and sunlight exposure and larval mortality.

DeLorenzo *et al.* (2018) studied the toxicity of two common dispersants, Corexit EC9500A and Finasol OSR 52, and their water-accommodated fractions of Sweet Louisiana Crude oil on seven estuarine species. Using static exposure tests with species-dependent durations, the group calculated LC50s for multiple life stages of each species. The concentration-based mean LC50 for all species tested was 261.96 mg/L (TEH) and 2.95 mg/L (TPAH) for Corexit-enhanced WAF, and 23.19 mg/L (TEH) and 0.49 mg/L (TPAH) for Finasol-enhanced WAF. The authors note that larval life stages were more sensitive than adults within a species.

Jasperse *et al.* (2018) compared the toxic effects of Corexit 9500, Louisiana sweet crude (LSC), and a Corexit-enhanced WAF of LSC using the eastern oyster (*Crassostrea virginica*). After static exposure for 1, 3, and

7 days, the group used immunological, physiological, histological endpoints to measure responses. The oyster was most sensitive to Corexit-enhanced WAF and clearance rate was the most sensitive endpoint. Tarrant *et al.* (2018) used RNA-sequencing of the starlet sea anemone (*Nematostella vectensis*) to assess effects of UV light on WAF of Macondo sweet crude oil, WAF of Corexit 9500, and CEWAF (Corexit and Macondo sweet crude oil). Treatment and control groups were exposed to, or sheltered from, UV radiation during the first 6 hours of 24-hour exposure period. The authors describe significant UV-enhanced toxicity among all treatment groups.

Wise *et al.* (2018) studied the genotoxicity and cytotoxicity of water accommodated fractions and chemically enhanced (Corexit 9527) water accommodated fractions of Alaskan North Slope crude oil on Sperm Whale (*Physeter macrocephalus*) skin cells. After 24-hour exposure, the group found WAF to be non-cytotoxic (measured in colonial survival) and mildly genotoxic (measured by chromosome aberrations) to the skin cells. CEWAF were both cytotoxic and genotoxic with concentration dependent toxicity.

Several studies were reviewed on the effects of dispersants on bacteria. Brakstad *et al.* (2018) assessed the efficiency of the oil biodegradation process in the presence and absence of a chemical oil dispersant commonly used in Europe (Slickgone NS). The study compared various ratios of dispersant and oil in seawater to mixtures of seawater and oil alone. The authors argue that the chemical dispersant does not inhibit biodegradation under normal conditions. Li *et al.* (2018) studied the combined effects of a chemical dispersant (GM-2) and a strain of petroleum hydrocarbon degrading bacteria (LSH-7<sup>+</sup>) on a light crude oil (Shandong, China) over a 38-day laboratory experiment. The study suggests that dispersants promote bacterial growth, the introduction of bacteria and nutrients can affect native bacteria, and that dispersants, emulsions, and bacteria interact in complex ways to influence the characteristics of oil over time.

Kamalanathan *et al.* (2018) reported on the successful development of a robust multivariate model using Fourier Transform Infrared spectroscopy to

determine if a sample of phytoplankton has been exposed to chemically enhanced water accommodated fractions of oil. The model performed well in the controlled experiment, detecting species-specific differences in molecular responses to exposed and non-exposed plankton. The authors note that improvements in the model are needed for this method to be applied to natural plankton samples.

Two studies focused on alternatives to traditional chemical dispersants. Shah *et al.* (2018) synthesized various formulations of two choline-based dispersants, choline myristate [Cho][Myr] and choline oleate [Cho][OI], and tested their ability to disperse Tapis crude oil. The authors report that these purportedly less toxic mixtures are comparably effective to commercial products. Zang *et al.* (2018) tested the efficacy of a shrimp waste-based dispersant under a multitude of environmental conditions (salinity, mixing energy, temperature) and compare the effectiveness of this green alternative to that of Corexit 9500 using light, medium, and heavy crude oils under high (22°C) and low (04°C) temperature conditions. The authors describe comparable dispersant effectiveness at both temperatures and across the crude oil spectrum.

### **Critiques of Dispersed Oil Testing Methods**

Several authors reviewed, critiqued and offered guidance on effects testing of dispersants and dispersed oil. Bejarano (2018) critically reviewed numerous aquatic dispersant toxicity studies generated during and after the 2010 DWH oil spill. The author combined these datasets with pre-DWH studies to develop dispersant-specific species sensitivity indices. The review concluded that, in general, and under normal field conditions (i.e. proper application and dilution), modern dispersants may not pose a significant threat to most aquatic species. Bejarano also provides guidance on proper toxicity testing and reporting. Hodson *et al.* (2018) provide a critical review of oil toxicity literature focusing on the experimental design elements affecting results, their interpretation, and legitimacy. The review offers various recommendations for improving test methods including exposure regimes, WAF and CEWAF solution preparation, oil handling and storage, and proper

reporting of toxicity tests. Bera *et al.* (2018) explored two passive dosing techniques and compared their analytical utility to that of standard (CROSERF) methods of producing WAF. The authors conclude test results from passive dosing are consistent with traditional methods and describe several advantages of passive dosing methods (increased stability of dissolved oil exposure, and limit exposure to droplets).

**Disclaimer.** The information in this paper reflects the views of the authors, and does not necessarily reflect the official positions or policies of NOAA or the Department of Commerce.

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