

Effects of Pollution on Marine Organisms

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ABSTRACT: This review covers selected 2016 articles on the biological effects of pollutants and 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, dredging and disposal etc. Special emphasis is placed on effects of oil spills and marine debris due largely

Mexico. Several topical areas reviewed in the past (ballast water and ocean acidification) were dropped this year. The focus of this review is on effects, not pollutant fate and transport. There is considerable overlap across subject areas (e.g. some bioaccumulation papers may be cited in other topical categories). Please use keyword searching of the text to locate related but distributed papers. Use this review only as a guide 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 pollution, Arctic, Antarctic, microbes, plankton, invertebrates, fish, birds, turtles, mammals, marshes, mangroves, inter-tidal.

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to the 2010 Deepwater Horizon oil blowout in the Gulf of

Bioaccumulation and Biomagnification

Tabular Data. Table 1 lists examples of the concentration of 15 trace elements and 14 organic contaminants in tissues of marine plants and animals in 2016. Data are presented alphabetically by element, then by organic compound and arranged by geographical area, and concentration and author. Whenever possible the data for concentration are presented as $\mu\text{g/g}$ dry weight. In some cases the data are recorded as wet weight, lipid weight, or indicated it was done under experimental laboratory conditions. The dates for the reference are for 2016 unless otherwise stated. Following are reviews of selected papers that report on body burdens, bioaccumulation and biomagnification.

Below are reviews of selected additional papers that report on marine animal concentrations of radionuclides, pharmaceutical compounds and other organic contaminants.

Radionuclides. Wada et al. (2016) reported the results of monitoring data collected between 2011 and 2015 to characterize the changes in radiocesium contamination in marine products following the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident. The highest concentrations of radiocesium were found in demersal fish, especially those living in shallow areas south of the FDNPP, compared to pelagic fish and other taxa. Demersal fish showed a slower than expected decreasing trend of radiocesium concentration, though concentrations were dramatically reduced after five years in most fish. Rates of radiocesium decline in multiple species were similar inside and outside of the FDNPP port, with some species-specific

differences based on eco-physiological differences and contamination of the surrounding habitat. The authors note their results suggest a low or negligible risk from fish consumption at present. Marine animals, seawater and sediment were contaminated with radiocesium also from the FDNPP accident (Wang et al. 2016). Assimilation of ^{137}Cs was 16% in polychaetes feeding on sediment, up to 56% in crabs fed polychaetes, and 80% in fish feeding on polychaetes. Loss of ^{137}Cs was faster in polychaetes and slower in fish.

Radiocesium originating from the 2011 FDNPP accident was measured in the fur sea seal and the marine species of wild celery off Alaska (Ruedig et al. 2016). ^{134}Cs and ^{137}Cs were below detectable limits in the wild celery. Radiocesium was present in low levels in the fur seal which is known to migrate from Alaska to Japan. Radiocesium was also measured in three species of fish with different feeding habits off Hong Kong by Pan et al. 2016. Uptake was greater in omnivorous fish than carnivorous species, but both feeding types showed a potential to biomagnify ^{137}Cs .

Isotopes of Sr, Pb, Po and Ra were measured in three species of seaweeds and mussels collected from the Black Sea (Nonova and Toshiro, 2016). Concentrations were lowest for ^{90}Sr and highest for all isotopes of radon. The data for mussels fell with these levels. The sources of radionuclides were from the rivers emptying in the Black Seas and from aerial fallout.

Heavy Metals. El Nemt et al. (2016) conducted detailed assessment of heavy metal concentrations in nearly 30 species of harvestable mollusks from 15 sites along the

Egyptian Red Sea Coast: ranges of values are shown in Table 1. Heavy metals were measured in sediment and marine organisms collected in 2003 from Admiralty Bay, Antarctica (Trevizani et al. 2016): sediment contained high concentrations of copper and zinc. Bioaccumulation of arsenic and zinc were noted in biota. A unique study by price et al. (2016) reported extremely high concentrations of Arsenic in hydrothermal vent gastropods.

See Table 1 for a more extensive list of values from selected papers.

Pharmaceuticals. Pereira et al. (2016) measured the concentrations of pharmaceuticals in a submarine outfall sewer located in a Brazilian coastal zone. Seven pharmaceuticals were present including, for example, ibuprofen and the illicit drug cocaine. Concentrations ranged from ngL^{-1} to μgL^{-1} indicating that these amounts are of environmental concern in a subtropical ecosystem. Twenty pharmaceuticals were analyzed in shellfish and fish from a coastal lagoon in Spain (Moreno-Gonzalez et al., 2016). Laboratory studies were also conducted using snails, clams and two species of fish. Concentrations were higher near the discharges in the lagoon. Concentrations were lower in snails than clams, and lower in gobies compared to mullets. As a result of the field and laboratory studies, it was considered that the mullet were serve as a biomonitor for pharmaceuticals

Other Organic Chemicals. The uptake of PAHs in transplanted oysters was used to monitor sewage discharges in Brazil (Lourenço et al. 2016). The concentrations of PAHs reached levels up to 1105 ng g^{-1} near the discharge but were not detected at the reference

site. The results indicated the value of the use of transplanting animals to monitor waste discharges.

Dahlberg et al. (2016) measured the concentrations of hydroxylated polybrominated diphenyl ethers and related brominated substances (i.e., polybrominated phenols, polybrominated anisoles, methoxylated polybrominated diphenyl ethers, and polybrominated diphenyl ethers) in herring *Clupea harengus membras* from two locations (Askö and Ängskärsklubb) in the Baltic Sea.

Marine mammals bioaccumulate and biomagnify persistent organic compounds (POC). Arias et al. (2016) reviewed the PCBs concentrations in Franciscana dolphins off Argentina. This dolphin has been used as an environmental monitor in the southern Atlantic Ocean. Levels of PCBs were lower than measured 20 years ago which is attributed to the international elimination program. Blubber was sampled from dolphins collected off Argentina by Durante et al. (2016). POCs were the dominant compounds followed by PCBs. There were differences in the amount of POCs in the common dolphin and Franciscana dolphin. Please see Table 1 for more citations.

Toxicity Methods And Protocols

Everaert et al. (2016) examined the growth rate of algae *Phaeodactylum tricorutum* in a 72-h algal growth inhibition test using a full factorial design with three nutrient regimes, two test temperatures, three light intensities, and three chemical exposures. Passive samplers were used to achieve exposure to mixtures of organic materials close to ambient concentrations. Nutrient regime,

temperature, and exposure time interval explained 85 percent of the observed variability in the experimental data and the variability explained by chemical exposure was about one percent. Libralato et al. (2016a) reviewed toxicity testing protocols and endpoints for brine shrimp *Artemia spp.* They examined endpoints, toxicants, and experimental design, including replicates, exposure times, number of exposed organisms, life stages, exposure conditions during hatching and testing, types of test chambers, quality assurance, and quality control criteria. They highlighted similarities and differences between approaches and identified *Artemia spp.* protocols they think should move forward with international standardization.

Pérez et al. (2016) used three limpet species (*Patella vulgata*, *P. depressa*, and *P. ulyssiponensis*) as model organisms in ecotoxicological tests. They described their procedures, successes, and challenges that remain in the development of their test protocols. Morroni et al. (2016) proposed improvements to the sea urchin embryo toxicity test based on the analysis of two developmental stages, at 24 and 48 h post-fertilization, and the differentiation between developmental delays and germ layer impairments. Their new index integrates the frequency of abnormal embryos with the severity of such abnormalities.

Toxicity Of Metals

Arsenic. Yu et al. (2016) examined valence-specific responses of As in juvenile mussels *Mytilus galloprovincialis* using a combined metabolomic and proteomic approach. Metabolic responses showed that

As (III) mainly caused disturbance in osmotic regulation and As (V) caused disturbances in osmotic regulation and energy regulation. As (III) elicited a proteomic response that exhibited a negative effect on cytoskeleton and cell structure and As (V) affected key enzymes involved in energy metabolism and cell development.

Copper. Araújo et al. (2016) used Cu as the contaminant as they studied avoidance response demonstrated by fish fry (cobia, *Rachycentron canadum*) and shrimp larvae (*Litopenaeus vannamei*). Avoidance of about 60 percent was observed for the fry at 1.0 mg Cu/L and about 80 percent by the larvae at 0.1 mg Cu/L. Manfra et al. (2016) carried out 24-48 h swimming speed alteration (EC50) and mortality (LC50) tests using *Artemia franciscana* as the test organisms and Cu sulfate pentahydrate, sodium dodecyl sulfate, and diethyl glycol as the toxicants. Llagostera et al. (2016) exposed seagrass *Cymodocea nodosa* to Cu for 21 d under laboratory conditions. At 5 mg/L Cu, they observed decreased quantum yield, increased leaf necrosis, and decreased shoot growth and shoot recruitment, while these effects were almost absent at 1 mg/L Cu. Jorge et al. (2016) exposed clams *Mesodesma mactroides* to Cu (1.6 µmol/L) for 96 h. They measured tissue Cu accumulation (hemolymph, gill, and digestive gland), hemolymph ionic (Na⁺, K⁺, Mg²⁺, and Ca²⁺) and osmotic concentrations, tissue (gill and digestive gland) ionic concentration, enzyme (Na⁺, K⁺, ATPase, and carbonic anhydrase) activity, and oxygen consumption. They evaluated succinate dehydrogenase activity in mitochondria isolated from gills and digestive gland. Their results indicated that Cu is an ionoregulatory

toxicant in *M. mactroides*. Weaver et al. (2016) measured the expression of mitochondrial gene cytochrome c oxidase I and antioxidant gene glutathione reductase, and production of red ketocarotenoid (astaxanthin) in response to sublethal Cu exposure of copepods *Tigriopus japonicus*. They found that mRNA of cytochrome c oxidase I and glutathione reductase was more abundant in Cu-exposed copepods and that Cu-exposed copepods produced less astaxanthin than controls.

Van Dam et al. (2016) used barnacle larvae (*Amphibalanus amphitrite*) in a 96-h test to examine the toxicity of Cu, Al, Ga and Mo. The larvae were sensitive to Cu and Al, but Ga and Mo did not elicit discernible effects. Holan et al. (2016) conducted 7-d toxicity tests using Cu, Zn, and Cd as the toxicants and six sub-antarctic invertebrates as the test organisms; *Gaimardia trapesina* (bivalve), *Harpacticus sp.* (copepod), *Tigriopus angulatus* (copepod), *Obrimoposthia ohlini* (flatworm), *Pseudopsolus macquartensis* (sea cucumber), and *Anasterias directa* (sea star).

Lithium. Ruocco et al. (2016) used sea urchins *Paracentrotus lividus* as test organisms in their investigation of lithium chloride. They found that sea urchin eggs exposed to Li (1-80 mM LiCl) prior to fertilization resulted in developmental malformations in embryos in a dose-dependent manner. They subsequently used real time qPCR expression levels for 37 genes to identify the molecular targets of LiCl.

Lead. Hariharan et al. (2014) conducted acute (96-h) and chronic tests (30-d) using Pb as the toxicant and mullet (*Mugil cephalus*) and perch (*Terapon jarbua*) as the

test organisms. The exposure to Pb rapidly induced changes in enzyme activity (catalase, glutathione) and histology (gill, liver, intestine).

Zinc. Niyogi et al. (2016) studied the effects of salinity (20%, 60%, and 100% seawater) on short-term Zn accumulation and sublethal toxicity in euryhaline crabs (*Carcinus maenas*). The maximum accumulation of Zn occurred in 20% seawater and gills appeared to be the primary site of Zn accumulation. The results indicate that acute exposure to Zn leads to disruption of Zn and Ca homeostasis in *C. maenas*, and these are more obvious at lower salinity.

Combined Metals. Boukadida et al. (2016) studied the effects of various temperatures (18, 20, 22, 24°C) on Cu and Ag toxicity (separate and combined) to mussels (*Mytilus galloprovincialis*) in 48-h embryolarval development tests. Higher temperatures appeared to increase the toxicity of both Cu and Ag. Using binary mixtures of Zn and Ni, de Figuerêdo et al. (2016) investigated whether the two metals act additively or interact with each other inside the mysid *Mysidopsis juniae*. Their results show that the metals acted additively. Fetters et al. (2016) examined the mobilization of Zn, Cu, Cd, Pb, Ni, and Cr during resuspension of a freshwater and two marine sediments and whether resuspension and redeposition resulted in toxicity to the experimental organisms: *Hyalella azteca* (amphipod, survival), *Daphnia magna* (cladocera, survival), *Neanthes arenaceodentata* (polychaete, survival, growth, tissue metal concentration), and *Pyrocystis lunula* (dinoflagellate, bioluminescence).

Overall, the net release of metals from suspended particles was limited and toxicity to organisms was minimal.

Toxicity Of Nano Materials

Deryabin et al. (2016) conducted a comparative analysis of four luminescent sensor strains to the toxic effect of 10 carbon-based nanomaterials and 10 metal nanomaterials. The bioluminescent inhibition assays with marine *Photobacterium phosphoreum* and recombinant *Escherichia coli* strains were varied in minimally toxic concentrations and EC50 values. The sensor strain *Bacillus subtilis* EG168-1 showed the highest sensitivity to carbon-based nanomaterials and metal nanomaterials. Saggese et al. (2016) investigated the responses to 5 nm metallic Ag nanoparticle exposure in the mussel *Brachidontes pharaonis*. In an 8-d mesocosmal exposure at three sublethal nanoparticle concentrations, changes in mussel respiration rate, heartbeat rate, and absorption efficiency were observed. Huang et al. (2016) studied the effects of Ag nanoparticles on diatoms *Skeletonema costatum*. They focused on effects on photosynthesis and associated mechanisms and observed induction of excess intracellular reactive oxygen species, reduced cell viability, reduced chlorophyll-a content, adherence of Ag nanoparticles to the cell surface, and genes of the photosystem II reaction center protein were down regulated. Magesky et al. (2016) examined the physiological states, main uptake routes, and cellular response to polymer-coated Ag nanoparticles (0.19-4.64 mM as Ag) in developmental stages of sea urchins *Strongylocentrotus droebachiensis*. Chronically exposed

metamorphic larvae had their morphogenic processes interrupted by polymer-coated Ag nanoparticles. Fluorescent markers showed that Ag nanoparticles could be transferred between consecutive developmental stages (swimming larvae and postlarvae). Gambardella et al. (2016) reviewed morphological and biochemical markers of stress in sea urchin (*Paracentrotus lividus*) life stages exposed to nanomaterials. They summarized submicroscopic particles, nanomaterials in the marine environment, sea urchins in nanoparticle assessment, monitoring for nanomaterials using sea urchins, toxicity tests, and distribution of nanomaterials inside sea urchins. Jemec et al. (2016) conducted an interlaboratory comparison of nanosilver characterization and hazard identification where they harmonized their methods and materials. The investigators presented a dataset of toxicity values and silver nanoparticle characteristics (i.e., hydrodynamic sizes of silver nanoparticle agglomerates and the share of Ag+-species). They used the following test organisms: rainbow trout (*Oncorhynchus mykiss*) intestinal cells in vitro, bacteria (*Vibrio fischeri*), protozoa (*Tetrahymena thermophile*), freshwater green algae (*Pseudokirchneriella subcapitata*), freshwater crustaceans (*Daphnia magna*), brine shrimp (*Artemia franciscana*), and freshwater fish (*Danio rerio*). The toxicity tests showed the same hazard ranking for silver nanoparticles and silver nitrate, specifically, the EC50 values were in the same order of magnitude.

Thakkar et al. (2016) studied the effects of single walled carbon nanotubes (concentration range of 0.1-20 mg/L) that had been carboxylated by microwave assisted

acid oxidation on marine algae *Dunaliella tertiolecta*. They evaluated growth, photosynthetic activities, oxidative stress, and intracellular glutathione in the algae, and physical interactions between the nanotubes and algae. Lee et al. (2016) examined cellular-damage responses to multi-walled carbon nanotubes using copepods *Tigriopus japonica* as test organisms. They observed that exposure to multi-walled carbon nanotubes resulted in down-regulation of reactive oxygen species and enzymatic activities of glutathione S-transferase and catalase, and induced extracellular signal-regulated kinase activation without p38 and c-jun terminal kinase activation.

Rocha et al. (2016) investigated histopathological alterations and inflammatory responses induced by Cd-based quantum dots in comparison with their dissolved counterparts in mussels *Mytilus galloprovincialis*. Mussels were exposed to Cadmium Telluride Quantum Dots and dissolved Cd at the same concentration (10 µg Cd/L) for 14 d and 15 histopathological alterations and 17 histomorphometric parameters were analyzed in the digestive gland along with the determination of histopathological condition indices. Mussel response to quantum dots was more related to exposure time, inflammatory conditions, and changes of cell-type composition when compared to dissolved Cd. Zhou et al. (2016) investigated the effect of CdSe/ZnS quantum dot nanoparticles on the growth rate of four microalgae (*Phaeodactylum tricoratum*, *Rhinomonas reticulata*, *Isochrysis galbana*, and *Dunaliella tertiolecta*) and effects (acute 48-h, chronic 4-d) on copepods (*Acartia tonsa*). Quantum dots in the range of 1 to 4 nM inhibited growth

rates in the algae and copepods showed increased naupliar mortality with an EC50 of 0.7 nM.

Wang, Zhu, and Lao et al (2016) examined the toxicity of nanoscale titanium dioxide on phytoplankton *Phaeodactylum tricoratum*. Results showed that 12.65 mg/L could affect phytoplankton growth, and that nanoscale titanium dioxide aggregates were found to entrap algal cells and damage cell walls. Direct physical effects could be the dominant factor causing nanoscale titanium dioxide toxicity in these phytoplankton. Banni et al. (2016) investigated the interactive effects of nanosized titanium dioxide and 2,3,7,8-tetrachlorodibenzo-p-dioxins (TCDD) in mussel (*Mytilus galloprovincialis*) digestive gland using an integrated approach transcriptomics/immunohistochemistry. Mussels were exposed to nanosized titanium dioxide and TCDD, alone and in combination, for 96 h. Transcriptomic analysis identified 48, 49, and 62 differentially expressed genes in response to nanosized titanium dioxide, TCDD, and nanosized titanium dioxide/TCDD, respectively. Nanosized titanium dioxide mainly up-regulated cytoskeletal genes, while TCDD up-regulated endocrine and signal transduction processes. Co-exposure induced transcriptional changes common to individual treatments.

Gallo et al. (2016) investigated the effects of Ni nanoparticle exposure on sperm quality of ascidians (*Ciona intestinalis*). They found that Ni nanoparticles generate oxidative stress that in turn induces lipid peroxidation and DNA fragmentation, and alters the mitochondrial membrane potential and sperm morphology. These particles also affect the sperm fertilization ability and cause

developmental anomalies in the offspring. Kanold et al. (2016) characterized metallic Ni nanoparticles by X-ray diffraction, high resolution transmission electron microscopy, and energy dispersive X-ray spectroscopy, and determined the solubility of free nickel ions from 3 mg/L metallic Ni nanoparticles in seawater by inductively coupled plasma mass spectrometry over 96 h, which was below three percent. Embryonic development of fertilized sea urchin (*Paracentrotus lividus*) eggs was studied for 48 h in the presence of Ni nanoparticles (3 mg/L) and no lethal effects were observed, although there was a reduction in size. Taze et al. (2016) evaluated the oxidative responses of mussels (*Mytilus galloprovincialis*) after exposure to Fe oxide nanoparticles and Fe oxide nanoparticles incorporated in zeolite for 1, 3, and 7 d. They found that both iron oxide particles induced oxidative stress in hemocytes as shown in increased reactive oxygen species production, protein carbonylation, lipid peroxidation, ubiquitin conjugates, and DNA damage.

Toxicity Of Organic Materials

Surfactants. Jackson et al. (2016) summarized marine environmental fate (monitoring, biodegradation, bioconcentration) and effects data of five surfactant groups: linear alkylbenzene sulfonates, alcohol ethoxysulfates, alkyl sulfates, alcohol ethoxylates, ditallow dimethyl ammonium chloride.

Anti-fouling Agents. Hannachi et al. (2016) conducted a long-term study to examine the effects of increasing biocide (Irgarol) concentrations (11.5 to 315 ng/g sediment dry weight) on meiofauna and benthic

prokaryotes. Irgarol impacted meiofaunal abundance, causing a decline in nematode abundance and an increase of the relative importance of oligochaetes. The molecular fingerprinting analysis showed that prokaryotic diversity was altered by the biocide. Liu et al. (2016) examined the natural degradation and ecotoxicity of the marine antifoulant Nonivamide. They studied photolysis and biolysis of Nonivamide in seawater, and generated 6-d EC50 growth data using marine microalgae, *Chlorella vulgaris* and *Platymonas sp.* Using Dubrovnik Port as the case study, Caric et al. (2016) presented an approach to assess antifouling environmental risk by combining calculations of antifouling emission and pollution cost, using data available to port authorities, and recognizing ecotoxicological endpoints that serve as indicators of environmental threat.

Benzo(a)pyrene. Jiang et al. (2016) investigated the embryo transcriptomic profile of mussels *Perna viridis* treated with benzo(a)pyrene via digital gene expression analysis. A total of 92,362,742 reads were produced from two groups, control and benzo(a)pyrene exposure, by whole transcriptome sequencing. The investigatory found whole transcriptome sequencing for transcriptome profiling of mussel embryos can contribute to better understanding benzo(a)pyrene's toxic effects on mussel embryos.

Bisphenol. Balbi et al. (2016) studied the effects of bisphenol A on mussel (*Mytilus galloprovincialis*) embryos at the molecular level by evaluating transcription of 13 genes that were selected based on their biological functions in adult mussels. Gene expression was first evaluated in trochophorae and D-veligers (24 and 48 h post

fertilization) in comparison with fertilized eggs. Exposure of fertilized eggs to bisphenol A (10 µg/L) induced general up-regulation at 24 h followed by down-regulation at 48 h. Estrogen receptors, serotonin receptor, and genes involved in biomineralization were the most affected by bisphenol A exposure. At 48 h, changes in gene expression were associated with irregularities in shell formation as shown by scanning electron microscope.

Chlorophenols. Noack et al. (2016) conducted 24-h and 48-h LC50 toxicity tests on copepods *Acartia tonsa* using 3,5-dichlorophenole as the toxicant. Vlastos et al. (2016) used bacteria (*Vibrio fischeri*), micronuclei induction in the erythrocytes of fish (*Carassius auratus*), and the cytokinesis block micronucleus assay in cultured human lymphocytes to examine toxic and genotoxic effects of 2-chlorophenol. They found that 2-chlorophenol induced dose-dependent genotoxic effects on selected tested concentrations under the specific experimental conditions.

Organochlorines. Nacci et al. (2016) provided an accounting (69 percent) through quantitative trait locus analysis of the genetic basis for dioxin-like compounds tolerance in killifish (*Fundulus heteroclitus*) inhabiting an urban estuary contaminated with PCB congeners, the most toxic of which are dioxin-like compounds. Consistent with mechanistic knowledge of dioxin-like compounds toxicity to fish and other vertebrates, aryl hydrocarbon receptor region accounts for 17 percent of trait variation; however, quantitative trait locus analysis on independent linkage groups and their interactions showed even greater explanatory power (44 percent).

Complex Organics Mixtures. Lemaire et al. (2016) examined the influence of high hydrostatic pressure on the effects of xenobiotics in the European seabass (*Dicentrarchus labrax*). Liver slices of *D. labrax* were co-exposed to the aryl hydrocarbon receptor agonist 3-methylcholanthrene at hydrostatic pressure levels representative of surface and deep-sea environments. High hydrostatic pressure precluded the aryl hydrocarbon receptor agonist-mediated increase of CYP1A mRNA expression, as well as that of glutathione peroxidase and reduced that of heat shock protein 70. Echeveste et al. (2016) examined the toxicity of naturally occurring complex mixtures of organic pollutants to temperate and polar phytoplankton communities. They monitored the cell abundance of different phytoplankton groups, chlorophyll a concentrations, viability of cells, and growth and decay constants in response to the addition of a range of concentrations of mixtures of organic pollutants obtained from seawater extracts. Almost all the phytoplankton groups were affected by the complex mixtures of non-polar and polar organic pollutants with toxicity being greater for these mixtures than for single persistent organic pollutants or simple organic pollutant mixtures.

Toxicity Of Contaminants Of Emerging Concern

Pharmaceuticals. Minguez et al. (2016) presented a risk assessment for freshwater and marine ecosystems for 48 pharmaceutical compounds, belonging to 16 therapeutic classes. Ecotoxicity data were obtained for freshwater (*Daphnia magna*, 48-h EC50 and *Pseudokirchneriella subcapitata*, 72-h EC50) and marine

species (*Artemia salina*, 48-h EC50 and *Skeletonema marinoi*, 72-h EC50). Measured environmental concentrations in the sea off Merville-Franceville (France) were compared to predicted environmental conditions. Predicted no-effect concentrations were derived from acute data for each compound and a risk assessment for each compound and the mixture was performed by calculating risk quotients. González-Ortegón et al. (2016) studied the combined effects of selected pharmaceutical compounds (diclofenac sodium, clofibric acid, clotrimazole) and environmental variability (salinity and temperature) on survival, development, and body mass of shrimp larvae (*Palaemon longirostris*). Clofibric acid affected development through an increase in intermolt duration and reduced growth without affecting larval body mass.

Anti-Androgenic Compounds. Watermann et al. (2016) exposed copepods *Acartia tonsa* to environmental concentrations of anti-androgenic compounds for 21 d, covering a full life cycle, and observed histological alterations in reproductive organs. The anti-androgenic compounds used were cyproterone acetate, linuron, vinclozolin, and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE). The authors observed: degeneration of spermatocytes, deformation of the spermatophore, ovotestis, enhancement of oogenesis in female, impaired spermatogenesis, and other effects.

Dumped Chemical Weapons. Greenberg et al. (2016) produced a review article on the environmental risks and occupational hazards of sea-dumped chemical weapons. They addressed the following: history and

geography of chemical weapons dumping at sea; chemical warfare agents; environmental concerns; human health concerns; and their conclusions.

Herbicides. Filimonova et al. (2016) examined the effects of the herbicide Primextra® Gold TZ and Cu on the fatty acid profiles of diatoms (*Thalassiosira weissflogii*), copepods (*Acartia tonsa*), and brine shrimp nauplii (*Artemia salina*). Their results suggest that Primextra® Gold TZ may affect changes in fatty acid contents of these test organisms and thus, their nutritive value. Wang, Lin, and Li et al. (2016) examined the physiological effects of the herbicide glyphosphate on 14 species of phytoplankton. Based on growth responses to different concentrations of glyphosphate under contrasting dissolved inorganic phosphorus conditions, they found the phytoplankton could be classified into 5 groups: 1) could utilize glyphosphate as sole phosphorus source to support growth in axenic culture, but in the presence of dissolved inorganic phosphorus were inhibited by both 36 µM and 360 µM glyphosphate; 2) could not utilize glyphosphate as a sole phosphorus source to support growth, and in the presence of dissolved inorganic phosphorus was not affected by 36 µM but inhibited by 360 µM glyphosphate; 3) glyphosphate consistently enhanced growth; 4) glyphosphate consistently inhibited growth; and 5) exhibited no measurable response to glyphosphate regardless of dissolved inorganic phosphorus condition.

Toxicity Of Complex Sources And Materials

Aquaculture. Ayer et al. (2016) compared the impacts of culturing salmon *Salmo salar* in copper-alloy

net-pens to industry-average Chilean nylon net-pens. Life cycle assessment was used to quantify the environmental performance of both systems in compliance with the ISO 14040 and 14044 standards for life cycle assessment.

Wastewater. Diaz-Garduño et al. (2016) conducted toxicity tests on effluents from three different wastewater treatment plants. The three plants used similar wastewater treatment technologies. Effluent samples for toxicity testing were taken before and after tertiary treatment (which used a combination of advanced oxidation processes). Bioluminescence inhibition (*Vibrio fischeri*, bacteria), growth inhibition (*Isochrysis galbana*, algae), fertilization and embryolarval development (*Paracentrotus lividus*, sea urchins), and larval mortality (*Sparus aurata*, fish) were endpoints utilized for examining effluent toxicity. Sea urchin larval development and fish larvae mortality were the most sensitive endpoints. Libralato et al. (2016b) evaluated the ability of phytoplankton *Phaeodactylum tricorutum* to discriminate the quality of domestic, municipal, and industrial wastewater samples. They aimed to establish a wastewater effect score based on phytoplankton exposed to wastewaters from activated sludge sequencing batch and ultra-filtration membrane biological reactors. Wastewater samples produced stimulation and inhibition effects. The results showed that nitrogen and phosphorus concentrations were not correlated to ecotoxicological values.

Chlorine. Last et al. (2016) exposed polychaetes *Sabellaria alveolata* to aqueous chlorine (0.02, 0.1, and 0.5 mg/L total residual oxidant) at mean and maximum summer temperatures, 18 and 23°C., respectively. A

combination of the higher temperature and higher chlorination levels showed increased mortality in comparison to controls and the lower chlorination level.

Ammonia. Kir et al. (2016) exposed fish (meagre, *Argyrosomus regius*) to different ammonia concentrations at three temperature levels. The fish were more tolerant of total ammonia nitrogen and unionized ammonia at lower temperatures. Bardou-Albaret and Saillant (2016) studied the effects of hypoxic conditions and elevated ammonia on the viability of red snapper (*Lutjanus campechanus*) embryos and newly hatched larvae. They found variation among different spawns and tolerance to hypoxia or elevated ammonia.

Ocean SAcidification/pH. Jager et al. (2016) applied a simplified dynamic energy budget model to previously published data on growth, feeding, and respiration of larval sea urchins (*Strongylocentrotus droebachiensis*) exposed to a range of pH values. They found the simplified dynamic energy budget model provided good explanation of larval traits over development from egg to maximum larval size. The observed effects of decreasing pH were best explained by the hypothesis that ocean acidification increases the maintenance costs of the larvae. In this study, the stress factor for ocean acidification shows an apparent tipping point around a pH of 7.5.

Adsorbents. Park et al. (2016) used Microtox assays to investigate the toxicity of adsorbent materials used to extract uranium from seawater. To test the potential toxicity of the adsorbent materials with or without bound metals, the Microtox assays were used to test both direct

contact toxicity and the toxicity of any leachate in the seawater. No toxic effects were observed for the adsorbents.

Photovoltaic Panels. Motta et al. (2016) investigated the potential toxicity of exhausted photovoltaic panels. They broke an exhausted panel into pieces and placed these pieces in water for 30 d. Crustaceans (*Daphnia magna* and *Artemia salina*) and sea urchins (*Paracentrotus lividus*) were exposed to the leachate and developmental effects were observed in the test organisms.

Applications Of Toxicity Data

Dong et al. (2016) attempted to develop globally applicable and spatially differentiated marine comparative toxicity potentials or ecotoxicity characterization factors for metals (Cd, Cr (III), Co, Cu(II), Fe(III), Mn, Ni, Pb, and Zn) in 64 large marine ecosystems for use in life cycle assessment. Their toxicity potentials are based on marine ecotoxicity data and account for metal speciation and bioavailability. Their results showed that the comparative toxicity potentials of a specific metal varies 3-4 orders of magnitude across large marine ecosystems, largely due to different seawater residence times. The highest toxicity potential for metals was found in the large marine ecosystems with the longest seawater residence times.

Machado et al. (2016) proposed an overview and conceptual model for the environmental fate of metals and their toxicity effects on aquatic organisms in estuaries. Their proposal includes the analysis and integration of biogeochemical processes and parameters, metal chemistry, and organism physiology. In 2011, 2012, and 2013, using

field observations in the intertidal zones of eastern Japan, Horiguchi et al. (2016) investigated the ecological effects of the Fukushima Daiichi Nuclear Power Plant accident that accompanied the 2011 Great East Japan Earthquake and Tsunami. They found the number of intertidal species had decreased with decreasing distance from the power plant and that *Thais clavigera* were not found near (approximately 30 km) the plant in 2012. They discussed factors (e.g., tsunami, radionuclides) that may have contributed to the change in numbers of species.

Balassone et al. (2016) used a multi-disciplinary approach, consisting of geological and biological studies, to assess the environmental status of the Falerno-Domitio littoral (Italy). They observed that a relationship between meiobenthos and Cr, Co, and V may exist. Mali et al. (2016) proposed a new hazard index for assessing sediments, the cumulative Normalized and Weighted Average Concentration index, that considers concentrations of hazardous, toxic, and bioaccumulative sediment contaminants. They verified their proposed index using a dataset derived from 42 samples collected at the Port of Bari (Italy) and by testing the samples by ecotoxicological assays using three species (*Vibrio fischeri*/bioluminescence, *Paracentrotus lividus*/fertilization, and *Phaeodactylum tricorutum*/growth inhibition) and comparing the test results to corresponding categories developed for the cumulative Normalized and Weighted Average Concentration index.

Moschino et al. (2016) evaluated environmental conditions in the Bay of Muggia (Italy) using two lysosomal biomarkers, lysosomal membrane stability and

lipofuscin accumulation in hepatopancreas cells, in mussels (*Mytilus galloprovincialis*). Mussels were transplanted to the Bay twice yearly for five years. The transplanted mussels were examined and the biomarkers were found to be appropriate for determining the responses of mussels to environmental pollutant loads over time, and more specifically, the variations in lysosomal membrane stability and lipofuscin content were mostly related to environmental total polycyclic aromatic hydrocarbons and metals, respectively. Gu et al. (2016) characterized the distribution, sources, and potential risks of polycyclic aromatic hydrocarbons (PAHs) in the surface sediments of Zhelin Bay (China). Total concentrations of PAHs ranged from 29 to 815 ng/g (dry wt), with the composition characterized by an abundance of low molecular weight PAHs. Testing their methodology in a salt marsh affected by agriculture effluents, Silva et al. (2016) demonstrated a weight of evidence approach to assess environmental quality and ecological risk of contaminated sites. Using spatial modeling tools and fuzzy logic and multi-criteria analysis, they accounted for four lines of evidence: 1) physico-chemical characteristics of water and sediment; 2) acute toxicity bioassays; 3) biomarkers; and 4) in situ alteration of benthic communities.

Field Studies Using Biomarkers

As noted in the section above, biomarkers are seeing increasing use as toxicity endpoints. Additional field studies follow below.

Invertebrates. A number of the biomarker field studies published in 2016 focused on refinements to

increase reliability and relevance of biomarker results, including developing a regression model to establish a baseline assessment criterion, quantifying annual and seasonal variability that may affect long-term studies, and relating biomarker results to results from other exposure regimes and lines of evidence.

Barrick et al. (2016) used data from the polychaete *Hediste diversicolor* collected from Authie estuary in France to identify a formula describing the relationships between the energetic reserves (glycogen and lipids) and confounding factors such as size, temperature and salinity. They then used the formula to predict glycogen and lipid levels expected, based on confounding factors, in polychaetes at a site in the Seine estuary. This helped them develop a graph that showed which values fell within an expected baseline (baseline assessment criterion), and which were likely to be associated with sublethal or lethal effects (environmental assessment criteria). They found that the formula was effective at describing the values for lipids but not for glycogen, and other environmental components, like food availability and reproductive cycles, may be necessary to adequately characterize the glycogen baseline.

Annual and seasonal variability were examined in several studies. Helmholz et al. (2016) transplanted mussels (*Mytilus spp.*) to Helgoland Island and Cuxhaven in the Elbe estuary, Germany, over three time periods lasting from 8 to 12 months each, sampling every 6 weeks, to analyze the repeatability and robustness of the resultant data. Condition index (CI), gonadosomatic index (GSI), and carbohydrates showed reproducible, sometimes seasonal,

trends that were distinct between locations, along with lipids and proteins to a lesser extent. Mitochondrial electron transport system activity was not reproducible. The authors recommended at least three point sampling over one year, including after a high-consumption phase (summer), after starvation (winter), and after potential reproduction (spring). Breitwieser et al. (2016) sampled the variegated scallop *Mimachlamys varia* at four sites during two seasons (before and after reproduction) along the Atlantic coast of France to investigate links between tissue levels of trace metals and organics, biomarkers, and population genetic diversity. Except for lipid peroxidation (LPO, oxidative cell damage biomarker), all biomarkers differed among sites in March (laccase activity [immune system biomarker], superoxide dismutase activity [SOD, antioxidant response biomarker], glutathione-S-transferase activity [GST, phase II detoxification and antioxidant biomarker], citrate synthase activity [mitochondrial respiration biomarker], phosphatase activity [general metabolism biomarker]), while only three showed differences in September (laccase activity, LPO, and SOD). Cadmium levels were significantly linked to effects on laccase activity and LPO, and organics were linked to effects on SOD, GST, laccase, citrate synthase and phosphatase activity. Genetic diversity was lowest at sites impacted by heavy metal pollution. Ramos et al. (2016) sampled the goose neck barnacle (*Pollicipes pollicipes*) monthly from the intertidal zone of the Douro river estuary in Portugal. Peduncle, cirri and haemolymph GST activity were higher in warmer months, as was LPO and AChE activity in the same tissues along with total hemocyte

count, but glycogen levels were significantly lower in warmer months. Madeira et al. (2016) collected the shrimp *Palaemon elegans* from tidepools in Cabo Raso, Cascais, Portugal in April and June to determine the effects of thermal stress on several biomarkers. Total ubiquitin (a heat stress biomarker involved in protein degradation), CAT, SOD, GST, LPO, and potassium all varied between spring and summer, suggesting that protein denaturation, oxidative stress, and ionic regulation may all be affected by season, which should be taken into account in environmental monitoring programs.

Comparative studies looked at both exposure regime and lines of evidence. Taylor and Maher (2016) examined results from Sydney cockles (*Anadara trapezia*) collected wild, transplanted (56d), and laboratory-exposed (56d) to sediments along a metal concentration gradient from a zinc-lead smelter site in Lake Macquarie, Australia. Total antioxidant capacity reduction, LPO, and lysosomal membrane stability (LMS, a biomarker for lysosomal cell death) reduction were strongly correlated with tissue metal concentrations in all three exposure types. While most biomarker responses were similar between transplanted and laboratory-exposed cockles, resident cockles had somewhat lower LMS reduction and significantly higher LPO than the other two exposure types. Condition index was significantly lower in the laboratory-exposed cockles than resident and transplanted cockles. De los Ríos et al. (2016) examined the relationships between five lines of evidence (contaminants in water, sediments and transplanted mussels, biomarkers in transplanted mussels and macroinvertebrate benthic communities) measured in

previous studies in 5 test and 3 reference sites in the Bay of Biscay on the North Iberian Peninsula. The most significant correlations existed between contaminants in water and biological effects (biomarkers and macroinvertebrate communities), followed by correlations between contaminants in mussels and biological effects. Levels of contaminants in sediments were not significantly correlated with biological effects. Polycyclic aromatic hydrocarbon (PAH), Bisphenol A, and perfluorinated compound concentrations in water were the most frequently correlated with biological effects. Metals and polychlorinated biphenyls (PCBs) in mussel tissue were correlated mainly with biomarkers. Among biomarkers, vitellogenin (VTG, an endocrine disruption biomarker) showed the highest number of significant correlations both with contaminants in water and mussels but not with response at the community level; LPO was the biomarker that correlated best with macroinvertebrate community indices. Tsangaris, Kaparou et al. (2016) examined relationships between four lines of evidence (biomarkers in harbor crabs [*Liocarcinus depurator*], metal contaminant levels in sediment and crabs, and benthic community indices) measured at several distances from a ferro-nickel slag disposal site in the North Evoikos Gulf, Greece. Nickel and Cr sediment concentrations were above effects range – median levels and Cu was above the effect range – low value in the disposal area. Catalase and especially GST activities, but not AChE activity, decreased with increased tissue metal concentrations as did the ratio of sensitive versus opportunistic benthic species. Cephalothorax/hepatopancreas index was higher in the disposal area,

however. By comparing metabolic responses in the shrimp *Crangon affinis* collected at two metal-contaminated sites and a reference site in Laizhou Bay, China, Xu, Ji et al. (2016) were able to tease out differential effects caused by the variable contaminant loads. Shrimp from both the site with Cd and As as the main metal contaminants and the site with Cu as the main metal contaminant had disturbances in osmotic regulation and energy metabolism and reduced anaerobiosis, lipid metabolism and muscle movement. But altered levels of osmolytes and decreased levels of arginine and glutamine indicated that the Cu-dominant site induced osmotic stress through different pathways than the Cd and As-dominant site.

Multiple Phyla. Two studies compared biomarker responses in several potential sentinel species for field efforts. A study by Tsangaris, Moschino et al. (2016) spanning the Adriatic, Aegean, Levantine and Black Seas aimed to investigate 1) whether the responses to pollution of a suite of biochemical biomarkers were consistent across study areas in a well-recognized sentinel species (*Mytilus galloprovincialis*); 2) to compare the biomarker responses in alternative sentinel species to those of *M. galloprovincialis* for areas in which the mussels are not found; 3) compare environmental stress levels across study areas by integration of biomarker responses using the “Integrated Biological Response version 2” (IBRv2) index. Responses in *M. galloprovincialis* catalase (CAT, an antioxidant biomarker), acetylcholinesterase (AChE, a neurotoxicity biomarker), and GST activity were consistently measured at impacted sites versus reference sites within the different geographical areas, but

metallothionein (MT, a metal response biomarker) responses were only measured at two sites exceeding effects range – low guidelines for metals. Catalase and AChE activities were consistently lower at impacted sites, whereas GST was either lower or higher compared to reference. Baseline levels of biomarkers were inconsistent across regions, however, making it difficult to compare values between geographical areas. Similar results were obtained in the mussel *Brachiodontes pharaonis*, and for CAT and MT in the veined whelk *Rapana venosa*, but results were inconsistent for the white seabream (*Diplodus sargus sargus*) and red striped mullet (*Mulhus surmuletus*) fish. Index IBRv2 results did not fully correspond to the characterization of sites by contaminant levels, but the highest stress levels were found at the three most-contaminated sites. Laitano and Fernández-Gimenez (2016) compared the responses of three intertidal invertebrates, the mussel *Brachidontes rodriguezii*, the limpet *Siphonaria lessoni*, and the barnacle *Balanus glandula*, collected in the port Mar del Plata and the reference site Punta Cantera in Argentina. The barnacle showed the greatest difference between sites, yielding higher phenoloxidase, peroxidase (immunological biomarkers) and GST activity in the port. The mussel only exhibited an increase in GST activity, and the limpet did not exhibit any differences between sites.

Vertebrates. A few reviewed studies focused on biomarker responses in fish. Yilmaz et al. (2016) collected red mullet (*Mullus barbatus*) and thinlip grey mullet (*Liza ramada*) from Mersin Bay, Turkey, and a reference location near Tisan. Ethoxyresorufin-O-deethylase activity (EROD,

a P450 CYP1A induction biomarker indicating exposure to planar aromatic and halogenated hydrocarbons) was 2.5 to 3 times higher in Mersin Bay fish, and was positively correlated with Σ dichlorodiphenyltrichloroethane (DDT) and Σ PCB in tissues of both species. Scaled mass CI was negatively correlated with both Σ DDT and Σ PCB in *L. ramada*, but only Σ PCB in *M. barbatus*. Histological abnormalities were present in liver and gonad tissues of both species from Mersin Bay. Crespo and Solé (2016) collected juvenile sole (*Solea solea*) from the Ebre Delta in Spain for one year pre- and two years post-removal of the upstream Flix chlor-alkali plant toxic dump site. Two years after the removal, after upstream flooding events, there were significant rises in EROD and benzyloxy-4-[trifluoromethyl]-coumarin-O-debenzyloxylase (an induction biomarker for P450 CYP3A, responsible for drug metabolism) and effects on antioxidant responses including increased gill glutathione reductase (GR) and decreased CAT activities, decreased carboxylesterases (CbE, involved in phase I metabolism of xenobiotics) and increased muscular AChE activities along with a highly significant increase in hepatic LPO levels. Alves et al. (2016) studied juvenile blue sharks (*Prionace glauca*) caught as by-catch by swordfishing vessels southwest of Portugal. In muscle tissue high LPO levels were associated with 1,2,5,6,9,10-hexabromocyclododecane enantiomers, polychlorinated dibenzofurans, perfluorinated compounds and Cr, whereas high DNA damage and low glutathione peroxidase activities (GPx, an antioxidant biomarker) were associated with PCBs, polybrominated diphenyl ethers, Hg and Cd.

Blood samples from green sea turtles (*Chelonia mydas*) were collected by da Silva et al. (2016) in the area of Ubatuba, São Paulo State, Brazil to determine possible correlations between fibropapillomatosis (FB, a disease characterized by multiple tumors), metal concentrations, and oxidative stress biomarkers. Turtles afflicted with FB had elevated Cu, Fe, and Pb serum levels, elevated LPO, and reduced serum cholesterol levels and 3-hydroxy-3-methylglutaryl-CoA reductase activity (a sterol biosynthesis enzyme) compared to non-afflicted turtles.

Two reviewed papers focused on marine mammals. Recent necropsy reports have suggested a link between marine mammal strandings and naval midfrequency sonar, with the mammals experiencing symptoms similar to decompression sickness. Fahlman et al. (2016) set out to determine if microparticles (MPs), cellular fragments between 0.3 and 1 µm derived from decompression stress and associated with vascular damage in mice, would be a reliable biomarker of decompression stress in marine mammals. They measured MPs in Stellar sea lions (*Eumetopias jubatus*) diving to 5 and 50 m, and following exercise and feeding experiments to assess confounding factors. Baseline variability along with an inconsistent increase with depth or duration of dive indicated that the biomarker had low reliability in marine mammals. Fossi et al. (2016) investigated the effects of microplastics on fin whale (*Balaenoptera physalus*) populations in two basins with different levels and forms of human pressure and abundance of plastic debris, the Pelagos Sanctuary in the Mediterranean Sea, and the Sea of

Cortez in Mexico. Surveys indicated much higher abundance of microplastics in the Pelagos Sanctuary versus the Sea of Cortez. P450 CYP2B activity and hexachlorobenzene levels were higher in the skin biopsies of fin whales from the Sea of Cortez, whereas the values for PCBs, DDTs, total organochlorines, mono-(2-ethylhexyl) phthalate, LPO and CYP1A activity were all higher in whales from the Pelagos Sanctuary.

Biomarker Laboratory Studies

Libralato et al. (2016) reviewed test methods for *Artemia* species. Biomarkers in use included those for oxidative stress (GST, GR, GPx, LPO), neuronal effects (AChE), heat shock proteins (HSPs, which help protect protein structure upon exposure to environmental stressors), and osmoregulation (ATPase and aldehyde dehydrogenase), along with proteomics and an enzyme inhibition assay called Fluotox. Their order of easy-to-standardize toxicity tests was 24-48 hour mortality > 14-28 day mortality > hatching test > behavioral endpoints > biomarkers.

Laboratory Studies: Metals. Four reviewed studies assessed effects of Cd exposure on biomarker responses. Naija et al. (2016) exposed the peacock blenny (*Salaria pavo*) to 2 mg/L Cd nominal (0.12-0.18 mg/L Cd actual) for up to 15 days. In gills, transcription of metallothionein 2 (mt2) and manganese SOD (mnsod) was induced, GPx activity increased, EROD and GST activity decreased, but there was no effect on LPO and effects on gill histology could be considered moderate and reversible. In the liver, GST, GPx, CAT activity and LPO increased at

all time points, along with EROD activity on day 15 only. The liver histopathology was significantly elevated from day 4 to 10 but began to decrease on day 15, possibly indicating the development of resistance to Cd exposure. Clams (*Meretrix meretrix*) exposed to 1.5 to 12 mg/L Cd nominal for 5 days demonstrated significant apoptosis and oxidative stress in the hepatopancreas (Xia et al. 2016). Both reduced glutathione (GSH, a free-radical scavenger) and GSH/glutathione disulfide (GSSG) ratios (the ratio of reduced/oxidized glutathione) were reduced, along with GPx activity, but LPO, apoptosis ratio, and caspase-3 activity (protease that plays a critical role in the initiation and execution of apoptosis) all increased. The activity of CAT and SOD was bell-shaped, with the highest activities at the middle concentrations. Bouraoui et al. (2016) examined the interaction of Cd and Cu in polychaetes (*Hediste diversicolor*) exposed to 1 μ M Cd and Cu nominal, alone and in combination, for 48 hours. LMS was decreased equally in all exposures, neutral lipid content (NL, indicator of unbalanced fatty acid metabolism) was increased in the order of Cd < Cu < Cd/Cu, and lipofuscin content (end product of LPO) was slightly increased in the Cu exposure and greatly increased in the Cd/Cu exposure, but not affected by Cd alone. Activity of Ca²⁺-ATPase (associated with cellular energetics) was depressed in the order of Cd < Cu < Cd/Cu. Liu et al. (2016) cloned hsp90 cDNA in the mussel *Mytilus coruscus* and examined gene expression. Haemolymph expression increased upon exposure to the pathogens *Vibrio alginolyticus* and *V. harveyi*, as well as exposure to 200 μ g/L Cd, 20 μ g/L Cu, and 40 μ g/L 180 CST fuel (all nominal concentrations),

with a peak in expression at day 10 for Cd and Cu, and day 15 for 180 CST fuel.

Studies performed with other metals include an examination of Hg-induced genotoxicity in the marine diatom *Chaetoceros tenuissimus* (Sarker et al. 2016). Exposure to 30 to 120 ng/L Hg nominal for up to 20 days caused a dose-dependent decrease in DNA integrity up to day 16, followed by a slight increase on day 20. A dose-dependent decrease in growth was also observed, with virtually no growth occurring in the 90 and 120 ng/L concentrations. Freitas, Salamanca et al. (2016) exposed manila clams (*Ruditapes philippinarum*) for 96 hours to 4 to 17 mg/L As nominal at salinities of 14 to 42 ppt to determine the effects of multiple stressors on biomarkers in whole body tissue. Arsenic exposure increased protein and glycogen content at all salinities tested, and salinity increases caused a decrease in SOD and GST Ω activities at all As concentrations tested. An increase of salinity and As concentrations combined induced oxidative stress, as measured by higher LPO and total glutathione levels and lower GSH/GSSG ratios. Edge et al. (2016) exposed the deep-water Arctic-boreal sponge *Geodia barretti* to two drilling mud components, barite and bentonite, along with a reference sediment at total suspended solids (TSS) concentrations of 10 to 100 mg/L for 12 hours. Lysosomal membrane stability decreased and GSH increased upon exposure to barite but not bentonite, which may have been caused by the significantly higher concentrations of Cu, Pb and Sr in the barite. When the sponges were exposed continuously to 10-30 mg/L TSS of barite for up to 14 days LMS was again reduced, but when the sponges were

exposed intermittently, as is often the case during drilling operations, toxicity was reduced, though not removed completely. Energetics measurements, LPO and GSH were found to be unreliable indicators of toxicity in this exposure.

Laboratory Studies: Nanoparticles. Three reviewed studies examined the effects of nanoparticles on marine organisms. Marisa et al. (2016) exposed Manila clams (*Ruditapes philippinarum*) to 1 and 10 µg/L nominal of nano ZnO (nZnO) for up to 7 days and compared the results to clams exposed to ZnCl₂. Though Zn accumulation was similar in the exposures to both compounds the responses were different. Both SOD and CAT increased with increasing time in both gills and digestive gland, but SOD was more responsive to ZnCl₂ in the gills and nZnO in the digestive gland, and CAT was responsive in both tissues to nZnO but only in the digestive gland to ZnCl₂. Hemocyte proliferation was more responsive to ZnCl₂, whereas hemocyte DNA damage was more sensitive to nZnO. Teles et al. (2016) exposed gilthead seabream (*Sparus aurata*) for 96 hours to gold nanoparticles (nAu) with different surface coatings, citrate and polyvinylpyrrolidone (PVP), at nominal concentrations of 4 to 1600 µg/L. The nAu-citrate immediately aggregated in seawater, whereas the nAu-PVP maintained its characteristics, possibly increasing its bioavailability, which may explain the increased expression of hepatic gpx, cat, gst3, gr, mt, interleukin 10 (immune function biomarker), transferrin (immune function biomarker), and Bcl-2 associated X protein (apoptosis biomarker) with nAu-PVP exposure but not nAu-citrate exposure. Most

effects occurred at low and intermediate doses but not the high dose, a response frequently found for endocrine disrupting compounds. Lee et al. (2016) exposed the copepod *Tigriopus japonicus* for 96 hours to multi-walled carbon nanotubes (MWCNTs) at nominal concentrations of 6.25 to 100 mg/L, to 1 to 32 mg/L Cu nominal, and to 4 to 100 mg/L MWCNT in combination 1 to 32 mg/L Cu. In all doses excretion of MWCNT was observed, but copepods exposed to 100 mg/L MWCNT also had surface attachment of MWCNTs and a significant decrease in reactive oxygen species (ROS) generation, CAT and GST activity. A transient increase in extracellular signal-related kinase was observed, indicating that MWCNT may activate genes related to apoptosis, inflammation, and fibrosis through direct interaction with a cell surface receptor and not through a ROS pathway. The addition of MWCNT decreased both the body burden and toxicity of Cu.

Laboratory Studies: Polycyclic Aromatic Hydrocarbons. When performing biomarker studies it is important to tease out how confounding factors may affect responses to toxicants. González-Fernández, Albentosa et al. (2016) explored the effects of reproductive status on biomarker responses in mussels (*Mytilus galloprovincialis*) exposed to fluoranthene (FLU) concurrently via water (3 and 60 µg/L nominal) and food (2 and 40 µg/mm³ nominal) for 3 weeks. When clearance rate, absorption efficiency, respiration rate, accumulation, CAT, SOD, and GPx activity were combined into an “Integrated Biomarker Response” (IBR) index, values for FLU-exposed mussels did not differ much from control in the reproductive phase, however during the resting phase there was a significant

dose-related effect, which may be in part due to FLU tissue concentrations approximately double those found in the reproductive mussels. Index values for reproductive stage controls were similar to resting stage mussels exposed to the high FLU concentration, demonstrating the strong effect of reproductive stage. Fluoranthene did have a negative effect on gonad histology during the reproductive phase. González-Fernández, Lacroix et al. (2016) performed a similar experiment examining the effects of diet quality on *M. galloprovincialis* response to FLU. Mussels were conditioned for 6 weeks by feeding with either the diatom *Chaetoceros neogracile* or the dinoflagellate *Heterocapsa triquetra*, then fed for one week with either food source spiked with FLU to provide a final nominal concentration of 30 µg/L, then depurated for 1 week to assess recovery. Larger amounts of FLU were accumulated in diatom-fed mussels, and phagocytosis capacity, hemocyte ROS production, CAT, SOD and GR activity were also higher in these mussels, but when GR activity was plotted against FLU concentration in tissues, there was still a clear delineation in diatom- vs. dinoflagellate-fed mussels, indicating that food type can affect biomarker response beyond its effect on contaminant accumulation alone. The percentage of dead hemocytes was higher in dinoflagellate-fed mussels, and this effect continued after depuration. Overall the results indicate that in large-scale field studies with mussels, variance in both reproductive status and the dominant food source between sites may act as confounding factors in biomarker response.

Other studies with PAHs included a 1 week exposure of Sydney rock oysters (*Saccostrea glomerata*)

fed 5x daily with 20 mg/L/oyster of rice flour spiked with nominal 17.96 mg/kg pyrene and 17.64 mg/kg FLU (Ertl et al. 2016). RNA-Seq analysis of pooled tissues indicated a suppression of GST, pathogen recognition and some protein synthesis processes (e.g. pattern recognition receptors, DNase II, laccase 1, nuclear respiratory factor-1, 5-methylcytosine rRNA methyltransferase nsun4) and an enhancement of PAH detoxification and clearance of cellular debris (e.g. Carbonyl reductase [NADPH], cytochrome P450s 49a1, 3a24-like, 3A11, uracil-DNA glycosylase, caspase-7). Higher concentrations of FLU than pyrene were measured in oyster tissues, suggesting either higher uptake of FLU or faster metabolism of pyrene. Chen et al. (2016) exposed pearl oysters (*Pinctada martensii*) to 1 and 10 µg/L nominal benzo[a]pyrene for 1 week to determine effects on proteins and metabolites in the digestive gland. Altered proteins included those involved in cytoskeleton, cell injury, oxidative stress, signal transduction and energy metabolism, such as actin, cathepsin L, proteasome, non-neuronal cytoplasmic intermediate filament protein, DNAJ-like protein subfamily B member 11, 78kDA glucose-regulated protein, and Cu/Zn SOD among others. Altered metabolites were mainly involved in the citric acid cycle, urea cycle and glycolysis, and included branched chain amino acids, threonine, alanine, glutamate, hypotaurine, dimethylglycine, malonate and acetoacetate which were decreased and phosphocholine, glycine and NADP⁺ which were increased. Bhagat et al. (2016) exposed gastropods (*Morula granulata*) for 24 hours to 10, 25, 50 and 100 µg/L nominal phenanthrene. There was a significant increase in

GST activity and LPO at all concentrations, and DNA damage at 25 µg/L and above, but CAT activity was only significantly affected (decreased) at 25 µg/L. The combination of biomarkers into an IBR index indicated significant responses in the 50 and 100 µg/L concentrations.

Studies with complex PAH mixtures included an exposure of juvenile marbled rockfish (*Sebastes marmoratus*) for 2 to 10 days to the water-accommodated fraction (WAF) of Weizhou crude oil (WAF ΣPAH = 9.64 µg/L actual, with exposures at 20 to 320 µg/L WAF) alone and in combination with Cd or Pb (0.8 to 80 µg/L alone or 4 to 40 µg/L with WAF, nominal) (Zheng et al. 2016). Concentrations of 80 to 320 µg/L WAF for 5 days and 40 µg/L WAF for 6 to 10 days significantly increased EROD, but the metals had no effect on EROD, neither inducing EROD on their own, nor affecting the EROD induction caused by WAF exposure. Xu, Mager et al. (2016) exposed mahi mahi (*Corphaena hippurus*) embryos for 24 to 96 hours post-fertilization (hpf, hatching occurs at 35-40 hours) to the High Energy WAF of Deep Water Horizon slick oil from surface skimming operations and source oil from the subsea containment system. 96-hour ΣPAH LC50s were 19.5 and 16.5 for the slick and source oil, respectively, based on initial concentrations. At 24 hpf, transcript profiles between slick and source oil were similar, but at 48 and 96 hpf transcript profiles diverged, with many more genes altered upon exposure to slick than source oil. Slick oil altered the eukaryotic initiation factor 2 signaling which predicted loss of cell viability at early development stages, and induced differentially-expressed

cardiac-associated genes at 48 and 96 hpf, which may be tied to the highly variable heart rate at all time points, decreased heart rate at 24 and 96 hpf, and increased pericardial area at 48 hpf observed in slick oil-, but not source oil-exposed embryos. At 96 hpf slick oil exposure caused pronounced effects on metabolism, steroid biosynthesis, visual, and P450 gene expression suggesting other targets which may be involved in developmental toxicity. Ben Naceur et al. (2016) set out to determine the potential of volume regulatory capacity (VRC) as a biomarker in oysters (*Crassostrea gigas*) exposed for 24 hours to 8.4 mg/L nominal of diesel oil. After diesel exposure hepatopancreas cells and oocytes from early reproductive-period oysters were excised and exposed to hyposaline solutions for up to 90 minutes and the cell volume measured to determine any adverse effects on osmoregulation. Diesel significantly lowered the VRC of both types of cells, though hepatopancreas cells produced cleaner results. The biomarker proved to be more sensitive than gill GST activity, which was not affected by the exposure.

A couple of reviewed studies examined effects of marine diesel on Arctic/subarctic species. Geraudie et al. (2016) performed a 7-day static exposure of Icelandic scallops (*Chlamys islandica*) to marine diesel (6.4 and 14.23 mg/L TPH actual at initiation) to examine effects on biomarkers and behavior. Muscle lipid contents, GSI, and digestive gland CAT activity were not significantly affected, but adductor muscle AChE was significantly decreased at the highest concentration, which may have been related to the significant decrease in escape response.

Sagerup et al. (2016) performed an interesting experiment exposing immature red king crab (*Paralithodes camtschaticus*) males and their prey items (Islandic scallops and blue mussels [*Mytilus edulis*]) together to Bunker Oil Ltd. marine diesel (flow-through exposure, 7.4 and 19.0 mg/L ΣPAH 24 hours into exposure) for 1 week followed by a 3 week recovery in uncontaminated water with uncontaminated food. Though changes in locomotion were observed, the crabs ate normally, possibly contributing to the higher bioaccumulation factor in the crabs than in the bivalves. Catalase (non-significant) and GPx (significant) activity were both increased at the end of the exposure but back to background after the recovery period, but AChE activity was not affected by the exposure, and LPO results were inconclusive.

Laboratory Studies: Biocides. Naqvi et al. (2016) injected Mozambique tilapia (*Oreochromis mossambicus*) with 0.0001 to 0.01 ppm of organophosphate and pyrethroid pesticides and an herbicide for 24 to 48 hours to determine effects on micronucleus (DNA damage) frequency in peripheral blood erythrocytes. At 24 hours the order of genotoxicity was cypermethrin < malathion < chlorpyrifos < lambda-cyhalothrin < buctril. At 48 hours the order of genotoxicity was cypermethrin < chlorpyrifos < malathion < lambda-cyhalothrin < buctril. Kondee et al. (2016) exposed hooded oysters (*Saccostrea cucullata*) for 48 hours to tributyltin. At 10 µg/L nominal, mucocyte numbers increased in the epithelia of the mantle, gills and stomach, perhaps providing a defensive mechanism which was not present at 50 and 150 µg/L nominal, where cell necrosis was induced. Proteomic results indicated oxidative

stress, cell structure and function alterations, and altered energy production and calcium homeostasis. Potential biomarkers of tributyltin exposure include actin, tubulin-alpha, tubulin-beta, which are involved in cell structure, and voltage-dependent anion-selective channel protein 3, which is involved in calcium homeostasis.

Laboratory Studies: Pharmaceuticals and Personal Care Products. Prichard and Granek (2016) reviewed 23 reviews and 73 primary studies performed on marine and estuarine organisms exposed to pharmaceuticals (excluding most studies with antibiotics, which were reviewed elsewhere) and personal care products. Fifty-five different species from 20 taxonomic classes were exposed to 27 active pharmaceutical ingredients, 9 endocrine-disrupting compounds, 22 personal care products and 4 antibiotics. Gaps in research identified by the authors included effects of chronic exposure, mixture effects, risks to mammals, birds and reptiles, bioconcentration and bioaccumulation, non-animal testing methods, multi-generational effects, how to bridge the gap between subcellular biomarkers and ecosystem-level effects, and effects of abiotic factors on bioavailability and uptake. Two 2016 studies actually delved into the effects of abiotic factors. Fonte et al. (2016) examined the effects temperature on the toxicity of microplastics and the antibiotic cephalixin to juvenile common gobies (*Pomatoschistus microps*) exposed for 96 hours. At 20°C 0.184 mg/L actual of microplastics caused a non-significant reduction in predatory performance and AChE activity. At 20°C, 5 mg/L actual and above cephalixin significantly reduced predatory performance and caused a

non-significant increase in LPO. Co-exposure with microplastics decreased the toxicity of cephalexin to a small extent, and an increase of temperature to 25°C increased the toxicity of both microplastics and cephalexin.

Other studies with antibiotics included an exposure of nauplius and adult ovigerous female copepods (*Tigriopus japonicus*) to 10-1000 mg/L nominal trimethoprim (Han et al. 2016). The 48-hour nauplius LC50 was 156 mg/L, and the 96-hour ovigerous female LC50 was 200 mg/L. In nauplii, development to adult stage was significantly delayed in a dose-dependent manner between 2.5 and 10 mg/L. In adults, 100 mg/L significantly reduced fecundity over 10 days, and significantly increased ROS levels and expression of GST-kappa, GST-sigma, Tj-CYP3024A2 and Tj-CYP3024A3 over 24 hours. Zhao et al. (2016) exposed marine medaka (*Oryzias melastigma*) for up to 24 hours to 36 and 197 µg/L actual of sulfamethazine (SM2). The liver, bile and gonads tended to have the highest uptake of SM2 and its metabolite, N-SM2, with higher accumulation in males than females. There was a time-dependent increase in SOD activity up to 12 hours with a subsequent drop at 24 hours. Catalase activity display a u-shaped trend over time at the low dose, and an inverted u-shaped trend over time at the high dose. Moreira et al. (2016) exposed the amphipod *Ampelisca brevicornis* for 10 days to sediments spiked with the antibiotic novobiocin and the anti-cancer and rheumatic drug methotrexate (MTX). Concentrations of 0.5 nominal to 500 ng/g actual novobiocin produced neither lethal nor sublethal effects. Concentrations of 10 nominal to 830 ng/g actual MTX caused significant mortality. At 100 nominal

and 830 ng/g actual MTX EROD activity, LPO, and DNA damage were significantly increased, and there was an increase in GST activity at 830 ng/g actual and GPx activity at 1 and 10 ng/g nominal.

Aguirre-Martinez et al. (2016) also examined the effects of MTX, along with the anti-cancer drug tamoxifen (TMX), this time measuring short-term toxicity in the bacteria *Aliivibrio fischeri*, the microalgae *Isochrysis galbana*, and the sea urchin *Paracentrotus lividus* (both MTX and TMX), and long-term effects on biomarkers in the clam *Ruditapes philippinarum* (TMX only). While environmentally-relevant concentrations of MTX did not cause short-term toxicity, TMX was found to be more toxic to all 3 species than MTX, and significantly decreased fertilization in sea urchins at environmentally-relevant concentrations (EC50 1.5 µg/L nominal). Fourteen-day exposure of clams to TMX caused an LMS EC50 of <0.1 mg/L nominal, and exposure to 0.1 to 50 µg/L increased EROD activity, GPx activity, and LPO, decreased dibenzylfluorescein dealkylase activity (phase I biotransformation biomarker) and AChE activity (50 µg/L only), and caused intermittent effects on GST activity, GR activity and DNA damage.

Studies on other compounds included a 14-day exposure of *Mytilus galloprovincialis* to 0.5 µg/L nominal of the non-steroidal anti-inflammatory drugs (NSAIDs) acetaminophen, diclofenac (DIC), ibuprofen (IBU), ketoprofen (KET) and nimesulide (NIM) (Mezzelani et al. 2016). Only DIC and NIM accumulated in mussel tissue. All NSAIDs decreased NL, IBU decreased LMS, KET increased both phagocytosis and granulocyte-hyalinocyte

ratio (immune system biomarkers) along with DNA fragmentation and micronucleus frequency, both DIC and NIM increased lipofuscin content and micronucleus frequency and NIM also decreased AChE activity in the hemolymph, but no NSAID had an effect on AChE activity in gills or Acyl-CoA oxidase activity (involved in fatty acid metabolism and peroxisome proliferator-activated receptor [PPAR] pathway signaling). None of the NSAIDs had an effect on antioxidant biomarkers either. Both KET and NIM modulated a large number of genes, including those associated with arachidonic acid metabolism, immune response, apoptosis, cell cycle and DNA repair. Capolupo et al. (2016) exposed *M. galloprovincialis* for 24 hours to 12.22 to 397.60 ng/L actual caffeine. While a reduction in LMS in hemocytes and digestive gland was observed, along with an increase in NL and GST activity in the digestive gland, there was no effect on digestive gland lipofuscin content, lysosome-cytoplasm volume ratio, CAT activity, DNA damage or LPO, or gill AChE, CAT or GST activity.

Laboratory Studies: Endocrine Disrupting Compounds. In vertebrates, estrogen induces the production of the egg-yolk precursor protein VTG via estrogen receptors (ERs) which bind to estrogen response elements (EREs) in the promoter region of the VTG gene. Tran et al. (2016) set out to determine if a similar mechanism exists in the Sydney rock oyster (*Saccostrea glomerata*). They found that the sgVTG gene lacks a consensus palindromic ERE but contains 3 half-EREs, consisting of two direct repeats and one inverted repeat, located in close proximity to each other and close to a

potential binding site for GATA factors. 17 β -estradiol (E2) was shown to stimulate expression of sgVTG both in vivo and in vitro, and an ER antagonist abolished the effect, indicating possible ligand-dependent activation of the sgVTG gene. Ye et al. (2016) examined endocrine-disrupting effects in marine medaka (*Oryzias melastigma*) embryos exposed for up to 9 days to 0.01 to 1 mg/L nominal of the plasticizer di(2-ethylhexyl) phthalate (DEHP) and 0.01 mg/L nominal E2. Hatching time was delayed by E2 but not DEHP. After 1-3 days of exposure, E2 decreased expression of CYP19b (aromatase) and PPAR β and increased expression of CYP19a, ER α , ER β , Vtg1, Vtg2, choriogenin (Chg) H and ChgL (egg envelope genes), but DEHP only decreased CYP19b expression. After 9 days of exposure ER α , ER β , PPAR α , PPAR γ , Vtg1, Vtg2, ChgH, ChgL, CYP19a and CYP19b were all induced by DEHP in a dose-dependent manner and E2 (except for PPAR α). After an 8-day recovery in clean seawater gene expression was no longer affected in DEHP-exposed fish, but in E2-exposed fish ChgL was still upregulated and ER α , ER γ , CYP19a, PPAR α , PPAR β , and PPAR γ were all downregulated. Von Hippel et al. (2016) made the case for the use of ninespine sticklebacks (*Pungitius pungitius*) as model organism for Arctic ecotoxicology. They determined that more Arctic contaminated sites lie within the distribution of the ninespine stickleback (about 818) versus the threespine stickleback (about 585). A genome sequence is available for ninespine sticklebacks to aid in transcriptomic studies, and a Vtg assay has been developed, and a thyroxine assay validated, to aid in endocrine disruption studies.

Laboratory Studies: Climate Change/ocean

Acidification. Several studies dealt with effects of climate change. Velez et al. (2016) exposed Manila clams (*Ruditapes philippinarum*) for 28 days to salinities of 14, 28 and 35 ppt in combination with a pH of either 7.8 or 7.3. At a pH of 7.8, clams exposed to a salinity of 14 ppt had lower Cl, Na, K, glycogen and protein concentrations and higher lipid content, LPO, and carbonic anhydrase, electron transport, SOD, CAT and GST activity than at higher salinities. A drop in pH appeared to reduce the metabolic state of the clams, which in turn appeared to decrease the physiological and biochemical changes induced by salinity changes. Within each salinity pH had little effect on biomarker responses, with the exception of carbonic anhydrase and electron transport activity at 14 ppt, and glycogen and lipid content at 28 and 35 ppt. Sogin et al. (2016) exposed coral (*Pocillopora damicornis*), along with their symbiotic partners, to ambient (26.5°C and 416 μatm pCO₂) and high (29°C and 805 μatm pCO₂) conditions for 1.5 months. Both lipid and primary metabolite profiles differed between the treatments, with primary metabolite profiles providing a clearer separation of the treatments and correlation with net photosynthesis. While many of the affected primary metabolites were unidentified, those that were identified were involved in energy storage, cell signaling, protein collagen, carbohydrate metabolism and energy metabolism.

Freitas, Almeida et al. (2016) evaluated the impact of ocean acidification on the effects of 3 $\mu\text{g/L}$ actual carbamazepine (CBZ), an antiepileptic drug, on the clam *Scrobicularia plana* exposed for 28 days. While a pH drop

from 7.8 to 7.1 had no effect on bioconcentration of CBZ, or the depression of CAT activity, induction of GST activity, increase in Na and protein content, decrease in electron transport activity and increase in long-term mortality caused by CBZ alone, it did mitigate effects on K content, and increase LPO over that caused by CBZ alone.

Effects of Dredging, Dumping, and Extraction

Impacts to the benthic invertebrate structure and function following dredged material disposal were examined by Bolam et al. (2016) in northern England. Using secondary production measurements and biological trait analysis (BTA), the authors found that benthic invertebrate communities at the disposal site shifted to organisms with higher bioturbative capacity, lower productivity, and longer life spans.

Hendrick et al. (2016) studied the tolerance of six benthic macroinvertebrate species, including a brittle star, tunicate, scallop, sea urchin, anemone, and polychaete, to smothering using a multifactorial experimental design that included a range of sediment fractions, burial durations, and burial depths. The authors found wide variation in survival and emergence across species, with the tunicate, *Ciona intestinalis*, the least tolerant to burial and the polychaete, *Sabellaria spinulosa*, the most tolerant. The tolerance of blue mussels (*Mytilus edulis*) to smothering by dredge disposal material was investigated by Cottrell et al. (2016). The authors found that mussel mortality increased with smothering from fine-grained material, increasing organic content, and increased temperature. Hutchison et al. (2016) also examined the behavioral response of two

species of mussels (*Mytilus edulis* and *Modiolus modiolus*) to sudden burial. Similar to Cottrell et al. (2016), Hutchison et al. (2016) found mortality increased with longer duration of burial, finer sediment fraction, and increased temperature but not burial depth. *M. modiolus* was better able to survive short duration (<8 day) burial than *M. edulis*, but only *M. edulis* was able to emerge from burial.

Crowe et al. (2016) studied the recovery of physical and biological characteristics of two sand borrow pits near an estuarine plume in South Carolina using a before-after-control-impact (BACI) study design. The authors found that following dredging, grain size in the borrow pits shifted to fine-grained material. The benthic macroinvertebrate community shifted to opportunistic species, with changes in faunal density, species composition, and species number. After 6–8 years, the physical and biological characteristics of the borrow pits were relatively unchanged.

To investigate how low light levels associated with high suspended sediment loads released during dredging and disposal operations affect marine sponges, Pineda et al. (2016) conducted a series of experiments on five species of sponges under varying light intensities. The authors found heterotrophic sponges were insensitive to decreases in light, but two out of three phototrophic sponge species experienced discoloration, with subsequent mortality in one of the discolored sponge species.

Ricardo et al. (2016) found no effects to survival and metamorphosis of embryonic and larval tropical coral species after exposure to a range of environmentally relevant suspended sediment concentrations, such as those

that occur during dredging and disposal operations. The authors suggest the lack of effects may be associated with mucus production and cilia activity; however, the same authors also reported that environmentally relevant suspended sediment concentrations could adversely affect broadcast coral spawning success. Ricardo et al. (2016b) found suspended sediment particles adhere to egg and sperm bundles and cause them to sink and never reach the surface of the ocean where fertilization occurs.

Effects of Wastewater Discharges

Amato et al. (2016) used $\delta^{15}\text{N}$ and C:N ratios in algal tissue as bioindicators of the link between nutrient sources in groundwater and coral reef health around the island of Maui. Reefs adjacent to sugarcane farms and waste water injection sites demonstrated high algal N concentrations, with increases in reef algal biomass and decreases in biodiversity. $\delta^{15}\text{N}$, %N, and %P content of mangrove leaves were evaluated as indicators of long-term eutrophication trends related to human sewage impacts in New Zealand. Gritcan et al. (2016) found anthropogenically derived N has had an impact on mangrove nutrient status for 100 years, with recent decreases in N thought to be related to sewage system improvements.

de-la-Ossa-Carretero et al. (2016) used the benthic opportunistic polychaetes and amphipods (BOPA) index to assess impacts to benthic communities from different types of sewage treatment. The impact of sewage effluent exposure was related to the level of treatment, flow rate, and depth of effluent release. The effects of sewage

effluent to functional traits of estuarine intertidal macroinvertebrates in Paranaguá Bay, Brazil, were examined using BTA and multivariate indices related to functional diversity. Gusmao et al. (2016) found abundance-based BTAs and functional indices reliably detected functional changes related to sewage effluent exposure.

The effects of sewage discharge to the abundance and composition of intertidal invertebrate communities on Terceira Island, Azorean archipelago, were examined by Cabral-Oliveira and Pardal (2016). Using an After Control/Impact study design, the authors found no significant effects of sewage discharge to the invertebrate assemblage in the intertidal zone. Sewage discharges influenced the distribution pattern of dominant species, with a decrease in barnacles and limpets and an increase in littorinids. Seventeen months after the cessation of sewage release, the eulittoral community had largely recovered. Becherucci et al. (2016) conducted a succession of experiments on rocky shoreline communities in Buenos Aires, Argentina, following physical disturbance. Proximity to a sewage outfall was associated with a lower time to recovery for macrobenthic communities.

Long-term monitoring data (1992–2013) were used to investigate the effects of sewage sludge disposal on benthic infauna abundance and relative composition off the Israeli coast. Consistent with previously reported results, Kress et al. (2016) found the extent of the area affected by the sludge disposal varied seasonally, though a marked change in the association between sewage and infauna abundance was detected after 2004. The authors believe

this change may be related to load reductions that began in 2000, biological processes, and environmental forcing.

Liu et al. (2016) found a higher relative proportion of particle-associated bacteria, compared to free-living bacteria, in marine waters affected by sewage effluent using quantitative polymerase chain reaction. The authors suggest the relative proportional shift may be related to adaptations in response to the chemical oxygen demand (COD) of the effluent.

Huber et al. (2016) conducted a “snap shot” risk assessment of pharmaceuticals, hormones, and additives in personal care products discharged into the marine environment from sewage lines in the Faroe Islands, Iceland, and Greenland. Risk to aquatic organisms was greatest from surfactants associated with personal care products, followed by risk from the hormones 17 β -estradiol and estriol. Risk ratios associated with concentrations of pharmaceuticals in receiving waters were generally much less than one, indicating no risk to the aquatic environment from the measured chemicals.

Garaffo et al. (2016) investigated the link between sewage effluent, environmental variables, and densities of the invasive polychaete *Boccardia proboscidea* in Mar del Plata, Argentina. The authors found *B. proboscidea* density was related to total organic matter, temperature, season, and site, but they were unable to develop a predictive model for all areas around the sewage outfall due to environmental variability not captured by the model.

Effects of Fishing and Aquaculture

Sciberras et al. (2016) studied the effects of chronic bottom trawl fishing on benthic infaunal community structure and the sediment biogeochemistry processes they mediate across different fishing pressure gradients and habitat types (mud, sand) in the North Irish Sea. Scallop dredging on sand had little effect on the infauna community and biogeochemical processes. Otter trawling on mud had no significant effect on the infauna community, but the frequency of otter trawling was associated with increased chlorophyll a concentrations in the sediment, increased porosity, increased NH₄⁺ concentrations in sediment deeper than 5 cm, and decreased NH₄⁺ concentration in sediment shallower than 2 cm.

Mercaldo-Allen et al. (2016) examined longer-term changes to benthic community structure following hydraulic dredging of hard clams or quahog (*Mercenaria mercenaria*) in Long Island Sound at sites representing 0-, 1-, 2- and more than 10-years post-harvest. The biomass of organisms and number of species were greatest at the sites 1- and 2-years post dredging, while sites left unharvested for more than 10 years had greater species diversity and evenness. Huvenne et al. (2016) examined the recovery of deep-sea corals in the Darwin Mounds Marine Protected Area west of Scotland. The area was closed to bottom-contact fishing in 2003, but the authors detected no recovery of the deep-sea corals in 2011.

Buhl-Mortensen et al. (2016) investigated effects to substrate and megabenthos in response to otter trawling on the shelf (50–400 m) and slope (400–2,000 m) of the Barents Sea off the coast of Norway. Fishing intensity was significantly and negatively correlated with megafauna

density and diversity, with five sponge taxa experiencing statistically significant declines in density. Murillo et al. (2016) conducted a survey of the epibenthic invertebrate assemblages of the Tail of the Grand Bank of Newfoundland and the Flemish Cap in the northwest Atlantic Ocean to characterize the composition, structure, and distribution of organisms and their relationship to environmental variables and bottom trawl fishing intensity. The authors found fishing intensity directly related to the presence of hard-shelled scavenging and predatory gastropods, a thick-shelled bivalve (*Astarte undata*), and an epibenthic assemblage associated with the least spatial cohesion. Pommer et al. (2016) examined the effects of bottom trawl fishing on benthic community composition in the Kattegat using vessel monitoring systems (VMS) data to quantify trawling intensity in conjunction with concurrently collected benthic faunal data. The multivariate analyses could not distinguish between lightly trawled and heavily trawled areas, because benthic community characteristics and trawling intensity both correlated with environmental gradients. Similarly, Szostek et al. (2016) investigated the drivers of benthic infaunal community composition of king scallop (*Pecten maximus*) fishing beds in the English Channel. These authors found effects from dredge fishing intensity could not be differentiated from natural variability in dynamic ecosystems. Fishing intensity was not correlated to species richness, species diversity, or species composition; rather species richness and diversity were negatively correlated with the environmental variable bed shear stress.

Öndes et al. (2016) examined the effect of king scallop dredge fisheries to brown crab (*Cancer pagurus*) mortality, as a result of bycatch, in the waters around the Isle of Man. The authors found that 84% of the bycatch was female crabs and 45% of the crabs were either severely damaged, crushed, or killed by the dredging. The impact of the scallop fishery to brown crabs varied seasonally and spatially but was generally limited.

Hiddink et al. (2016) examined the effect of bottom trawling frequency on food intake, condition, and biomass of target fish in the Kattegat as a function of prey biomass. They found prey were affected by trawling gear, and the ratio of prey to consumer biomass determined whether the condition and food intake of the target species would increase or decrease with increased trawling frequency. Also examining community dynamics, Duplisea et al. (2016) used extinction debt theory (EDT) to study fish and invertebrate communities on George's Bank in the northwest Atlantic Ocean. These communities demonstrated a change in occupancy away from competitor-dominated species towards colonizer-dominated species. The authors report that this shift is consistent with habitat fragmentation and destruction associated with trawling, though these effects may be exacerbated by climate changes and fishing.

In a preliminary effort to evaluate impacts from bottom trawl fisheries off the coast of Brazil, Port et al. (2016) estimated the spatial footprint of industrial trawling by fishing strategy and found the shrimp trawling industry uses the most extensive habitat area and potentially disturbs more habitat to obtain less landed biomass. Rijnsdorp et al.

(2016) developed a framework to estimate trawling pressure and ecological impact using readily available information on the area trawled, the size and type of trawl gear used, habitat type, and the vulnerability, recovery rate, and ecological role of the benthic population. The authors applied the framework to three habitat types in the North Sea as a preliminary illustration of the approach.

Coll et al. (2016) assessed the effects of fishing pressure on three Mediterranean commercial fish species using Ecospace, the spatial-temporal module of the Ecopath with Ecosim (EwE) food-web model that incorporates a new habitat foraging model. While the authors acknowledge the analysis is limited by a lack of empirical data at matching spatial and temporal resolution to validate the model, the model confirmed a decline in abundance of the three commercially harvested fish species and indicated an increase in the abundance of non-commercial invertebrates as a result of decreased pressure on those populations from predatory commercial fish species. Ecospace also identified fishing pressure as the largest driver of the abundance of the commercially harvested species examined, followed by primary production and depth.

Clark et al. (2016) published a review of deep-sea fishing impacts on benthic communities. Topics reviewed included changes to the seabed environment, impacts on epifauna, impacts on infauna, changes in community characteristics, faunal sensitivity, recovery potential, and management implications.

Kaiser et al. (2016) reported the results of an international effort to identify and prioritize

“knowledge-needs” necessary to develop “best practices” approaches for bottom trawl fisheries. Fifty-two stakeholders, including “research scientists” and practitioners from the fishing industry, the fish processing industry, non-governmental organizations, government agencies, and an intergovernmental organization, participated in the effort, along with an “expert task force” consisting of twelve scientists. The top priority knowledge-need identified by the group was an understanding of the extent and distribution of different seabed habitat types. The authors conclude that continuing collaborative discussions across multiple stakeholder groups will draw upon combined knowledge and ensure priority knowledge-needs are met and new knowledge gaps are identified.

Low-frequency pulsed direct current used in electric fishing of brown shrimp was found to cause no significant behavioral or radiographic changes in any of the five species of fish studied (Desender et al. 2016). Significant increases in melano-macrophagecenters were found only in the spleens of exposed Atlantic cod (*Gadus morhua*).

Kalantzi et al. (2016) compared the effects of copper alloy (brass) aquaculture nets (CuN) to traditional aquaculture nets (NN) painted with Cu₂O by measuring Cu concentrations in water, sediment, and particulate matter as well as measuring biochemical markers in mussels (*Mytilus galloprovincialis*) and gilthead sea bream (*Sparus aurata*) tissues. An initial exponential release of Cu was found near the CuN nets, but after approximately 6 months, the concentrations of dissolved Cu were similar to those at the

NN nets. The concentration of Cu in mussels followed the same trend as for dissolved water concentrations. Sediment and particulate matter were not affected by the type of aquaculture net. Biochemical indicators for fish were not significantly different between all exposure scenarios (control, CuN, NN). The relationship between mariculture and Hg contamination of marine waters, sediment, and fish tissue in a Chinese bay was examined by Liang et al. (2016). Concentrations of MeHg were higher in the bottom layer of the water column and top layer of porewater in areas beneath mariculture facilities compared to reference areas in the same bay but not beneath mariculture operations. The authors suggest high summer temperatures stimulate MeHg production and higher fish tissue concentrations of Hg in blackhead seabream (*Acanthopagrus schlegelii*) are associated with fish feed rather than environmental exposure.

Effects of Noise Pollution

A number of articles addressing noise pollution in the marine environment were published in 2016 as part of a book entitled, *The Effects of Noise on Aquatic Life II* (Popper and Hawkins 2016). Among these, Aguilar de Soto (2016) reviewed the published literature reporting effects of noise pollution to marine invertebrates and found the literature was relatively scant compared to the growing body of literature on noise pollution effects to fish. Reported effects to invertebrates of different species and life stages ranged from no effects to behavioral/physiological effects to mortality. In an effort to understand the effect of noise pollution to invertebrate

species, André et al. (2016) found that sound exposure causes whole body vibration in scallops and cephalopods, which is consistent with the theory that particle motion causes the whole body to move with the same amplitude and frequency. de Jong et al. (2016) used acoustic modeling of shipping traffic and trailing suction hopper dredging to characterize potential risks to harbor porpoises (*Phocoena phocoena*), harbor seals (*Phoca vitulina*), and various fish species in the Port of Rotterdam, the Netherlands. Dredging activity increased sound exposure in the area by ~4 dB, but due to uncertainty in dose-response relationships, the authors could not determine if the increase in sound exposure was physiologically significant to the species considered. Nehls et al. (2016) reported an approximate 90% decrease in the harbor porpoise disturbance area when a bubble curtain was used around pile driving operations during the construction of an offshore wind farm in the North Sea. Radford et al. (2016) reviewed the published literature reporting effects to a variety of organisms from noise pollution and highlighted the need to better understand differences in responses between species, within populations, and in light of the context of the exposure, repeated exposure and prior experience, and recovery and compensation. To better understand the implications of noise pollution beyond single-species effects, Sabet et al. (2016) reported the results of a laboratory experiment to determine whether sound exposure affected the predator-prey relationship between the water flea (*Daphnia magna*) and the zebra fish (*Danio rerio*). Though experimental results were not presented in the chapter, the authors reported sound exposure had no

apparent impact on water flea behavior, but fish feeding efficiency was negatively impacted by sound exposure. Thomsen et al. (2016) reported a risk assessment framework and technical guidance developed by the World Organization of Dredging Associations' Expert Group on Underwater Sound (WEGUS) that highlights the need to standardize terminology and methods for data collection and analysis and development of dose-response relationships from which to characterize risk.

Impacts to invertebrates from noise pollution were featured in two focused review articles and laboratory studies. Edmonds et al. (2016) conducted a review of the published literature focused on reported effects to crustaceans from noise pollution. The authors found current research indicates crustacean sensitivity to sound is related to particle motion, and an effort to understand impacts to commercially-important shellfish should involve development of species-specific particle motion audiograms. Tidau and Briffa (2016) identified and reviewed eleven peer-reviewed articles describing behavioral effects of noise pollution on aquatic crustaceans. Some common patterns of response were identified among the studies, but distinct differences between the published reports underscore the need for clearer and more consistent methods application and reporting. Using laboratory studies and outdoor simulations of pile driving, Roberts et al. (2016a) found the common marine hermit crab (*Pagurus bernhardus*) responded with clear behavior modifications in response to substrate vibrations of a similar magnitude to those associated with anthropogenic noise pollution. Filiciotto et al. (2016b) conducted laboratory experiments

with the common prawn (*Palaemon serratus*) to examine behavioral and biochemical responses to boat noise. Prawns exposed to boat noise spent significantly more time outside the shelter and exhibited significantly reduced locomotor activity. Analysis of brain biochemistry also revealed significantly higher plasma total protein, upregulation of two heat shock proteins, and evidence of apoptotic fragmentation of DNA.

Behavioral responses of brown meagre (*Sciaena umbra*) to noise pollution from boat traffic were studied by La Manna et al. (2016) in a marine protected area of the Mediterranean Sea. Boat noise increased the duration of flight reactions, the number of individuals performing flight reactions, and the frequency of hiding behaviors, but boat noise did not affect fish activity levels and sound emission from the fish. Bruintjes et al. (2016) examined the stress response of black seabream (*Spondyliosoma cantharus*) and European plaice (*Pleuronectes platessa*) to simulated pile driving. Black seabream significantly increased their oxygen consumption in response to pile driving, while the oxygen consumption of European plaice was not significantly affected.

Filiciotto et al. (2016a) evaluated oxidative status and immune system response of gilthead sea bream (*Sparus aurata*) juveniles to aquaculture noise pollution. Noise associated with offshore aquaculture operations and the sea soundscape produced significant increases in bioindicators of immune response compared to control exposures. Levels of bioindicators measured in fish exposed to onshore aquaculture noise were between those of fish exposed to offshore aquaculture soundscapes and those of fish exposed

to control conditions and not statistically significantly different from either. In a related study, Celi et al. (2016) measured a series of biochemical indicators of stress response in one year old gilthead seabream exposed to boat noise. Significant changes in response to boat noise were detected in almost all the biochemical parameters measured, but no significant differences in weight or fork length were found.

Several studies examined the effects of noise pollution to toadfish vocalizations. Alves et al. (2016) found that noise pollution from small motor boats and ferryboats reduced the communication active space of Lusitanian toadfish (*Halobatrachus dydactylus*), with a larger effect associated with noise from the small motor boat compared to the ferryboat. Krahfurst et al. (2016) investigated the effects of a variety of soundscape noises to male oyster toadfish (*Opsanus tau*) calling rates. Outboard motorboat noise had an insignificant impact on oyster toadfish calling behavior compared to significant changes resulting from exposure to inboard motorboat noise, bottlenose dolphin biosonar, and inboard motorboat noise combined with dolphin biosonar. Conducted in conjunction with the experiments reported by Krahfurst et al. (2016), Luczkovich et al. (2016) found that male oyster toadfish demonstrated the Lombard effect, an involuntary increase in vocal levels in noisy environments, in response to experimentally introduced vessel noise, dolphin biosonar, and snapping shrimp noises.

Blair et al. (2016) identified significant effects on the feeding behavior of ten humpback whales (*Megaptera novaeangliae*) in response to ship-based noise in North

Atlantic coastal waters. Increasing ship noise was associated with slower descent rates and fewer bottom-feeding events per dive. Parks et al. (2016) investigated the impact of ship noise on social sound production in six humpback whales in the same area of the North Atlantic. The five male whales decreased social sound production in the presence of ship noise, but the single lactating female whale in the study, who also had her calf with her, increased social sound production in the presence of ship noise, which continued at an elevated rate after the ship passed.

Several papers undertook a retrospective analysis of the current literature on the effects of noise pollution to identify future research needs. Aguilar de Soto et al. (2016) reviewed examples of observed physiological effects of noise pollution to a wide variety of organisms. The review highlighted the importance of developing an integrated approach to assessment and management of noise effects on marine organisms that considers hearing and non-hearing related effects as well as direct and indirect effects. With a goal of understanding how noise pollution may impact commercial shellfish in the United Kingdom, Kunc et al. (2016) also reviewed the current understanding of the impacts of noise pollution to aquatic organisms. The authors identified existing challenges and directions for future research that include the need to develop approaches that extrapolate beyond individual effects to address ecological effects. Hawkins and Popper (2016) described the problems of understanding and regulating the impacts of noise to fish and invertebrates by highlighting the

existing data gaps in the science and suggesting directions for future research.

Cox et al. (2016) undertook a meta-analysis of the published literature to describe the role of noise pollution in altering fish behavior and physiology. All studies included in the analysis reported negative effects of noise to behavior and physiology. This indicates that negative effects are not limited to specific responses and that negative effects could occur regardless of species, habitat, or experimental conditions.

Effects of Recreation and Tourism

Gridley et al. (2016) examined whether common bottlenose dolphins (*Tursiops truncatus*) in Walvis Bay, Namibia, vary their whistles in response to the presence of boats. The authors found statistically significant increases in the production rates of whistles, signature whistles, and some whistle frequency parameters.

The effects of recreational fishing and diving to marine biodiversity were studied by Riera et al. (2016) in Tenerife, part of the Canary archipelago in the northeast Atlantic Ocean. In general, consistent differences in invertebrate and fish assemblages in relation to pressure from recreational activities was not detected. The authors suggest the impoverished condition of the marine ecosystems of the Canary archipelago due to overfishing may mask effects from recreational pressure to the environment. Prato et al. (2016) used the EwE model with the Ecotroph plug-in to explore the influence of recreational fisheries and artisanal fisheries on the status of two high-trophic-level predator (HTLP) groups in the

Portofino Marine Protected Area in the Mediterranean Sea. Artisanal fisheries had a larger impact on the biomass accessible to fisheries than recreational fisheries, but recreational fisheries had a larger impact on the composition of the fish assemblage.

The effects of tourism to marine mammals were reported for sites in Australia, east Africa, and several Asian countries. Osterrieder et al. (2016) examined the responses of the Australian sea lion (*Neophoca cinerea*) to visitation from tour boats to two differently managed sites. Distance between the tourist and sea lion was a key metric related to response, and “aggressive” and “retreat” behavior was observed more frequently when approaches were less than 10 m. Pérez-Jorge et al. (2016) used Pollock’s closed robust design capture-recapture model to evaluate the influence of oceanographic variables, anthropogenic disturbance, and artisanal fisheries on the demography of an Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) population off the southern coast of Kenya. The authors found human disturbance from tourism activities and seasonal temperatures influenced the probability of animals emigrating to and from the study area. Okemwa et al. (2016) developed a modified Driver-Pressure-State-Impact-Response (DPSIR) framework to examine the risk from tourism to harm, displace, or extirpate dolphins. Using data associated with seven dolphin-watching sites in six countries in Asia, the authors found high risk to the sites in India and Indonesia and intermediate risk at the site in Cambodia. Additional ecological data is necessary to confirm low risk from the sites in Thailand, the Philippines, and Malaysia.

Lester et al. (2016) evaluated the environmental and economic costs associated with the next generation of larger cruise ships visiting the island of Bermuda. Three scenarios were considered: two alternative viable routes that could be dredged to accommodate the larger boats on the island and a third scenario that does not include dredging and would prohibit larger cruise ships from visiting the island. The authors found that the “no dredging, no larger cruise ships” scenario performed well but came with a risk of a significant loss of tourism revenue. Of the two dredging scenarios, upgrading the south channel had lower costs to the coral community, but dredging the north channel was significantly less costly if dredged material was not reused. While the government of Bermuda chose to dredge the north channel, the authors argue that the south channel option was equally as good or better because, among other attributes, it has a lower potential ongoing impact to coral communities from sediment resuspension during cruise ship passage.

Assessment Methods and Pollution Indicators

Roberts et al. (2016b) developed an innovative approach to study the behavioral responses of individual wild, unrestrained fishes to an acoustic sound array using baited remote underwater video and motion analysis. Four species were included in the initial field survey, which employed two noise signatures: a large container ship and a synthetic pile-driving stimulus. To better understand the impact of noise and bubbles from open circuit SCUBA on fish surveys, Gray et al. (2016) compared the results of fish surveys undertaken using open circuit SCUBA and closed

circuit rebreathers in Hawaii. The authors found that at sites with low to moderate fishing pressure, the survey method was generally inconsequential to the results; however, at sites with high fishing pressure, the open circuit SCUBA surveys consistently underestimated the biomass of several target fish groups.

Vinagre, Pais-Costa, Gaspar, et al. (2016) collected macroinvertebrate and macroalgal abundance and taxonomic composition data to assess a gradient of anthropogenic disturbance along two rocky Portuguese shores. Both macroalgal and macroinvertebrate communities demonstrated parallel shifts along the nutrient enrichment gradient, with macroinvertebrate density and macroalgae biomass metrics better capturing disturbance gradients at each site. In conjunction with their work comparing macroalgal and macroinvertebrate indices, Vinagre, Pais-Costa, Hawkins et al (2016) separately reported their efforts to test the ability of several macroinvertebrate indices to distinguish sites affected by anthropogenic disturbances along rocky intertidal habitat in Portugal. Of the 23 ecological indices calculated, the biomass-based Shannon-Weiner and Hurlbert indices as well as the Bentix biotic index showed the greatest performance consistency. In both papers, the authors suggest benthic macroinvertebrate community metrics could be integrated into a yet undeveloped multi-metric assessment tool that would satisfy European Water Framework Directive (WFD) requirements.

Effects of Chemical Warfare Agents

Greenberg et al. (2016) undertook a review of 64 published scientific papers regarding the environmental effects and health concerns associated with dumping of chemical warfare agents in the ocean. The authors report that following World War II large quantities of chemical weapons, including sulfur mustard, Lewisite, and nerve agents, were dumped in the offshore waters of Europe, Russia, the United States, Canada, and Japan, with the largest levels of dumping in the Baltic and North Seas. The physical condition of the munitions casing determines the extent of release of the chemical agents in the water, and available evidence suggests casings are in a mixed condition, with some heavily corroded and others fully intact. Concentrations of the parent and breakdown products of the chemical agents in water and sediment are minimal. Tissue concentrations in marine organisms are not at levels that would indicate risk, though evidence of chronic toxicity has been reported. Populations of microorganisms have also been found to be significantly altered at dumping sites. As part of the Chemical Munitions Search & Assessment (CHEMSEA) project, Grzelak and Kotwicki (2016) investigated the nematode community of chemical munitions dumping sites at Gotland Deep, Bornholm Deep, and Gdansk Deep in the Baltic sea. The authors identified 42 nematode genera and found approximately 20% of the *Halomonhystera disjuncta* (complex) females carried eggs and/or juveniles in their ovaries, which is a form of reproduction previously observed in the species under harsh environmental conditions. The authors hypothesize that the species has adapted to unfavorable conditions at the dumping site

stations that could be related to factors such as toxic compounds or low oxygen levels.

Offshore Wind Energy Effects

Kaldellis et al. (2016) reviewed the published literature to evaluate the environmental effects of offshore wind power and compare these to onshore wind power operations. The authors described published impacts from offshore wind power to birds, marine mammals, fish, and benthic invertebrates as well as impacts from associated noise and visual effects. Three key conclusions were posited by the authors. First, there is a lack of in-depth knowledge of the environmental and social impacts of offshore wind power. Second, there is no clear indication offshore wind power is more beneficial to flora and fauna than onshore wind power operations. Finally, implementation of new technologies, materials, and construction methods affect the environmental effects and efficiency of energy generation.

The impact of offshore wind farms to populations of the common shore crab (*Carcinus maenas*) were investigated at Lillgrund offshore wind farm in the Öresund Strait between Sweden and Denmark. Langhamer et al. (2016) found no significant effects in catch per unit effort, crab size, and crab body condition from the offshore wind farm, including a hypothesized positive effect from the creation of more complex habitat. Differences in population sizes between Lillgrund and the two control sites were not possible because of very low recapture rate.

Effects of Marine Debris

In 2016, a number of research papers and reports were published on the full suite of interactions between types of marine debris and the resulting effects on organisms that live in the marine environment. Here, we summarize key studies that moved the state of the science forward, and refer the reader to additional research for further details. Perhaps the notable trends included less research on entanglement of marine organisms in derelict fishing gear, and an abundance of research reporting ingested debris in a number of novel locations and/or species across the world. The following text is divided into sections on entanglement, ingestion, toxicity of ingested debris, and biofouling of debris by potentially toxic organisms.

Entanglement. This section documents published interactions between marine organisms and various types of debris, including debris utilized in nesting material of several avian species.

Stelfox et al. (2016) reviewed global reports of marine mammal, reptile, and elasmobranch entanglement in derelict fishing gear, and found 76 reports of 40 individual species entangled in or otherwise associated with derelict fishing gear. Also focused on large marine megafauna, Farmer et al. (2016) evaluated a number of potential management actions aimed to decrease the entanglement of federally-protected North Atlantic right whales (*Eubalaena glacialis*) in commercial fishing trap gear used in the black sea bass (*Centropristis striata*) fishery. While this paper does not specifically focus on derelict trap gear, it

contextualizes the impact that lost gear may have on particularly sensitive marine mammal populations (Farmer et al. 2016).

Fujisaki and Lamont (2016) published a study on the presence of large natural and anthropogenic debris items on the number of successful nesting attempts by sea turtles nesting along a stretch of beach in Gulf County, Florida. Along the stretch of beach where debris was removed, the incidence of successful nesting attempts tripled while there was no change in nesting attempts along the stretches of beach where debris was not removed (Fujisaki and Lamont 2016). This study provides evidence for the success of debris removal as a restoration action that may assist threatened and endangered sea turtle populations.

Petersen et al. (2016) recorded field observations of plastic utilized by sooty terns in nesting material on Trindade Island, a remote location off the Brazilian coast in the Atlantic Ocean. Petersen et al. documented 78 plastic fragments in 54 or 1,800 nests (accounting for 3 percent of the breeding group), and observed a higher incidence of blue plastic fragments than any other color (white, green, and red). Similarly, Tavares et al. (2016) documented marine debris in nesting material produced by the brown booby (*Sula leucogaster*) on two Brazilian islands. Here, fishing gear and hard plastics were most frequently observed, which mirrored the debris types found in nearby coastal waters. Approximately 61 percent of the 203 surveyed nests contained some form of marine debris (Tavares et al. 2016).

Ingestion. A large number of papers were published on the topic of marine debris ingestion, covering a wide variety of species and organism groups. This section focuses on several key studies and refers the reader to additional papers that may be of interest.

Clark et al. (2016) review the mechanisms by which microplastics may interact with marine organisms, and propose a research plan to determine the prevalence of these interactions to better understand whether the proposed “missing” microplastics (i.e., the mis-match between observed amounts of plastic debris in the global ocean with modeled predictions) is accounted for by interactions with marine organisms.

Cole et al. (2016) provide supporting evidence that polystyrene microplastic particles (measuring 20.6 μm) are egested by the copepod *Calanus helgolandicus* within fecal pellets, and that those fecal pellets had a more than two-fold reduction in sinking rate and a higher fragmentation rate. The authors suggest this ability to egest microplastics may remove this material from surface waters (Cole et al. 2016).

Gusmao et al. (2016) detected microplastic fibers in the gut contents of three species of the sandy beach annelid *Saccocirrus*, potentially due to the non-selective suspension-feeding behavior of these species, though observations within the laboratory did not detect immediate physical injury to the organisms. Additional studies focused on marine invertebrates, particularly ingestion of debris by crabs and lobsters, include Watts et al. (2016), Welden and Cowie (2016a, 2016b), and Wojcik-Fudalewska et al. (2016). Lastly, Rosas-Luis (2016) determined that 12

percent of the jumbo squid (*Dosidicus gigas*) caught off the coast of Ecuador contained plastic fragments, particularly polyethylene and polyvinyl chloride fragments which may be fishing-related.

A single paper focused on determining the most reliable analytical methods for analyzing microplastic content in seafood samples. Dehaut et al. (2016) published a methodological comparison of six techniques used to separate microplastic particles and fibers from biological tissues, and discussed the digestion efficiency of each approach related to the many polymers that have been found in mussel, crab, and fish tissues. Dehaut et al. (2016) recommend one approach as the best compromise for achieving tissue digestion without dissolution or decomposition of multiple polymer types.

Tanaka and Takada (2016) detected microplastic particles, including fragments and microbeads, in the digestive tracts of 77 percent (49 of 64) of the sampled Japanese anchovy (*Engraulis japonicas*) collected from Tokyo Bay. The size distribution of particles (150-1,000 μm) is smaller than many particles found at the sea surface, and is possibly explained by the anchovy's sub-surface foraging behavior.

In another study that elucidated a connection between foraging behavior and plastic ingestion, Amelineau et al. (2016) measured microplastic abundance in the zooplankton and little auk (*Alle alle*) communities off the eastern coast of Greenland in 2005 and again in 2014. There was a noted increase in microplastic particles over time (from 0.99 ± 0.63 particles/ m^3 to 2.38 ± 1.11 particles/ m^3), which could be the result of increased plastic

production or lower sea ice conditions in 2014. Within the little auk colony, a total of 44 gullet pouches were sampled to analyze the diet fed to auk chicks for microplastics; all adult birds had consumed microplastic filaments, with an average of 9.99 and 8.99 pieces per chick meal in 2005 and 2014, respectively (Amelineau et al. 2016). The authors suggest that melting sea ice may be the source of the plastic filaments observed in this study.

In Ireland, Acampora et al. (2016) conducted months of beached bird monitoring surveys as a proxy/indicator for marine debris levels. Approximately 27 percent of beached birds (33 of 121 individuals) had ingested litter, with an average mass of 0.141g, which exceeds the policy target of less than 0.1g used by the Marine Strategy Framework Directive (Acampora et al. 2016).

In China, Zhao et al. (2016) investigated the digestive tract contents of 17 birds that were obtained opportunistically, including both terrestrial and coastal species. The authors found microplastic fibers and natural fibers in the esophagus, stomach, and intestine of 16 birds, thus confirming that such varied species as the common buzzard (*Buteo buteo*) and the common sandpiper (*Actitis hypoleucos*) had ingested microplastics regardless of feeding preferences. Zhao et al. (2016) is one of the first documented reports of terrestrial birds ingesting both natural and microplastic fibers.

Additional studies investigated debris ingestion by the following species, and in multiple instances made the case for utilizing bird pellets for debris monitoring: white-faced storm petrels (predated by gulls) in Furtado et

al. (2016); great skuas, Hammer et al. (2016); Newell's and wedge-tailed shearwaters, Kain et al. (2016); Laysan albatross and Bonin petrels, Lavers and Bond (2016); kelp gulls, Lenzi et al. (2016); and multiple coastal and marine species of eastern Australia were investigated by Roman et al. (2016).

A number of studies added to the growing body of literature documenting and quantifying the effect of marine debris on sea turtle species. Schuyler et al. (2016) conducted a global risk analysis that cross-referenced simulated marine debris distribution with known sea turtle habitats to determine likely hotspots where sea turtles are likely to interact with marine debris. Specifically, the probability of debris ingestion was the modeled outcome, and the parameters included parameters such as life history stage, species, and date of observation as possible factors affecting the analysis. The authors determined that the oceanic life stages are the most vulnerable, and olive ridley turtles are the most at-risk species. In addition, the following studies documented debris ingestion in sea turtles around the globe: de Carvalho-Souza et al. (2016); Fukuoka et al. (2016); Gama et al. (2016); Nelms et al. (2016); Nicolau et al. (2016); Oros et al. (2016); Ryan et al. (2016); van Houtan et al. (2016). A noteworthy review of the documented interactions between marine turtles and plastic debris highlighted the fact that all seven species of sea turtles are known to ingest or become entangled in marine debris, which can result in reduced growth rates, reduced reproduction, lacerations, increased drag, starvation, and death (Nelms et al. 2016).

Lastly, an important report by Unger et al. (2016) documented large amounts of marine debris in each of the 22 analyzed sperm whales (*Physeter macrocephalus*) that stranded along the North Sea coast in early 2016. Findings highlight the high degree of exposure to marine debris, though it was not determined that any of the ingested debris items were responsible for the strandings (Unger et al. 2016).

Toxicity. Marine debris has increasingly been the subject of toxicity studies, elucidating the relative magnitude of effects that ingestion of various synthetic polymers has on marine organisms. In 2016, Bakir et al. conducted a modeling study focused on the pathways that transfer hydrophobic organic chemicals to organisms that ingest plastic fragments. Bakir et al. (2016) varied pH, temperature, and gut surfactants in models of dichlorodiphenyltrichloroethane (DDT), phenanthrene (Phe), and bis-2-ethylhexyl phthalate (DEPH) uptake to multiple trophic levels, and found that the most important transfer pathways included food and water and that plastic was not predicted to be a major route of exposure. In addition, Bakir et al. (2016) did not observe a significant difference in these scenarios based on the type of plastic (polyethylene and polyvinyl chloride) tested as an exposure pathway. Li et al. (2016) studied the effects of water containing chemicals leached from seven commonly used plastic polymers on the survival and settlement of the barnacle *Amphibalanus amphitrite*. When exposed to leachate from each of the seven polymers, Li et al. (2016) documented larval toxicity and inhibited larval settlement

in a 24-hour exposure at concentrations ranging from 0.10-0.50 mg/L.

In a trophic transfer study, Batel et al. (2016) developed a food chain model with *Artemia sp.* nauplii and *Danio rerio* to investigate uptake and transfer of 1-20 µm microplastic particles from *Artemia* to *Danio*. Experiments tracked uptake and transfer to zebrafish ingesting the nauplii, though effects of exposure were inconclusively demonstrated by an analysis of EROD activity in zebrafish liver.

Jang et al. (2016) conducted a study to fill a gap in research on the uptake of plastic-associated chemicals from polystyrene foam to marine organisms. The authors compared levels of brominated flame retardants (hexabromocyclododecanes, HBCDs) in mussels living on four different types of substrates, including polystyrene, high-density polyethylene, metal, and rocks (Jang et al. 2016). Mussels using polystyrene buoys as substrate had measured concentrations of HBCD ranging from 23.1-5,160 ng/g lipid weight, concentrations that are among the highest reported measurements of HBCD in mussels. Jang et al. (2016) also found that mussels ingested polystyrene particles; comparatively, mussels growing on other substrates accumulated less HBCD.

Sussarellu et al. (2016) looked at the effect of polystyrene particle (2-6µm) exposure on the physiology of the Pacific oyster (*Crassostrea gigas*), and determined that the oysters preferentially ingested 6-µm particles instead of 2-µm particles. After the two-month exposure, exposed oysters had significant decreases in oocyte number and diameter; a decrease in D-larval yield (i.e., a significant

reduction in larval yield when oocytes from exposed oysters were crossed with control spermatozoa, as compared to oocytes from control oysters); and increases in energy allocation from reproduction to structural growth (Sussarellu et al. 2016). Rist et al. (2016) exposed Asian green mussels (*Perna viridis*) to polyvinylchloride particles measuring 1-50µm that were suspended in the water column, and found that filtration and respiration rates were lowered with increasing exposure to the particles. After an exposure period of more than 90 days, mussel survival rates also declined (Rist et al. 2016). In a similar study, Silva et al. (2016) tested the toxicity of leachates from virgin polypropylene and beached plastic pellets to brown mussel (*Perna perna*) embryonic development, and determined increased toxicity from beached pellet leachate, likely from chemicals that adsorbed to the pellets during environmental transport.

In a field-based study, et al. (2016) measured a suite of trace elements and persistent organic pollutants in the tissues of Antarctic prions, and found notably high concentrations of BDE-209 in some birds, which the author suggest could be the consequence of plastic debris ingestion. Fromant et al. (2016) found debris ingestion in the stomach contents of five of the ten individual birds analyzed, though the authors did not provide direct evidence of a correlation between debris ingestion and levels of BDE-209.

To relate field-based and laboratory-based studies, and compare the experimental parameters commonly utilized in laboratory settings to real-world concentration, Phuong et al. (2016) conducted a review of

microplastic particles found in the environment (seawater, sediment/sand, and biota) and compared the resulting list to those particles utilized in laboratory exposure experiments. In general, Phuong et al. describe the complexity of conducting realistic exposures given the wide variety of sizes, three-dimensional shapes, concentrations, exposure times, and associated chemicals that could influence the toxicity of microplastic particles on marine organisms.

Results of laboratory leaching-based studies were reported in the literature, including Brennecke et al. (2016) which examined the leaching of copper and zinc from antifouling marine paint and subsequent uptake to polystyrene and polyvinyl chloride microplastics, and describe the kinetics of the copper and zinc adsorption to both types of particles. Adsorption to polyvinyl chloride particles was significantly greater than to polystyrene particles, which the authors hypothesize was the result of greater surface area and polarity (Brennecke et al. 2016). Wu et al. (2016) determined the linear sorption coefficients of several chemicals associated with personal care products (e.g., carbamazepine and triclosan) to polyethylene plastic measuring 250-280 μm . Staniszewska et al. (2016) conducted experiments to determine leaching rates for three chemicals (bisphenol A (BPA), 4-tert-octylphenol (OP), and 4-nonylphenol(NP)) from polycarbonate and recycled tire granules. Zhan et al. (2016) investigated the sorption of PCB77 to polypropylene plastic, varying particle size, temperature, and the composition of the seawater medium; the authors found sorptive capacity increased with decreasing particle size and temperature. Lastly, Turner et al. (2016) characterized 70 samples of foamed plastic

collected from a beach in England, and found that most samples contained detectable levels of bromine, iron, and zinc, indicating perhaps the presence of halogenated flame retardants, iron oxides, and zinc-based additives to polyurethane and polystyrene foams. Additional modeling experiments determined a bioaccessible amount of lead mobilized from the polyurethane foam (Turner et al. 2016).

Biofouling. The presence of marine debris acts as a hard surface in the coastal and open ocean, and serves as a substrate for microbial communities and an attachment point for various organisms. There has been increased research over the past few years to elucidate the possible capability for some debris items to serve as vectors of invasive, harmful, and pathogenic species. Adding to the growing body of literature, Oberbeckmann et al. (2016) characterized microbial film communities that formed on deployed polyethylene terephthalate (PET) drinking bottles exposed to natural conditions in the North Sea. The communities that formed on PET bottles were significantly different from free-living communities in seawater collected from the same locations but were not significantly different from other particle-associated communities or those communities that attached to glass as a substrate (Oberbeckmann et al. 2016). Kirstein et al. (2016) collected microplastic particles from the North and Baltic Seas, isolated bacterial colonies from biofilms present on these particles, and found potentially pathogenic *Vibrio* species on polyethylene, polypropylene, and polystyrene particles. The presence of potentially pathogenic species in biofilms on microplastics makes the case for increased understanding of the biogeographical origins and pathways

of debris on local and global scales. For additional research into the presence of biofilms and hitchhiking organisms on marine debris, see Fazey and Ryan (2016), Gil and Pfaller (2016), Keswani et al. (2016), Li et al. (2016), Maso et al. (2016), Nauendorf et al. (2016), Rech et al. (2016), and West et al. (2016).

Effects of Oil Spills: 2010 Deepwater Horizon in Gulf of Mexico

Reviews. Beyer et al. (2016) reviewed and summarized the findings of the peer reviewed literature published through January 2016 about the Deepwater Horizon oil spill, covering the fate and effects of the spilled oil and gas in offshore, nearshore, and coastal sites, as well as effects on long-lived organisms like deep-sea corals, sea turtles and marine mammals. Biological effects included significant alterations in microbial communities in habitats ranging from the pelagic ocean, pelagic benthos, nearshore waters, oiled beach sediments, and marsh sediments; incorporation of oil derived carbon into pelagic food webs; adverse effects on both deep-sea and shallow water coral communities; increased erosion in heavily oiled marshes, reduced growth rates of brown shrimp at heavily oiled sites, a shift to a higher trophic position for red snapper (*Lutjanus campechanus*) at studied reefs, developmental effects on embryos of various fishes, acute mortality of numerous birds exposed to oil, particularly to shorebirds, as well sub-lethal effects on bird health and behavior; as much as a 5x increase in sea turtle strandings, particularly of Kemp's ridley (*Lepidochelys kempii*) turtles, significantly greater

and more severe disease occurrences in dolphins in the oiled areas as compared to unoiled, among many others.

Fisher et al. (2016) conducted a review of the impacts of the Deepwater Horizon spill on the deep-sea ecosystems of the northern Gulf of Mexico. Impacts range from incorporation of oil derived carbon into the epipelagic, mesopelagic, and bathypelagic food webs, likely loss of a year class of epipelagic predators, shifts in sperm whale distribution, reductions in benthic species richness and diversity, and injuries to deep-sea colonial coral and mesophotic reefs, among many others.

Rabalais and Turner (2016) summarized the research on the effects of the Deepwater Horizon oil spill on coastal marshes and associated organisms. In heavily oiled areas, there was extensive die back of marsh vegetation, and recovery time varied considerably from as few as 17 months to >4 years. In some areas with heavy oiling, shoreline erosion rates tripled over background. *Juncus roemerianus* (black needrush) was more sensitive to oiling than *Spartina alterniflora* (marsh cordgrass) in both laboratory and field studies. In oiled marshes, the diversity of the microbial community decreased significantly with increasing concentrations of total petroleum hydrocarbons (TPH), but diversity increased over time as TPH concentrations declined. Marsh invertebrates like fiddler crabs (*Uca spp.*), salt marsh snails (*Littoraria irrorata*), horse fly, and terrestrial arthropods (e.g. predators, sucking herbivores, stem-boring herbivores etc.) displayed significant population losses in oiled sites with variable recovery rates ranging from 1 year to >4 or more. Seaside sparrow (*Ammodramus maritimus*) nests were less likely to

fledge successfully in oiled areas for >2 years post spill, and increasing PAH concentrations in the blood of common loon (*Gavia immer*) resulted in reduced body mass. Marsh and seagrass nekton populations showed no adverse impacts.

Exposure in the Water Column. Wade et al. (2016) evaluated over 20,000 water quality samples collected during and after the Deepwater Horizon oil spill in both State (nearshore) and Federal waters (offshore) from the southeast coast of Florida to Texas, and at depths ranging from 0 to >1600 meters (m). 84% of 13,172 total petroleum hydrocarbon (TPH) water samples reflected background (<1 µg/L) concentrations, as did 79% of 16,557 PAH water samples (0.056 µg/L). The highest TPH (>3000 µg/L) and PAH (>100 µg/L) concentrations were collected near the wellhead (<25 kilometers) and either within 1 m of the surface or in subsurface plumes (800 to 1400 m).

Effects on Organic Matter Composition. Prouty et al. (2016) documented significant changes after the Deepwater Horizon oil spill in the marine sourced organic matter (OM) sampled from sediment traps in the north-central Gulf of Mexico, indicating reduced primary production and carbon export to the deep-sea. Marine-sourced sterols and n-alkanes (e.g. C17-C19) decreased significantly between 2008-2009 to 2010-2011. Specifically, there were significant reductions in the sterols 22-dehydrocholesterol (226 reduced to 97 µg g⁻¹), cholesterol (1868 reduced to 696 µg g⁻¹), and brassicasterol (315 reduced to 193 µg g⁻¹). Marine sourced n-alkanes shifted from from 76% bulk composition in

2008-2009 to 48% in 2010-2011. In addition, %Corg significantly decreased from 4.45±1.10% (avg) to 2.82±0.57% (avg).

Plankton Blooms. Using sediment traps deployed near the Deepwater Horizon wellhead site, Yan et al. (2016) documented a massive diatom (*Skeletonema sp.*) bloom and associated sedimentation event in Sept. 2010 that deposited 109 mg particulate organic carbon (POC) ·m⁻²·d⁻¹ to the seafloor along with ~120 µg·m⁻²·d⁻¹ of the sum of n-alkanes C22 to C36 (ΣSH), 8.40 mg ·m⁻²·d⁻¹ black carbon, 1.6 mg ·m⁻²·d⁻¹ barium, and 3.70 mg ·m⁻²·d⁻¹ olefins. POC deposition was >5x that in the fall of 2011 and 2012. Deposition of Deepwater Horizon derived hydrocarbons continued for at least five months following the capping of the Macondo well, with the potential that 7.2 (±0.6) x 10⁸ grams ΣSH or 0.14 (±0.01%) of the total crude oil released was deposited to the seafloor via co-sedimentation with diatoms.

Microbial Community Impacts. Kleindienst et al. (2016) described shifts in the oil degrading bacterial community associated with the deepwater plume generated by the Deepwater Horizon oil spill. The use of oligotyping illustrated the diversity of the bacterial taxa, primarily associated with *Oceanospirillaceae*, *Cycloclasticus*, and *Colwellia*, most of which had not been previously recognized. These bacterial communities shifted rapidly in response to the changing biogeochemical conditions in the hydrocarbon enriched plume.

Yang, Nigro, Gutierrez et al. (2016) observed that the bacterial community in the water column near the Macondo wellhead changed dramatically during the

Deepwater Horizon (DWH) oil spill. Pre- and post-DWH, Alphaproteobacteria (SAR11), SAR406 lineage, deltaproteobacteria (SAR324), and gammaproteobacterial lineages including Oceanospirillales and Alteromonadales dominated the population (76%) with miscellaneous others comprising the remainder. During the DWH spill, *Cycloclasticus*, *Pseudoalteromonas*, *Alteromonas*, and *Cowellia* dominated surface slick populations, while Oceanospirillales dominated the deep hydrocarbon plume initially, but was later overtaken by *Cycloclasticus* and *Cowellia*. The bacterial community was approaching its pre-spill composition by September 2010, although small numbers of oil degrading bacteria remained detectable. Yang, Speare, McKay et al. (2016) also documented significant differences in bacterial communities in oil contaminated versus non-oiled seafloor sediments following the Deepwater Horizon oil spill. *Roseobacter*, *Desulfobacteraceae/ Desulfobulbaceae*, *Bacteroidetes*, *Verrucomicrobia*, and *Cycloclasticus* dominated in oil contaminated sediments, but were only minimally represented or not at all in unoiled sediments. By November 2010, bacterial community composition began to resemble pre-spill conditions.

Ziervogel et al. (2016) observed increased microbial activity in the top 0-2 cm of sediment cores collected in November and December 2010 near the Macondo wellhead as evidenced by strong gradients in porewater dissolved organic carbon (DOC), dissolved ammonium (NH₄⁺), and inorganic phosphate (HPO₄³⁻) concentrations, and significantly enhanced β-glucosidase,

lipase activity, and bacterial protein production (3x), as compared to cores collected from a distant site.

Shoreline Impacts. Using data collected from a wide variety of sources, Nixon et al. (2016) determined that 2,113 km of shorelines across Texas, Louisiana, Mississippi, Alabama, and Florida were oiled by the Deepwater Horizon oil spill, a 19% increase over previously reported estimates. The majority of the shoreline oiling occurred in Louisiana (65%), with the least in Texas (2.7%). Of the 2,113 km, just over half were wetlands (52% of total), and just under half were beaches (46%), with the remaining 2% being other shoreline types. The vast majority of wetland oiling occurred in Louisiana (95%).

By comparing size-frequency distribution data of sub-adult and adult salt marsh snails (*Littoraria irrorata*) from the years before, during, and after the Deepwater Horizon oil spill, Pennings et al. (2016) determined that there was a 90% reduction in the proportion of sub-adult snails in Louisiana as compared to pre-2011 numbers. The authors suggest that the significant reduction in this age/size group was a result of widespread recruitment failure during the Deepwater Horizon oil spill.

Zengel, Montague, Pennings et al (2016) recorded significant reductions in marsh periwinkle (*Littoraria irrorata*) total densities between reference and oiled sites, at both the marsh edge (reference = 34±14; oiled untreated = 7±2; oiled treated = 3±1) and marsh interior (~9m inland) (reference = 95±28; oiled untreated = 47±12; oiled treated = 45±18), corresponding to reductions of 80-90% and 50%, respectively. Furthermore,

significantly reduced adult periwinkle densities (1 ± 0.4) occurred in the oiled and treated marsh edge areas, along with a significant shift to smaller sub-adult snails in both the treated oiled marsh edge and treated oiled marsh interior. Zengel, Pennings, Silliman et al. (2016) observed significant reductions in Fiddler crab (*Uca sp.*) burrow size and density on oiled shorelines in the aftermath of the Deepwater Horizon oil spill. Burrow size was significantly smaller in the marsh interior landward of the main oiling band (2010) and at the oiled marsh edge (2011) with reductions by 44 and 21%, respectively. Burrow density was reduced by an average of 39% across oiled sites examined (marsh edge, oiled marsh interior, marsh interior landward of oiling band), with statistically significant reductions at the oiled marsh edge through 2014. There was also a statistically significant shift in *Uca spp.* community structure from *U. longisignalis* to *U. spinicarpa* through at least 2013.

Effects on Fish. Hernandez et al. (2016) found significantly worse body condition (skinnier and weighed less) in red snapper (*Lutjanus campechanus*) larvae (≥ 3.7 mm, ≤ 8 mm) collected during (2010) and after (2011, 2013) the Deepwater Horizon oil spill as compared to those collected in 2007-2009. After adjusting for environmental conditions, worse body condition persisted. Red snapper larval abundance was not significantly different between years.

Ransom et al. (2016) found that Spanish mackerel (*Scomberomorus maculatus*) larvae (≤ 7 day) collected in 2010 during the Deepwater Horizon oil spill were in better body condition as compared to larvae

collected in 2007 and 2009. Larval abundance was not significantly different between years, and there was no notable change in stable isotope signatures in larvae between years to suggest incorporation of oil derived carbon into body tissues.

Using assemblage data for near-coastal fish collected pre- and post-Deepwater Horizon oil spill, Schaefer et al. (2016) found that catch per unit effort (CPUE) increased by 2.5x in 2011 (mean = 256.3) as compared to other years (mean range = 26.4 – 103.1), and that medium-sized species showed the greatest increase in abundance. There were no significant differences in species diversity between years. Abundance and CPUE returned to pre-DWH levels within one year of the resumption of fishing. The authors suggested that the fishing closures associated with the spill increased adult survival, resulting in greater abundance.

Impacts to Deep Sea corals. Etnoyer et al. (2016) documented a significant decline in the health of gorgonian octocorals located on the deep-water rocky reefs of the Pinnacle trend area in the northern Gulf of Mexico where surface oil slicks were present for 19-39 days during the Deepwater Horizon oil spill. Injury rates were 10.8 times higher post spill and 6.9 times higher for injuries $>10\%$. Overgrowth by hydroids or sedimented material was the predominant injury, but corals also displayed eroded polyps, discoloration, and bare branches, among other injuries. Marked colonies continued to decline in health between 2011 and 2014.

By comparing still photography images taken pre- and post-Deepwater Horizon (DWH) at two

mesophotic coral reef sites in the northern Gulf of Mexico, Silva et al. (2016) found that six taxa of octocoral and antipatharian corals including *Hypnogorgia pendula*, *Swiftia exserta*, *Thesea nivea*, *Bebryce spp.*, *Antipathes sp.*, and *Stichopathes sp.* displayed significantly more numerous and more severe injuries post-DWH. The injuries ranged from retracted polyps with mucus secretion to necrotic tissues with biofilm or hydroid overgrowth to denuded and broken branches. Sediment tPAH levels exceeded background concentrations in both 2010 and 2011 by at least a factor of two at both locations sampled (Alabama Alps Reef (AAR), background = 26, 2010 = 101 (43.9), 2011 = 41.7 (21.8); Roughtongue Reef (RRT), 2000=10, 2010 = 20.1 (16), 2011 = 20 (10.9, all units ppb dry weight). Coral tissues also contained detectable concentrations of tPAH in 2010 and 2011 (AAR = 151 and 345 (791.2) in 2010, 2011 respectively; RRT = 179 (252) and 51 (88), in 2010, 2011 respectively).

Impacts to Turtles. By measuring stable isotope concentrations of carbon and nitrogen in scute tissues collected from Loggerhead (*Caretta caretta*) turtles before and after the Deepwater Horizon oil spill, Vander Zanden et al. (2016) concluded that the turtles did not shift their foraging grounds during or after the spill.

Effects of Other Oil Spills

Australia Montara Spill. Burns and Jones (2016) documented total hydrocarbons (THC) and polycyclic aromatic hydrocarbons (PAHs) in reefs and shoals ranging in distance from 2.5 km to 155 km from the Montara wellhead, site of the Montara well blowout in

2009. THC and PAHs in reef sediments ranged from 0.3 to 2.6 $\mu\text{g g}^{-1}$ and 1.8 to 11.2 ng g^{-1} , respectively, while in shoal sediments, THC ranged from 10.4 to 1.2 $\mu\text{g g}^{-1}$ and PAHs from 31.0 to 0.29 ng g^{-1} . The highest THC and PAHs concentrations were nearly double that of background. However, overall levels were low and below the level predicted to observe biological effects.

Italy. Fiori et al. (2016) observed short-term, negative effects of hydrocarbons on marine algae as sampled from oiled surface waters in the Northern Tyrrhenian Sea in the Mediterranean Sea, particularly nanoplankton and micro-phytoplankton ($>20 \mu\text{m}$) at low concentrations ($<100 \mu\text{g L}^{-1}$ of THC) including decreased abundance (low cell counts) and changes in species composition. In particular, there was a large decrease in centric diatoms (*Cyclotella sp.* and *Chaetoceros spp.*) representation, as well as of Dinophyceae and Gyrodinium sp.

South Korea. Two years after the Hebei Spirit (2007) oil spill, Hong et al. (2016) found that Manila clams (*Ruditapes philippinarum*) collected from oiled areas exhibited enhanced cellular immune activity as expressed through significantly higher granulocyte concentrations, phagocytosis activity, generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and significantly lower parasite loads. In addition, clams from the oiled areas had significantly lower glycogen reserves as compared to the control (un-oiled) sites.

France. Troisi et al. (2016) reported a weak, but statistically significant negative relationship between PAH exposure and plasma thyroid-stimulating hormone (TSH)

concentrations in guillemonts (*Uria aalge*) oiled by the 2002 MV Tricolour spill in Dunkirk Harbor. Mean plasma PAH concentration was 98.1 ± 8.3 ng/ml wet weight (18.8-345 ng/ml, n=50), and mean TSH concentration was 0.13 ± 0.02 ng/ml (<0.01-0.5 ng/ml).

New Zealand (MV Rena spill). Battershill et al. (2016) summarized the research and results of the various environmental studies completed or underway surrounding the MV Rena grounding and oil spill (2011), and presented lessons learned to guide future responses. Three years post spill, PAHs remain above background levels at the Astrolabe Reef, but on the Bay of Plenty coastline, there was little evidence of the oil. Contamination of endemic surf clams (*Paphies subtriangulata*) by PAHs occurred, but the clams appear to have rapidly depurated them (within four months). Rocky intertidal communities recovered within one month of oiling. The most persistent impacts to Astrolabe Reef are associated with contamination by copper and tributyltin. Smith et al. (2016) recommended that assessments of biosecurity risk be incorporated into emergency response planning to minimize and mitigate the introduction of invasive and non-native species because of maritime accidents like the grounding of the MV Rena (2011) and associated oil spill. Surveys of salvage vessels brought from Australia documented 12 non-indigenous species on the vessels, six of which had never before recorded in New Zealand. Surveillance via dive surveys and shoreline searches in Tauranga Harbor, Mōtītī Island, and Astrolabe Reef through 2013 found no evidence of these species.

Intentional Research Oil Spills. Brussaard et al (2016) monitored the fate and effects of two intentionally released 4-5 m³ crude oils at offshore sites within the Netherlands North sea EEZ. They also conducted environmental monitoring around surface slicks observed in heavily used shipping routes. The authors reported concentrations at depth over time of hydrocarbons and PAHs, most notably observed no visible sheen on the water surface but detected oil components at a depth of 8m and lateral distance of 500m at 20 hours post controlled spill and a series of assays that indicated that the spills provoked toxic responses within 1 day of release, concluding that even small spills can cause local injuries in the upper water column.

Background and Risk Studies. Carls et al. (2016) concluded that there was little cancer risk associated with consuming shrimp and shrimp eggs captured near the Port Valdez Alyeska Alaska Marine Terminal. The authors recorded low TPAH levels in shrimp (*Pandalus platyceros* (Spot), *P. hypsinotus* (Coonstripe), and *P. borealis* (Pink)) eggs (median = 12.1, 46.3, and 89.7 ng/g dry weight (wt.) for Spot, Coonstripe, and pink respectively), cephalothoraxes (median = 11.2, 4.7, and 88.0 ng/g dry wt. for Spot, Coonstripe, and pink, respectively), and muscle (median = 2.9, 0.6, and 10.9 ng/g dry wt. for Spot, Coonstripe, and pink, respectively). There was no evidence of petrogenically derived hydrocarbons in shrimp muscle or cephalothoraxes, but low levels petrogenic PAHs were present in coonstripe and pink shrimp (median = 90 ng/g dry wt.) eggs.

Nesvacil et al. (2016) found very low TPAH concentrations (≤ 42 ng/g dry weight) in sediments sampled in 2011-2014 from St. Paul Island, Alaska (AK) and Cook Inlet, AK. Water column TPAH and tissue samples collected from marine gastropods (*Macoma sp.*) and mussels (family Mytilidae) generally reflected similarly low concentrations with most samples at ≤ 178 ng/g device and < 500 ng/g dry weight, respectively. The authors concluded that TPAH concentrations in these habitats are not large enough to cause adverse effects to the Pribilof Island rock sandpiper (*Calidris ptilocnemis ptilocnemis*).

Using oil spill probability of occurrence information for Canada's Pacific coast, overlapped with areas of overall importance to, and distribution and density information of, multiple marine bird species and groups, Fox et al. (2016) determined that the highest risk areas are the northern Hecate Strait and eastern Dixon Entrance, particularly adjacent to the mainland, and the area adjacent to the Scott Islands and extending south into the Queen Charlotte Strait, off northern Vancouver Island. In general, those species with high densities near the mainland coast of British Columbia and the northeast coast of Vancouver Island had the highest exposure risk, including large gulls, cormorants, Pigeon Guillemot, grebes, and small gulls.

Effects of Oil, Dispersants and Dispersed Oil on Marine Organisms

Review and Overview. Incardona and Scholz (2016) reviewed publications reporting on cardiotoxicity affects, specifically edema, and linked similarities among freshwater, marine, and pelagic fish species. The authors

summarize exposure of embryonic and early life stage fishes to PAHs and other toxicants found in freshwater and marine environments impact normal heart morphogenesis leading to reduced hatch success and other effects observed in later life stages making the fish more vulnerable. Dornberger et al (2016) evaluated available literature to determine the best toxicity model, specifically linear, step-wise, hockey stick, and exponential, using data reported on juvenile, adult, and pelagic fish health and growth effects post PAH exposure. The authors report the hockey stick model as the best fit while acknowledging current available data which use multiple test mediums, species, and conditions make the model open to improvements as new information becomes public. An overview of dispersants available, chemical processes that occur when dispersants are applied to oil in water is provided by Vijay et al (2016) and the authors describe the fate of oil/dispersant mixtures in the environment and its impact in the role of spill response. The authors conclude continued laboratory research should be wary of exposure concentrations and duration of exposure due to the immediate dilution factor occurring in the environment and that dispersants are a reasonable tool to use in appropriate oil spill scenarios.

Coral. Negri et al (2016) conducted acute laboratory toxicity tests to assess the effect of exposing North West Shelf condensate WAFs to the coral, *Acropora tenuis*, and the sponge, *Rhopaloeides odorabile*, larvae for 24 hours with varying degrees of UV light exposure. Initial concentration of tPAH's reported for both organism exposures are reported as 157 μ g/L and 224 μ g/L for coral

and sponge, respectively. The authors report IC_{50} values as 339 μ g/L and 132 μ g/L TPAH for coral with no UV alteration and increased UV light, respectively and 16,000 μ g/L and 13000 μ g/L TPHA for sponge with no UV alteration and increased UV light, respectively. Acute toxicity tests using *Paramuricea* type B3, *Callogorgia delta*, and *Leiopathes glaberrima* exposed to field collected oil from the Deepwater Horizon incident as oil-water mixtures, water-accommodated fractions (WAFs), and chemically-enhanced water accommodated fractions (CEWAFs) prepared with Corexit 9500A for 96 hours was published by DeLeo et al (2016). The authors report effect of treatments on *Paramuricea* type B3 to be mortality observed in highest test dispersant concentration of 25ppm, mortality in all WAFs treatments ranging 50-250 μ M, and mortality in the high concentration 250 μ M of oil/dispersant treatments. The effect on *C. delta* was reported as no observed effect in oil only treatments, and a decrease in vitality when exposed to Corexit 9500A and CEWAF mixtures. The authors reported observed mortality effects on *L. glaberrima* at high concentrations of oil exposure, no significant observations for dispersant only treatments, and effects at medium and high concentrations of oil/dispersant mixtures.

Copepods. Alemda et al (2016) conducted laboratory toxicity tests on *Acartia tonsa*, *Temora turbinata*, and *Pseudodiaptomus pelagicus* exposed to Light Louisiana sweet crude oil only, LSC/Corexit 9500A mixture, and Corexit 9500A only treatments, in addition exposed UVB radiation to each treatment. Acute 48 hour tests were completed using the following exposure

concentrations, with and without UVB, for *Acartia tonsa* and *Temora turbinata* a range of 0.5 - 2 μ LL⁻¹ oil only, all three organisms were exposed to crude oil alone at 1 μ LL⁻¹, 0.05 μ LL⁻¹ dispersant, and a mixture of 1 μ LL⁻¹ of oil and .05 μ LL⁻¹ of dispersant. The authors report LC50 values of oil only tests with *A. tonsa* as 1.88 \pm 0.03 μ LL⁻¹ and 1.25 \pm 0.05 μ LL⁻¹ without and with UVB, respectively, as well as LC50 for *T. turbinata* as 1.95 \pm 0.12 μ LL⁻¹ and 1.34 \pm 0.09 μ LL⁻¹ without and with UVB, respectively. The authors did not report LC50's or EC50's for the remaining tests, but provided statistical data confirming the organisms were significantly affected by the combination of UVB exposure and toxicant mixtures than toxicant mixtures alone.

Amphipods. Olsen et al (2016) exposed the deep-sea benthic amphipod *Eurythenes gryllus* to Brut Arabian Light oil dispersed with Finasol® at nominal concentrations of 0, 90, 188, 313, 500, and 625mgL⁻¹ oil solutions and 0, 5, 9, 16, 25, and 31 mgL⁻¹ dispersants solutions under laboratory conditions for 72 and 96 hours. The authors reported LC₅₀ concentrations of measured total petroleum hydrocarbon concentration (TPH) as 101mgL⁻¹, 24mgL⁻¹, and 12mgL⁻¹ at 24, 72, and 96 hours, respectively. A 28 day laboratory toxicity study was completed by Lotufo et al (2016) exposing *Leptocheirus plumulosus* to sediments contaminated with field collected weathered oil sampled during DWH with tPAH₅₀ concentrations ranging from 0.3 to 24 mg/kg. The authors were unable to calculate an LC50 due to inconsistent lethal effects across test concentrations; however, an EC50 value related to growth of the organisms was reported at 1.13mg/kg tPAH₅₀.

Redman et al (2016) conducted 48 hour laboratory acute toxicity tests exposing *Daphnia magna* and *Americamysis bahia* to Endicott crude oil and oil/Corexit 9500 mixtures by utilizing two different dosing systems to compare effects associated between oil droplet size and dissolved oil on organism toxicity. The authors report no significant difference in the two methods of exposing dissolved oil versus droplet oil, for both organisms and assert these two methods of toxicity testing and analysis output communicating toxicity via toxic units is preferred to understand effects of dispersed crude oil in the environment.

Shrimp and Crab. DeLorenzo et al (2016) published laboratory toxicity tests using adult and larval stage grass shrimp, *Palaemonetes pugio*, exposed to Corexit 9500 (C) and Finasol OSR 52 (F) nominal concentrations of 37, 111, 333, and 1000mg/L at salinity concentrations of 5, 20, and 30ppt. The authors reported larval grass shrimp 96 hour LC50 values as 8.21mg/L (F) and 35.4mg/L (C) at 5 ppt, 16.83mg/L (F) and 40.1mg/L (C) at 20ppt, and 29.35mg/L(F) and 67.9mg/L (C) at 30ppt. The adult grass shrimp 96 hour LC50 toxicity values as 15.1mg/L(F) and 377mg/L(C) at 5ppt, 32.2mg/L(F) and 419mg/L(C) at 20ppt, and 64.5mg/L(F) and 447mg/L(C) at 30ppt. Sagerup et al (2016) investigated the effect of marine diesel exposures to Arctic red king crab (*Paralithodes camtschaticus*) at concentrations of 7.4 ± 1.9 $\mu\text{g/L}$ and 17.4 ± 2.2 $\mu\text{g/L}$ THC, in a flow through design for one week with a 3 week depuration and observation period. The authors reported observations of avoidance behavior in exposure treatments, no reduced food

consumption, and observed PAH uptake in organism tissues after exposure and reported bioaccumulation to be at non detect levels after depuration period.

Mollusks. Geraudie et al (2016) completed a 7 day laboratory toxicity test using the Icelandic scallop, *Chamys islandica* exposed to marine diesel under static conditions. The authors report the low exposure group as 6.4mg/L and high exposure group as 14.23mg/L measured TPH during initial exposure and reported TPH decreased over exposure period. The authors investigated changes in behavioral response, PAH concentration in residues, acetylcholinesterase (AChE) activities, catalase activity, and lipid content and reported no gender-specific significance due to exposure, bioaccumulation of PAH in hemolymphs, no changes in lipid content, and significant AChE inhibition of exposure groups. The Icelandic scallop, *Chlamys islandica*, was used as a test organism by Frantzen et al (2016) in 48hour laboratory toxicity tests exposed to WAFS prepared from Troll crude oil and CEWAFS prepared from Troll crude oil and Dasic NS mixtures reported as nominal test concentrations of 3.33mg oil/L, 66.7mg/L, and 333.3mg/L seawater. The authors reported no observed mortality during the 48 hour test however did observe sublethal effects and reported mortality over the 73 day post-exposure observation period.

Finch et al (2016) evaluated toxicity of 3 life stages and sperm of the eastern oyster, *Crassostrea virginica*, using 48 hour acute laboratory toxicity tests and toxicant solutions prepared from field collected weathered samples of Macondo crude oil solutions diluted to 12.5, 25, 50, and 100% seawater and UV-A light intensities of

3,6,12,18W/m². The authors report LC50 values as 14.9µg/L for sperm at all light frequencies tested, 12.4µg/L, 11.9µg/L, 5.03µg/L for early stage larvae, >12.4µg/L, 12.2µg/L, 7.6µg/L for mid stage larvae, and >12.4µg/L, >12.2µg/L, >12.4µg/L for adult larvae at respective light intensity treatments. Similarly, Volety et al (2016) conducted acute toxicity tests exposing *Crassostrea virginica* spermatozoa and oocytes to field collected Deepwater Horizon crude oil high energy water accommodated fractions (HEWAFs), oil and Corexit 9500A CEWAFs, and Corexit only solutions for 30 mins at which point treatments underwent FCM analysis and observations on fertilization success were evaluated. Treatment concentrations were reported as 16.53, 36.94, 57.50, 94.47, 248.89µg/L tPAH₅₀ HEWAF, 1.29, 3.32, 6.43, 14.24, 26.14µg/L tPAH₅₀ CEWAF, and 0.625, 1.25, 2.5, 5, 10mg/l nominal concentration Corexit 9500A. The authors report fertilization was significantly impacted at 57.5µg/L tPAH₅₀ HEWAF, 26.14µg/L tPAH₅₀ CEWAF, and 10mg/L tPAH₅₀ Corexit 9500A and no impact on spermatozoa at any treatment except for 10mg/L Corexit 9500A where a decrease in viability was observed. Three larval stages of the Eastern oyster, *Crassostrea virginica*, were used in 96 hour acute toxicity tests using toxicants collected from the Deepwater Horizon incident HEWAFs, oil and Corexit 9500A CEWAFs, and Corexit 9500A only test solutions by Vignier et al (2016). Test concentrations of HEWAFs were reported as 95.3, 202.0, 389.9, 761.7, 1605.4, 2985.2µg/L tPAH₅₀ for veliger and umbo stage exposures and 47.8, 112.9, 191.0, 399.1, 719.0µg/L tPAH₅₀ for pediveliger stage exposure, as well as, CEWAF concentrations of 14.0, 25.3,

44.9, 91.2, 178.5 µg/L tPAH₅₀ for veliger and umbo stage and 10.1, 19.1, 43.6, 80.9, 177.3µg/L tPAH₅₀ for pediveliger. The authors report LC50 values for the veliger stage as 715 nominal value HEWAF, 41.8µg/L tPAH₅₀ CEWAF, and 22.9mg/L Corexit 9500A, umbo stage values reported as 2814 nominal concentration HEWAF, 72 nominal concentration CEWAF, 58mg/L Corexit 9500A, and pediveliger 1530µg/L tPAH₅₀ HEWAF, 88µg/L tPAH₅₀ CEWAF, and no observations for Corexit 9500A. Langdon et al (2016) conducted 10 and 28-day chronic static renewal toxicity tests exposing *Crassostrea virginica* to field collected Deepwater Horizon fresh and weathered oil WAFs, field oil samples and Corexit 9500A CEWAFs, and Corexit 9500A only exposures with growth and settlement as endpoints. The authors report LC50 for 10-d survival as >141, >15, >3.49µg/L for 3 WAFs, 5.32, 2.32, 0.747µg/L for 3 CEWAFs, and LC50 for 28-day survival as >15µg/L for 1 WAF, and 0.397µg/L for 1 CEWAF. The authors report results from chronic studies indicate chronic toxicity studies are valuable indicators of toxic exposure and are more sensitive than acute toxicity studies.

Echinoderms. Alexander et al (2016) conducted laboratory toxicity tests exposing 3 life stages of the Antarctic sea urchin, *Sterechinus neumayeri*, to physically dispersed IFO 180, chemically dispersed IFO 180/Slickgone NS mixtures, and Slickgone NS only. The authors reported EC50 values based off nominal concentrations for the unhatched blastula 48hour exposure as 62 and 71µL/L for IFO 180 WAF and 5985 and 12026µL/L for CEWAF. The EC50s for the 10 day exposure test with the gastula stage are reported as 32 and

340 μ L/L for IFO WAF and 2340-4713 μ L/L CEWAF. The EC50s for the 16-18 day exposure of the 4-armed pluteus stage is reported as 26 and 98 μ L/L IFO WAF and 2351 and 766 μ L/L CEWAF. Echols et al (2016c) reported results from a 96 hour acute laboratory toxicity study on the effect of Macondo crude oil and weathered field samples, Corexit 9500, and Macondo crude oil/Corexit mixtures on the ephyrae survival of the jellyfish, *Aurelia aurita*. The authors measured TPAH₄₆ for test solutions at test initiation ranging from 0.096 \pm 0.012 thru 152 \pm 104 μ g/L and reported no mortality in all crude oil and field collected weather oil only tests and an average LC50 of Macondo crude oil/Corexit CEWAF as 131 \pm 65 μ g/L TPAH/L and an LC50 in Corexit 9500 only exposures at 32.3 \pm 3.6 μ g/L. The moon jellyfish, *Aurelia aurita*, was also investigated by Gemmell et al (2016) in laboratory experiment conducting exposures of the organism to emulsified Light Louisiana Sweet Crude oil at a concentration of 1000ppm. The authors report an increase in mucus production and bacterial density as a result of oil exposure and may impact oil spills in the environment by increasing natural degradation.

Fish. Akah et al (2016) conducted laboratory 96 hour acute toxicity studies exposing freshwater species, *Tilapia guineensis*, and brackish water species, *Sarotherodon melanotheron* to artificial mixtures of Corexit 9527 and Forcados crude oil at concentrations of 5, 10, 35, 70, 100, and 450 mgL⁻¹. The authors report LC₅₀'s for *Tilapia guineensis* as 197.00 \pm 6.12, 132.87 \pm 4.33, 76.46 \pm 5.04, and 62.15 \pm 4.50 mgL⁻¹ for 24, 48, 72, and 96 hours, respectively. Additionally, LC₅₀'s reported for *Sarotherodon melanotheron* were 121.34 \pm 6.77, 100.75 \pm 4.28, 55.34 \pm 4.40,

and 45.63 \pm 4.46 for 24,48,72, and 96 hours, respectively. A study investigating effects on Juvenile European sea bass, *Dicentrarchus labrax*, exposed to test solution comprised of 0.08g/L weathered Arabian light crude oil and Corexit 9500A for 48 hours and monitored fish for a 4 month depuration period recording observations such as growth, survival, and swimming performance was published by Maudit et al (2016). The authors report uptake of 21 PAHs in white muscle tissue after 1 month exposure but non-detect thresholds at 4 month post exposure and also reported some impairment of exposed fish occurred during hypoxic challenge and swimming performance tests post 1 month exposure but when tested 11 months post exposure no significant difference from controls were recorded. Nahrgang et al (2016) completed laboratory toxicity tests exposing embryo stage polar cod, *Boreogadus saida*, to concentrations of 0.5, 3, 6g oil per kg of gravel for 37 days in an oiled-rock column experimental design. The authors report treatment concentrations of THC and tPAH were mostly at or below non-detect levels for the entirety of the study and observed significant impairment of fish spinal development and growth, as well as effects to yolk sac was measured in all treatments.

Weathered (slick) oil may be more toxic to fish larvae than fresh oil. Stieglitz et al (2016a) exposed young adult mahi-mahi, *Coryphaena hippurus*, to field collected oil from Deepwater Horizon incident at concentrations of 2.30 \pm 10 μ g/L and 8.40 \pm 0.59 μ g/L PAH₅₀ for 24 hours and observed effects on parameters such as impact to critical swim speed, metabolic rates, and kinematics. The authors report significant differences observed at the 8.40 \pm 0.59

$\mu\text{g/L}$ PAH₅₀ treatment for swimming performance and metabolic rates, and no significant difference among treatments for cost of transport and swimming kinematics measurements. Esbaugh et al (2016) conducted 96 hour laboratory toxicity tests on embryonic mahi-mahi, *Coryphaena hippurus*, using source oil from the Macondo wellhead, field collected weathered surface slick samples, and Corexit 9500 to determine if crude oil source, fresh or weathered, or chemically dispersed mixtures impacted the viability of fertilized embryos. The authors report LC50s as tPAH for high energy oil only WAFs as 45.8, 12.3, 8.8 $\mu\text{g/L}$ for source, weathered, and slick A treatments, respectively, Corexit mixtures as 25.3, 8.7, 9.5 $\mu\text{g/L}$ for source weathered and slick A, respectively, and Corexit only treatment as 3.9 $\mu\text{g/L}$. In a separate study, Stieglitz et al (2016b) reported 96 hour laboratory toxicity tests using a pelagic embryo-larval exposure chamber (PELEC) to account for pelagic embryo's inherent buoyancy and observed effects after exposure to toxicants derived from Slick A DWH Macondo Crude oil HEWAF collected during the incident in combination with full spectrum UV light. The authors reported LC50s with light exposure as 0.7 $\mu\text{g/L}^{-1}$ PAH(50), without light exposure as 6.5 $\mu\text{g/L}^{-1}$ PAH(50), and increased control survival of test organisms using the PELEC design. Similarly, Alloy et al (2016) conducted four 48hour laboratory toxicity tests using *Coryphaena hippurus* embryos exposed to CEWAF and HEWAF mixed from DWH slick oil and artificially weathered CEWAF and HEWAF under two to three UV light concentrations. The authors reported significant effects observed at 100% UV light intensity and the following tPAH₅₀ concentrations

>2.7 $\mu\text{g/L}$ CEWAF, >4.3 $\mu\text{g/L}$ HEWAF, 49.9 $\mu\text{g/L}$ weathered CEWAF, and >4.09 $\mu\text{g/L}$ weathered HEWAF.

Raimondo et al (2016) exposed fertile adult sheepshead minnow, *Cyprinodon variegatus*, and their offspring in a 19-week chronic laboratory toxicity study using artificially weathered South Louisiana crude oil spiked sediments with concentrations measured at 50, 103, 193, 347, and 711mg tPAH/kg sediment in larvae tanks and 52, 109, 199, 358, and 751mg tPAH/kg sediment in juvenile and adult tanks. The authors reported tPAH LOEC concentrations as 50mg/kg and 103mg/kg for larval fish, 199mg/kg and 109mg/kg juvenile/adult, and 109mg/kg and 109mg/kg spawners for standard length and wet weight post exposure measurements. The Caspian roach, *Rutilus caspicus*, was utilized as a test organism by Lari et al (2016) to conduct 96 hour semi-static laboratory toxicity tests using Tehran crude oil WAFs and tested water renewal intervals of 6 and 24 hours. The authors report LC50 values as 62.54mg/L and 35.75mg/L at 24 and 6 hour water renewal intervals, respectively. Finally Xu, Majer et al (2016) also measured biomarker responses in slick and fresh DWH oil exposures: please see Biomarker section, above, for details.

Multiple Species. Echols et al (2016a) completed 96 hour toxicity studies with inland silversides, *Menidia beryllina*, and mysid shrimp, *Americamysis bahia*, using Macondo crude oil collected at the source and two field collected weathered samples of spill oil and varied test design to assess if sealed test chambers, diluent source, and WAF preparation effect toxicity. Due to the primary study objective of testing design variables, PAH ranges for

toxicant solutions were varied for the three tested oils and the authors concluded LC_{50} values were higher in open chamber mysid tests, diluent source did not impact toxicity, and WAF preparation using increased energy procedures increased toxicity. In a separate study, Echols et al (2016b) conducted 21-28 day chronic laboratory toxicity studies exposing mysid shrimp, *Americamysis bahia*, and inland silversides, *Menidia beryllina*, to Macondo crude oil and field collected weathered surface slicks with mortality and growth inhibition as endpoints. The authors reported TPAH₄₂ ranges for exposure concentrations as $2.26 \pm 0.268 \mu\text{g/L}$ to $165 \pm 17.2 \mu\text{g/L}$. LOEC survival and growth concentrations for *A. bahia* as 132, >17.9, >4.75 $\mu\text{g/L}$ TPAH₄₂ for Macondo crude, Slick A, and Slick B WAFs, respectively, and LOEC survival for *M. beryllina* as 132, 12.3, >5.00 $\mu\text{g/L}$ TPAH₄₂ and LOEC growth as 44.1, 7.61, 2.02 $\mu\text{g/L}$ TPAH₄₂ for Macondo crude, Slick A, and Slick b WAFs.

Stefansson et al (2016) tested multiple species in 48 and 96 hour acute toxicity studies using echinoderm larvae of *Strongylocentrotus purpuratus* and *Dendraster excentricus*, as well as mollusks *Crassostrea virginica*, *Crassostrea gigas*, *Mytilus galloprovincialis*, and *Mercenaria mercenaria* exposed to field collected samples from the Deepwater Horizon incident WAFs, CEWAFs prepared with Corexit 9500A, and Corexit 9500A only test solutions. The authors reported no LC_{50} for WAFs, LC_{50} s of 9.6%-32.2% for CEWAFs, and LC_{50} of 28.8%-100% Corexit 9500A only treatments.

Birds. Fiorello et al (2016) investigated oil impacts to birds in a laboratory toxicity test with field

captured common murre, *Uria aalga*, exposed to test solution for 90 seconds at concentrations ranging from 1.5mg/L-1066mg/L Prudhoe Bay crude oil, 9.8mg/L - 944.5mg/L Corexit 9500A, 93.5mg/L PBCO 7.4mg/L Corexit 9500A mixture, and 1128.0mg/L PBCO 78mg/L Corexit 9500A mixture added to pool water. Authors report no significant effect regarding tear production or intraocular pressure after exposure and birds exposed to toxicants were likely to develop conjunctivitis. Fritt-Rasmussen et al (2016) exposed feathers collected from the Common eider seabird, *Somateria mollissima*, to fresh Grane crude oil, fresh IFO 30 crude oil, and burnt oil residues from each respective sample. Feathers were exposed to for 15s, removed, and replaced for another 15s with photographs taken to assess the integrity of the feathers microstructure. The authors reported a alteration of feather structure determined by weight parameters were higher in treatments exposed to Gran oil rather than IFO30.

Mangroves. Reinert et al (2016) investigated chlorophyll *a* fluorescence as an indicator of the health of the mangrove species *Laguncularia racemosa* under laboratory conditions by applying 185mL of marine fuel oil onto the sediment in each microcosm containing a mangrove propagule and observed the effect for 17 days. The authors reported 80% decrease of total photosynthetic performance index by the exposed plants which complimented the fluorescence kinetics analysis model, JIP-test, and making it a viable model to detect stress on mangroves exposed to oil spills.

Cell Cultures. Bhattacharya et al (2016) conducted laboratory toxicity tests exposing DWH field

collected tartrate WAF solutions to H19-7 neuronal rat hippocampal cells, MCF10A epithelial cell line and HEK293 kidney cell lines as well as observed multiple biological effects on glutathione content, lipid peroxidation content, and superoxide dismutase activity. The authors reported results of a 24 hour exposure of cell lines to 25, 50, 75 and 100 μ L WAF solution as observing 40% of reduced cell structure in neuronal cells and kidney cells at 25 μ L and 100 μ L, respectively, and 60% of reduced cell structure in epithelial cells. Pi et al (2016) exposed LSH Bacteria maintained in field collected sediment and water to 0.5%w/v crude oil, temperature changes, and nutrient enhancements including additions of $(\text{NH}_4)_2\text{SO}_4$ and K_2HPO_4 to determine biodegradation rates of oil in the presence of various conditions. The authors concluded the elevation of temperature and nutrients to optimal levels were the primary variables which resulted in observed increases of biodegradation rates.

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Table 1—Chemical residues reported in marine organisms from selected publications in 2016. Do not cite from this table but go directly to source citation. Year of all references is 2016. See footnotes regarding actual concentrations.

Chemical	Type of Organism	Concentration $\mu\text{g/g}$ dry weight	Location	Reference
Aluminum	Bivalves	0-5.3 shell	Persian Gulf	Karbasdehi et al.
		1-41.4	Tunisia	Lahbib et al.
Arsenic	Bivalves and Gastropods	56-1,513	Brazil	Vilhena et al.
		4.3-22.3	Egypt Red Sea	El Nemr et al.
	Phytoplankton	6.5	Brazil	Vilhena et al.
		1.57-15.4	Italy	Bonanno and Di Martino
	Sea grass	0.09-2.2	Singapore	Calbet et al.
		2.5-5.8	India	Srichandan et al.
	Bivalves	35-179	Tunisia	Lahbib et al.
		16-118	Arabian Gulf	Krishnakumar et al.
	Gastropods	2.4-7.3	Brazil	Vilhena et al.
		4.5 – 15.7	Bismark Sea vent	Price et al.
	Decapods	22 -5558	Bismark Sea vent	Price et al.
		59-75 ^a	Hawaii	Koide et al.
	Fish	11-134	Arabian Gulf	Krishnakumar et al.
		7.6-456	China	Zhang et al.
Bismuth	Mammals	0.3-0.6 ^a	Portugal	Monteiro et al.
	Green algae	0.59 ^c	UK	Kearns et al.
	Brown algae	4.19 ^c	UK	Kearns et al.
Cadmium	Red algae	1.68 ^c	UK	Kearns et al.
	Phytoplankton	1.2	Brazil	Vilhena et al.
Green algae		2.4-23	Senegal	Diop et al.
	Sea Grass	0.06-0.3	Italy	Bonanno and Di Martino
Zooplankton		0.02-0.14	Singapore	Calbet et al.
	Bivalves	0.8-1.5	India	Srichandan et al.
Gastropods		1-31	Med. Sea	Vázquez-Luis et al.
	Crustaceans	2.2-615	China	Gao et al.
Decapods		8.6-115	Senegal	Diop et al.
	Fish	2.5-12	India	Chakraborty et al.
Bivalves and Gastropods		0.1-19.2	Tunisia	Lahbib et al.
	Gastropods	1.2-2.2	Brazil	Vilhena et al.
Crustaceans		0.04-1.7	Egypt Red Sea	El Nemr et al.
	Decapods	70	China	Gao et al.
Fish		1.6-20	China	Gao et al.
	Mammals	3.7-233	Senegal	Diop et al.
Bivalves		0.05-3.8	China	Gao et al.
	Crustaceans	18-149	Senegal	Diop et al.
Decapods		0.1-6.47	Tunisia	Ben Salem et al.
	Fish	0.01-13 ^a	Ecuador	Araújo and Cedeño-Macias
Mammals		0.02-0.47	Bay of Bengal	Saha et al.
	Phytoplankton	0.01-2.39	Portugal	Monteiro et al.
Green algae		0.4-72 ^a	UK	Gajdosechova et al.
	Sea grass	16.6	Brazil	Vilhena et al.
Zooplankton		0.06-8.2	Senegal	Diop et al.
	Bivalves	1.5-5.7	Italy	Bonanno and Di Martino
Crustaceans		2-4.5	India	Srichandan et al.
	Decapods	0.03-2.04	Singapore	Calbet et al.
Fish		0.001-0.24	Persian Gulf	Karbasdehi et al.
	Mammals	2.6-3.2	China	Gao et al.
Bivalves		0.01-11	Senegal	Diop et al.
	Crustaceans	0-5.8	Tunisia	Lahbib et al.
Decapods		5.67-6.5	Brazil	Vilhena et al.
	Phytoplankton	1.0	China	Gao et al.
Green algae		1.9	China	Gao et al.
	Sea grass	0-.01-0.6	Senegal	Diop et al.
Zooplankton				

	Fish	3.3-4 2-4.5 1.27-4.66	China India Bay of Bengal	Gao et al. Srichandan et al. Saha et al.
Cobalt	Zooplankton	0.2-0.6	India	Srichandan et al.
	Bivalves	0-0.022 shell	Persian Gulf	Karbasdehi et al.
	Fish	0.2-0.56	Bay of Bengal	Saha et al.
Copper	Phytoplankton	16	Brazil	Vilhena et al.
	Green algae	287	China	Gao et al.
	Sea grass	0.3-11	Italy	Bonanno and Di Martino
	Zooplankton	2-15	India	Srichandan et al.
		4.7-119	Singapore	Calbet et al.
	Bivalves	1-261	Red Sea	Vázquez-Luis et al.
		1.9-5.9	China	Gao et al.
		0.01-2	Senegal	Diop et al.
		0-0.6	Tunisia	Lahbib et al.
		3.7-66	Brazil	Vilhena et al.
	Bivalves and Gastropods	0.9-137	Egypt Red Sea	El Nemr et al.
	Gastropods	0-3.2	China	Gao et al.
	Crustaceans	59-158	China	Gao et al.
	Decapods	14.4-44.3	Senegal	Diop et al.
		5.3-11 ^a	Hawaii	Koide et al.
	Fish	1.5-5.3	China	Gao et al.
		74-1753	Senegal	Diop et al.
		0-40.8	Tunisia	Ben Salem et al.
		8.54	Bay of Bengal	Saha et al.
	Mammals	2-7.1 ^a	Portugal	Monteiro et al.
Iron	Phytoplankton	21,887	Brazil	Vilhena et al.
	Zooplankton	100-6,000	India	Srichandan et al.
		53-627	Med. Sea	Calbet et al.
	Bivalves	0.5-6.8 shell	Persian Gulf	Karbasdehi et al.
		7,706-21,887	Brazil	Vilhena et al.
	Fish	1.2-563	Tunisia	Ben Salem et al.
Lead	Mammals	8-363 ^a	UK	Gasjdosechova et al.
	Phytoplankton	10	Brazil	Vilhena et al.
	Green algae	0.38-43	Senegal	Diop et al.
	Zooplankton	0.5-4	India	Srichandar et al.
		3-90.7	Singapore	Calbet et al.
	Sea grass	0.69-8.3	Italy	Bonanno and Di Martino
	Bivalves	1-28	Med. Sea	Vázquez-Luis et al.
		1.9-5.9	China	Gao et al.
		0.1-2	Senegal	Diop et al.
		0-0.6	Tunisia	Lahbib et al.
		2.5-4.88	Brazil	Vilhena et al.
	Bivalves and Gastropods	0.2-17	Egypt Red Sea	El Nemr et al.
	Crustaceans	1-2.9	Senegal	Diop et al.
	Decapods	0.01-0.16	Senegal	Diop. et al.
		0-0.06 ^a	Hawaii	Koide et al.
	Fish	0.08-0.4	China	Gao et al.
		0.4-4.8	Senegal	Diop et al.
		0.17-5.06	Tunisia	Ben Salem et al.
		0.04-0.23 ^a	Ecuador	Aráuja and Cedeño – Macias
		6.33	Bay of Bengal	Saha et al.
Magnesium	Mammals	0.01-0.02 ^a	Portugal	Monteiro et al.
	Phytoplankton	510	Brazil	Vilhena et al.
	Green algae	0.04-12	Senegal	Diop et al.
	Zooplankton	5-50	India	Srichandan et al.
		1.5-32.4	Singapore	Calbet et al.
	Bivalves	0.07-1.02 shell	Persian Gulf	Karbasdahi et al.
		51-214	China	Gao et al.
		0.2-2.8	Senegal	Diop et al.
		949-3,521	Brazil	Vilhena et al.
	Gastropods	8	China	Gao et al.

Mercury	Crustaceans	5.7-6.8	China	Gao et al.
	Decapods	0.02-1.14	Senegal	Diop et al.
	Fish	7-19	China	Gao et al.
		0.04-32.5	Senegal	Diop et al.
	Mammals	3.6-17.8	Bay of Bengal	Saha et al.
		0.2-1.2 ^a	Portugal	Monteiro et al.
	Phytoplankton	0.15-21 ^a	UK	Gajdosechova et al.
		0.014-0.09	China	Bastamante et al.
	Algae	0.054	China	Qiu and Wang
		0.044	China	Qiu and Wang
	Green algae	0.03-0.06	China	Bastamante et al.
	Bivalves	0.5-1	Med. Sea	Vásquez-Luis et al.
		0.15-0.18	China	Bastamante et al.
	Bivalves and Gastropods	0.17	China	Qiu and Wang
		<0.005-0.2	Egypt Red Sea	El Nemr et al.
	Decapods	0.099-0.11	China	Qiu and Wang
	Fish	0.25-3.15	N. Atlantic O.	Lee et al.
		0.05-13.1	Med. Sea	Vásquez-Luis et al.
	Mammals	0.05-13.2	Malaysia	Jeevanaraj et al.
		0.005-2 ^a	Ecuador	Araúje and Cedeño-Macias
	Bivalves and Gastropods	0.18-0.49 ^a	Croatia	Cervený et al.
0.018-0.45		China	Qui and Wang	
Mammals	0.0005-0.013	Atlantic Pac. O.	Hammerschlag et al.	
	0.2-10	California	Peterson et al.	
Phytoplankton	0.2-0.9	Malaysia	Jeevanaraj et al.	
	0.48-21 ^a	UK	Gajdosechova et al.	
Green algae	7.5	Brazil	Vilhena et al.	
	0.38-3.8	Senegal	Diop et al.	
Sea grass	2.17-4.89	Italy	Bonanno and Di Martino	
Zooplankton	1.5-3	India	Srichandan et al.	
Bivalves	0.002-0.23 shell	Persian Gulf	Kashasdehi et al.	
	3.5-12	China	Gao et al.	
Bivalves and Gastropods	0.002-0.037	Red Sea	Ele et al.	
	0.02-0.77	Senegal	Diop et al.	
Gastropods	0.1-4.6	Tunisia	Lahbid et al.	
	1.5-2.1	Brazil	Vilhena et al.	
Crustaceans	7.8-41	Egypt Red Sea	El Nemr et al.	
	0.5	China	Gao et al.	
Decapods	1.1-2.5	China	Gao et al.	
Fish	0.02-0.12	Senegal	Diop et al.	
	0.32-0.88	China	Gao et al.	
Mammals	0.02-11.5	Senegal	Diop et al.	
	0.08-3.7	Tunisia	Vilhena et al.	
Phytoplankton	1.88-7.56	Bay of Bengal	Saha et al.	
	0.1-1.1 ^a	Portugal	Monteiro et al.	
Zooplankton	1.4	Brazil	Vilhena et al.	
	1-1.7	India	Srichandan et al.	
Bivalves	0.1-3.24 ^a	Singapore	Calbet et al.	
	1-2.3	Brazil	Vilhena et al.	
Fish	1.2-4	Bay of Bengal	Saha et al.	
	0.8-4 ^a	Portugal	Monteiro et al.	
Zooplankton	2.3-71.2a	UK	Gajdosechova et al.	
	0.14-1.78	Singapore	Calbet.	
Bivalves and Gastropods	0.28 – 53.9	Egypt Red Sea	El Nemr et al.	
	38	Brazil	Vilhena et al.	
Green algae	028-2.2	Senegal	Diop et al.	
	23.7-55	Italy	Bonanno and Di Martino	
Zooplankton	10-4,100	India	Srichandan et al.	
Bivalves	1,200-4,000	Med Sea	Vázquez-Luis et al.	
	0.002-6.9 shell	Persian Gulf	Karbasdehi et al.	
	175-21,741	China	Gao et al.	

		0.6-14	Senegal	Diop et al.
		3.19-363	Tunisia	Lahbib et al.
		74-201	Brazil	Vihlena et al.
	Bivalves and Gastropods	41-192	Egypt Red Sea	El Nemr et al.
	Gastropods	2,575	China	Gao et al.
	Crustaceans	120-273	China	Gao et al.
	Decapods	1.27-14	Senegal	Gao et al.
	Fish	41-110	China	Gao et al.
		2-25	Senegal	Diop et al.
		196-284	Tunisia	Ben Salem et al.
		13.2-74.36	Bay of Bengal	Saha et al.
	Mammals	10.9-46.6 ^a	Portugal	Monteira et al.
Biphenyl diglycide ethers	Mammals	0.04-1.6 ^a	California	Xue et al.
Chlordecone	Fish	0.075-0.76	Lesser Antilles	Dromard et al.
Chloronated parafins	Gastropods	0.0035-0.25	Antarctica	Fu et al.
pp' DDE	Mammals	0.0036-0.9 ^b	Pacific coast	Lundin et al.
DDT	Fish	0.01	Indonesia	Dwiyitno et al.
	Mammal	1.56-9.5 ^b	Arctic Canada	Morris et al.
HCB	Fish	0.008-0.134 ^a	Arctic O.	Evenset, at al.
	Mammal	0-0.34 ^b	Arctic Canada	Morris et al.
HCH	Fish	0.0005-0.065 ^b	Indian O.	Dirtu et al.
	Mammal	0.01-0.08 ^a	Arctic Canada	Morris et al.
	Mammals	0.0003-0.045 ^b	Indian O.	Dirtu et al.
Irgarol	Aquatic plants	0.05-0.24	Florida	Fernandez and Gardinali
Mirex	Fish	0.0002-0.0017 ^a	Arctic Ocean	Evenset et al.
	Mammals	0.08-0.17 ^b	Arctic Canada	Morris et al.
Organochlorine	Fish	0.003-0.14	Guadeloupe	Charlotte et al.
Organic compounds	Fish	0.31 ^a	Atlantic O.	Alves et al.
PAHs	Zooplankton	0-1.1	Iran	Ziyaadini et al.
	Bivalves	0.13-0.45	Japan	Onozuto et al.
		0.03-0.43	Tunisia	Barhoumi et al.
	Decapods	0.1-0.3 ^a	China	Sun. et al.
	Fish	0.1-0.52 ^a	China	Sun et al.
		0.11-0.13	Tunisia	Barhoumi et al.
ΣPBDE	Fish	0.003-0.06 ^a	NY-NJ	Smalling et al.
PCBs	Bivalves	0.06-0.14 ^a	Italy	Giandomenico et al.
	Gastropods	0.18-0.29 ^a	Italy	Giandomenico et al.
	Fish	0.026-0.37 ^a	NY-NJ	Smalling et al.
		0.02-0.34	Italy	Glandomecicico et al.
		0.0003-0.06	Med. Sea	Cresson et al.
		0.036-2.1	Indian Ocean	Dirtu et al.
	Birds	0.1-67	Indian Ocean	Dirtu et al.
	Mammals	0.005-0.2	Canada	Cadieux et al.
		3.86-7.5 ^b	Arctic Canada	Morris et al.

^aWet weight

^bLipid weight

^cExperimental study