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Effects of Pollution on Marine Organisms

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ABSTRACT: This review covers selected 2019 articles on the biological effects of pollutants, including human physical disturbances, on marine and estuarine plants, animals, ecosystems and habitats. The review, based largely on journal articles, covers field and laboratory measurement activities (bioaccumulation of contaminants, field assessment surveys, toxicity testing and biomarkers) as well as pollution issues of current interest including endocrine disrupters, emerging contaminants, wastewater discharges, marine debris, dredging and disposal etc. Special emphasis is placed on effects of oil spills and marine debris due largely to the 2010 Deepwater Horizon oil blowout in the Gulf of Mexico and proliferation of data on the assimilation and effects of marine debris microparticulates. Several topical areas reviewed in the past (e.g. mass mortalities ocean acidification) were dropped this year. The focus of this review is on effects, not on pollutant sources, chemistry, fate or transport. There is considerable overlap across subject areas (e.g. some

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

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

Effects of Dredging, Dumping, and Extraction

Species' tolerances to increased sedimentation were investigated in multiple studies conducted in Australia. Abdul Wahab et al. (2019) found that the tropical sponge larvae (*Carteriospongia foliascens*) was relatively tolerant to the suspended sediment concentrations tested in the laboratory, though changes in swimming behavior and in larval body shape, which reduced swimming ability and settlement success, were observed. Laboratory mesocosm studies of the effects of sedimentation on two tropical seagrass species (*Zostera muelleri* and *Halophila ovalis*) found shoot density declines at burial depths of 5–7.5mm and low levels of growth above 10mm burial depth; however, field studies of the same species revealed that mitigating factors and adaptive strategies by the plants lessen the effects of sedimentation (Benham et al. 2019). Jones et al. (2019) investigated the impacts of a capital dredging project in Western Australia on nearby hard corals

and found that the sediment deposition field affecting corals was approximately ten times less than the distances covered by the sediment plumes, with the response of corals was highly dependent on the species and the growth form within a species.

Time series studies in arctic and tropical regions evaluated both regional and local causes of adverse benthic community changes associated with dredging operations. Benthic community data collected between 1945 and 2013 in the high-Arctic Baydaratskaya Bay (southwestern part of the Kara Sea) revealed no evidence of long-term changes in benthic invertebrate communities in response to local and regional climate variations (1945-2013); however, marked changes in benthic communities were detected in 2013, which the authors attribute to direct impacts of dredging and dumping of sediments during burial of an underwater a gas pipeline between 2012 to 2013 (Azovsky and Kokarev, 2019). Using a repeated-measures coral monitoring program and published models relating either disease or dredging to coral mortality, Gintert et al. (2019) found that the regional disease event was the determining factor of most coral mortalities between 2013-2016 in the study area, with local dredging stress predicting only a small proportion of coral mortalities.

Two studies reported effects to benthic communities from dredging operations in the Romanian Black Sea after cessation of dredging operations to remove sand for coastal beach nourishment projects (Muresan et al. 2019; Teaca et al. 2019). Comparison of results collected in 2016, three months after dredging operations ended, to results collected in 2018 showed that some species characteristic of the area had not returned (Teaca et al. 2019) and a lower diversity of nematodes were measured in the center of the dredge area than at the edges of the study area (Muresan et al. 2019).

Impacts to phytoplankton and algae from dredging operations were also reported. Remote sensing methods measured a decrease in chlorophyll-a concentrations in the South China Sea associated with dredging operations during Mischief Reef island building (Smith et al. 2019), and dredging operations to widen and deepen the Savannah River Estuary in Georgia, USA,

resulted in increased fine sediment load, decreased diatom species richness, and increased abundance of filamentous algae four months into the operations (Thomson and Manoylov, 2019).

Submarine tailing disposal in Franfjord on the west coast of Norway was reported to cause lower species numbers closer to the tailings outfall, though the observed decrease in functional diversity of infauna and epifauna was found to be less than the decrease in species diversity along the tailings gradient (Trannum et al. 2019).

A qualitative comparison of environmental impacts from standard dredging technologies and newer methods reported that traditional dredging has the greatest environmental impacts (e.g., impacts for flora and fauna), while sand by-passing plants have the least environmental impacts, despite possible higher noise levels and higher carbon dioxide emissions if a water pump is used rather than a water jet system (Bianchini et al. 2019).

Effects of Wastewater Discharges

Several studies examined the long-term impacts of sewage disposal in the marine environment. For example, seven years of sewage disposal on the west coast of Scotland resulted in an increase in benthic community species richness and changes to benthic community composition associated with organic enrichment, though four multi-metric indices with pre-defined quality classifications (Infaunal Quality Index [IQI], Benthic Quality Index [BQI], AZTI Marine Biotic Index [AMBI], and Benthic Opportunistic Polychaete Amphipod Index [BOPA]) did not detect negative impacts until seven years after the outfall began operating, despite community composition changes and organic enrichment that occurred throughout the seven years (Culhane et al. 2019). Results of an 18-year benthic community monitoring study associated with the installation and operation of deepwater ocean outfalls off the coast of Sydney, Australia indicate that sediment granulometry has a greater effect in shaping benthic community structure than accumulation of chemicals of concern near the deepwater ocean outfalls, which Besley and Birch (2019) conclude demonstrates that the three Sydney deepwater ocean outfalls have not caused

significant ecological impact. Analysis of a 40 year time series of annually collected living benthic assemblages from the Palos Verdes (southern California, USA) shelf and comparison to present-day bivalve shell assemblages collected in the same area revealed historical changes in the macrobenthic community over time in response to wastewater disposal and near recovery of the present-day macrobenthic community to its pre-effluent condition (Leonard-Pingel et al. 2019).

The distance or location from sewage outfalls did not result in any consistent pattern of nematode distribution in sediments historically affected by abandoned industrial plants in the Bagnoli-Coroglio area of southern Italy, and no multivariate or univariate patterns of meiofauna distribution were apparent from the data collected that differed from local variability (Bertocci et al. 2019). However, proximity to industrial wastewater discharge points was found to be positively correlated with increased total sediment metals concentrations in the Bizerte Lagoon, Tunisia, which were identified as the primary influence on meiofaunal and bacterial communities (Saidi et al. 2019).

Living foraminifera species were used as ecological indicators in the southwestern Atlantic Ocean and the Adriatic Sea. The proximity of coral reefs to sewage-impacted river discharge from the Buranhém River, Brazil, was found to be associated with lower density of foraminifera (*Amphistegina gibbosa*) populations and higher frequency of bleaching (Marques et al. 2019), while differences in total density, species richness, dominance, and ecological quality indices were observed between a sewage impacted site and a marine reserve location in the Gulf of Trieste (Adriatic Sea) (Melis et al. 2019).

Long-term responses of phytoplankton communities to sewage, nutrient, and climate factors were documented in the Arabian Gulf of Kuwait (Devlin et al. 2019) and in the East China Sea (Chen et al. 2019). Three macroalgae taxa (*Ceramium spp.*, *Corallina spp.* and *Halopteris scoparia*) were found to be significant contributors to the dissimilarity between rocky intertidal communities located near wastewater treatment plant (WWTP) outfalls compared to control areas along French and Spanish portions of the southeastern Bay of Biscay;

however, no clear evidence of impact from the WWTPs was detected (Huguenin et al. 2019).

Burns et al. (2019) investigated the residency times of four species of demersal fish (*Citharichthys sordidus*, *Pleuronichthys verticalis*, *Parophrys vetulus*, *Sebastes miniatus*) at two sites (near a wastewater outfall and at a reference site) in southern California over one year to collect fish behavioral information that can support interpretation of tissue contaminant levels relative to inter- and intra-specific differences in space use and habitat association; the authors found that only *S. miniatus* spent more than 10% of the study duration at the outfall site (nearly 40% of the study duration) and may be the most susceptible of the species considered to wastewater effluent effects. Using ordinary least squares analysis and publicly reported data, Ghanem and Alnafissa (2019) reported a relationship between increased untreated sewage waste in Saudi Arabia and decreased production of marine fisheries.

Llanos et al. (2019) found that during both spring and autumn experiments the non-native tube-dwelling spionid polychaete, *Boccardia proboscidea*, dominated early succession in experimental settlement plots near a sewage outfall on the north coast of Mar del Plata city, Buenos Aires, Argentina, over the native bed-forming mussel, *Brachidontes rodriguezii*, though high energy storms substantially removed the polychaete tubes, allowing the mussels to become more dominant.

Gornati et al. (2019) reported that treatment of oily wastewater discharges with a biofilm system (BioFilm Membrane BioReactor) reduced impacts to morphological organization, mRNA expression, immunohistochemistry and metabolomics in the gills and digestive gland of mussels (*Mytilus galloprovincialis*).

Granulated coal ash (GCA), a by-product of coal-fired electric power plants, was reported to support a higher species richness in GCA than clean gravel or sediments impacted by untreated wastewater in Fukuyama inner harbor, Japan (Morimoto et al., 2019).

Effects of Fishing and Aquaculture

Fishing nets, lines and fences were reported to adversely impact marine fish and coral populations. Exton et al. (2019) reported that artisanal fish fences, which funnel fish into a holding structure, cause habitat fragmentation, create physical barriers that interrupt natural movement of mobile species and benthic community structure, and remove large numbers of juvenile fish, which produces a corresponding decline in fish abundance on adjacent coral reefs. Gorgonian coral species in canyon systems of the western Ligurian Sea (north-western Mediterranean Sea) were found to be negatively impacted by one or more types of derelict fishing gear, with fishing nets causing the greatest impact at most study sites (Giusti et al., 2019).

McConnaughey et al. (2019) reviewed nine different management measures and voluntary industry actions to reduce adverse impacts from bottom trawl fishing and found that risks and benefits of such efforts depend on the specific fishery and the characteristics of the ecosystem; no universal best practice was apparent due to the diversity of policy drivers that influence the management of fisheries and their environmental impact. In the Northwestern Hawaiian Ridge and Emperor Seamounts, despite significant evidence of adverse impacts from decades (1960s-1980s) of bottom trawling and coral tangle net fishing including scarred hard bottom habitat, coral rubble, and lost fishing gear, Baco et al. (2019) reported multiple signs of recovery in benthic seamount communities that have been protected for more than 30 years.

A meta-analysis of the impacts of marine and freshwater aquaculture found that aquaculture facilities attract a variety of species, particularly wild fish, which were found to be on average 1.2x larger and 1.7x heavier than fish from reference sites; marine mammals showed no consistent response to the presence of aquaculture facilities, though pinnipeds were found to experience higher mortality from aquaculture facilities due to culling and entanglement (Barrett et al. 2019). Bottlenose dolphins (*Tursiops truncatus*) off the northwestern coast of Spain, however, were found to forage predominantly inside shellfish farming areas, with such behavior correlated to

both environmental conditions (sea surface temperature and time of day) and the presence of shellfish farms (Methion and Díaz López, 2019).

Using observations from diving surveys and the local ecological knowledge (LEK) of fishers and scuba-divers, Öndes et al. (2019) report that illegal trawl fishing in shallow waters may be a large component of fan mussel bycatch affecting the density and distribution of the endangered fan mussel, *Pinna nobilis*, on Turkish coasts.

Effects of Light Pollution

Effects from artificial light at night (ALAN) was reported for a variety of different types of marine organisms. For example, oxidative stress and physiological effects from exposure to ALAN were observed for the scleractinian corals, *Acropora eurystoma* and *Pocillopora damicornis*, from the Gulf of Eilat in the Red Sea, with more severe effects observed from exposure to blue LED and white LED lights (Ayalon et al. 2019). ALAN was also found to inhibit hatching of common clownfish (*Amphiprion ocellaris*) eggs in the laboratory (Fobert et al. 2019). Manriquez et al. (2019) reported that juvenile Chilean abalone (*Concholepas concholepas*) exposed to ALAN in the laboratory experienced longer self-righting times, higher metabolic rates, and were less frequently found near food sources. Convict surgeonfish (*Acanthurus triostegus*) larvae exposed to ALAN in the laboratory experienced changes in swimming behavior and significantly higher mortality rates compared to control fish, but they also grew faster and heavier than control fish (O'Connor et al. 2019). Juvenile ALAN-exposed intertidal rockfish, *Girella laevis*, had increased oxygen consumption and activity and no longer displayed the natural activity cycles compared to control fish (Pulgar et al. 2019). Zapata et al. (2019) reviewed the state of the science relative to the impacts of ALAN on coastal estuaries.

Effects of Noise Pollution

Noise pollution from seismic surveys were reported to impact marine species. Seismic air gun exposure in a field study conducted in Tasmania, Australia,

caused significant damage to the mechanosensory statocyst organ of rock lobster (*Jasus edwardsii*) and resulted in impaired righting reflex, damage which remained 365 days after exposure (Day et al. 2019). While over 8,000 hours of marine mammal observer data from seismic and control vessels in the eastern Atlantic Ocean revealed a significant effect of seismic surveys on marine mammal sightings, with an average decrease of 88% in sightings of baleen whales and an average 53% decrease in sightings of toothed whales (Kavanagh et al. 2019). A review of the current state of knowledge for evidence of impact from airgun acoustic exposure on fish and the potential for extrapolation of existing data to understand population-level consequences found that data are limited, with no species for which there are adequate data sets to assess the potential for population-level effects (Slabbekoorn et al., 2019).

Vessel noises were similarly found to affect marine organisms. For example, physiological changes (e.g., increased heart rate) were observed in coral reef damselfishes (*Amphiprion melanopus* and *Acanthochromis polyacanthus*) following laboratory exposure to boat vessel noise, though species-specific differences were noted (Fakan and McCormick 2019). The specific type of vessel noise, whether from a 2-stroke or 4-stroke outboard engine, was also found to produce different responses in juvenile whitetail damselfish (*Pomacentrus chrysurus*; Pomacentridae), with noise from 2-stroke engines having a short duration effect reducing swimming speed and noise from 4-stroke engines significantly impacting the fast-start response (McCormick et al. 2019). Mikkelsen et al. (2019) found some evidence of functional behavior interruption in North Sea seals coinciding with high-level vessel noise using long-duration audio and 3D-movement tags.

Cominelli et al. (2019) reported on a geospatial method to overlay noise exposure levels in Puget Sound and locations of Southern Resident Killer Whale (SRKW) sightings to develop exposure hot spot maps and probabilistic level exposure predictions to support analysis of alternative shipping route scenarios to reduce noise exposure of SRKW population.

Effects of Recreation and Tourism

Recreational activities, including beach visits and SCUBA diving, were assessed for impacts to several different types of marine organisms. Tire ruts from vehicles driving on beaches of Boa Vista Island, Cape Verde were experimentally found to increase the frequency of disorientation, misorientation, and crawl time in newly hatched loggerhead sea turtles (*Caretta caretta*) (Aguilera et al. 2019). While, the density of human occupancy at tourist beaches near Rio de Janeiro, Brazil did not result in significantly different nematode biological indexes (average number of genera per sample, Shannon index, Pielou's evenness, and rarefaction index) compared to beaches that are less affected by human presence (Santos et al., 2019). Sites that are frequented by recreational SCUBA divers were found to have up to ten times more incidence of coral fragments accumulated at the base of cliffs within the Portofino Marine Protected Area (after normalization to colony density), compared to reference sites (Betti et al. 2019).

Assessment Methods and Pollution Indicators

Pilo et al. (2019) evaluated dredging effects on macrobenthic communities of the Ria Formosa coastal lagoon, Portugal, using the difference in species composition between local and regional assemblages (beta diversity), partitioned into turnover (species replacement) and nestedness (species lost), in conjunction with multivariate analyses and benthic ecological status assessment (AMBI and M-AMBI); the authors found that combined uni- and multivariate analysis including detailed beta diversity information may be an effective approach to identify the effects of dredging on benthic communities.

The Nested Environmental status Assessment Tool (NEAT) was applied to the Saronikos Gulf, in the Eastern Mediterranean Sea using time series data on different spatial and temporal time scales that included nine biological and chemical ecosystem components, 24 indicators, and eight descriptors of the Marine Strategy Framework Directive (MSFD); the study demonstrated that there is no loss of information using an integrated analysis, as NEAT was able to identify specific areas/resources with

poorer conditions, while concluding overall that the gulf met the criteria for good status (Pavlidou et al. 2019).

Effects of Micro-plastic Debris

Research on the incidence and impacts of microplastic debris continues to grow in both breadth and depth, with published studies from across the world investigating ingestion of microplastics by invertebrates, fish, birds, turtles, and marine mammals. Many studies provided context for growing concern among seafood consumers, and began to fill the information gaps related to microplastic ingestion in species commonly eaten by humans. Laboratory and modeling studies continued to evaluate sublethal impacts of microplastic ingestion and mechanisms of toxicity, and addressed questions about bioaccumulation and food chain impacts (e.g., Akhbarizadeh et al. 2019). For example, Besseling et al. (2019) reviewed laboratory studies and used the available information from studies to-date to conduct a risk assessment as a proof of concept for future assessments. Nor and Koelmans (2019) simulated gut conditions in aquatic biota and determined that for plastics dosed with polychlorinated biphenyls (PCBs), transfer kinetics depended somewhat on the other food inside the gut, implying that chemical contamination could occur if gut contents were less contaminated, while gut cleaning could occur if gut contents were more contaminated. Several studies provided nuanced results that begin to contextualize the reports of microplastic particle ingestion in the wild, and move forward the scientific understanding of microplastic impacts on marine organisms.

Invertebrates. Many studies investigated the concentration of microplastics in wild-caught invertebrates, from bivalves in Oregon (Baechler et al. 2019) and southeastern India (Naidu 2019), to deep sea invertebrates in the northeast Atlantic (Courtene-Jones et al. 2019) and around the world (Jamieson et al. 2019), to coral reefs in the South China Sea (Ding et al. 2019), Australia (Jensen et al. 2019), and cold-water corals off the coast of Scotland (La Beur et al. 2019). Wang, Wang, Ru and Liu (2019) measured the abundance and characteristics of microplastics found in sediments and benthic organisms

from the South Yellow Sea, China, and documented 1.7-47.0 particles per gram (ww). Concentrations were positively correlated with water depth, and fibers were the most prevalent type of particle identified. Similarly, Saley et al. (2019) measured microplastics in a benthic community near a remote marine reserve in California, USA and found microplastics adhered to macroalgal surfaces at between 2.34 and 8.65 particles per gram. Saley et al. also measured uptake in an herbivorous snail (*Tegula funebris*) at higher concentrations than other benthic organisms, 9.91 ± 6.31 particles per gram, implying possible bioconcentration.

Several studies focused on documenting microplastic ingestion in commercially important invertebrates, such as mussels and oysters. For example, Abidli et al. (2019) measured concentrations in six molluscs from Bizerte, Tunisia, where concentrations ranged from approximately 700 to more than 1,400 microplastic particles per kg wet weight (ww), with an average abundance of 1,031.1 particles per kg (ww). Fibers were the most common type of microplastic in each species, and further analysis confirmed polyethylene and polypropylene polymers.

In coastal China, Teng et al. (2019) collected oysters from 17 sites and documented microplastic presence in 84% of sampled organisms. The authors measured an average 2.93 microplastic particles per individual, the majority of which were fibers, and saw a difference in abundance based on site location. Fish and oysters were collected from the Maowei Sea, China for microplastics analysis, and the authors identified microplastics in gastrointestinal tracts and gills of all fish and oysters collected (Zhu, Zhang, Li et al 2019). Abundance was greater in the gastrointestinal tracts (2-14 particles per individual) compared to the gills (0-8.5 particles per individual) in fish, and ranged from 3.2-8.6 particles per oyster, with results mirroring microplastic abundances in local waters.

Birnstiel et al. (2019) collected wild and farmed mussels from the southwestern Atlantic, and detected microplastics in all 40 organisms. A maximum concentration of 31.2 ± 17.8 microplastics (≥ 0.45 μm) per

individual was measured; the authors compared organisms that were depurated and non-depurated and found that a 93-hour depuration significantly reduced microplastic concentration in tissues. In Korea, Cho et al. (2019) conducted a survey of fishery markets to collect four species (oyster, mussel, clam, and scallop) to analyze for microplastic content. Cho et al. (2019) measured a mean of 0.97 ± 0.74 microplastics per individual, with fragments and particles smaller than 300 μm accounting for the majority of shape profiles, and calculated the annual human dietary intake of microplastics based on typical shellfish consumption rates. It seems likely that future studies will focus on this type of analysis as it relates human and marine organism health.

In a laboratory experiment, Goncalves et al. (2019) exposed the Mediterranean mussel (*Mytilus galloprovincialis*) to polystyrene microplastic at concentrations ranging from 10-1,000 particles per mL. The authors found that mussels rapidly clear the water column of particles while filter feeding, which was not dependent on particle size, ingested the particles into the gut, and excreted particles through feces. Goncalves et al. (2019) measured no particles in gills or digestive gland diverticula and did not find biochemical indicators of oxidative stress in the different treatment groups. Harris and Carrington (2019) measured clearance rates in mussels (*Mytilus trossulus*) in a laboratory experiment using treatments that compared algae, microplastics and algae, and silt and algae across multiple concentrations to investigate whether natural abiotic particles are cleared at similar rates as microplastics. The authors measured a 62% decrease in clearance rate at high microplastic concentrations ($> 1,250$ particles per mL) but not at the same concentration of silt particles, implying a differential impact of plastic than naturally occurring particles. Revel et al. (2019) exposed blue mussels (*Mytilus edulis*) to polyethylene and polypropylene particles at environmentally relevant concentrations (0.008-100 μg per L). The authors only identified microplastics in the digestive gland of mussels exposed to the highest concentration, with a mean of 0.75 particles per organism after a 10-day exposure, but found particles in biodeposits

following all exposure scenarios, confirming exposure and ingestion. They also found significant increases in superoxide dismutase and catalase activities in mussel digestive glands exposed to low and medium concentrations, and in gills of mussels in the high exposure group. Differences in clearance rate and histopathological metrics were not observed.

Blue mussels and eastern oysters were used in a laboratory experiment to compare ingestion and egestion of spherical and fibrous microplastics (Ward et al. 2019). Both species rejected larger spheres (1000 μm) at a higher rate than smaller spheres, and rejected a higher proportion of large microspheres than long microfibers. The authors found that the physical characteristics of the particles plays a role in determining the number of type of microplastic particles found in bivalves, and due to the particle selection noted in these organisms, they may not be suited as indicators of microplastic pollution.

Fish. As with invertebrates, many studies published in 2019 relayed information on microplastic concentration in fish tissues and more broadly incidence of microplastic ingestion. For example, studies documented microplastics in fishes from coastal waters of the Red Sea (Al-Lihaibi et al. 2019), the Canary Islands (Herrera et al. 2019), Bay of Bengal (Hossain et al. 2019), and the Yellow Sea, China, as well as estuarine waters in Argentina (Arias et al. 2019), southeastern Brazil (Dantas et al. 2019), and Portugal (Rodrigues et al. 2019) and deep-sea waters, such as those in the South China Sea (Zhu, Wang, Chen et al. 2019).

Several studies focused on species important to commercial fisheries around the world, providing baseline information on the prevalence of debris ingestion in species most likely to be consumed by humans. Zhang et al. (2019) studied multiple fish and crustacean species captured from a fishing area near the East China Sea, and found microplastic particles in the gills and gastrointestinal tissues of the organisms analyzed. The authors believe microplastic pollution is concentrating in higher trophic level species. Also in China, Feng et al. (2019) reviewed microplastics in six wild fish species captured at a fish farm and mariculture area in Haizhou Bay. Abundance ranged

from 13.54 ± 2.09 to 22.21 ± 1.70 particles per fish, and particle abundance increased with decreasing particle size. Su et al. (2019) examined stomach and gill tissues in 13 species of estuarine fish in Chinese waters, as well as muscle and liver from Asian seabass (*Lateolabrax maculatus*), and detected 0.3-5.3 particles per fish (gut) and 0.3-2.6 items per fish (gill). Smaller particles were detected in gill tissues, and no particles were detected in liver or muscle samples. Su et al. point to a need for additional quality control to assess microplastic concentrations in commercial fish species.

Near Vancouver Island, Canada, Collicutt et al. (2019) collected juvenile Chinook salmon (*Oncorhynchus tshawytscha*) as well as surrounding water and sediment samples for microplastics analysis. The authors measured 1.2 ± 1.4 particles per fish, 659.9 ± 520.9 particles per m^3 water, and 60.2 ± 63.4 particles per kg dry weight sediment, with no statistical differences among sites except for sediment concentrations. The Chinook ingestion rate appears lower than other rates (e.g., Feng et al. 2019, Su et al. 2019), though smaller particles less than 100 μm were not measured as part of the experiment and could present a risk. Another study of three common commercial and sustenance fish in Newfoundland, Canada, found no incidence of microplastic ingestion for Atlantic salmon (*Salmo salar*) and capelin (*Mallotus villosus*) ($n=419$), and less than 2% incidence of ingestion in more than 1,000 Atlantic cod (*Gadus morhua*) examined.

In the Mediterranean, more than 200 demersal fish were analyzed for microplastics and characterized using microscopy techniques (Giani et al. 2019). Plastic particles (0.10-6.6 mm) were detected, with an incidence of 23.3% fish containing microplastics, a majority of which were fibers. Renzi et al. (2019) analyzed the stomach contents of sardines and anchovies caught in the Adriatic Sea in 2013-2014. Authors found more than 90% of sampled organisms (160 individuals) contained marine litter and microplastics, ranging from 1.25 to 4.63 particles per fish, with a greater number ingested by sardines. In another study of anchovies and sardines in the Mediterranean (Gulf of Lions), Lefebvre et al. (2019) documented ingestion in 11% anchovies (at 0.11 ± 0.31

microplastic particles per organism) and 12% sardines (0.20±0.69 particles per organism), implying variance in ingestion rates based on location.

In a study of juvenile blueback herring in the Hudson River, Ryan et al. (2019) analyzed microplastic content in the fish's diets (12%) and the surrounding water column (21%), and based on selectivity analysis, described selective feeding on zooplankton. Feeding strategy likely influences the ability of certain species to preferentially select prey items and avoid microplastics, though more research across commercial species, locations, and seasons is necessary.

To evaluate uptake of five hydrophobic organic chemicals in fish, Lee et al. (2019) used simulated intestinal fluid in a modeled bioaccumulation experiment. The authors found if the contaminant was close to equilibrium conditions in microplastics and the environmental medium, accumulation in the fish is not as likely, and suggested future studies focus on plastic additive chemicals that likely exist within plastic particles at higher concentrations than surrounding media. Pannetier et al. (2019) used the rainbow trout liver cell line to evaluate toxicity of chemicals sorbed to microplastics collected on beaches around the world. Results indicated that EROD activity was altered depending on the microplastic particle's contaminant profile (e.g., sorbed chemicals and components of the plastic such as additive chemicals) and DNA damage was possible based on results of a comet assay. In another laboratory experiment, Wang, Li, Lu et al. (2019) exposed medaka (*Oryzias melastigma*) to relevant concentrations of 10 µm polystyrene particles in a long-term (60 day) test, and found uptake in the gill, intestine, and liver, as well as induction of oxidative stress, changes marked by histological parameters, delayed gonad maturation, and decreased fecundity in female fish. Together, results of these experiments indicate varied responses and risks based on the type of microplastic, associated contaminants, and species investigated.

Seabirds. As part of a review of published interactions between birds and litter, Battisti et al. (2019) compiled a list of 258 affected species that included 206 species of seabirds. That list undoubtedly is longer today,

based on the results of the studies described here. Multiple studies in 2019 focused on the incidence of debris as nesting material. Jagiello et al. (2019) reviewed 25 published reports of anthropogenic materials in bird nests, and found of the more than 10,000 nests studied, 31% contained debris items. The authors determined an association between human activity and debris prevalence in nests, and described that the published literature is currently skewed toward surveys of marine birds. O'Hanlon et al. (2019) surveyed the nest materials used by Northern Gannets (*Morus bassanus*) across their range and recoded debris in 46% of 7,280 nests, which accounted for all except one of the 29 colonies that were investigated. The authors noted that threadlike plastic, assumed from fishing activities, was the most prevalent debris in nests. In West Africa, Tavares et al. (2019) investigated seabird nests and found litter in 15% of Caspian Tern and 40% of Great Cormorant nests. The plastic most commonly incorporated into nests was different from plastic litter found in nearby waters.

In the western Indian Ocean, Cartraud et al. (2019) surveyed the stomach contents of nine seabird species (n=222) and found particles in each species, with the highest prevalence in tropical shearwaters (79%). In Brazil, Rossi et al. (2019) found synthetic debris in all 24 American Oystercatchers (*Haematopus palliatus*) necropsied, with plastic fragments and pellets most prevalent. Roman, Paterson, Townsend et al. (2019) necropsied 348 petrel carcasses across 20 species, and found that 90% of the rigid items ingested were between 2-10 mm and the size of the ingested items is positively correlated with the bird's size. Tanaka et al. (2019) individually analyzed 194 plastic fragments and pellets retrieved from Northern Fulmars (*Fulmarus glacialis*), Laysan Albatross (*Phoebastria immutabilis*), and Black-Footed Albatross (*Phoebastria nigripes*) for UV stabilizers, brominated flame retardants, and styrene oligomers, which were all detected in fewer than 5% of samples. Ingestion may be a vector of these hazardous substances to seabirds.

Caldwell et al. (2019) assessed the relationship between foraging strategy and plastic ingestion rate, using generalist (Herring Gull, *Larus argentatus*) and

specialized (Great Black-backed Gull, *Larus marinus*) species. Herring gulls feeding in the Gulf of Maine were more frequently exposed to dietary plastic (63% incidence in pellets) compared to Great Black-backed Gulls (approximately 23% incidence in pellets), suggesting differential risk to these species based on foraging behavior. Similarly, in the Bay of Biscay, Franco et al. surveyed fifteen species of seabirds (n=159) to evaluate their usefulness as monitors of plastic pollution, and suggested the Common Guillemot (*Uria aalge*) and Atlantic Puffin (*Fratercula arctica*) may be suitable monitors based on ingestion occurrence, species abundance, and stranding prevalence.

In a modeling study that incorporated seawater microplastic concentrations, seasonal information on Cassin's Auklet (*Ptychoramphus aleuticus*) foraging areas in the Canadian Pacific region, and necropsy results from recovered carcasses, O'Hara et al. (2019) derived seasonal exposure predictions that were consistent with the results of field studies to move toward understanding population level impacts to particular species. Roman, Bell, Wilcox et al. (2019) examined ecological factors associated with debris ingestion in 51 species of Procellariiform seabirds located near the Australasian / Southern Ocean boundary, and found four main factors help determine the incidence of debris ingestion in a species: taxonomy, foraging strategy, diet, and exposure to debris based on foraging range. Additionally, Roman, Hardesty, Hinal and Wilcox (2019) demonstrated a relationship between the amount of ingested debris and mortality (i.e., a dose response) in Procellariiformes seabirds. The authors estimated approximately 20% chance of mortality from ingesting one debris item, where balloons were the highest-risk item, and a 100% likelihood of mortality after ingesting 93 debris items.

In an investigation of sublethal effects of ingested plastic in Flesh-footed Shearwaters (*Ardenna carneipes*), blood chemistry metrics were investigated (Lavers et al. 2019). The authors found associations between ingested plastic and morphometrics and blood calcium (significant, declining trends with more ingested plastic) as well as uric acid, cholesterol, and amylase production (increasing levels

with more ingested plastic), and concluded analysis of physiological parameters is critical to better understanding the sublethal impact of plastic debris. Puskic et al. (2019) took a similar approach and investigated potential sublethal impacts to Flesh-footed (*Ardenna tenuirostris*) and Short-tailed Shearwaters (*Ardenna carneipes*), focusing on fatty acid analysis for the connection to energy usage; while the study did not find a relationship between fatty acids and ingested plastic, it furthered the concept of investigating sublethal impacts.

Sea Turtles. The loggerhead sea turtle (*Caretta caretta*) has been proposed by the European Union as an indicator of pollution in the Mediterranean Sea. Matiddi et al. (2019) published a protocol for quantifying the amount of litter ingested by marine turtles, to assist groups are conducting assessments related to the EU standard. Domenech et al. (2019) quantified debris ingested by *C. caretta* the western Mediterranean (northeast Spain) in order to provide a comparison to the metric listed by the EU. Domenech et al. (2019) found 71% of turtles surveyed from 1995-2016 contained plastic debris, and similarly to other areas in the EU, the amount of plastic ingested by late juveniles has decreased in the past ten years. Arcangeli et al. (2019) analyzed a five-year time series of sea turtle and marine litter observations along transects in the Mediterranean Sea to detect seasonal overlap and assess exposure risk, though results indicated species and floating litter observations were variable even along fixed transects. Duncan, Arrowsmith, Bain et al. (2019) conducted a study to test whether green turtles in the eastern Mediterranean selectively ingest debris that resembles dietary items, and found selectivity toward certain types, colors, and shapes of plastic (e.g., sheets and threads; black, clear, and green items; linear shapes) which may signal selection of items similar to sea grass, a main dietary item. The authors believe more investigation is necessary, but did find a significant negative relationship between turtle size and the number and mass of ingested plastic pieces, implying shifts in diet over time. In a separate, related study, Duncan, Broderick, Fuller et al. (2019) isolated and quantified ingested microplastics in 102 individual sea turtles (100% of samples) captured in the Atlantic, Mediterranean, and

Pacific Oceans. The study confirmed ingested synthetic materials such as thermoplastics and elastomers, and noted the majority of identified particles were fibers.

In southern Brazil, Rizzi et al. (2019) quantified ingestion of plastic litter in 49 of 86 sampled sea turtles, and noted that omnivorous turtles had higher rates of ingestion than carnivorous turtles. As part of a citizen science effort in the United States, Martin et al. (2019) compiled density maps of marine debris on Jekyll Island, Georgia, beaches and local sea turtle nesting activity to evaluate the likelihood of interactions and focus citizen science efforts in high-risk locations. In Florida, Garrison and Fuentes (2019) monitored marine debris at sea turtle nesting beaches to provide a baseline assessment of debris items, materials, and density per square kilometer in an effort to better understand the impacts to sea turtles and nestlings.

Marine Mammals. Multiple papers explored the ability of marine mammals scat to provide information on the prevalence of microplastic ingestion in marine mammals. Nelms, Parry et al. (2019) published non-invasive methods using molecular techniques to analyze scat for diet composition and isolation methods to quantify microplastics in scat samples. Nelms, Barnett et al. (2019) measured microplastics in digestive tracts of 50 marine mammals (representing 10 species) stranded in the United Kingdom, and detected particles in all 50 individuals. Nelms et al. () found a mean ingestion rate of 5.5 particles per individual, and a majority of fibers compared to fragments. Hudak and Sette (2019) processed fecal samples collected in the northwestern Atlantic from harbor (*Phoca vitulina vitulina*) and gray (*Halichoerus grypus atlantica*) seals. Microscopic debris (>500 μm) was detected in 6% of harbor seal and 1% of gray seal samples, and included cellophane, synthetic resin, and rubber. In the eastern Pacific, Donohue et al. (2019) evaluated northern fur seal (*Callorhinus ursinus*) scat and documented a 55% incidence of plastic fragments with a range of 1-86 fragments per sample, and a 41% incidence of microplastic fibers. These techniques seem promising for data collection and analysis, to produce a clearer understanding of the

factors that influence microplastic prevalence and abundance in marine mammal scat.

Effects of Oil Spills

Ten years after the Deepwater Horizon oil spill (2010), the volume of literature related to the biological effects of that spill have plummeted. In fact, there was an overall reduction in the number of papers related to the effects of oil spills on marine life and ecosystems. However, the 2019 papers published cover a wide breadth of topics ranging from how oil may have altered biofilm communities on historic shipwreck hulls, increasing their rate of degradation to how restoration actions after oil spills in tundra environments can cause more harm than benefit if not approached holistically. A common theme is an increasing recognition of the importance of the microbial and benthic communities in the degradation of oil compounds.

Gulf of Mexico Deepwater Horizon 2010. Salt marsh invertebrate communities had not fully recovered six and a half years following oiling by the Deepwater Horizon (DWH, (Fleeger et al. 2019). Although the regrowth of *Spartina alterniflora* and benthic microalgae significantly enhanced the recovery of numerous species, poor soil quality (e.g. reduced live below ground biomass, bulk density, elevated Total Petroleum Hydrocarbon (TPH) concentrations) appears to be a significant factor in the lack of recovery of the kinorhynch *Echinoderes coulli*, the polychaete *Manayunkia aestuarina*, ostracods, and juvenile gastropods. Girard et al. (2019) estimated that deep-sea coral colonies adversely impacted by the DWH oil spill displayed low annual growth rates ranging from 0.14 to 2.5 cm/year/colony. After 2014, the most impacted sites displayed the highest rates of growth, although not enough to offset the initial loss of branches. Return to pre-impact size was expected to take at least 50 years.

Tilefish (*Lopholatilus chamaeleonticeps*) caught in proximity to the DWH wellhead and the mouth of the Mississippi River (between 47 and 87km) had significantly lower concentrations of methylmercury (MMHg $1.52 \pm 0.82 \mu\text{g g}^{-1}$) in tissues than tilefish located >109 km east of the site (MMHg $4.09 \pm 2.04 \mu\text{g g}^{-1}$). A combination of

high sediment load from the Mississippi River and increased marine snow post DWH are the most likely explanation (Perrot et al. 2019).

Accelerated metal loss occurred in the aftermath of the DWH oil spill from the submarine wreck, U-166, located within 20 km of the Macondo well head in an area heavily impacted by oil flocculant residue. Biofilm community composition was significantly different between oil impacted and reference sites with a greater presence of oil degrading genes in the impacted areas (Mugge et al., 2019).

In a modeling effort, Kocmoud et al. 2019 predicted that turtle populations may be resilient to big oil spills, such as the DWH, assuming that other factors are favorable (e.g. food availability, quality of habitat). The authors evaluated three scenarios in an age structured population model: a “pulse” scenario, a “press” scenario, and a “density-dependent remigration.” The best fit model seemed to be the density-dependent remigration model which reproduces population dynamics observed after DWH

Other Spills. Of 2,063 seabird carcasses collected during New Zealand’s M/V Rena spill (2011), 1,376 were oiled. Most of the birds died of hypothermia, drowning, or starvation (Hunter et al. 2019). Of the live oiled birds, 87.1% were successfully rehabilitated and released. Little penguins (*Eudyptula minor*) fared the best with nearly 80% survival. Poor body condition upon arrival resulted in higher mortality, while degree of oiling had no significant impact on survival (Gartrell et al. 2019). Other researchers documented adverse impacts to birds from oiling and rehabilitation beyond survivorship. In San Francisco Bay, California brown pelicans (*Pelecanus occidentalis californicus*) oiled by the Cosco Busan (2007) IFO-380 heavy fuel oil spill in San Francisco Bay, and rehabilitated transitioned to breeding plumage (yellow crown feathers) significantly slower and had less intense gular coloration than non-oiled, non-rehabilitated birds (Jacques et al. 2019). Interestingly, the presence of Global Positioning System (GPS) transmitters on brown pelicans, regardless of oiling and rehabilitation status, resulted in similar adverse impacts. Golightly et al. (2019) found that

surf scoters (*Melanitta perspicillata*) oiled and rehabilitated after the 2015 Refugio crude oil spill near Santa Barbara California, displayed smaller home ranges, frequented more sheltered and deeper habitats, and had more restricted movement patterns than non-oiled, non-rehabilitated birds, most likely as a result of time spent in captivity and as a result of rehabilitation practices.

Neethu et al. (2019) examined oil-induced shifts in bacterial and fungal community structures in seawater and underlying sediments following a 2017 heavy bunker fuel oil spill in the Bay of Bengal, India. The group observed distinct differences between pre and post spill community dynamics; distinct differences between seawater and sediment community dynamics, albeit spikes in oil degrading fungi and bacteria in both matrices; and major differences between bacterial responses to oil in tropical (current study) and temperate climates (previously reported).

Other Studies Related to Petroleum Contamination. Oil spills are among the sources of sediment PAG contamination in Trinidad. Balgobin and Singh (2019) reported a high risk of cancer (mean incremental lifetime cancer risks of $>1 \times 10^{-4}$) to 14% of Trinidad’s population because of PAH exposure through seafood consumed from areas with PAH contaminated sediments. PAH levels on the western coast of Trinidad ranged from 15 to 2735 $\mu\text{g kg}^{-1}$. Sediment PAH levels were significantly higher in nearshore areas than offshore, and during the wet season (mean 481.2 $\mu\text{g kg}^{-1}$) than in the dry season (mean 410.2 $\mu\text{g kg}^{-1}$).

Tong et al. (2019) found that bioturbation by the polychaete *Perinereis aibuhitensis* of sediments contaminated with heavy crude oil (Caofeidian drilling platform, China) significantly reduced the amount of TPH in both highly contaminated ($2.67 \pm 0.33 \text{ g/kg dry wt TPH}$) and less contaminated ($1.48 \pm 0.19 \text{ g/kg dry wt TPH}$) sediments. There was less removal in the highly contaminated sediments than in the lower, possibly due to inhibition of the microbial community.

Oil spills and inappropriate associated restoration activities in permafrost environments result in extensive damage to the tundra including higher humidity of the

active layer, frost heaving, cracking, and thermokarst. Chelombitko (2019) notes that restoration of permafrost areas impacted by oil spills should include consideration of specific features of the terrain and vegetative community lest the remediation cause additional damage.

Egris et al. (2019) found a strong relationship between sediment type, PAH concentration, and benthic assemblages. Specifically, benthic assemblages at sites with higher PAHs and finer sediments generally had short life cycles, small size, high reproductive rates, and were deposit feeders (r-strategist), resulting in disturbance tolerant populations.

Ghizelini et al. (2019) documented distinctly and significantly different fungal communities at four mangrove sites sampled in Rio de Janeiro (three in Guanabara Bay and one at the Restinga da Marambaia), ranging from the most PAH polluted mangroves in the world (Duque de Caxias; $18888 \pm 8559 \text{ ng}\cdot\text{g}^{-1}$ to $19194 \pm 2352 \text{ ng}\cdot\text{g}^{-1}$) to pristine (Restinga da Marambaia, non detect). Species richness was greatest at the most highly PAH contaminated sites, indicating a positive effect, while community composition varied with the type of contamination.

González-Gaya et al. (2019) found that less than 1% (0.008 Tg/yr-1) of the total atmospheric PAH inputs (1.09 Tg/yr-1) to surface ocean waters makes it to the ocean floor. Microbial degradation accounts for the vast majority of the loss. Gene analysis found that PAH degrading organisms are ubiquitous in ocean waters worldwide.

Laboratory Studies on Effects of Crude Oils

Birds. Dorr et al. (2019) assessed the adverse effects of repeated, sublethal, external exposure of artificially weathered Deepwater Horizon (DWH) crude oil on metabolites extracted from the plasma and liver of *Phalacrocorax auritus* (Double-crested Cormorants). 6.5 g of oil were applied to the backs and breasts (13 g total) of birds every three days throughout the 15-day exposure period. The authors demonstrated that repeated (as little as two) sublethal exposures had detrimental effects on the

avian metabolome, ultimately affecting reproduction, migration, and survival.

Matcott et al. (2019) studied the effects of surface film thicknesses of crude oil and condensate oil on the feather structure of 12 taxa of tropical and sub-tropical birds. The group measured changes in feather mass proportional to feather size, as well as observed clumping of distal barbules resulting from oil exposure. The authors note that significant effects from these oil types were seen when oil film thickness reached and/or exceeded 0.3 to 3 micrometers on the surface of the water.

Fish Embryos and Larvae. Early life stages of fish are especially sensitive to petroleum hydrocarbons. McGruer et al. (2019) used high-energy Water Accommodated Fractions (WAFs) of naturally weathered surface oil, collected during DWH skimming operations, to assess cholesterol biosynthesis in larval *Mahi mahi* after 96-hour static exposure. The authors describe significant transcriptomic effects in genes related to cholesterol biosynthesis when larvae were exposed to TPAH concentrations ranging from 1.69 to 5.99 $\mu\text{g/L}$. The highest concentration used (8.3 $\mu\text{g/L}$) resulted in significant decreases in total cholesterol as well as an increase in mortality.

Juvenile and Adult Fish. Johansen and Esbaugh (2019) proposed a novel theory describing the physiological mechanism by which swimming is impaired in oil-exposed *Sciaenops ocellatus* (red drum). The authors exposed young adult *S. ocellatus* to environmentally relevant (29.6 $\mu\text{g/L}$) and much higher (64.5 $\mu\text{g/L}$), proof-of-concept concentrations of naturally weathered DWH crude oil, collected during response operations. After 24-hour static exposure, Johansen and Esbaugh examined mitochondrial performance in red and heart muscle tissue; results suggest intracellular impairments of red muscle, rather than mitochondrial dysfunction, may be responsible for impaired swimming performance in at least *S. ocellatus*.

In a transgenerational study, Jasperse et al. (2019) exposed a group of *Cyprinodon variegatus* (sheepshead minnow) to WAFs of crude oil to study somatic and reproductive effects of direct PAH exposure (f0), as well as the inherited effects of the indirectly-

exposed (f1) and non-exposed (f2) generations of offspring. After 14 days of continuous, flow-through (2 turnovers per day) exposure, PAH body burden, liver somatic index (LSI), and gonad somatic index (GSI) were assessed for f0. Egg production, fertilization success, heart rate, hatch rate, time-to-hatch, larval length at hatch, length and prey capture ability at 10 days post hatch, and survival were measured in f0, f1, and f2 generations. The authors demonstrated that exposure to PAHs can produce significant negative effects on the development and reproductive capabilities across multiple generations of unexposed offspring.

Invertebrates. Tapia-Morales et al. (2019) measured the effects of hydrocarbon exposure on gene expressions in the gonad of *Crassostrea virginica* (saltwater oyster). Oysters were exposed to WAFs (200 µg/L) of a light crude oil for 7, 14, and 21 days with daily water exchange and adjustment of WAFs every two days. The group noted negative effects on gonad maturation as well as significant sub-expression of genes in the bivalve's immune system. The study contributed to a growing body of work describing effects of hydrocarbon exposure on bivalve reproduction, and further developed the use of *C. virginica* as a bioindicator of hydrocarbons in the marine environment. Focusing on nematodes and copepods, Monteiro et al. (2019) examined changes in meiofauna distribution and community structure after short (1 week), medium (3 week), and long-term (9 and 15 week) exposure to high, medium, and low concentrations of WAFs of crude oil. Evaporated water was replaced with non-polluted seawater in all cases except for a group of mesocosms that were replenished with the *medium* stock of WAF throughout the experiment. The authors noted that copepods and amphipods were among the more sensitive taxa. Nematodes showed mostly sub-lethal short-term effects, with significant changes in species composition only becoming apparent several weeks after initial exposure.

Augustina et al. (2019) measured spinoid polychaete abundance at different depths of field-collected benthic sediment to assess oil-induced stress caused by low (1g/kg) and high (20g/kg) concentrations of Escalante crude oil. The laboratory experiment consisted of sediment

cores and water samples collected from the field in San Jorge Gulf, Argentina. The researchers demonstrated that while certain species of spinoids can tolerate exposure to crude oil (*B. proboscidea*), other species are much more sensitive to the experimental doses (*Rhynchospio glutaea* and *Scolecopsis* sp.) and may be effective bioindicators of crude oil exposure.

Silva et al. (2019) tested the performance of a beautifully engineered mesocosm facility designed to conduct toxicity tests under field-like conditions with laboratory-like control of parameters. The group used *Millepora alcicornis* (sea ginger, a colonial fire coral), in a proof-of-concept experiment, exposing the hydrocoral to low and high concentrations of Marlim crude oil. The study demonstrated the facility's ability to be used as either an open, flow-through system, or a reduced flow, closed cell. While this study focused on the utility and proof of design, data showed a decrease in photosynthetic algae associated with corals exposed to crude oil.

Luminescent Marine Bacteria. Kang et al. (2019) used the inhibition of luminescence in the marine bacteria *Aliivibrio fischeri* to measure photo-enhanced toxicity of common constituents and degradation products of crude oil (namely naphthalenes, and alkylated naphthalenes) under different temperature regimes (20, 25, 30, and 40 °C). While toxicity of parent compounds generally decreased with weathering, photodegradation byproducts contributed significantly to an increase in overall toxicity of the resulting solutions. Calculated EC50s are as follows: naphthalene (0.52 mg/L), 1-methylnaphthalene (0.37 mg/L), 2-methylnaphthalene (0.32 mg/L), 1-ethylnaphthalene (0.14 mg/L), and 2-ethylnaphthalene (0.16 mg/L).

Toxicity of Fuel Oils

Seaweeds. Liu et al. (2019) exposed the intertidal green algae, *Ulva pertusa*, to various concentrations of WAFs of diesel fuel, and to those derived from an intermediate fuel oil, for 96 hours. Endpoints included growth rate, chlorophyll-a production, and fatty acid synthesis; stable Carbon isotope comparisons were also analyzed. The authors note algal growth rate increased with

low exposure levels but was inhibited with higher concentrations of WAF. Fatty acid synthesis, and the ratio of stable Carbon isotopes were significantly affected by oil exposure, suggesting future utility of this method as a means to assess petroleum induced stress on coastal environments.

Invertebrates. Lemcke et al. (2019) investigated the effects of WAFs of IFO180 on the primary productivity of arctic phytoplankton, as well as the foraging capability of *Calanus finmarchicus* (copepod). The group also examined the potential for enhanced phototoxic effects associated with this fuel oil. Plankton (36-hour static exposure) and copepods (40-hour static exposure) were subjected to low (0.07 mg/L), medium (0.28 mg/L), and high (0.55 mg/L) concentrations of WAFs. These exposures reduced primary production by 6, 52 and 73%, and copepod foraging capability (fecal pellet production) by 18, 51 and 86% respectively. Phototoxic effects reduced primary production further to 71 and 91% of the control group when exposed to the medium and high WAF concentrations respectively.

Muller et al. (2019) studied the aquatic microcrustacean, *Daphnia magna*, and the marine bacterium *Alivibrio fischeri*, to compare the toxicity of WAFs of diesel and biodiesel. The group examined acute (*D. magna* and *A. fischeri*) and chronic (*D. magna* only) exposure to various concentrations of the two toxicants, calculating LC50, EC50, LOEC, and NOEC. The authors suggest that WAFs of diesel are 2.5 - 4 times more acutely toxic than WAFs of biodiesel, and have chronic negative effects on *D. magna* longevity, reproduction, and growth; biodiesel did not produce chronic effects at tested concentrations.

After cloning the full length of CYP4 cDNA from the marine polychaete *Marphysa sanguinea*, Li et al. (2019) analyzed CYP4 gene expressions after exposure to three concentrations (0.5, 5, and 50 $\mu\text{g/L}$) of benzo[a]pyrene for three, seven, and twelve days with daily renewal of test solutions. Results indicated that CYP4 gene may play an important role in the polychaete's ability to resist benzo[a]pyrene toxicity, and that *M. sanguinea* was an effective bioindicator of coastal marine pollution.

Silva et al. (2019) tested the performance of a beautifully engineered mesocosm facility designed to conduct toxicity tests under field-like conditions with laboratory-like control of parameters. The group used *Millepora alcicornis* (sea ginger, a colonial fire coral), in a proof-of-concept experiment, exposing the hydrocoral to low and high concentrations of Marlim crude oil. The study demonstrated the facility's ability to be used as either an open, flow-through system, or a reduced flow, closed cell. While this study focused on the utility and proof of design, data showed a decrease in photosynthetic algae associated with corals exposed to crude oil.

Fish. Imbery et al. (2019) analyzed gene expressions in the liver and caudal fin of juvenile *Oncorhynchus kisutch* (pacific coho salmon) exposed to various concentrations of WAFs of low sulfur marine diesel (33, 1067, and 3333 mg/L diluted 30%). The group observed significant changes in exposed fish, specifically noting the 3-methylcholanthrene responsive cytochrome P450-1a (cyp1 a) as being a particularly effective, non-lethal, and sex-independent bioindicator of PAH exposure. Ahmed et al. studied the histopathological, biochemical, and molecular effects of used engine oil on *Oreochromis niloticus* (Nile Tilapia) in the presence and absence of supplemental vitamin C. The study used high energy mixing to create WAFs of 0.25 ml/L for continuous 21-day exposure with weekly water exchange. Hematological and biochemical analyses, oxidative stress biomarkers, and histopathological examination showed negative effects caused by exposure to used engine oil. Many of these effects were lessened in fish introduced to supplemental vitamin C during exposure period. The group calculated 96-hour LC50 for used engine oil on *O. niloticus* to be 2.5 ml/l.

Khursigara et al. (2019) summarized a body of work on the ability of oil-exposed fish to cope with common environmental stressors (e.g. hypoxia, fluctuations in temperature, pH, and salinity), and to interact with other conspecific individuals (mate, compete for food, space etc.). The authors found a general decrease in the ability of oil-exposed fish to successfully adapt to changes in the environment, and cite several examples of oil exposure

affecting intra-species interactions. The authors advocate for more studies quantifying these holistic organismal responses to oil exposure.

Toxicity of Oil Spill Dispersants Alone

Stroski et al. (2019) compiled a comprehensive review of current knowledge of Corexit EC9500A toxicity, focusing on marine species but including several freshwater species as well as human effects. The authors conveniently tabulate reported endpoints (e.g. LC50, EC50, etc.) for tested species and find, in general, the specific dispersant formulation to be more toxic to plankton and early life stages of many species; less toxic to fish, crustaceans, and corals.

Toxicity Of Dispersed Crude Oils

Turtles. Three days post hatching, Harms et al. (2019) exposed *Caretta caretta* (loggerhead sea turtles) to crude oil (0.833 mL/L), crude oil and Corexit EC9500A (0.833 mL/L and 0.083 mL/L respectively), and to Corexit alone (0.083 mL/L) for 1 or 4 day static exposure periods. The authors measured changes in body weight, carapace length, and carapace width, and examined a suite of hematological analytes following exposure. Significant sublethal effects were observed in animals exposed to oil, oil and corexit, and to corexit alone. Bembenek-Bailey et al. (2019) used Nuclear Magnetic Resonance Spectroscopy (NMR) to compare the metabolic signatures of aqueous and lipophilic fractions of tissues extracted from hatchling *Caretta caretta* cutaneously exposed to crude oil, crude oil and Corexit EC9500A, and to the dispersant alone. Three days after hatching, turtles were exposed to aforementioned substances for 4 days before euthenasia and analysis. Hatchlings exposed to crude oil displayed significant variation in metabolic signatures compared to unexposed control groups; variation between tissues from animals treated with crude oil, crude oil and Corexit, and Corexit alone were insignificant.

Fish. Mauduit et al. (2019) investigated the short-term response and long-term recovery of year-old *Dicentrarchus Labrax* (European sea bass) after 48-hour static exposure to various concentrations of chemically

(Finasol OSR 52) dispersed Arabian Light Crude oil. The group measured hypoxia tolerance and temperature susceptibility of fish 1 month before, and 1 and 11 months after exposure. In addition, liver concentrations of 20 PAHs were assessed 24 hours and 1 month after exposure period. The authors demonstrated that all oil-exposed groups recovered to background levels within 31 days post exposure. No long term effects on hypoxia tolerance nor temperature susceptibility were observed. Philibert et al. (2019) examined the toxicity of various stages of weathered crude oil, unweathered crude oil, unweathered crude oil with dispersants, and dispersants only, on the early life stages of *Cyprinodon variegatus variegatus* (saltwater sheepshead minnow), and *Danio rerio* (freshwater zebrafish). Exposures commenced shortly after embryo fertilization and continued through embryological and larval stages (7-10 days) with mortality measured daily. LC50s for TPAHs of were calculated at 207 µg/L, and 265.1 µg/L for *C. variegatus* and *D. rerio* respectively. The authors also examined gene expressions, muscle deformities, and cardiac effects, showing significant differences in responses between species; cautioning against using freshwater species to model marine oil spills without understanding their limitations.

Beirao et al. (2019) examined the lethal and sub-lethal toxicity of WAFs and Chemically Enhance Water Accommodated Fractions (CEWAFs) of Hibernia crude oil as well as the effects of Corexit EC9500A alone on the embryo development of the forage fish *Mallotus villosus*. Embryo survival, hatching success, heart rate, larvae measurements, and gene expressions were analyzed to determine effects. The group demonstrated that the higher concentrations of TPAH in CEWAF had adverse effects on embryo development and survival. The authors also state that exposure to Corexit alone produced similar results. Karam et al. (2019) examined the effects of Kuwait crude oil and three chemical dispersant formulas (Corexit EC9500A, Corexit EC9527A, and Slickgone NS) on the embryonic and larval stages of *Epinephelus coioides* (Orange Spotted Grouper). The authors calculated LC50s for WAFs of crude oil alone, as well as those resulting from the addition of the chemical dispersants, after 48-hour

(embryonated egg) and 96-hour (larvae) static exposures. The study demonstrated that while more petroleum hydrocarbons are transferred to the water column when any chemical dispersant is used, there is substantial variation in toxicity between dispersant formulas; WAF of oil with Corexit EC 9527A were the most toxic, and WAFs from oil dispersed with Slickgone NS did not appear to be more toxic than WAFs from the oil alone.

Phytoplankton. Bretherton et al. (2019) examined the response of natural phytoplankton communities to 72-hour static exposure to WAFs and CEWAFs (Corexit) of a Macondo crude oil surrogate. Phytoplankton biomass and photophysiology were not significantly affected by exposure to non-chemically dispersed oil. However, exposure to CEWAF resulted in several negative effects including significant declines in photosynthetic rates and quantum yields. The group also examined the effects of chemically dispersed oil on phytoplankton community structure and diatom aggregate formation. Phytoplankton and bacteria secrete extracellular polymeric substances (EPS) that aggregate to form microgels, integral to the formation of marine snow (and marine oil snow) and other biogeochemical processes. Chiu et al. (2019) studied the effects of WAF and CEWAF (Corexit) of crude oil, as well as the effects of Corexit alone, on the aggregation rates of EPS microgels. The authors show that microgel size is enhanced by both WAF and CEWAF. However, Corexit alone was shown to not only inhibit EPS aggregation, but also disperse pre-existing microgels.

Severin and Erdner (2019) assessed the taxon-specific resiliency of phytoplankton (dinoflagellates and diatoms), and their associated bacterial communities, to sweet Louisiana crude oil and/or Corexit EC9500A. The authors also assessed the ability of these phycospheres (phytoplankton and bacteria) to degrade hydrocarbons. Dinoflagellates (*Amphidinium carterae* and *Peridinium sociale*) were more resistant to oil/dispersant exposure and harbored more oil degrading bacteria than the diatom *Skeletonema sp.*

Le and Yu (2019) examined the effects of various ratios of topped Sweet Louisiana Crude oil and Corexit

EC9500A on denitrification and organic matter mineralization in salt water marsh sediments. The study examined immediate (hours), short term (46 days), and long term (137 days) effects of three oil and dispersant ratios (1000:1, 100:1, and 10:1) in aerobic and anaerobic conditions. While even low levels of dispersant use were correlated with as much as 20% reduction in denitrification, sediments recovered quickly. However, the increased mobilization of stored nitrogen associated with stimulated organic matter mineralization caused lasting effects with negative implications for salt water marsh ecosystems.

Toxicity of Chemically Dispersed Fuel Oils

Tairova et al. (2019) subjected *M. villosus* (capelin) embryos to 72-hour static exposure to five escalating concentrations of WAF and CEWAF (Corexit EC9500A) of a heavy fuel oil (IFO30). Total hydrocarbon content ranged from 0.02-14.5 mg/L (WAF) and 0.5-304 mg/L (CEWAF). Mortality, hatching success, larvae malformations, growth and enzymatic activity were monitored over a 4-week period. The authors conclude that 72-hour exposure to environmentally relevant concentrations of WAFs and CEWAFs have significant negative effects on embryo development and early life stages of *M. villosus*. In addition, concentrations of CEWAFs were significantly correlated with CYP1A/EROD activity in newly hatched larvae.

McDonnell et al. (2019) examined the effects of WAFs, and CEWAFs, of two diluted bitumen blends (Access Western Blend and Cold Lake Blend) on gene expression and embryonic development of *Perca flavescens* (freshwater perch). After 16 days of static exposure (daily renewal), the authors observed embryonic malformations and altered gene expressions.

Uttiere et al. (2019) investigated the role of microscale biological processes that alter the distribution of oil droplet sizes in the water column following an oil spill. After combining non-toxic paraffin oil with a non-ionic, saccharide-based surfactant (used in COREXIT), adult female copepods (*Paracartia grani*) were introduced to the comparatively benign oil-water mixture. The study monitored changes in oil droplet size over a 24-hour period.

The authors noted a significant shift to smaller oil droplets, contributing this observation to the energetic movement of copepod appendages when swimming and feeding (mechanically separating oil droplets), and to the ingestion/egestion of oil after being mistaken for prey.

Oil Biodegradation. Schreiber et al. (2019) focused on microbial degradation of diluted bitumen under various conditions (i.e. summer, winter, chemical dispersant application, dispersant without oil). While monitoring hydrocarbon loss over 42 days in experimental microcosms, the group genetically characterized changes in biospheres associated with each treatment. In general, chemically dispersed diluted bitumen (and interestingly, Corexit EC9500A alone without oil) promoted more abundant and diverse oil degrading microbial communities

Risk Assessment Models. Liu and Callies (2019) created an interactive Bayesian network model to aid in dispersant use decision making in the North Sea. The probabilistic model uses location, weather conditions, and time of year to summarize potential environmental benefits and consequences associated with dispersant use. While this study focused on bird concentrations in the German Bight, the model is flexible and could be adapted to other locations and biological parameters. Kim et al. (2019) developed a method to assess initial risks associated with oil and hazardous materials spilled into various types of marine environments (marina, commercial harbour, open ocean etc.). Focusing on major South Korean ports, the study calculated hypothetical concentrations of 158 potentially spilled substances under different environmental conditions. Reduced water circulation associated with neap tides caused higher, more harmful concentrations of water soluble products when spilled in semi-enclosed basins.

Fahd et al. (2019) used arctic environmental and geophysical parameters, along with the unique physiology of Polar cod (*Boreogadus saida*) in a model linking enzyme activity in liver and other organs to potential toxicological effects in *B. saida*. The authors offered an approach to ecological risk assessments that circumvents the need to acquire toxicity assays when estimating toxicity in aquatic species.

Biomarkers for Measuring Effects of Pollutants: Field Studies

Biomarkers are measureable substances in living organisms that respond to infections and chemical pollutants. Dozens of biomarkers are being used to assess the health of marine organisms in the field and laboratory, many of which are useful in determining contaminant toxicity modes of action. They are replacing conventional lethal and sublethal toxicity tests.

Mollusks. Counihan et al. (2019) sampled mussels (*Mytilus trossulus*) over two summers from three sites in each of two national parks in Alaska, United States, in order to develop a baseline for future studies. They measured condition index (CI, weight/length), shell thickness, five biomarkers in the hemolymph including hemocyte count (immune function), hydrogen peroxide production (immune function), RNA:DNA ratio (metabolic condition), cytochrome p450 activity (CYP, metabolism/xenobiotic biotransformation), and heat shock protein 40 content (HSP40, protects proteins when under thermal and other stress) as well as the transcription of 14 genes in gill tissue. Differences were detected between the sites despite the lack of anthropogenic stressors, possibly due to differences in nutrient input or exposure to wave activity, indicating the importance of measuring natural variation in order to properly identify climate or anthropogenic effects. Blanco-Rayón, Guilhermino et al. (2019) were also focused on techniques to aid interpretation of field studies with mussels. In a review of sampling and transport techniques, they found that while most studies reported biomarker responses that occur in at most a few hours, less than a fifth reported *in situ* dissections and about half transported mussels to the laboratory without taking into account site conditions. To remedy the discrepancy the authors performed a study to determine the best available methods for collection and transport of field-collected mussels (*M. galloprovincialis*), focusing on effects on the activity of the enzyme biomarkers catalase (CAT, antioxidant), glutathione peroxidase (GPx, antioxidant), glutathione-S-transferase (GST, detoxification/phase II metabolism and antioxidant), glutathione reductase (GR,

recycles antioxidant glutathione) in the gills and digestive gland and acetylcholinesterase (AChE, neurotoxicity) in the foot, as well as lipid peroxidation (LPO, oxidative lipid damage), lysosomal membrane stability (LMS, cellular stress) and structure and tissue-level biomarkers in the digestive gland. Results indicated that mussels should be collected from the low intertidal zone and processed at the site as soon as possible (within 1 hour). If that is not feasible, then mussels should be transported in air (not water) at the ambient temperature of the collection site. Collection, transport and time elapsed before dissection should always be reported to aid interpretation of results.

Lehtonen et al. (2019) transplanted caged mussels (*M. trossulus*) for two months at 10 sites off the coast of Finland. Biomarker responses (CAT, GR, GST activity and LPO in digestive gland, AChE activity in gills), and CI were combined with chemical characterization of water (some compounds via passive sampling), bioaccumulation, benthic community status and water quality in a weight-of-evidence approach to determine the health status of the sites. They found effects on biomarkers (especially GST and AChE activity) and CI that appeared to be caused by contaminant exposure, primarily certain polycyclic aromatic hydrocarbons (PAHs), but the magnitude of the effect was influenced by extreme salinity conditions and food availability. Özdilek et al. (2019) measured superoxide dismutase (SOD, antioxidant enzyme) activity in gill, hepatopancreas and mantle of mussels (*M. galloprovincialis*) collected off the coast of Turkey, correlating it with stable nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) as indicators of anthropogenic inputs. Negative correlations were observed between isotopes and SOD activity overall, and the isotopic composition in the hepatopancreas appeared to be a better indicator of pollution than the composition in other tissues. Drif et al. (2019) measured DNA damage (via comet assay), LPO and reduced glutathione (GSH, antioxidant) in mussels (*M. galloprovincialis*) and clams (*Donax trunculus*) collected from three sites in the Gulf of Annaba, Algeria. All three biomarkers appeared to be affected by pollution exposure as well as the sex of the mollusk. The highest DNA damage in clams was in those exposed to

mixed industrial-domestic inputs, whereas in mussels it was highest in those exposed to untreated sewage.

Moreira et al. (2019) exposed caged clams (*Anomalocardia flexuosa*) and oysters (*Crassostrea rhizophorae*) for 28 days both during and at the end of dredging operations in Mucuripe Bay, Brazil, measuring contaminant bioaccumulation and effects on biomarkers. Copper, PAHs and linear alkylbenzenes bioaccumulated in both species. Significant changes were measured in ethoxyresorufin-*O*-deethylase (EROD, planar PAH and polychlorinated biphenyl [PCB] phase I metabolism), GR and GST activity, LPO and DNA damage (via alkaline precipitation assay) in the gills of both species, in addition to GPx activity in the digestive glands of oysters. The timing of response (during vs. post dredging) was species dependent for EROD and GST. Aguilera et al. (2019) collected clams (*Chione elevata*) from four locations in Laguna Madre, Mexico. AChE, butyrylcholinesterase (BChE), carboxylesterase (CbE, all neurotoxicity biomarkers), alkaline phosphatase (ALP, inflammation) and GST activity and cellular antioxidant capacity were incorporated into an Integrated Biomarker Response (IBR) index to determine the relative health of clams at the four sites. The lowest CI and greatest IBR (i.e. highest effect) was not in the site with the highest sediment contaminants, but in the site with the poorest water quality (salinity 40 ppt, pH 6.03, lowest organic matter), though this was driven by cellular antioxidant capacity, ALP and GST. Neurotoxicity was higher at the more contaminated sites. Ji et al. (2019) collected clams (*Ruditapes philippinarum*) during three seasons at three sites in Laizhou Bay, China. Seawater and sediment PAH concentrations were measured, and gill and digestive gland biomarkers (aryl hydrocarbon receptor [*AhrR*, PAH metabolism], P-glycoprotein [*P-gp*, detoxification], *GST* and *SOD* expression, EROD, GST and SOD activity, DNA strand breaks [via *F* value], LPO and protein carbonylation [PC, oxidative protein damage]) were incorporated into three biomarker indices (IBR, multi-biomarker pollution index [MPI] and biomarker response index [BRI]) to determine the relative health of clams. In the gills, IBR indicated that *F* value and LPO content, *SOD*, *P-gp* and *GST* expression

and EROD and GST activity were responsive biomarkers, while in the digestive gland *F* value and LPO content, *AhR* and *SOD* expression and EROD, GST and SOD activity were responsive. Both MPI and BRI indicated that the reference site was the least impacted and the site with the highest PAHs was the most impacted (MPI = moderately contaminated category, BRI = moderate alteration category). At the intermediate site there was disagreement between the MPI results for gills versus digestive glands, with the digestive gland biomarker responses indicating a higher impact (lightly contaminated category) than the gill biomarker responses (favorable category), though BRI placed this site in the no/slight alteration category based on biomarker responses from either tissue.

Fish. Cullen et al. (2019) collected secondary-consumer bonnethead sharks (*Sphyrna tiburo*) and tertiary-consumer bull (*Carcharhinus leucas*) and blacktip (*Carcharhinus limbatus*) sharks from Galveston Bay, Texas, USA, measuring PAH and PCB burdens along with biomarker response in livers. EROD activity was higher in bonnethead than blacktip or bull sharks, and GST activity was lower in bull than blacktip or bonnethead sharks. In general, EROD activity was positively correlated with bibenz[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene and PCB 126 and negatively correlated with PCB 153. In blacktips only, there was a negative correlation of hepatic toxic equivalent PCBs with EROD activity. GST activity was positively correlated with PCB 167 and negatively correlated with PCB 105. Lima et al. (2019) collected mullet (*Mugil curema*) from five estuaries in Pernambuco State, Brazil plus a reference estuary in a marine sanctuary in Paulo State for analysis of DNA damage in blood via comet assay (DNA strand breaks) and micronuclei (chromosome damage). Results indicated that fish from all sites except the Formoso River estuary, a protected area, had DNA damage significantly elevated above the reference site as measured by either endpoint. Valskienė et al. (2019) measured genotoxicity and cytotoxicity in erythrocytes of herring (*Clupea harengus membras*), flounder (*Platichthys flesus*) and Atlantic cod (*Gadus morhua*) from 52 stations in the southern Gotland Basin of the Baltic Sea between 2010 and 2017. As compared to

established background levels, herring, flounder and cod had high genotoxicity risk at 96%, 92% and 39%, respectively, of the stations in Polish waters and 100%, 86% and 33% of the stations in the Lithuanian waters. Those with high risk were generally clustered together, indicating the presence of hot spots in the region. Cytotoxicity was higher in the Polish EEZ while genotoxicity was higher in the Lithuanian EEZ. Fish collected near oil and gas platforms did not show the time-related decrease in effects that were observed in fish collected from other areas.

Walter et al. (2019) compared the relative abundance of gut bacterial communities in Atlantic cod from northern vs. southern Norway. PAH-degrading bacteria, including the genera *Novosphingobium*, *Sphingobium*, and *Spingomonas*, were only present in the gut microbiome southern cod, likely due to the higher anthropogenic input in this area. Operational taxonomic units of other hydrocarbon-degrading bacteria were also only present in southern cod.

Partial removal of a large field of creosote treated pilings at an abandoned rail trestle in Puget Sound, Washington, increased rather than decreased the exposure of Pacific Herring (*Clupea harengus*) eggs to PAHs and lead to continued embryonic stimulation of CYP 1a (West et al. 2019). The authors cautioned that removal of creosoted pilings needs to be complete in order to full reduce exposure of fish eggs to PAHs.

Mammals. Pedro et al. (2019) determined the correlation between persistent organic pollutants (POP) and vitamins A and E in the blubber of 14 killer whales (*Orcinus orca*) caught by subsistence fisherman in Greenland. No correlations were observed for vitamin A. In the inner blubber layer, vitamin E concentrations were negatively associated with Σ POP, but the association appeared to be dependent upon diet. In the outer blubber layer similar results were found for Σ dichlorodiphenyltrichloroethanes (DDT), Σ chlordanes and Σ endosulfan, with no correlation in killer whales with diets high in fish but a negative correlation in killer whales with diets high in marine mammals, with the Σ DDT and Σ chlordanes correlations being significant.

Multiple Phyla. Madeira et al. (2019) measured the cellular stress response (CSR) proteins HSP70, ubiquitin (tags proteins for destruction), CAT, GST and SOD activity, and LPO in a variety of marine taxa off the coast of Portugal to test the hypotheses that 1) sessile organisms have higher basal levels of CSR proteins, 2) season is a confounding factor, and 3) habitat affects intraspecies response. Organisms included fish, shrimp, crabs, hermit crabs, sea anemones, gastropods, bivalves and chitons. In general, biomarker levels, followed a pattern of mollusks > cnidarians > fish = crustaceans. HSP70 was highest in mollusks, CAT activity and LPO were highest in cnidarians and ubiquitin levels were higher in cnidarians and fish. There was a trend towards higher levels of biomarkers in sessile organisms, with HSP70 being significant. Organisms collected in spring had higher levels of LPO and lower levels of other biomarkers compared to those collected in summer. Mussels (*M. galloprovincialis*) and sea anemones (*Actinia equina*) collected in an estuary had lower overall biomarker levels than those collected on the coast. Crustacean and fish species along with one sea anemone (*Anemonia sulcata*) were the species with the lowest biomarker variation, while GST, SOD and HSP70 were the biomarkers with the lowest intraspecies variation, indicating they may be the best species and biomarkers to use in future studies.

White et al. (2019) reviewed the use of fatty acids to determine the exposure of wild marine species to waste from fish farms. In field-collected fish there was a general trend toward increasing C₁₈ fatty acids (linoleic, oleic and α -linolenic acids) in fish feeding on aquaculture waste. Biomarkers of exposure in invertebrates were more difficult to identify due to their generalized feeding strategies and higher fatty acid metabolism over that in fish. Confounding factors such as exposure to sewage or stormwater inputs that also release terrestrial fatty acids into the marine environment need to be taken into consideration when interpreting results.

Modeling. Regoli et al. (2019) demonstrated the use of the weight-of-evidence model, SediquaSoft, to provide quantitative hazard indices using data collected at three off-shore oil platforms in the southern Adriatic Sea.

Data included sediment chemistry, bioaccumulation and biomarkers (digestive gland metallothionein [MT, metal exposure], CAT, GST, GPx, GR, glutathione, total antioxidant capacity and LPO and hemocyte AChE, LMS, phagocytosis, granulocyte:hyalinocyte ratio [immune function], and DNA damage [comet assay and micronuclei]) in native and transplanted mussels (*M. galloprovincialis*), bioaccumulation in laboratory-exposed polychaetes (*Hediste diversicolor*), elutriate bioassays with four species (*Vibrio fischeri*, *Acartia tonsa*, *Phaeodactylum tricorutum* and *Paracentrotus lividus*), and benthic community analyses, resulting in about 7000 individual data points from 60 sites. For biomarkers the hazard quotient was calculated from the number of changed altered biomarkers, their toxicological relevance, statistical significance and magnitude of variance. The biomarker hazard indices in this study ranged between slight and moderate risk based mostly on MT, CAT, LPO, DNA damage, total antioxidant capacity and granulocyte:hyalinocyte ratio. Lines of evidence were assigned weights as follows: 1.0 for sediment chemistry, 1.2 for bioaccumulation in native and transplanted mussels, 0.6 for bioaccumulation of metals in polychaetes, 1.0 for biomarkers, 1.2 for elutriate bioassays, and 1.3 for benthic community composition. The overall weight-of-evidence risk level was determined to be slight, as determined by the combination of hazard indices ranging from absent (elutriate bioassays and benthic communities) to moderate (biomarkers).

Fahd et al. (2019) developed a Bayesian belief network model to estimate the probability of cell damage in polar cod (*Boreogadus saida*) from an oil spill. The model took into account environmental and physical factors that would affect exposure and uptake including season, ice thickness and openings, feeding activity, and PAH concentration, among others. It focused only on expected phase I (EROD) and phase II (GST) PAH metabolism. While the model presents a mechanistic approach to estimate the potential toxicity in Arctic fish, taking into account other measures of effect such as lipid peroxidation and environmental factors such as the effect of ultraviolet

light on PAH toxicity could increase the accuracy of the results.

Biomarkers for Measuring Effects of Pollutants: Laboratory Studies

Reviews of Laboratory Methods. Bolognesi et al. (2019) reviewed the use of the comet assay in studies with mussels (*Mytilus* sp.). They covered application of the method and its role in the assessment of genotoxic pollutants both in the laboratory and the field. Of particular interest are the recommendations for experimental design and interpretation. The cell type, such as hemocytes versus gill cells, affects the observed result and should be carefully chosen, particularly in nanoparticle exposures. Environmental factors need to be carefully controlled and reported, as do cell preparation of and electrophoresis techniques, as all can affect DNA damage results. Cell viability must always be measured in order to properly interpret the data. Interpretation of results should also take into account that DNA strand breaks may not be a direct effect of a chemical but rather a byproduct of DNA repair enzymes whose action may modulate the observed results.

Blanco-Rayón, Ivaniva et al. (2019) tested the effects of food type on biomarkers in mussels (*M. galloprovincialis*). The quality of the food affected the aerobic/anaerobic capacity, as measured by the pyruvate kinase/phosphoenolpyruvate carboxykinase ratio. Fate and distribution in the digestive gland was dependent on type of microalgae. Small microalgae easily reached the digestive alveoli and were intracellularly digested whereas larger microalgae remained in the stomach and digestive ducts. This in turn affected enzyme activity and lysosome function in the digestive gland, skewing biomarker results. The presence of food also interfered with measurement of some biomarkers such as LPO due to background pigment. It is important that detailed descriptions of food type and feeding conditions are reported to aid in comparison and interpretation of biomarker response in mussels.

Zhang et al. (2019) explored more effective methods for measuring endocrine-disrupting effects on vitellogenin (Vtg, egg yolk precursor protein) by focusing on lipovitellin (Lv), a component of Vtg with higher

stability than the parent protein. They found that a simple single water precipitation was effective at purification of Lv from Japanese flounder (*Paralichthys olivaceus*) ovaries, saving approximately 8 hours of work from the more traditional purification processes. Antibodies developed using the purified Lv were then used to measure Vtg induction in fish exposed to the birth control synthetic hormone 17 β -ethinylestradiol (2 to 50 ng/L nominal) for 21 days. Enzyme-linked immunosorbent assay of plasma, immunohistochemistry and immunofluorescence of liver tissue were all effective at measuring Vtg production using the anti-Lv IgG. Tarrant et al. (2019) reviewed the use of biomarker expression and transcriptomics in copepod research. Expression of antioxidant enzymes such as SOD, CAT, GPx and peroxiredoxins have been studied, but the pattern observed was dependent upon time period, concentration and type of exposure. *CYP*, *HSP70* and *GST* expression have also been measured in multiple studies, as well as effects on Vtg production. The authors provided suggestions for best practices including minimizing the influence of confounding factors such as handling stress, developmental stage and variant species sensitivities.

Assessment of Contaminated Sediments.

Ghribi et al. (2019) exposed sole (*Solea senegalensis*) for 28 days to sediment from three sites in the Gulf of Gabes, Tunisia along with a reference site in Portugal. Sediment from the site with the highest metal and PAH concentrations, near fish farms, induced both gill and liver CAT, total GPx and GST activity and LPO, induced liver selenium-dependent GPx (Se-GPx) activity, and reduced both brain and muscle AChE activity compared to reference. Sediment from the site with intermediate contaminant levels, near an untreated wastewater discharge, induced gill CAT activity but reduced liver CAT activity, induced liver total GPx and gill Se-GPx activity, induced both gill and liver GST activity and gill LPO, and reduced brain AChE activity. At the cleanest site, characterized as having high conservation value but still impacted by petrochemical plant wastewater, only GST activity was affected, with higher activity in both gills and liver. There were no significant impacts on gill and liver histopathology at any of the sites. Yáñez-Rivera et al. (2019) exposed female

fiddler crabs (*Uca princeps*) for 21 days to sediments from four sites in the Urias estuary, Mexico, and a negative control sediment from the crab collection site in the Sabalo estuary. The site with highest contaminant load, located near fish processing plants, lead to the highest overall effect in crabs, with the highest mortality and lowest vitellogenesis (egg yolk protein production), gonadal development and hepatosomatic index. However, hemolymph DNA damage (measured by comet assay) was lower in crabs exposed to this sediment than sediment from sites located near thermoelectric plant and sewage effluents. The authors speculated that the lower DNA damage found in surviving crabs at the highest-impacted site might be due to the shunting of energy to DNA repair mechanisms at the expense of reproduction.

Metals. Jeong et al. (2019) exposed rotifers (*Brachionus koreanus*) to five metal-contaminated seawater samples from Youngil Bay, South Korea. A 24-hour exposure to the sample with the highest concentrations of Cu, Ni and Zn (10.9, 63.9 and 97.1 µg/L, respectively) led to the highest increase in reactive oxygen species and highest upregulation of *CYP*, *GST* and 2 out of 3 *HSP* genes (*HSP21* and *27*). The sample with the highest Pb (20.74 µg/L) also increased ROS levels and led to the highest upregulation of *HSP70*. Population growth was most affected by the sample with the high Pb concentration, but life span and reproduction were unaffected by any sample. Falfushynska et al. (2019) exposed mussels (*M. edulis*) for 14 days to suspended ZnO nanoparticles (10 and 100 µg Zn/L, nominal), suspended ZnO nanorods (10 and 100 µg Zn/L), immobilized nanorods and ionic Zn²⁺ (10 and 100 µg/L) to assess effects on energy metabolism and cellular and molecular stress responses. While oxidative stress and bioenergetic biomarker responses were similar between ionic Zn²⁺ and the nanostructures, apoptosis and inflammation biomarkers (*caspase 2*, *Bcl-2*, *TAK1* and *NF-κB* expression) were highly sensitive and specific to nano-ZnO. The common commercial nanoparticles elicited a stronger response than the novel nanorods developed as a potential antifouling coating. Overall stress was also lower for the suspended nanorods, indicating that nanorods may be a potential low non-target toxicity alternative to other

antifouling paints. The immobilized nanorod exposure (simulating hull paint) was the only one that caused mortality (12%).

Vernon et al. (2019) exposed salt and freshwater mussels (*M. galloprovincialis* and *Dreissena polymorpha*) to Cu for 10 days. Concentrations of 25.2 to 51.6 µg/L (actual) significantly induced DNA strand breaks (measured by comet assay) and micronuclei in gills of both species, with little interspecies difference. DNA damage was correlated with an increase in immunostaining foci of γ-H2AX, a histone modification involved in DNA damage signaling and repair, as well as cell-cycle checkpoints and apoptosis. The foci were present at even at concentrations below those causing significant DNA damage (12.5 – 14.8 µg/L), indicating that γ-H2AX presents at damaged sites for DNA repair initiation even at low Cu concentrations. Istomina et al. (2019) exposed scallops (*Mizuhopecten yessoensis*) to Cu for up to 11 days. Enzyme biomarker response was phasic. At the starting stage of exposure (4 days, 10 µg/L Cu nominal), CAT, acid phosphatase (AcPase, lysosomal enzyme) and ALP activity increased in both gill and digestive gland tissue. However as exposure progressed a tendency towards lower enzyme activity was observed. At 11 days and 30 µg/L Cu activity either reached control levels or fell significantly below (digestive gland AcPase and gill CAT and AcPase). Oxidative damage did not follow the same pattern. At 4 days, 10 µg/L Cu digestive gland LPO increased but gill LPO and PC decreased. It is possible that the increased CAT activity in the gills, which was higher than in the digestive gland, prevented oxidative damage.

Harayashiki et al. (2019) orally exposed juvenile yellowfin bream (*Acanthopagrus australis*) to 0.7 to 6 mg/kg (actual) inorganic Hg for up to 16 days. Mercury exposure did not affect brain LPO levels or behavior, but growth was lower in exposed fish. Brain GST activity was higher in Hg-exposed fish. Brain CAT activity was higher in exposed fish on Day 4, lower on Day 8 and similar to control on Day 16. Jiang et al. (2019) exposed Manila clams (*R. philippinarum*) for up to 21 days to inorganic Hg and benzo(a)pyrene (BaP) alone and in combination. Hepatopancreatic SOD, CAT, GST and AChE activity,

GSH, LPO and glycogen content, and oxygen consumption, ammonia-N excretion and ingestion rates were compiled into an integrated biomarker response to assess effects. The order of IBR value, and thus effect, was 10 µg/L Hg + 3 µg/L BaP (nominal) > 2 µg/L Hg + 3 µg/L BaP > 3 µg/L BaP > 10 µg/L Hg > 3 µg/L Hg. In the Hg-alone treatments the relative weight of GST activity was higher than that of other biomarkers, whereas in the 10 µg/L Hg + 3 µg/L BaP treatment LPO had higher weight.

Organic Chemicals. Höher et al. (2019) exposed *M. trossulus* to chemical warfare agents that had been disposed in the Baltic Sea after World War II. The mussels were exposed for 96 hours to the phenyl arsenic compounds Clark I (sneezing and vomiting agent) and Adamsite (nausea and vomiting agent) as well as the tear gas α -chloroacetophenone in 13 mixtures of various concentrations. Geno- and cytotoxic effects in gill cells were observed in almost all mixtures. Decreased digestive gland GR activity was observed in four of the mixtures, decreased gill AChE activity in two and stimulated hemolymph phagocytosis in five with no clear correlation to either exposure or tissue concentrations. There was no marked effect on the oxidative stress biomarkers CAT, GST (measured in gill and digestive gland) and LPO (digestive gland).

Liu et al. (2019) orally exposed rainbow trout (*Oncorhynchus mykiss*) acclimated to seawater to the polybrominated diphenyl ether (PBDE) flame retardant 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) for up to 21 days. Fish were fed 2% of their body weight twice daily feed spiked with 50 and 500 ng/g dry weight, nominal. LPO in the head kidney increased with time and concentration. P450 *CYP3A*, pregnane X receptor and *GST*, all known to be involved in PBDE detoxification, initially increased expression then fell with time and concentration. The nuclear transcription factor *Nrf2*, involved in the antioxidant pathway, increased in expression with concentration and time. Expression of *CAT* and *SOD* increased with concentration and time in the higher concentration, but enzyme activity initially increased then fell with time and concentration. Martínez-Morcillo et al. (2019) determined the activity of AChE and the

pseudocholinesterases BChE and propionylcholinesterase (PChE) as well as CbE *in vitro* in muscle, liver and brain tissue of three fish: horse mackerel (*Trachurus trachurus*), blue whiting (*Merluccius merluccius*) and pouting (*Trisopterus luscus*). Of the cholinesterases AChE had the highest activity but the tissue with the highest activity varied by species. The activity of BChE and PChE was always lower in the brain than the other two tissues, and in general PChE activity was higher than that of BChE. In all species liver was the tissue with the highest CbE activity, followed by muscle and brain. The organophosphate insecticide chlorpyrifos inhibited AChE and to a lesser extent CbE activity *in vitro* but both enzymes were highly sensitive to chlorpyrifos-oxon in all tissues. As the phosphorylation that is involved in the inhibition of CbE stoichiometrically destroys the pesticide molecule, it was thought that CbE could play a protective role for other enzymes like AChE.

Microplastics. Magara et al. (2019) assessed the toxicity of polyethylene beads (PE) and the biodegradable biopolymer polyhydroxybutyrate (PHB), both alone and in combination with fluoranthene (Flu), either adsorbed onto the microplastic (MP) or coadministered with the plastic, to mussels (*M. edulis*). A 96-hour exposure to 100 µg/L Flu and/or 1000 MP/L (nominal) caused a reduction in gill CAT, Se-GPx, and GST activity in most treatments. In the digestive gland SOD, CAT, Se-GPx activities were also reduced in most exposures, but GR increased in PE, PE + Flu co-exposure, and PHB + adsorbed Flu. Total GPx activity fell and GST activity increased with Flu alone. For the most part biomarker responses in the gills and digestive glands exposed to both Flu and PE or PHB MPs (co-exposure and adsorbed) were similar to PE alone or PHB alone, and no combined effect was apparent. Revel et al. (2019) exposed mussels (*Mytilus* spp.) for 10 days to 0.008, 10 and 100 µg/L (nominal) of a mixture of PE and polypropylene MPs. The MPs were only observed in the digestive gland tissue (100 µg/L) and the feces/pseudofeces (all concentrations). There was little effect on clearance rate, CI, histopathology or reactive oxygen species production at any concentration. At 0.008 and 10 µg/L digestive gland CAT and SOD activity and hemolymph

AcPase increased. At 100 µg/L digestive gland GST activity decreased and gill CAT, SOD and GST activities increased. DNA damage (via comet assay) significantly increased with concentration, but the overall value was low.

Wang et al. (2019) determined the reproductive toxicity of polystyrene MPs to Japanese medaka (*Oryzias melastigma*) exposed to 803 to 74943 n/L (actual, 2 to 200 µg/L nominal) for 60 days. MPs were present in the gills, intestines and, to a lesser extent, liver but not gonads. SOD, CAT and GST activities and GPx and GSH content mostly decreased in all tissues (intestines, liver, gill, gonads) with the exception of SOD in gill, GST in intestines and gill and GSH in intestines and gill which increased. LPO increased in all tissues. Transcription of reproductive hormones and enzymes in the reproductive steroidogenesis pathway decreased in females which likely led to the decrease in estradiol, testosterone, Vtg and choriogenin (egg envelope protein) observed. This in turn may have led to the observed decrease in the number of eggs produced, increase in hatching time and perhaps the decrease in offspring heart rate. Interestingly, in males reproductive hormone expression increased along with estradiol and testosterone concentrations, demonstrating a sex-specific endocrine disruption.

Pharmaceuticals. Gomes et al. (2019) exposed polychaetes (*Hediste diversicolor*) to acetylsalicylic acid, the active ingredient in aspirin, for both 96 hours (2.5 to 250 mg/L nominal) and 28 days (0.005 to 2 mg/L) to assess acute and chronic effects on oxidative stress biomarkers and histology. An increase in CAT activity was observed in the highest concentration of the acute exposure, but no other significant effects on CAT, GR, GPx and GST activity or LPO content were observed. The only histological alteration observed was an increase in mucous cells in the tegument of chronically-exposed polychaetes. Dallarés et al. (2019) determined the effects of the antiviral Tamiflu® active ingredient oseltamivir phosphate (OST) on mussels (*M. galloprovincialis*) and Manila clams (*R. philippinarum*). After a 48-hour exposure to 100 µg/L (nominal) OST was detected in the hemolymph of both bivalves while its metabolite, oseltamivir carboxylate, was only detected in the mussels. Carboxylesterases, believed to

be involved in the metabolism of OST, had elevated activity in the digestive gland of exposed mussels and clams, somewhat dependent upon the substrate used in the assay, but basal levels were lower in the clams than in the mussels which may explain lack of detectable quantities of the OST metabolite in clams. In exposed mussels GR activity was elevated in gills and LPO was reduced in digestive glands. In exposed clams GST and CAT activities were elevated in digestive glands and LPO was elevated in gills. No other effects on measured oxidative stress biomarkers, including GPx activity, were observed.

Świacka et al. (2019) reviewed the use of mussels in both lab and field pharmaceutical toxicology experiments. Studies with non-steroidal anti-inflammatory drugs revealed effects on osmosis, energy balance, membrane stability, byssal thread strength and abundance, metabolism and general condition factors. Endocrine disrupting chemicals affected gamete maturation and release, digestive gland function and general condition factors. Antibiotics accumulated in shells and, along with antihistamines and β-blockers, affected metabolism and general condition factors. Rodrigues et al. (2019) exposed juvenile gilthead seabream (*Sparus aurata*) to the antibiotic erythromycin for 96 hours (0.3 to 323 µg/L actual) and 28 days (0.7 to 8.8 µg/L). There was no effect on the biotransformation biomarkers EROD (liver), GST (gills and liver) and uridine-diphosphate-glucuronosyltransferase (gills and liver) in either exposure, nor was there an effect on AChE activity in eyes or muscle, or the activity of the anaerobic metabolism biomarker lactate dehydrogenase in muscle. For oxidative stress biomarkers, while there was no effect on gill or liver CAT activity in either exposure, liver GPx activity was enhanced at the highest acute concentration, liver GR activity was enhanced at the intermediate chronic concentration, and liver LPO was reduced at the lowest and intermediate chronic concentrations. Erythrocyte DNA damage measured by comet assay increased at the two highest acute concentrations and the low and intermediate chronic concentrations, but there was no effect on erythrocytic nuclear abnormalities, indicating that the observed DNA damage may have been repairable.

Minovski et al. (2019) used quantitative structure-toxicity relationship models integrated with structure-based toxicity and molecular docking studies to develop a screening device to identify active pharmaceutical ingredients with the potential to affect AChE function in fish. A screening of 60 chemicals identified 23 with the ability to bind to Pacific electric ray (*Torpedo californica*) AChE. Among the chemicals estimated to be highly toxic were several pharmaceuticals used to treat central nervous system disorders. It's worth noting that human AChE is a central nervous system enzyme targeted and inhibited by anti-Alzheimer's and anti-Parkinson's drugs.

Climate Change. Almroth et al. (2019) examined the effects of temperature (5 to 18°C) and CO₂ (pCO₂ 400 µatm, ~pH 8.0, and pCO₂ 1000 µatm, ~pH 7.7) in juvenile Atlantic halibut (*Hippoglossus hippoglossus*) exposed for 96 days. Hepatic SOD activity was affected by temperature while CAT activity was affected by CO₂ levels, and there was a slight trend towards increased activity of GR, GST and GPx with increased temperature. EROD activity and PC were both strongly induced by CO₂. Both AChE and BChE increased with increasing temperature, though the effect on AChE activity was ameliorated somewhat by increased CO₂. Other authors focused on the effects of climate change on the toxicity of pollutants.

Cao et al. (2019) set out to determine whether ocean acidification (pH 8.1, 7.8 and 7.6) affected the toxicity of 25 µg/L Cu (nominal) in oysters (*Crassostrea gigas*) exposed for 28 days. A decrease in pH increased Cu bioaccumulation in a dose-dependent manner. This increase in bioaccumulation appeared to correlate with an increase in gill damage and an increase in IBR index. At 14 days, decreased pH led to higher IBR index scores but this effect was somewhat reduced at 28 days. However in Cu-exposed oysters not only were the scores higher than with decreased pH alone, they also increased with time. IBR scores were primarily driven by increases in GST and SOD activity, LPO, and increased pyruvate kinase and hexokinase activity, both glycolytic enzymes.

Mauvault, Camacho, Barbarosa, Alves, Anacleto et al. (2019) assessed the interactive effects of dietary exposure to the antimicrobial compound triclosan (actual 15.9 µg/kg dry weight), warming (+5°C) and pCO₂ (+1000 µatm, - 0.4 pH) in juvenile white seabream (*Diplodus sargus*) exposed for 28 days. Triclosan accumulated primarily in the muscle and both warming and to a lesser extent pCO₂ decreased the accumulation. Length and weight were negatively correlated with triclosan concentration in the liver. Triclosan increased LPO and decreased AChE brain activity and Vtg liver content, but effects on antioxidant (GST, SOD, CAT) activity were less apparent. These effects were either exacerbated or attenuated by climate effects with the combination of triclosan and acidification resulting in the highest effects overall (LPO, SOD, HSP70, ubiquitin, and Vtg). Mauvault, Camacho, Barbarosa, Alves, Anacleto, Pousao-Ferreira et al. (2019) performed a similar experiment looking at effects of warming and pCO₂ on the toxicity of the antidepressant venlafaxine (Vfx) in diet (actual 161.7 µg/kg dry weight) and water (actual 20.2 µg/L) in juvenile meagre fish (*Argyroxomus regius*) exposed for 28 days. IBR index scores indicated that acidification negatively impacted gills (increased ubiquitin and LPO) but effects were less evident in muscle, liver and brain compared to those of warming and/or Vfx exposure. Warming appeared to have a higher effect in the brain as measured by CAT, GST, AChE activities and LPO and HSP70 content. Warming or acidification alone either exacerbated or attenuated Vfx effects. Co-exposure to all three stressors (Vfx via water), however, resulted in higher overall IBR values.

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

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