International Conference on Marine Pollution and Ecotoxicology

Issues of legacy pollutants: P-1

Study on taxon-toxicity sensitivity of fish to representative transition metals

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Transition metals, an important class of metals, are a group of elements in groups IIIB to IIB of the Periodic Table of the elements. The last electron of the transition metal is often filled in the *d* orbital of the outer layer of the atomic orbital, which leads to a variable valence state for transition metals. As a result, transition metal plays a transitional role during the periodic characteristic change of elements. Transition metals widespread in aquatic environments that can seriously affect the species diversity and species distribution of aquatic organisms when concentrations exceeding threshold values. For example, cadmium, a representative toxic metal, can affect the embryonic development, the larvae survival and the reproduction of aquatic organisms. The United States and many other countries/organizations have worked on water quality criteria (WQC) of metals to protect aquatic life since the 1960s. However, different taxa are protected by use of unified WQC thresholds, which are derived to protect most creatures and rarely considers the ecological status and economic value of different species. At present, there are lots of experimental researches of aquatic toxicity of metals to different taxa, especially fish. It is the time to study taxon-toxicity sensitivity to metals through the dose-effect relationships and massive biotoxicity data obtained from laboratory exposure experiments. The present study established the species sensitivity distributions of nine representative transition metals (Cr(VI), Mn, Fe, Ni, Cu, Zn, Ag, Cd, Hg) for protecting Chinese fish based on non-parametric kernel density estimation models and then derived their HC5 values. The results showed that Ag has the lowest HC5 value, which has the highest toxic potency to fish; Ni has the largest, the least. Moreover, it suggested that the toxic potency of the transition metals to fish may be periodic and *Cyprinidae* is more sensitive to these nine metals than other fish. The study will provide some reference for future research on the toxicity sensitivity of fish to other transition metals.

Applicability of using the biological effects on *Ptilohyale barbicornis* and *Perinereis aibuhitensis* in evaluating metal contaminated sediments along the coast of Taiwan

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Chemical contaminants discharged into estuaries and coastal areas are often adsorbed to sediment particles and deposited on the sea bed. Contaminant-laden sediments may pose potential threats to coastal ecosystems, the sustainability of aquatic resources, and human health (via the food chain). We selected the local amphipod *Ptilohyale barbicornis* and polychaete *Perinereis aibuhitensis* as our experimental benthic organisms. Acute and chronic toxicity endpoints were 10 d / 28 d survival and growth for *Ptilohyale barbicornis* and 10 d / 28 d survival and bioaccumulation effects for *Perinereis aibuhitensis* for understanding their sensitivity in detecting metal contamination at ten (KY, DC, LG , JJ, CL, CT, AGD, HJ, CJ, LP) collected estuarine sediment samples. The metals lead, zinc, nickel, chromium, and arsenic were detected in all samples. The highest average detected concentration was for zinc, and cadmium was not detected. In *Ptilohyale barbicornis* acute toxicity tests, survival at sites DC, LG, CL, CT, AGD, HJ, CJ, and LP was significantly different from controls (p <0.05). Growth (body length and weight) for *Ptilohyale barbicornis* was mostly inhibited, with body lengths in particular being significantly different from controls (p <0.05) when exposed to field sediments. Overall, these contaminant levels affected the survival or growth of these small benthic aquatic organisms. In *Perinereis aibuhitensis*, survival showed no statistically significant differences from controls at all sampling sites (p >0.05). The 28 d sediment chronic toxicity test showed that 80% of the individuals had lower survival rates than controls, but this was not statistically significant (p >0.05). Bioaccumulation test results showed that there was only a significant difference (p <0.05) between the concentration of copper in the organism and in controls. However, the concentrations of metals in environmental samples had bioaccumulation factor (BAF) and biota-sediment accumulation factor (BSAF) values <1. This information will be used to elucidate the biological effects of long-term exposure for these two species to metal contaminated estuarine sediments.

International Conference on Marine Pollution and Ecotoxicology

Issues of legacy pollutants: P-3

Tissue concentrations of iron, manganese, zinc and copper in four Taiwanese toothed cetaceans

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Concentrations of Fe, Mn, Zn, and Cu in the muscle, pulmonary, hepatic, and renal tissues of 43 cetaceans, including *Grampus griseus* (*Gg*, *n*=12), *Kogia sima* (*Ks*, *n*=11), *Lagenodelphis hosei* (*Lh*, *n*=10), and *Stenella attenuata* (*Sa*, *n*=10) from 2001 to 2012 in Taiwan were measured. Significant species differences in the metal tissue concentrations were found. All *Ks* Fe tissue concentrations exhibited the highest concentrations (mean range =781-1789 ug/g dry wt.), while *Ks* showed the lowest Mn, Zn, and Cu tissue concentrations. Accordingly, Kogiid and Delphinid groups were significantly categorized by MDS analysis. Fe muscle concentrations were found to be positively correlated with *Ks*, *Gg*, and *Lh* body length, but negatively correlated with their muscle carbon isotope ($\delta^{13}C$) level, which may relate to their diving ability. These metal concentrations were similar to the samples of the same species collected in 1994-1995, indicating the homeostasis regulation of essential elements in cetaceans.

Keywords: Toothed cetaceans, Essential element, Iron, *Kogia sima*, Species difference

Review of trace metal pollution status in East China Sea sediment

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This study reviewed the published papers and employed the ERL and ERM guideline values (Long et al., 1995) to assess the pollution status of potential toxic metals (As, Cd, Cr, Cu, Ni, Pb, Zn, Hg) in East China Sea sediment (ECS). The percentages of metal concentrations exceeding the ERL value are as follows: Ni, 94.1 %; As, 90.2%; Cr, 35.3%; Cu, 10.4%; and Hg, 0.65%. No metal concentrations exceeded the ERM value. All data for Cd, Pb, and Zn concentrations in ECS sediment are less than the ERL value. The potential ecological risk from these sedimentary metals could be reduced because they were mainly present in the residual fraction, which is not available for marine organisms. It seems that the Ni ERL value is too low (20.8. mg kg^{-1}), close to the value (18.6 mg kg^{-1}) of the upper continental crust, to obey.

Keywords: trace metals; East China Sea; ERL value

International Conference on Marine Pollution and Ecotoxicology

Issues of legacy pollutants: P-5

Trace metal contamination in Hong Kong mangroves: Where are we now? 2000-2018

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Mangrove forests are under siege from a number of direct and indirect anthropogenic stressors. One of the major causes of mangrove loss over the last 50 years has been the increasing volume of chemical-runoff from urban, industrial and agricultural areas. Mangroves act as physical and biochemical barriers to contaminant transport, and their fine-grained sediments sequester trace metals. Therefore, high levels of heavy metals, such as Cu, Zn, Pb, Fe, Mn and Cd, have been reported from mangrove sediments all over the world, especially in Southern China and Hong Kong.

To understand the trends of trace metal pollution for Hong Kong mangroves, the present study compares heavy metal contamination in mangrove sediment recorded in 2000, during the most recent survey, to the ones collected by our team in 2018. Furthermore, this study characterizes mangrove sediments at 8 mangrove sites around Hong Kong not surveyed in 2000. The sediment samples were collected between August and October of 2018 at 11 different mangrove sites throughout the Northeast, Deep bay, Lantau Island, and Sai Kung regions of Hong Kong. These sites all have various levels of industrial and domestic waste inputs and hydrological influences. Six samples were taken at each site, 3 from the seaward side of the mangrove forest and 3 from the landward side. Sediment particle size was determined for each sample using laser diffraction and heavy metal analyses (Al, Cu, Cd, Cr, Fe, Mg, Mn, Zn, NI, Pb, and As) were carried out using an ICP-MS machine. The results of this study helps illuminate the changes occurring in these mangrove stands in the nearly twenty-year gap between surveys and it is of critical importance for future management of water quality and mangrove conservation in Hong Kong.

Keywords: Heavy metal contamination, Mangrove, Sediment particle size, Hong Kong

Heavy metal contamination in Hong Kong mangroves and its accumulation in local flora and fauna

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The mangroves of Hong Kong cover ~350 hectares and are exposed to multiple and intense anthropogenic stressors related to urban development. One of the major threats faced by Hong Kong mangroves is heavy metal pollution introduced via industrial activities. Mangroves are particularly vulnerable to heavy metal pollution as their fine-grained sediments and levels of organic matter result in a high binding affinity for contaminants. The extent and concentration of heavy metals in marine sediments are known to vary across the Hong Kong coastline thanks to the monitoring of marine sediment quality by the Environmental Protection Department (EPD). However, there is limited knowledge concerning the concentration of metals in Hong Kong mangroves specifically and current literature fails to consider the accumulation of metals in mangrove fauna including crabs, which are vital to the health and resilience of mangroves by performing key ecological roles (e.g. soil aeration through burrowing and feeding behaviour). To understand the extent of heavy metal pollution in Hong Kong mangroves and gain insight to their bioavailability, we assessed the concentration of Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pn and Zn in sediment, plant and crab species in four Hong Kong mangroves using Induction Coupled Plasma Mass Spectrometry (ICP-MS) for the first time in Hong Kong.

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Issues of legacy pollutants: P-7

Metal contamination and ecological risk in river sediment impacted by aquaculture

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Although aquaculture is an important food source and provide valuable economic support to many countries, it may cause critical environmental problems, such as aquatic pollution of surrounding waters. Aquaculture often result in discharge of particulate matter (e.g. feeds and faeces) containing high concentrations of potentially toxic metals. Since metals are non-biodegradable and persistent, they bioaccumulate and could result in adverse effects in aquatic organisms. Environmental monitoring and characterization of potentially toxic metals in rivers impacted by aquaculture is crucial for food safety and environmental risk assessment. In this study, we investigated the accumulation of selected heavy metals (Cu, Zn, Cd, Cr, Hg, As and Pb) and total organic matter in river sediments. Sediment samples were collected upstream and downstream the discharge point of an aquaculture area. Pollution levels were characterized using geo-accumulation index (I_{geo}) and single factor evaluation method. Ecological risks associated with heavy metals were assessed using the potential ecological risk index (RI).

The distribution and speciation of mercury in water and sediment of the Danshui Estuary in the Northern Taiwan

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Dissolved (DHg)and particulate (PHg) mercury in the estuarine waters of the Danshui Estuary, northern Taiwan, were analyzed by the cold vapor atomic fluorescence spectrometry (CVAFS) combined with amalgamation system. In addition, the Hg speciation in the estuarine sediments were analyzed by the sequential extraction method which chemically divided the speciation into five fractions: water soluble (F1); human stomach acid soluble (F2); organo-chelated (F3); elemental Hg (F4); and mercuric sulfide (F5). The concentrations of DHg and PHg ranged within 28-303 ng L^{-1} and 0.12-1.69 μg g^{-1}, respectively, within the estuary. The distributions of DHg and PHg exhibited no specific trend with the salinity. The values of distribution coefficient, log (K_D), between the particulate and dissolved phases ranged within 3.76-4.57 and was independent of the salinity in the estuary. Total Hg concentrations in the estuarine sediment ranged within 15.65-475.21 ng g^{-1}. The sedimentary mercury was dominated by the element Hg fraction which contributed 39.26-82.33% (average 65.72%) of the total Hg pool. The mercury sulfide and organic chelating were the second and third important fractions which account for 1.90-31.84%(average 14.67%) and 0.22-25.69%(average 10.80%), respectively.

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Issues of legacy pollutants: P-9

The geochemical distribution of trace metals in suspended particulate matter at the Doha Bay, exclusive economic zone, Qatar

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Trace metals are essential component in the organisms of the marine environment. Some play essential role in the biochemical processes (e.g. Fe, Cu, Mn, Zn, N and P) and are needed in minute concentration, while others have no known biological functions (such as As, Pb, Cd, Ba and Hg), and are toxic to marine life at any concentration. Suspended particulate matters (SPM) play significant role in the transport and distribution of elements in the environment. We investigated the distribution of 16 trace metals (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, Ti, V, Zn) associated with SPM in the Doha Bay in the east coast of Qatar (central Arabian Gulf) in the surface and near bottom water down distance from the shoreline. A negative correlation was evident between the concentration of selected metals and the amount of SPM (r = -0.77, -0.81, -0.81, -0.79, -0.79, -0.81, -0.8, -0.77 for As, Al, Co, Cu, Ba, Fe, Ni and V, consequently). Higher concentrations of metals were detected associated with the near bottom SPM. A significant relationship was found between As, Al, Ag, Ba, Cr, Cd, Co, Cu, Fe, Mn, Ni, Sn, Ti and V, that could point to the same origin and they showed similar trends in the waters of the Doha Bay. Nutrients and chlorophyll decreased with distance from shore, indicating high inputs of nutrients from discharge points, the same with chlorophyll-a. Chlorophyll-a decreased with increasing distance due to the increased amounts of available nutrients at the waters of the shores and indicated by the increased amount of dissolved oxygen as well. Furthermore, a significant inverse relationship was found between nutrients and trace metals.

Geochemical migration and transformation of thallium and accompanied trace metals in sediment profiles of the Beijiang River, China

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Thallium (Tl) is a non-essential heavy metal with severe toxicity. Due to excessive smelting activities using Tl-bearing minerals in the Beijiang River Basin, a serious Tl pollution incidence burst out in the surface waters of the Beijiang River in the year of 2010. Ever since then, very strict control of Tl pollution has been enacted, which has greatly reduced the Tl level in the surface waters. However, enriched levels of Tl have been observed in the sediments from the river basin. As a part of an ongoing investigation, the aims of this study were to further investigate the migration and transformation of Tl in the two sediment profiles from the river basin impacted by the smelting activities onshore. The results showed that Tl was mainly resided in the geochemically-mobile fractions (average percentage 57.06 ± 14.18%), among which the reducible (average 24.32 ± 9.84%) were predominant. These forms of Tl can easily be released into the environment under certain environmental conditions, causing potential ecological risks to the surrounding environment. Further correlation analysis showed that a significant correlation between Tl and Pb in the sediments. Therefore, source apportionment of Tl and other heavy metals in sediments was established using Pb stable isotope tracer technique. It was found that 60% - 90% of the heavy metal pollution in sediments in the area was mainly attributed to local Pb and Zn smelting activities.

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Issues of legacy pollutants: P-11

Contrasting behavior and speciation of mercury in two lagoons with different eutrophication conditions

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Distribution and behaviour of mercury species in the shallow and semi-enclosed Chiku and Dapong lagoons with different levels of eutrophication in southwestern Taiwan were investigated. The condition of eutrophication was more severe in the Dapong Lagoon than in the Chiku Lagoon, leading higher concentration in former than later. Distribution was spatio-temporally variable in both lagoons, ranging from 4.4 to 9.2 ng l^{-1} for total Hg (unfiltered), from 2.0 to 5.6 ng l^{-1} for total dissolved Hg, from 1.7 to 2.8 ng l^{-1} for reactive Hg and from 2.5 to 7.6 ng l^{-1} for particulate Hg in Chiku Lagoon. Also, the distribution ranged from 6.66 to 12.4 ng l^{-1} for total Hg (unfiltered), from 1.79 to 3.75 ng l^{-1} for total dissolved Hg, from 1.59 to 2.67 ng l^{-1} for reactive Hg and from 2.51 to 9.45 ng l^{-1} for particulate Hg in the Dapong Lagoon. Such ranges are similar to those found previously in most lagoons subjected to cultural impacts. The abundance of particulate Hg is positively correlated with chlorophyll *a*, and total dissolved Hg and reactive Hg are inversely correlated with Chlorophyll *a.* Such relationships indicate that the distribution of Hg species was primarily controlled by the processes of biological uptake and/or particulate adsorption-desorption. Reactive Hg (Hg^{2+}) also correlates positively with dissolved oxygen concentration suggesting the biological effect on modulating the distribution of Hg^{2+}. Particulate Hg also shows positive correlations with total suspended matter and particulate organic carbon, primarily due to biological uptake and particle adsorption-desorption. Such inferences were supported by poor correlations between particulate Hg and particulate (Al, Fe, Mn) derived mainly from terrestrial detritus.

Metal speciation in marine sediment from Mai Liao industrial area, central Taiwan

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The speciation of trace metals (Co, Ni, Pb, Cu, Zn, Cr, Mn, Fe, and Al) of marine sediments from Mai Liao industrial area coast, located in the central Taiwan, was seasonally investigated by means of Tessier's five-step sequential extraction method, dividing the speciation into five fractions: exchangeable (F1); bound to carbonates (F2); bound to Fe-Mn oxides (F3); bound to organic matter (F4); and residual (F5). In addition, the grain size and total organic carbon (TOC) in the sediment samples were also analysed. Totally, eighty-eight sediment samples were analysed for the four seasons. The analysed data show that the average concentration of the analysed elements were generally lower than the ERL value (Long, 1995), except Ni. The average concentration of Ni was 44.8 mg kg^{-1}, exceeding the ERL value (20.9 mg kg^{-1}). The values of the enrichment factor (EF), element normalized to Al, of the analysed elements ranged within 1.01-3.75 with the sequence Co>Ni>Pb>Fe>Zn>Cr>Mn>Cu. According to the speciation studied, the concentrations of Al, Co, Cr, Fe, Ni and Zn were dominated in the residual fraction, averagely exceeding 70% of the total pool. Copper and Pb was also dominated in the residual fraction, but the percentage decreased to ca. 35% of the total pool. The second important fraction of both metals was F4, accounting for ca.30% of the total pool. The concentration of Mn was slightly dominated in the F2 fraction with an average of 37 % of the total pool. The percentage of F3 and F5 nearly equalized and accounted for ca.27% of the total pool. Finally, it is found that the total concentrations of trace metals in the sediment samples generally positively correlated with TOC, suggesting that TOC played an important role in controlling the metal concentrations in the study area.

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Issues of legacy pollutants: P-13

Leaching behavior of heavy metals from steelmaking slag concretes into seawater

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The cyclic utilization of steel slag has always been the focus of attention for steelmaking industry. In order to know about the influence of steel slag applied to marine engineering on marine ecological environment, the leaching behaviour of heavy metals from the concretes of different steelmaking slag contents had been studied in this work by means of a tank test using natural seawater in accordance with GB/T 7023-2011, China.

Four weight-percent types of concrete blocks were made with different contents of steel slag as 0%, 35%, 40% and 45%. A control tank without any concrete block was also set and filled only with natural seawater. The leaching process was lasted for 90 days in indoor environment with the room temperature of 21~24℃.

The results mainly indicate that: (1) the pH values of seawater with the concretes are generally higher than that in the control tank with only natural seawater; (2) the amount of heavy metals leached into seawater is small as a whole for both with and without steel slag; (3) for different contents of steel slag, the amount of heavy metals leached out differentiated insignificantly with each other. It's meant that the application of steel slag concrete to marine engineering plays little influence on marine ecological environment.

Evaluation of bioavailability of heavy metals by acid volatile sulfide and simultaneously extracted metals in sediments from Kaohsiung Harbor, Taiwan

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A total of 20 surface sediments from Kaohsiung Harbor (Taiwan) were collected for acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) analysis to evaluate the bioavailability and potential toxicity of heavy metals (Cu, Pb, Cd, Zn, and Ni) for the aquatic organisms. Results showed that [AVS] and Σ[SEM] in sediments were ranges of 2.1–90.0 μmole/g and 1.4–20.6 μmole/g, respectively, and the higher concentrations appeared near the river mouth. The [SEM_{Zn}] accounts for 62–72% of the Σ[SEM] and plays the main part affecting the SEM distribution. The [AVS] showed a significantly positive correlation with Σ[SEM] ($r = 0.74$, $p < 0.05$) and SEM of each metal ($r = 0.69–0.80$, $p < 0.05$), indicating that AVS is an important factor affecting the accumulation of heavy metals in sediments. The bioavailability and potential toxicity of heavy metals in sediments were evaluated using Σ[SEM]-[AVS], Σ[SEM]/[AVS], and Σ[SEM]-[AVS]/f_{oc} modes, where f_{oc} is the fraction of organic carbon. All sediments showed Σ[SEM]-[AVS] < 2, Σ[SEM]/[AVS] < 2, and Σ[SEM]-[AVS]/f_{oc} < 130, indicating that heavy metals in sediments of Kaohsiung Harbor did not show the adverse effects on aquatic organisms. However, storm flooding and digging activities occur periodically or irregularly in Kaohsiung Harbor, which may release heavy metals associated with AVS and adversely affect on the environment. Further studies on the bioavailability and mobility of heavy metals in sediments are necessary for storm floods and dredging activities. Laboratory biotoxicity tests should also be performed to confirm on-site monitoring and evaluation.

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Issues of legacy pollutants: P-15

Variation of 210-Polonium in the cephalopod community from the Bay of Biscay, Northeastern Atlantic

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Among natural radionuclides, ^{210}Po is the major contributor to the radiation dose received by marine organisms (Cherry and Shannon 1974). In cephalopods, ^{210}Po is concentrated in the digestive gland, which contains over 90% of the whole body burden of the nuclide. Although previous studies showed that ^{210}Po was taken up independently of ^{210}Pb, its parent nuclide (Smith et al. 1984), very little is known about the factors influencing its levels in cephalopods. To the best of our knowledge, no studies investigated ^{210}Po in many different species at the same time. In the present study, ^{210}Po was analysed in 62 individuals from 11 species representing a large range of feeding ecologies and habitats, including oceanic species such as *Teuthowenia megalops* and *Histioteuthis reversa*. Among species, the highest activity was measured in *Loligo vulgaris* (1475 Bq/kg) and the lowest in *T. megalops* (22 Bq/kg). However, considering the habitats (benthic vs pelagic and neritic vs oceanic), no significant differences appeared. At the species level, no differences among sexes were found so both genders were plotted together to test the size effect for the species with at least 8 individuals (i.e., *Eledone cirrhosa*, *L. vulgaris*, *L. forbesi* and *Sepia officinalis*). In the 3 former species, ^{210}Po levels decreased significantly with increasing size or weight but not in *S. officinalis.* In squids, this might be related to ontogenic changes of the diet that includes a large proportion of crustaceans (with high Po content) in small individuals to fish (with low Po content) in larger individuals whereas the strong dietary plasticity of *S. officinalis* at all stages of its life cycle (Chouvelon et al. 2011) might explain the lack of decrease of ^{210}Po with size. In comparison to the few data, from the literature, the levels of ^{210}Po activity in the cephalopod community of the Bay of Biscay were below than those reported in other cephalopods such as *L. vulgaris* (1700-6000 Bg/Kg), *S. officinalis* (1600-2300 Bg/Kg) and *Octopus vulgaris* (810 Bq/kg) by Heyraud and Cherry (1979), and were far below the values reported for the squid *Nototodarus gouldi* from Japan (i.e., 4800-24200 Bq/kg; Smith et al. 1984).

Influence of food increased Hg bioaccumulation in oyster when feeding on protozoans

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Food is an important route of mercury uptake in marine organisms. Trophic transfer of mercury throughout the food webs may be influenced by various factors, including diet composition. Bivalves such as oysters are widely used as bioindicators of metal contamination such as mercury. Nevertheless, our current knowledge regarding their ability to accumulate mercury from their food is mainly based on experiments using phytoplankton as food. In natural environment, oysters are also feeding on a variety of organisms, including ciliates, in addition to phytoplankton. Understanding the influence of diet composition in the bioaccumulation of mercury in oysters is therefore important to improve management of biomonitoring programs. The present study aimed at examining the influence of the diet on the trophic transfer of organic and inorganic mercury in the Pacific cupped oyster *Crassostrea gigas*. The pulse-chase feeding method was used with two-radiolabelled natural prey: one ciliate (*Uronema* sp.) and one phytoplanktonic diatom (*Thalassiosira pseudonana*). Depuration of dietary mercury in the oysters was followed for 50 days. Kinetic parameters such as the Assimilation Efficiency (AE) and the efflux rate constant were calculated. Our results showed that trophic transfer of mercury both on organic (CH_3Hg) and inorganic (iHg) forms is dependent of the food type. Thus, oysters fed on ciliates assimilated 96.16 ± 1.39 % and 30.67 ± 2.40 % of the ingested CH_3Hg and iHg respectively while 71.72 ± 3.42 % and 84.03 ± 4.01 % of the ingested CH_3Hg and iHg were assimilated in the oysters fed on phytoplankton. Interestingly, we also found that biological half-lifes ($T_{b1/2}$) are also food-dependent, showing a strong retention time of CH_3Hg in oyster fed on ciliates. The discussion of the results focuses on the digestive and depuration mechanisms used by bivalves for the elimination of mercury. Perspectives from our results in the context of biomonitoring programs for metals, in particular mercury, are also presented.

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Issues of legacy pollutants: P-17

Evidence of trophic transfer from two intertidal rocky shore ecosystems in different biogeographic regions

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Pollution of the oceans by means of metals and organochlorine pesticides pose a great threat to the biodiversity of intertidal rocky shores, especially sedentary filter-feeders since they are well known to accumulate a wide range of metals in their soft tissues, as well as to consumers of these species. Past research focused on the use of bio-indicator species to determine concentrations of compounds found within the environment. This hinders the comparison of uptake and transfer between species and results in a knowledge gap in terms of most intertidal rocky shore food webs and species. By examining sites that are as near as possible to a natural ecosystem, the degree of uptake and transfer can be determined. This study aims to determine the degree of trophic transfer of these substances in the intertidal ecosystems to allow for comparison of the biomagnification potential of metals and OCPs from two biogeographic regions. This was achieved through stable isotope analysis of carbon and nitrogen isotope signatures in a variety of organisms from the intertidal rocky shores, as well as ICP-MS and GC/μECD analysis for metals and OCPs, respectively. The results were then compared between sites and a correlation between compounds and the trophic structures of the two regions were determined. The warm temperate Tsitsikamma revealed a nutrient enriched food web and a carbon increase, compared to the subtropical Sheffield Beach. This can be attributed to the different ocean currents associated with the specific stretch of coastline, as well as upwelling and nutrients, temperature, and freshwater input. The metal concentrations detected in the present study correlates with past research within the Tsitsikamma section. A positive regression was calculated for all of the elements at Tsitsikamma but only for Cu, Zn, As, Se and Cd at Sheffield Beach. For specific food chains, there was an obvious trend of biomagnification from primary producers to primary consumers and a definite decrease from secondary to tertiary consumer. The sea surface temperatures are predicted to have great influence on the patterns observed. The concentrations detected at Tsitsikamma can be explained by the feeding habits of the specific species, where tertiary consumers consisted of omnivorous organisms that depict a variety of prey source concentrations rather than the direct magnification of just one secondary consumer species. Organochlorine pesticides were also analysed in selected species, where in most cases the concentrations were higher at Tsitsikamma compared to Sheffield Beach. With regards to the trophic magnification factor calculated for OCPs, total HCH, total DDx and total Chlordane were determined for each trophic group in order to establish the overall degree of magnification which occurs. All of the compounds however, indicated biodilution rather than magnification from the trophic group comparison. From the results obtained during the study, magnification of compounds within the marine environment under consideration is rather the exception than a general trend.

Salinity-dependent influence of mesophyll conductance on photosynthesis in two mangrove species

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The photosynthesis of mangrove plants growing in tidal regimes with high and varying salinity has been an interesting topic, but the role of mesophyll conductance (g_m), which characterizes the transport efficiency of Co_2 from substomatal internal cavities to carboxylation sites in chloroplasts and is thus considered as an important component of photosynthesis, has rarely been investigated. In the present study, two mangrove species with different salt management strategies, *Kandelia obovata* and *Aegiceras corniculatum*, were cultured for a period of four weeks under a gradient of varying salinity (0, 10, 20, 30 and 40 ppt) to assess how g_m, and net photosynthetic rate (A_n) vary with salinity. We observed that g_m and A_n of both species increased first and then decreased with increasing salinity. Salinity corresponding to g_m maximum in *K. obovata* and *A. corniculatum* were 20 and 10 ppt, respectively, while optimal salinity for A_n was 10 ppt for both species. Significantly, photosynthesis limitation analysis revealed that g_m posed considerable limitation on A_n, varying between 13% and 60% depending on salinity. As such, our work provides the first quantitative demonstration of salinity-dependent influence of g_m on photosynthesis in mangroves. Future work will explore leaf anatomical basis for the observed g_m-salinity responses.

International Conference on Marine Pollution and Ecotoxicology

Issues of legacy pollutants: P-19

Designed hydrocyclone for harbor sediment separation

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Contaminated sediments in the harbor remain being significant issues during dredging operations. Numerous methods have been suggested in recent years for remove, treating and beneficial uses of harbor sediments. In this research, the basic characteristics and the geochemistry of the sediments were studied. The content of heavy metals contaminants might be primarily governed by the grain size. The optimum separation process was approached through hydrocyclone separation, which is designed to separate coarse and fine-grained particles by high settling velocity of particles. Furthermore, the FLUENT flow simulations are helping to calculate the cut-off size and fractional efficiencies in hydrocyclone separators. The designed hydrocyclone performance validations are achieved by comparing the results from experiments with simulating results predicted by the FLUNET. The validation is set on target particle (63 μm fine particle) could reached separation efficiency better than 80%.

Results from the FLUNET simulation showed that the optimum designed hydrocyclone parameters were nominal diameter of hydrocyclone of 156mm, inlet pipe diameter of 75mm, splitoff diameter of 33mm, inletvelocity 4m/s. Under upon conditions, a separation rate 82.1% for 63μm particle (fine particle) was calculated by FLUENT. Furthermore, hydrocyclone separation experiments were conducted, and the results showed that the 63μm particle separation efficiency was 82.3% for Kaohsiung harbor sediments.

It is concluded that comparing FLUENT simulation and experimental data, the designed hydrocyclone were effective in fine particles capture (63 μm) for sediments. The coarse and fine-grained particles could be separated well by hydrocyclone operation.

Keywords: Sediment, Hydrocyclone, Separation

Occurrence and spatial distribution of perfluoroalkyl substances (PAFSs) and their precursors and alternatives in coastal environment of Korea

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Per- and polyfluoroalkyl substances (PFASs) have been used in a variety of commercial and industrial applications such as fluoropolymer, semi-conductor, textile, carpet, and firefighting foam since 1950s. Based on regulation by the Stockholm Convention, perfluorooctaonic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) were replaced into short, long, and novel PFASs in many countries. Despite this, several alternatives of PFASs have been reported as toxic potential to wildlife and human. In this study, 25 fluorinated compounds, comprising of 12 perfluoro carboxylic acids (PFCAs), four perfluoro sulfonic acids (PFSAs), six precursors, and three alternatives were measured in seawater and sediment collected along the Korean coasts. Perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), PFOS, and PFOA were predominantly detected in seawater, while PFOA, PFOS, perfluoroundecanoic acid (PFUnDA), and perfluorotridecanoic acid (PFTrDA) were dominant in sediment samples. This finding suggests the matrix-dependent distribution of PFASs. The total concentrations of PFASs in seawater and sediments ranged from 0.41 to 23.0 (mean: 4.49) ng/L and from 0.05 to 1.14 (mean: 0.35) ng/g dry weight, respectively. Precursors and alternatives such as 3H-perfluoro-3-(3-methoxy-propoxy) propanoic acid (ADONA) and hexafluoropropylene oxide dimer acid (HFPO-DA; GenX) were not detected in all seawater samples, but 8:2 fluorotelomer sulfonic acid (8:2 FTSA) was detected in most of sediment samples. The highest concentrations of total PFASs in seawater samples were found at the locations (Gunsan, Incheon, and Asan) from western coastal waters of Korea. The highest concentrations of PFASs in sediment samples were found at the locations from western (Gunsan and Asan) and eastern (Sokcho)coastal waters. These coastal regions are characterized by high population density and/or high industrialization, indicating that the contamination by PFASs is associated with industrial activity and population density and suggests the need for management of these regions. Significant correlations for PFOA and PFOS were found between seawater and sediment. The mean partition coefficients of PFOA and PFOS between sediment and seawater were calculated as 1.78 and 2.39 L/kg, respectively. Total organic carbon in sediment was significantly correlated with longer-chain PFASs such as PFUnDA, PFDoDA, and PFTrDA.

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Emerging chemicals of concern (1): P-21

Physiological and biochemical responses of *Kandelia obovata* leaves under stress of diethyl phthalate

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Diethyl phthalates (DEP) widely use as plasticizers are ubiquitous in the environment, particularly in coastal areas such as mangrove wetlands. DEP are likely to undergo biodegradation and produce products that can potentially harm living organisms. However, little is known about the toxicity of DEP and their metabolites, especially in higher plants. The present study aims to evaluate the effects of DEP on *Kandelia obovata*, a dominant plant species in mangrove wetlands in South China. Healthy propagule of *K. obovata* were exposed to six concentrations of DEP, that is, 0.02, 0.2, 2, 5 and 10 mg/L, under hydroponic culture for six months. The same set-up but without any DEP was also prepared as the control. At the end of the exposure, the contents of chlorophyll (chl) and malondialdehyde (MDA), and activities of three anti-oxidative enzymes in leaves were determined. Results revealed that chl content, including chl a, chl b and chl a+chl b, increased with DEP concentrations, implying DEP would pose toxicity (or stress) to *K. obovata* seedlings, while the increasing chl content in leaf could counter-balance the toxic oxidative stress. The content of MDA and activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in low (0.02 and 0.2 mg/L) and medium DEP (2 and 5 mg/L) were also significantly higher than that in the control. However, these values decreased at high DEP concentration (10 mg/L). The maximum activity of the three anti-oxidative enzymes was found in treatments with medium DEP concentrations (2 and 5 mg/L). These findings indicated that DEP posed toxicity to *K. obovata* but seedlings could tolerate DEP stress through enhancing their chlorophyll content and anti-oxidative enzyme activities. Nevertheless, such defence mechanism may be collapsed if the concentration of DEP is too high. The present study is first time reporting the effects of DEP on mangrove plants and how leaves respond to DEP stress.

Keywords: Diethyl phthalate (DEP); Mangrove; *Kandelia obovata*; Chlorophyll; Antioxidant enzymes

Occurrence and distribution of organophosphorus pesticides in overlying water and surface sediments of Guangzhou urban waterways: Potential ecological risk to aquatic systems

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Occurrence of organophosphorus pesticides (OPs) in overlying water and surface sediments and ecological risk to aquatic systems were investigated, where paired water and surface sediments were collected during dry and wet periods in Guangzhou urban waterways. Four target OPs (i.e., malathion, chlorpyrifos, terbufos and diazinon) were ubiquitously detected in water with malathion and chlorpyrifos as major components. Significant decreases of ΣOP (sum of four OPs) concentrations were observed in both water and sediment from the dry period to the wet period, possibly ascribing to the dilution effect of heavy rains during the wet periods. A special emphasis was placed on the potential effects of both individual OPs and their mixtures in three trophic levels (i.e., algae, daphnia and fish) using Toxic Units (TUs) and Risk Quotients (RQs) for water and sediments. The toxic units showed acute risks to daphnia and fish in most water but exhibited acute effects to daphnia in several sediments, where daphnia was the most sensitive species. The risk assessment points out that a chronic toxicity (RQ index) caused by OPs in three trophic levels (algae, daphnia and fish) exists, especially for *Daphnia magna* in water.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (1): P-23

Monitoring organochlorine exposure and effects using SPMDs and mussels in two South African sub-tropical harbours

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Marine pollution monitoring has been active in South Africa for the past 40 years, but with large periods of neglect where no research has been done. Although a mussel watch programme is active along the South African coastline, they only focus on metals. Previous studies have shown that the levels of organic pollutants along the coast are low. This project focused on the organic contamination in Durban Harbour and Richards Bay Harbour using passive and active biomonitoring methods. The aim of this study was to successfully implement passive and active biomonitoring methods using semi-permeable membrane devices (SPMDs) and indicator organisms (mussels) for chemical and biochemical analyses. Brown mussels (*Perna perna*) were transplanted from Sheffield Beach to both harbour sites for two exposure periods of six weeks (March/April rainy season and June/July dry season) together with SPMDs containing PCB 8 spiked triolein lipid. Biomarkers exposure (acetylcholine esterase - AChE, cytochrome P450 - CYP450) and biomarkers of oxidative stress (superoxidase dismutase - SOD, catalase - CAT, lipid peroxidase – LP and protein carbonyls - PC) were conducted to elucidate responses and oxidative effects in the presence organochlorine pesticides (OCPs). The results showed low levels of OCPs exposure in mussels, while the SPMDs were able to detect slightly higher levels in Durban Harbour. Both mussels and SPMDs were able to successfully accumulate PCBs and PAHs at all sites. Both harbours had higher levels of these pollutants than at Sheffield Beach, and Richards Bay Harbour had higher levels of PAHs during the dry season survey due to an oil spill a few weeks earlier. The biomarkers were able to confirm oxidative stress and exposure effects due to organic pollutant exposure, which were confirmed by chemical analyses. The biomarkers were also able to confirm oxidative stress due to other environmental factors (freshwater runoff from high rainfall, tidal influences, food scarcity) at Sheffield Beach, which are not associated with organic pollutant exposure. The data collected from this study will contribute to baseline data on the state of Durban- and Richards Bay Harbours concerning persistent organic pollutants, and therefore open the door to further marine pollution monitoring studies along the east coast of South Africa.

Tissue distribution of persistent organic pollutants in sperm whale

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Presence of persistent organic pollutants (POPs) in marine ecosystems may pose a health risk to marine mammals. Most studies on bioaccumulation of POPs in marine mammals focused on their presence in the blubber, and a few studies investigated tissue distribution of POPs. In this study, the concentration of twelve POPs compounds including ΣPCBs, 13C-chlorobiphenyl (CB) 141, ΣDDTs, ΣHCHs, ΣCHLs, mirex, hexachlorobenzene, heptachlor, aldrin, pentachlorobenzene, dieldrin, and endrin was determined in the thymus gland, adrenal gland, kidney, muscle and other tissues of sperm whale using GC-MS. The sperm whale was found stranded in Daya Bay, China in 2017. The results of this study could help us better understand the adsorption, distribution, metabolism and elimination of POPs in different tissues, and to verify the hypothesis that the concentration of POPs is affected by the physicochemical properties of the contaminants and the biological function and lipophilic content of the target tissue.

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Emerging chemicals of concern (1): P-25

The toxicological effects and mechanisms of BDE-47 and its on immortalized pygmy killer whales epithelial cells

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Polybrominated diphenyl ethers (PBDEs) and their analogues, such as OH-BDEs and MeO-BDEs are of interest since their bioaccumulation potency and was suspected to be toxic on cetaceans. Even though related toxic effect and mechanism of PBDEs and their analogues have been well studied in fish, rodent and even human, their potential toxic effect on cetaceans are still not clear. To further understand the potential effect of PBDEs and their analogues on Pygmy killer whales, immortalized cell line from pygmy killer whale was treated with BDE-47, 6-OH-BDE-47 and 6- MeO-BDEs-47, which are the predominant PBDEs and analogues in cetaceans. After treatment, measure the cell apoptosis ratio, the reactive oxygen species (ROS) level and the enzyme activities of three kind of enzyme (7-ethoxyresorufin O-deethylase[EROD], Glutathione-S-transferase[GST], UDP-glucuronosyltransferase[UGT]) about toxicant metabolism. Furthermore, the abundance of proteins and the gene expression level of AhR (*aryl hydrocarbon receptor*), EROD, GST and UGT were also detected at molecular level. With the data, the mechanism of toxicant metabolism of PBDE to cetaceans could be inferred probably.

Historical trends in halogenated flame retardants in dated sediments from a semi-enclosed bay of Korea

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Flame retardants (FRs) are substances added to polymers for preventing fire. Polybrominated diphenyl ethers (PBDEs), representative FRs, have been widely used in electronics and electrical equipment. Due to domestic and global regulations on PBDEs associated with toxic effects to wildlife and human, several alternatives such as novel brominated flame retardants (NBFRs) and dechlorane plus (DP) are introduced into industrial market. To date a few studies have conducted on historical trends in PBDEs and their alternatives in coastal environments worldwide. Twenty-two PBDE congeners, eight NBFRs and four DP were measured in dated sediments collected from a semi-enclosed bay of Korea, using a gas chromatography coupled to triple quadrupole tandem mass spectrometer. BDE 209, 1,2-bis(pentabromophenyl)ethane (DBDPE), and *anti*-DP were predominant compounds for PBDEs, NBFRs, and DP, respectively. Concentrations of PBDEs, NBFRs, and DP in all dated sediments ranged from 0.01 to 46.5 (mean: 5.62) ng/g dry wt, 0.01 to 3.56 (0.37) ng/g dry wt, and 0.01 to 1.08 (0.13) ng/g dry wt, respectively. The highest concentrations of halogenated FRs for sediments were found at the location close to the outfall of a wastewater treatment plant, suggesting a potential contamination source of FRs in coastal environment. Historical trends in PBDE concentrations showed clear increase since 1980s and the highest peak in 1990s, consistent with consumption patterns of PBDEs. Similar trends in PBDEs were found for 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) and DP, suggesting contemporary consumption for these FRs. However, the concentrations of DBDPE gradually increased since 2000s and then slightly decreased to the present, implying consumption of DBDPE as alternatives for regulated FRs. Gradual increase in the ratio of DBDPE/BDE 209 over time indicates the effectiveness of regulation on PBDEs in Korea.

Keywords: PBDE; DBDPE; BTBPE; Dechlorane; Regulation

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Emerging chemicals of concern (1): P-27

Concentration and distribution of polybrominated diphenyl ethers in mesozooplankton in the Gaoping waters off southwestern Taiwan

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Polybrominated diphenyl ethers (PBDEs), one of the persistent organic pollutants (POPs), are toxic and ubiquitous in the environment and pose great risks. To date, little is known about the distribution and accumulation of PBDEs in field mesozooplankton in the world. This study examined the composition and concentration of PBDEs of mesozooplankton in the Gaoping waters off southwestern Taiwan during August 2015 and April 2016. The PBDEs concentrations of mesozooplankton ranged between 0 and 1415 ng/g dw, with the higher levels near the transect Kaohsiung Harbor. BDE-002, BDE-015, BDE-017, BDE-099, BDE-190, and BDE-209 were the most dominant PBDEs compounds in mesozooplankton. Higher PBDE concentrations were found in mesozooplankton with lower biomass. In addition, PBDE concentrations in mesozooplankton showed a negative correlation with the precipitation. These results indicated that the biomass dilution and metabolism of PBDEs by mesozooplankton probably determine the fate of PBDEs in the water column.

Toxic equivalency factors of brominated dibenzofurans based on Japanese medaka early-life stage toxicity test

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Polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDFs) have been detected from biotic and abiotic matrices. Since PBDFs are contaminated in brominated flame retardants as impurities, they can be released into environment via wastewater discharged from flame retardant fiber manufacturing or e-waste recycling processes. Brominated dioxins are thought to cause similar biological effects as chlorinated dioxins, however, information of their effects on aquatic organisms is limited. In the present study, we conducted early-life stage toxicity tests using Japanese medaka (*Oryzias latipes*) in order to derive toxic equivalency factors (TEFs) of PBDFs. First, we exposed medaka embryos to 2,3,7,8-tetrachlorinated dibenzo-*p*-dioxin (TCDD) at different doses and durations to fix the experimental conditions. Then, using the fixed conditions, the toxic effects of 2,3,7,8-tetrabrominated dibenzofuran (tetraBDF), 2,3,8-tribrominated dibenzofuran (triBDF) as well as TCDD were evaluated, and TEFs of each brominated compound were calculated. TCDD-exposed fish showed typical symptoms called blue-sac disease, including yolk sac and pericardial edema, subcutaneous hemorrhages, and craniofacial malformations. EC_{50} for normal hatching and LC_{50} at 21 or 28 days post-fertilization in TCDD exposure test were 249, 220, or 78.3 pM, respectively. TetraBDF exposure caused similar phenotypic effects to TCDD in medaka embryos and larvae, and its EC_{50} and LC_{50} values were 8720, 7500, or 4580 pM, respectively. On the other hand, triBDF induced none of the effects observed in the TCDD exposure test. Based on these results, TEF values of tetraBDF or triBDF ranged from 0.0171 to 0.0293 or <0.000119, respectively. Previous *in vitro* studies demonstrated that tetraBDF had relatively high potency (REP: 0.10–0.97) causing TCDD-like effect, while triBDF had much less (REP: 0.00003–0.0013). These results agreed with our present study. TEF values obtained in the present study will be used for risk assessment and management of brominated dioxins in aquatic environment.

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Emerging chemicals of concern (1): P-29

Assessment of fitness of the manila clam *Ruditapes philippinarum* five years after the *Hebei spirit* oil spill

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In December 2007, a crane vessel collided with the oil tanker Hebei Spirit moored approximately 8 km off Taean on the west coast of Korea, leaking 10,900 tons of crude oil. Five years after the accident, while oil and polycyclic aromatic hydrocarbons (PAHs) in the water column had declined to pre-oil spill level, residual PAHs persisted in intertidal sediments. Burrowing organism, such as Manila clam Ruditapes philippinarum, were therefore continuously exposed to the residual oils during this time. Although the low PAH levels in sediment were too low to be lethal to clam, long-term sub-lethal effects on growth, reproduction, energy balance, and pathology were unknown. In the present study, the biological performances including condition index (CI), reproductive cycle, energetic reserves, and parasite infection levels in Manila clam were assessed monthly from April to December 2013 at Uihangri and Padori beaches in Taean, one of the most heavily damaged areas by the spilled oil, five years after the accident. Manila clams from Uihangri followed the pattern of an annual CI, reproductive cycle, and biochemical composition of normal clams in oil-spill free areas. The observed biological performances of clams at the Uihangri suggested their recovery of the physiological status to normal level after five years of the oil spill accident. On the other hand, the clams from Padori exhibited constantly low physiological status in CI and glycogen level while reproductive cycle was not altered. The modified energetic metabolism in Padori clams may reflect long-term sub-lethal physiological stresses caused by the residual oils in the sediment. The prevalence (3-26%) and infection intensity (9,000-61,000 cells/g gill tissue) of protozoan parasite Perkinsus olseni in the clams was considered to be low to interfere with physiological condition of clams.

10 years variation of polycyclic aromatic hydrocarbon in Xiamen coastal seawaters: Inversion of coastal urban development and land use in Southeast China

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Polycyclic aromatic hydrocarbons (PAHs) are considered as one important class of persistent organic contaminants, which mainly originate from oil spills and anthropogenic combustion around the world. Coastal sea area, especially estuary, plays a key role in the transportation of such organic pollutants (like PAHs) from land to open sea, where their environmental behaviors are affected by hydrodynamic and environmental variations. Xiamen is a typical southeast coastal city, which locates in the Jiulong River Estuary and was designated as one of the first four “special economic zones” in China. Benefits from location and policy advantages, Xiamen has experienced an extensive urban expansion since the early 1980s, however, at the cost of coastal environmental degradation. For effective coastal management, it is necessary to understand the relationship between environmental pollution and socio-economic development in a scientific and quantitative way. With these concerns, the level, distribution pattern and risk assessment of PAHs in the seawater from Western Xiamen Bay and Jiulong River Estuary were analyzed in 2006 and 2017, separately. The phase distribution of PAHs with different in different sampling sites varied obviously. We tried to explore the change characteristics of PAHs in the past decade and examine the possible socio-economic reasons (such as population, land use or environmental policy) by multiple statistics analysis. It is necessary to understand the relationship between environmental pollution and socio-economic development in a scientific and quantitative way. This study hopes to provide not only an epitome of the consolations between socioeconomic development and persistent organic pollution in the typical urban coastal area in China, but also hints for future management.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (1): P-31

Distribution characteristics of PAHs in water and sediments of canal received effluents from industrial park: A case of Taiwan

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Salt River is an artificial river running through the Linhai Industrial Park, which is the largest industrial park in southern Taiwan, and eventually it flows out of the Kaohsiung Harbor into sea. Salt River is mainly used to as a drainage channel to receive the sewage from Linhai Industrial Park and local residents. This study designed a total of 10 sampling sites in Salt River and its estuary to collect the water and sediments samples and then measure their basic properties and 16 PAHs (polycyclic aromatic hydrocarbons) concentrations. The measured PAHs concentrations in the drainage basin of Salt River can understand their distribution pattern and assess the levels, possible source, and potentially ecological toxicity of PAHs. Results indicated that the total PAHs concentrations in sediments and water respectively ranged of 0.28–16.5 mg/kg dw and 43.1–1580 ng/L. The spatial distribution of PAHs in sediments showed that the highest concentration was found at the estuary and decreased to directions of the upper stream and the sea. The phenomenon indicated that PAHs discharged into Salt River is finally injected and stably accumulated in sediments of the estuary through the river transport. As the view of the spatial distribution of PAHs concentration in water, the concentration decreased from the upstream to the middle stream and the high peak values were observed in the downstream and estuary and then decreased to the direction of sea region. The partitioning coefficient (log K_{oc}) of the PAHs between sediment to water were 2.8–4.5, increasing with the ring number of PAHs. A significantly positive was found between log K_{oc} and log K_{ow} (r = 0.78, $p < 0.05$). PAHs in sediments were mainly composed of the 3-4 ring with 58–77% at most sampling sites except that at Site 5 was dominantly composed of the 2-3 ring with 85%. The 2-3 ring was found the major species of PAHs in water with about 51–93%. According to the estimates of diagnostic ratios, the possible source of PAHs in sediments for most of the sampling sites was coal combustion except that for Site 5 was the mixed combustion of oil and coal. By calculating the BaP toxicity equivalent and mean effect range median quotients (ERM-Q), the PAHs level in sediment at the upstream and middle stream posed the low potentially ecotoxicity was posed, while that at the downstream, estuary, and sea region showed the moderated potentially ecotoxicity.

Naphthacene, 4,5-methanochrysene and 11h-benzo[a]fluorene as novel AhR active compounds in sediments of an industrial area

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Environmental pollutants are found to potently disturb the dynamics of environmental microbiota. However, whether and how perfluoroalkyl acids (PFAAs) will affect microbial community remain largely unknown. In this study, effects of PFAAs on environmental microbiota of various habitats (i.e., sediment, surface seawater and teleost intestines) were investigated. A shelf cruise was launched in 2017 to sample surface seawater and sediment around PRD region. Concentrations of total PFAAs in seawater were measured between 131–1563 pg L^{-1}, among which PFOS, PFOA, PFHpA, PFBA, PFBS, PFHxS, PFPeA and PFHxA homologues were ubiquitous. In sediment, total PFAA concentrations were 24.2–181.4 pg/g dry weight and PFOS was the dominant homologue. Compared to previous monitoring data, PRD PFAA pollution in current cruise showed a remarkable decrease, indicating an effective management of PFAA manufacture and uses. Microbial community in seawater and sediment was also profiled using 16S amplicon sequencing. Correlation analysis between environmental factors and microbiota found that PFAA pollutants were positively and significantly associated with *Fluviicola*, *Nitrosopumilus*, *Limnohabitans*, *Sediminibacterium*, *C39* and *Polynucleobacter* genera in seawater, while dissolved oxygen was the major shaper of sedimentary microbiota. However, whether PFAAs pose a direct effect on microbial community needs a large-scale long-term monitoring.

Following the phase-out of PFOS, PFBS is an emerging PFAA pollutant of concern. In this study, marine medaka were exposed to environmentally realistic concentrations of PFBS (0, 1, 3 and 10 μg/L) for an entire life-cycle. Then, F0 adult medaka were depurated in clean water for two months. F1 offspring were cultured in clean seawater until sexual maturity. Effects of PFBS on gut health from F0-exposed, F0-depurated and F1 medaka were examined. Although PFBS accumulation was only detected in F0 intestines, results showed that PFBS exposure significantly impaired a series of physiological activities in both F0 and F1 generations, including gut-brain neurotransmission, gut epithelial barrier integrity, inflammatory induction, endotoxin secretion, oxidative stress and hepatic lipid metabolism. Clustering analysis and PCA based on bacterial community composition and abundances found that PFBS had a dramatic and long-lasting disruption on intestinal microbiota, which could not be restored after depuration. Furthermore, gut microbiota of F1 medaka from exposure groups showed a high similarity to that of F0 parents, highlighting a transgenerational dysbiosis despite that F1 offspring was cultured in clean seawater.

Overall, results of current study underlie the disruptive effects of PFAA pollutants on environmental microbial community structure, especially in intestinal environments. Future research is warranted to elucidate the interactive modes between pollutants and microbiota as well as the biological meaning to host health.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (1): P-33

Characterization of estrogen-related receptor (ERR) gene in the brackish water flea *Diaphanosoma celebensis* and its expressions in bisphenol A and its analogous exposure

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Bisphenol A (BPA) is a widely used plastics constituent that associated with endocrine, immune and metabolic disrupting effects in organisms. Evidence for how BPA acts disruptor on biological effects at chronic levels has remained unclear. In vertebrate, however, many studies already demonstrated that BPA exposure are highly associated with expression of estrogen-receptor (ER). In arthropod, estrogen-related receptor (ERR) is a nuclear receptor associated with the reproductive system. To investigate the role of ERR during reproduction and the effect to environmental stressors on their expression in aquatic organisms, we isolated and characterized ERR associated genes from the brackish water flea *Diaphanosoma celebensis* exposed to BPA and its analogous (bisphenol F and bisphenol S). First, we identified three sex-steroid genes (ERR, Vitellogenin (Vtg), and Vitellogenin receptor (VR)) from the *D. celebensis*. We further investigated the transcriptional modulation of these genes according to developmental stages and under endocrine disrupting conditions by BPA and its analogues for 48 h. High similarity of ERR with conserved domain in alignment with those of *Daphnia magna*, *Chironomus riparius Tigriopus japonicas, and Daphnia pulex* suggesting that this gene plays role in development. The mRNA expression pattern of *ERR* was similar to that of showing high expressions of *VR* on the 5th day of growth, and it seems to be highly involved in the breeding of adult. When it comes to BPA and its analogous exposures, only *ERR* was significantly increased in BPA, while the pattern in the others (BPF and BPS) were similar in three genes. This information will be helpful in understanding the reproduction disrupt mechanism in response to BPA, BPS and BPF.

Characterization of ecdysone receptor (EcR) in the brackish water flea *Diaphanosoma celebensis* and expressions after exposure to bisphenol A and its analogues

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Bisphenol A (BPA) is a representative endocrine disrupting chemical (EDC) that has estrogenic effects in aquatic animals. Due to harmful effects and continuous usage of BPA, its analogues have been developed as alternative substances to replace its use. However, studies of the effects of BPA on molting and reproduction in crustaceans at the molecular level are scarce. The molting process is a pivotal point in the development and reproduction of crustaceans. Ecdysone receptor (EcR) belongs to the nuclear receptor, is associated with the regulation of arthropod reproductive development and molting. In the present study, we isolated and characterized EcR genes from the brackish water flea *Diaphanosoma celebensis* and studied mRNA expression pattern in different developmental stages and in an exposure to BPA and two analogues, bisphenol F (BPF) and S (BPS). Sequencing and phylogenetic analyses revealed that these four genes are highly conserved among arthropods and may be involved in development and reproduction in the adult stage. We further identified four ecdysteroid pathway-related genes (*cyp314a1*, *EcRA*, *EcRB*, and *USP*) in *D. celebensis*, and investigated the transcriptional modulation of these genes through their development (molting) and after exposure to BPA and its analogues for 48 h. The mRNA expression patterns of *cyp314a1*, *EcRA* and *USP* were matched with the molting cycle, suggesting that these genes may play a role in the molting process in the adult stage. Following quantitative real-time polymerase chain reaction (qRT-PCR) analyses, BPA and its analogues were found to modulate the expression of each of these four genes differently, indicating that these compounds can disrupt the normal endocrine system function of *D. celebensis*. This study improves our understanding of the molecular mode of action of BPA and its analogues in *D. celebensis*.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (1): P-35

Toxicity effect of environmental estrogen (EEs) on reproductive endocrine activity of the juvenile Japanese common goby, *Acanthogobuus flavimanus*

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In the coast waters, environmental estrogens (EEs) are widely accumulating and inhibiting the reproduction and next generation production on wildlife. EEs show a high potential of feminization inducing on aquatic organisms, however, limited information is currently available on EEs effects on endocrine system of seawater fish. In this study, we conducted the field survey in Japanese coastal waters using the juvenile Japanese common goby, *Acanthogobius flavimanus*, focusing the effect of estrogenic contamination on fish in the coastal waters. Moreover, in order to obtain the information of the titer to induce a reproductive feminization, wild Japanese common goby were captured from the coast and treated with E_2 in vitro and in vivo. The exposure test was conducted at a grads dose of E_2 (1, 10, 100 ng/L) for 1 week and culture test was conducted at the dose of E_2 (1 and 10 ng/ml) and HCG (mimic as GTH, 1 IU/ml and 100 IU/ml) using the gonad for 24 hours, to clarify the defence mechanism response to different EEs via hypothalamus – pituitary – gonadal axis (H-P-G axis) regulated by reproductive endocrine system.

Japanese common goby, as a good target species to evaluate the impact of estrogenic contamination, were captured from Taira River in Nagasaki (control site) and Yokojyukken River and Tsukudabori in Tokyo (the sites influenced by the domestic wastewater) in autumn of 2012 - 2014. The juvenile Japanese common goby captured from Taira for 1-week E_2 exposure test and 24-hours E_2 culture test. Gonad, liver and blood samples were collected in exposure test and the culture mediums were collected in culture test. Vitellogenin (VTG), as a good biomarker for indicating the EEs effects, was detected to evaluate the toxicity potential of EEs pollution. Plasma E_2 and testosterone (T) levels were measured by enzyme-linked immunosorbent assay (ELISA).

In this field survey, testis-ova, which is abnormal testis, was not observed in all male fish. However, VTG concentration in plasma indicated high levels in fish collected in Tokyo. In the E_2 exposure experiment, VTG concentration significantly increased to high levels by E_2 treatment. In the culture test, high E_2 synthesis was induced by high exogenous E_2 and high HCG in juvenile female. These results indicate that the endocrine system of wild goby in each gender is affected by EEs pollution.

Combinatorial immune and stress response, cytoskeleton and signal transduction effects of graphene and triphenyl phosphate (TPP) in mussels

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Because of their unique surface properties, graphene nanomaterials can absorb environment pollutants and affect their environmental behavior, but little is known about their interaction with other pollutants. Therefore, a systematic study on the combined toxic effects of graphene and contaminants is needed to effectively evaluate the ecological risk of graphene. Triphenyl phosphate (TPP) is a high production flame retardant that has been detected in various environment media and biota. In this study, the toxicity of graphene, TPP and graphene + TPP exposures were studied by the determination of histopathology, antioxidant enzyme activity and functional gene expression changes. The results showed that the graphene exposure caused slight damage to the digestive gland tissues of mussel *M. galloprovincialis*. Both graphene and TPP induced oxidative stress in digestive gland tissues and had inhibitory effects on antioxidant enzymes, while their combined exposure reduced the oxidative stress. The combination of graphene and TPP can reduced the expression of genes associated with immune response, stress response, cytoskeleton and signal transduction, but has no significant effect on the expression of reproductive related genes in the digestive gland tissues of *M. galloprovincialis*. The interacting partners of proteins for the selected functional genes were identified using STRING 10.5 database and generated a visible protein-protein interaction (PPI) network using Cytoscape. The genes with PPI relationships were found to affect the cell cycle, signal transduction and reduce the expression of immune inflammatory factors. In summary, combinatorial graphene and TPP exposure could interfere with the signaling and immune function of digestive gland tissue of *M. galloprovincialis*.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-37

A new cause of microplastics fragmentation in Hong Kong waters

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Microplastics collected from different environments vary in composition due to the variation in sources and factors that brought these pieces to a particular location. In order to characterize the types of microplastics present in Hong Kong waters, microplastics (size of 1 – 5 mm) were collected from 11 beaches. Out of more than 6000 pieces of microplastics analyzed, 56% (by pieces) were plastic fragments with a predominance of white or transparent (42%) and blue (28%) in color. More importantly, a special type of fragment which has not been reported before is revealed. These fragments, composing about 10% of the fragments collected, are interestingly triangular in shape with at least two of the three sides being characteristically straight and resembling a cut made by compression, often coupled with signs of tearing. Objective measurements and observation from some special pieces have made apparent differences between these “trimmed fragments” and those triangular fragments that were fractured randomly as the plastic degraded. Therefore, we proposed that this type of “trimmed fragment” was formed by biological causes, presumably by biting of macrofauna, instead of natural weathering. Preliminary tests with the bite mark of some local fish species have given some clue on the causes of these trimmed fragments. The finding here could therefore have wide implications on current ecotoxicological and modeling studies for microplastics since the active biting of large plastic debris has generally not been considered as a factor of microplastics fragmentation.

Micro-plastic found on the recreational beach at Bagan Lalang and Morib, Selangor, Malaysia: A baseline data

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The occurrence of microplastic has vastly distributed around the globe. Ranging size from 1mm up to 5 mm in diameter, this plastic is extensively found on the coastal beach resulting from the fragmentation of marine debris. Present study was conducted at the recreational beaches (Bagan Lalang and Morib, Selangor) with the objectives 1) to identify the type of microplastics present on both study locations and 2) to provide baseline data on the occurrence and distribution of microplastics. Sampling session was conducted on the dry season of February 2019 at five different sites representing both recreational beaches. Samples from the surface sand beaches were collected by using the sieving and wet floating methods. Results demonstrated that Morib beach has the higher counted number of microplastics found from the sand beach compared to Bagan Lalang beach. Meanwhile, microplastics found consisting of a high number of fragments, followed by films, pellets, fibres and the least, foams. All the data were statistically analyzed by using one-way ANOVA. Microplastic was highest at Morib beach as this site was less disturbed by the human activities (e.g., cleaning beach activities) compared to Bagan Lalang beach. As a conclusion, the study on sand beaches fraction need to be conducted in order to know the distribution of microplastic in the deeper depth of sand beaches.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-39

Spatial and seasonal distribution of microplastics on sandy beaches along the coast of Kenting National Park, Taiwan

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Microplastics (MPs) have raised great concern globally. Many studies have reported microplastic pollution of beaches around the world. However, microplastic pollution of beaches in Taiwan is still poorly understood. No study has been conducted in southern Taiwan yet. Therefore, we collected sand samples from eight beaches in June and November in 2017 along the coast of Kenting National Park, which is one of the major tourist attractions in Taiwan. MPs in the sand samples were extracted using a saturated NaCl solution and were quantified and characterized under a stereomicroscope. The average density of MPs in the sand samples ranged from 80 to 480 particles/kg dw. The most abundant type of MPs was fiber (>97%) and the most common color was white/transparent (57%). There was no significant seasonal difference except for one site (Dawan). The highest density of MPs was found at the beaches with high tourist activities. Statistcal analysis showed that MPs were significantly more abundant at the popular tourist beaches. Our results show that MPs are ubiquitous along the coast of Kenting National Park, and the major factor associated with the abundance of MPs is tourist activity.

A territory-wide study of microplastics in barnacles of Hong Kong

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Microplastic pollution is an emerging problem in the marine environment and assessment of microplastic abundance in wild organisms is essential for risk assessment. The occurrence of microplastics in barnacles *Amphibalanus amphitrite*, *Tetraclita japonica japonica* and *Capitulum mitella* was determined for 30 sites, including rocky shores, sandy shores and mudflats. The mean number of microplastic abundance ranged from 0.26 to 10.26 items g^{-1} of body wet weight, or from 0.13 to 1.72 items $individual^{-1}$ with fibers being the most abundant type of microplastics. The chemical composition of 152 pieces of potential microplastics was analysed using micro-Fourier transform infrared spectroscopy (μ-FTIR). Fifty two of them were synthetic polymers (45 fibers, 6 fragments and 1 bead), 95 natural cotton fibers and five unknown. Eight types of polymer were identified with cellophane being the most abundant (58%). Compared with animals from other taxonomic groups, the problem of microplastic ingestion was relatively minor in barnacles.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-41

Flame-retarded polystyrene debris as a source of HBCDD contamination in corals

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Following its high production, use and inappropriate disposal, polystyrene has become a major constituent of marine debris throughout the world. Highly persistent and transported by oceanic currents, it can threaten sensitive ecosystems such as coral reefs. Hexabromocyclododecane (HBCDD), a now banned brominated flame retardant (BFR) extensively used as an additive of polystyrene foams, is present in the marine environment and biomagnifies in the food chain. Previous studies conducted in our group indicated that polystyrene was the dominant foam-like floating debris encountered in the French Riviera coastline and that 50 % of the studied debris contained high levels of HBCDD. In this framework, the aim of this study was: (1) to evaluate the ability of plastic-contained HBCDD to become available to marine biota by leaching to the dissolved phase; (2) to evaluate the kinetics of transfer of HBCDD to *Stylophora pistillata* corals and (3) to assess bioaccumulation of HBCDD and toxic effects of polystyrene leachate to this species.

In a flow-through leaching experiment, ΣHBCDD from the beached polystyrene was found to migrate to the dissolved phase at rates reaching more than 150 ng per g of polystyrene per day in our conditions. Polystyrene ΣHBCDD concentrations at the end of this 32 d experiment was not significantly different to its initial concentration, suggesting a release over much longer periods of time. *Stylophora pistillata* coral nubbins exposed to HBCDD contaminated seawater showed a rapid accumulation, with less than 15% of the initial ΣHBCDD concentration left in the dissolved phase after 6 h. Vessels without corals, used as negative controls, showed stable concentrations over 24h. Over a 4 day exposure to a polystyrene leachate filtered at 0.7 µm (ΣHBCDD: 232 ± 48 ng/L), coral nubbins were found to accumulate ΣHBCDD at levels reaching 30 ± 12 ng/g dw. After a 9 d depuration period, ΣHBCDD concentrations only decreased to 20 ± 6 ng/g dw, showing a slow decontamination of the corals in clean water. The transfer of HBCDD from the beached debris to the coral was found to be isomer specific as the α-HBCDD compositions were 41% in the polystyrene, 90% in the dissolved phase of the leachate, 98% on the corals after the accumulation phase and 99.5% after the depuration phase. ΣHBCDD levels in corals exposed to the leachate-derived HBCDD were statistically comparable to the concentration levels measured in the corals exposed to HBCDD enriched seawater. Neither treatment significantly influenced the coral photosynthetic activity, symbiont concentration and chlorophyll content. Consistent polyp retraction was observed in coral nubbins exposed to the polystyrene leachate, but not those exposed to HBCDD alone, suggesting that the organisms were stressed by another constituent of the leachate. As such, changes at the metabolic or cellular levels are not excluded and should be considered in future studies.

This study showed for the first time that widely occurring HBCDD containing polystyrene can release additives into the dissolved phase. We also showed that HBCDD is rapidly accumulated and slowly depurated in corals, whilst unidentified additives might likely cause the coral polyps to retract.

Adsorption of Ag, Cd and Cs to polyethylene microspheres and sediment: A preliminary case study on the importance of microplastics as vectors of contaminants

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Plastic pollution is a major concern worldwide, in particular in downstream aquatic ecosystems that have become globally polluted with nano- and micro-sized plastic particles. There is growing evidence that such plastics are important vectors for the transport of contaminants in marine systems. However, our understanding of the sorption/desorption kinetics of waterborne chemical compounds to plastic particles remains incomplete. Nuclear techniques (e.g., radiotracers) provide unique tools for rapid, accurate, sensitive and reproducible measures of the biokinetic and toxicodynamic of radioelements, such as metal radioisotopes and radionuclides. In this context, these techniques are particularly powerful to determine the fate of radiolabelled contaminants adsorbed by plastic particles in aquatic ecosystems. The distribution coefficient (Kd) of two metal radioisotopes (Cd, Zn) and one radionuclide (Cs) to microplastics (MP) and sediment particles of similar size (40-70 μm) in seawater were examined in this study. Observations revealed that sediment particles absorbed Cd, Cs, and Zn more so than MP, with several orders of magnitude difference in the observed Kd values. Such results suggest that MP may play a lesser role in the scavenging of these metals under such experimental conditions. Significant variations in the scavenging efficiencies were however observed between the metals, and further studies are needed to better resolve how physico-chemical characteristics, MP composition, and surface biofilms all affect these biokinetic processes. Observations demonstrate how nuclear technics can allow us to identify important knowledge gaps that will advance our understanding of MP surface complexation reactions and contaminant transfer mechanisms into marine food webs.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-43

Phthalate esters in plastic express bags and their leaching potential

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In recent years, rapid development of the express industry not only brings convenience but also creates serious environmental problems. The massive use of plastic express bags and inadequate management measures input a large amount of wasted plastic express bags into the environment. Plastic express bags can break down into microplastics in the environment, which are considered to be contaminants of emerging concern. Plastic additives contained in those bags can also be released, many of which are extremely toxic. In this study, 43 plastic express bags were gathered from major express companies in China to determine the phthalates (PAEs) contents and their leaching potential. Fourier-transform infrared spectroscopy (FTIR) showed that all plastic express bags are polyethylene (PE) or recycled PE. Sixteen PAEs in the plastic express bags and their leachates were analyzed by gas chromatography-mass spectrometry (GC/MS). The total contents of PAEs (∑ PAEs) in the plastic express bags range from 11.16 ± 2.80 to 309.70 ± 19.37 mg kg^{-1}. Despite significant differences in the contents of ∑PAEs, the composition of PAEs in different plastic express bags is similar. Dibutyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP), and diisobutyl phthalate (DIBP) were found in all samples while butyl benzyl phthalate (BBP) and diphenyl phthalate (DPhP) were not detected. The contents of ∑PAEs in leachates of the plastic express bags ranged from 4.95 to 91.96 mg kg^{-1}. The proportion of PAEs released from the plastic express bags (0.5g plastic express bag into150 mL ultrapure water) ranges from 16.89% to 68.72%. Our results suggest that plastic express bags are an important source of PAEs. Wasted plastic express bags should be better managed to minimize the releasing of PAEs and other plastic additives into the environment.

A preliminary screening of HBCD enantiomers transported by microplastics in wastewater treatment plants

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Hexabromocyclododecane (HBCD), a commonly used flame retardant, causes public concern due to its potential negative effects on organisms. Microplastics are suspected to contain certain amounts of HBCD. Wastewater treatment plants (WWTPs) are believed to be one of the largest sources of microplastics and a sink for micropollutants, providing opportunities for interactions between them, especially for hydrophobic micropollutants such as HBCD. There is a lack of studies focusing on the prevalence of microplastics and HBCD they carry. In addition, WWTPs are one of the major pollution sources of costal marine waters of Hong Kong. The present study investigated two typical WWTPs in Hong Kong, Stonecutters Island WWTP (SCI) and Shek Wu Hui WWTP (SWH), which employ different treatment technologies. The abundance of microplastics decreased with the treatment flow, and the microplastic concentrations in effluent were at intermediate levels (0.40 and 0.27particles/L) compared with the levels reported in previous studies. The concentrations of HBCD transported by microplastics reached 4184.4 ng/g in the effluent, whereas that in sewage water (dissolved phase) was 0.8 pg/L. For microplastics, 7.32×10^7 and 2.24×10^7 particles per day were estimated to be released from SCI and SWH, respectively into the environment; the release of HBCD carried by microplastics potentially reached 15.5 g per day, whereas the dissolved HBCD in the effluent may reach 0.067 g per day. A preliminary risk assessment of HBCD transported by microplastics showed that HBCD posed negligible risk; nevertheless, attention should be paid to the continual discharge of microplastics from WWTPs.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-45

Heavy metal contamination of sedimentary microplastics in Hong Kong

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This is the first study of heavy metal (As, Cd, Ni, Mn, Cu) contamination of microplastics on sandy beaches in Hong Kong. Three study sites are located in the eastern waters (Pak Lap Wan, Stanley Bay, Tung Lung Chau Island) and the remaining sites in the western waters (Tai Pai Tsui, Ha Pak Nai and Shui Hau Wan). The mean concentration of Mn (52.88 mg kg^{-1}) was the highest and followed by Cu (4.09 mg kg^{-1}), Ni (0.36 mg kg^{-1}), As (0.07 mg kg^{-1}), and Cd (0.06 mg kg^{-1}). Western sites have significantly higher concentrations of Ni but eastern sites higher concentrations of Cd. Inter-site differences were found with a significantly higher concentration of Cd (0.15 mg kg^{-1}) at Shui Hau Wan than Tai Pai Tsui (< LOD) and Ha Pak Nai (< LOD). The concentration of Ni was significantly higher at Ha Pak Nai (1.09 mg kg^{-1}) than Tai Pai Tsui (0.08 mg kg-^{1}) and Pak Lap Wan (0.04 mg kg^{-1}). The results indicate that the source was different for different metals. As a large number of marine biological species are known to ingest microplastics, the study of heavy metal contamination of microplastics will help assess the potential risk of bioaccumulation and biomagnification of heavy metals through trophic transfer.

Accumulation of microplastics and its reproductive effect on medaka fish

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This study was performed to elucidate the uptake and bioaccumulation of MP in Japanese medaka (*Oryzias latipes*) and Java medaka (*Oryzias javanicus*) and assessed the effects of the bioaccumulated MP on survival rate, reproduction and gene expression of Japanese medaka. We exposed medaka to 2 treatments: Control (No MP) and exposed 2 μm fluorescent PS-MP (10^7 beads/L) for 3 weeks. Results showed that PS-MP were accumulated in intestines of Japanese and Java medaka. The calculated bioaccumulation factor in Java medaka (marine water fish) was estimated to ca. 4 x 10^2 that higher than Japanese medaka (ca. 1 x 10^2). Further, there were no significant impacts of 2 μm PS-MP (10^7 beads/L for 3 weeks) on survival rate, and reproduction of Japanese medaka spawning pairs (each group consists of 4 replicates containing 6 fish, 2 males, and 4 females). From mRNA-seq analysis, a few changes were detected in the gene expression of medaka intestine after 3 weeks of exposure. Our results suggest that virgin MP was potential to accumulate in an intestine and has limited toxicity on medaka fish.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-47

Toxic effects of polyethylene microplastic on *Daphnia magna*

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The concern of microplastic pollution in aquatic environment has been risen in recent years. Microplastics, which include broken-down plastic waste and synthetic fibers, are defined as plastic pallets with size less than 5 mm. They are known to harm life since the animals may mistake them as food. Among different plastic pallets, polyethylene (PE) is the most popular one in the world. Yet, research on the exact toxic effects brought by PE on marine organisms is still limited. Daphnia, which can be easily cultured in the laboratory and is sensitive to many toxic chemicals, is widely used in toxicity tests. The aim of this study is to use *D. Magna* as a model organism to study the toxicity of PE.

Adult *D. magna* were treated with polyethylene (PE) microplastics for 96 hours. The PE pellets used are 10-22 μm with green light under fluorescence microscope. Serial dilutions of plastic stock solution were conducted into low (0.002 mg/L), medium (0.02 mg/L) and high concentration (0.2 mg/L) of PE. Concentrations used in the experiment were relevant to environmental concentrations of PE microplastics. For each treatment, 3 replications were done with 20 *D. magna*.

After the 96-hour exposure, confocal microscopy observation confirmed the uptake of PE in D. magna. However, no lethality nor effect on reproductive rate was found in the exposures. Real-time quantitative PCR (qPCR) was conducted to examine the molecular effect of PE exposure in D. magna. And differential expressions of some toxic response genes were observed in this study. Results showed that significant downregulations of FLOT (flotillin), GST (Glutathionin-S-transferase), JHE (juvenile hormone esterase), MTA (metallothionin A) and MTB (metallothionin B) were observed after exposing D. magna to PE. And upregulation of HSP60 (heat shock protein 60) was documented in D. magna after the 96-hour exposure of 0.002 mg/L PE. But no significant change was found in the expression of HSP70 (heat shock protein 70) in our experiment. On-going work will focus on examining the effect of long-term exposure to PE in *Daphnia magna*.

The potential of using marine mussels as bioindicators of microplastics: A field transplantation experiment

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Green-lipped mussel Perna viridis was transplanted to three sites in Sai Kung, namely Pak Sha Wan, Tsoi Wo Hang and Yim Tin Tsai, with different intensities of human activities. Bioaccumulation of microplastics was studied on Day 7, 19, 33 and 47 post transplantation. Number of microplastics increased with time for all the sites and the highest concentration of 32 items individual-1 was recorded on Day 47 at Pak Sha Wan, the site with the highest intensity of human activities, as compared with 18.8 items individual-1 at Tsoi Wo Hang and 17.6 items individual-1 at Yim Tin Tsai. Only fiber and film were found with > 90% of them being the former. The potential of using marine mussels as bioindicators of microplastics is discussed.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-49

Plastic particle exposure investigation using planktonic larvae of *Amphibalanus amphitrite* and *Spirobranchus krausii*

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Microplastics and nanoplastics may enter the marine environment either as manufactured particles or as products from degradation of larger plastic wastes. Such microparticles have been shown to present ecological hazards when ingested by marine organisms. In this project, we are exploring the environmental impact of micro- and nanoplastics to various marine organisms. Common plastic particles in the size range of 100 nm to 20 microns were used for exposure studies. Day-old larvae of *Amphibalanus amphitrite* and *Spirobranchus kraussii* exposed to synthetic polymer particles at different concentrations were observed to readily ingest and quickly translocated within the organism's body. However, the responses from exposure of different polymer particles using two marine species were not identical, indicating that polymer structure and functional groups are important for understanding environmental risks to marine ecosystems.

Pollution characteristics and source analysis of microplastics in farmland soils of China

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Microplastics in environments usually refer to polymer particles with the size of less than 5 mm. A number of studies have found the occurrence of microplastics in oceans, sediments, freshwaters, estuary environments, and even in Arctic and Arctic glaciers. However, there are only a few studies focusing on microplastics in soil environment, especially the farmland soils. Microplastics in farmland soils can enter the food chain through different crops, thus the farmland soil environment may affect human health. In northern China, plastic mulching is widely used to ensure the growth of crops and also possibly have an adverse effect on soil performance at the same time. In addition, the broken mulch remaining in farmland soils can directly lead to microplastic pollution in soil and this urgently needs a well-rounded study. In this study, a large vegetable and fruit planting base in Tianjin was selected. The microplastics in three kinds of soils were monitored, including soils where strawberries were planted with a long-term coverage of mulching film, soil where celeries were planted with a coverage of film only in the germination period and soil where cabbages were planted without a coverage of film. The microplastics were divided into fibers, fragments, films and spheres according to their shapes. The results show that there were microplastics in all soil samples, and the concentrations of microplastics in strawberry soil, celery soil and cabbage soil were 455.53 particles/kg, 695.43 particles/kg and 405.52 particles/kg, respectively. Fibers occupied the largest proportion of microplastics with the percentage range of 26.49%-37.04%, while films made up the smallest proportion with the percentage range of 5.79%-11.11%. The main particle size range of microplastics in this study was 500-2000 μm with the contributions of 34.71%-52.91% to total microplastics. The FTIR analysis demonstrated that a large amount of polyethylene, polypropylene and polystyrene, all together accounting for more than 85% of all type microplastics in soils. This study determined the distribution and composition of microplastics in farmland soils, suggesting the importance of soil microplastic pollution. Further research is needed to investigate the effects of pollution sources such as agricultural mulching, irrigation and aerial deposition on microplastic pollution in farmland soils, and the potential mechanism of microplastics transportation in soils as well as the adverse effects caused by microplastics on crops and ecological environments.

International Conference on Marine Pollution and Ecotoxicology

Plastic pollution: P-51

Occurrence and characteristic of microplastics in surface water of Qiangtang River, China: The implication of anthropogenic inputs to Hangzhou Bay

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Microplastics (MPs, particles <5 mm in size) are present in rivers, lakes, and oceans all over the world and are of particular concern. They are made from synthetic polymers used in consumer and industrial products, or result from breakdown of larger plastic debris. As potential carriers of organic contaminants, MPs are released to the aquatic environment by many routes of discharge such as improper waste disposal, insufficient waste management, and urban runoffs. Meanwhile, MPs will be ingested by organisms and cause negative ecological impacts. In this study, we report the abundance and composition of MPs at the surface of the Qiantang River (Hangzhou City section), the largest river discharge into Hangzhou Bay in Zhejiang Province of China. Measurements were made at 10 locations along and across the river in June 2018. The abundance of MPs in water ranged from 0 to 6517 particles/m^{-3} with 1461 particles/m^{-3} on average. The spatial variability of concentrations was correlated to different anthropogenic activities. The highest MPs abundance was found in Puyang River (QTJ09 station) which is one of the major tributaries of Qiantang River and also is the main supply for industrial water usage. There are many steel mills, chemical fiber companies around it. The MPs concentrations at QTJ01 and QTJ03 were also higher than the other stations, which can be explained by the distribution of high-density residential area and light industrial area. Analysis of MPs by Fourier Transform Infrared Spectroscopy (FTIR) showed a large abundance of polyester (PES), polyethylene (PE) and polypropylene (PP), which covered about 60% of all polymer types identified in the surface water. The most frequent geometries were fibers in water samples, underlining the potential sources of clothing production or washing processes in the city. This study emphasizes the importance of rivers as carriage systems of microplastics, and its relations with terrestrial anthropogenic activities. Moreover, we also discuss the river transport impact of MPs on offshore marine environment.

Occurrence and characteristics of microplastics in Haihe River: Preliminary study of an urban river in a megacity of northern China

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Microplastic pollution has become one of the major marine pollutants attracting international concern, especially in China where plastic production and emission were in high level in this region. Freshwater system is an important source and transportation route for marine plastic pollution, but the current situation of microplastic pollution has not paid adequate attention. Moreover, the related data on the pollution of microplastics in typical seagoing rivers in Northern China is fairly lacking. Here we investigate the distribution and characteristics of microplastic in surface water in mainstream Haihe River, one of the main rivers passing through the most developed regions with high population density and industrialization, and entering the Bohai Sea. Microplastics were detected by two types of manta trawls (50 μm and 333 μm) with concentrations ranging from 0.83 to 6.17 particles/m^3 and 2.52 to 8.79 particles/m^3, respectively. Among the five shape types (fiber, film, foam, fragment and granule) we divided, fibers predominated microplastic types in surface water of Haihe River, which accounted for 18.46-43.48% of the total microplastics. Microplastic sizes distribution is concentrated on the size-fraction of 200-1000μm, accounted for 59.04% of the total microplastics size. Micro-Fourier transform infrared spectroscopy analysis showed that the main types of microplastics were polypropylene, polystyrene, polyethylene and resin. The results of this study confirm the presence of high abundance microplastics in an urban river, filling the gap in the study on microplastics in the natural rivers from Northern China, and indicate that non-point source inputs are important influencing factors for microplastic pollution in urban rivers. Further studies are recommended to focus on the influences of microplastics on the environmental behavior of other aquatic pollutants.

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OMICS: P-53

Transcriptomic responses in Japanese medaka (*Oryzias latipes*) exposed to individuals or mixtures of polycyclic aromatic hydrocarbons

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In the present study, we assessed the effects of single or mixtures of polycyclic aromatic hydrocarbons (PAHs) by transcriptome analysis. Four-week-old Japanese medaka (Oryzias latipes) were exposed to phenanthrene (5, 50 ppb), pyrene (3, 30 ppb), or their mixtures for 96 hours. Fish were sampled at 0, 24, or 96 hours after the exposure test started. Total RNA extracted from whole body of medaka was subjected to microarray experiment. Principal component analysis of transcriptome data at 24-hr exposure showed that three groups were formed and were separated from each other, indicating that PC1 (32.1%) and PC2 (12.3%) reflected the effects of mixtures and single PAHs, respectively. The profiles of altered gene expressions at 24-hr exposure were similar between phenanthrene- or pyrene-exposed groups; however, the effects of their mixture were completely different from those of individual compounds. Exposures to phenanthrene or pyrene induced the genes related to immune response (GO:0006955) and induction of apoptosis (GO:0006917) in a dose-dependent manner, which seemed to be an acute response to PAH exposures. Meanwhile, exposures to mixtures of phenantherene and pyrene dramatically suppressed the expression levels of genes associated with collagen catabolic process (GO:0030574) and glycolysis (GO:0006096), although each individual chemical showed no effect on the expressions of these genes. On the other hand, the PCA result of 96-hr exposed groups did not show the similar trend, and the gene expression patterns exhibited neither dose-response nor PAH mixture-specific effects. The effects of fasting, detected as significant changes in gene expression profiles of control fish among time points. Data analyses using GO terms revealed that 96-hr fasting significantly suppressed collagen catabolic process and glucose homeostasis (GO:0042593), which were also observed in fish exposed to mixtures of PAHs. The effects of fasting seemed larger than those of PAH exposures at 96-hr, which may be a major factor of interference with the detection of biological responses to PAH exposures. Thus, our analysis can detect mixture toxicities of PAHs, as well as the effects of individual PAHs. However, some effects of PAHs were overlapping with the effects of fasting. Therefore, for precise evaluation of chemical effects by microarray experiment, fasting should be considered as a factor to affect the gene expression profiles in experimental animals.

Effects of phenanthrene and/or pyrene on metabolic profiles in medaka larvae

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Phenanthrene and pyrene are common polycyclic aromatic hydrocarbons (PAHs) generally release by hydrocarbon combustion or oil spill. These widely distribute even the aquatic environment and sometimes high concentrations of them are found especially in the coastal area closed to big city or industrial area. Generally, the various kinds of PAHs were residue at the site, and, as a result, the aquatic organisms have been exposed by multiple PAHs. Some PAHs are known to cause deformities, growth retardation, and other adverse effects in fish including the embryos by single exposure. However, little is known about their combined effects to fish. Therefore, in the present study, the effects of phenanthrene and/or pyrene on the metabolic profiles in larvae of Japanese medaka (*Oryzias latipes*) were examined.

One-month old medaka were exposed to phenanthrene (5 and 50 ng/g), pyrene (3 and 30 ng/g) and their mixtures for 96 hr. Metabolites were extracted from the whole medaka, and measured by GC/MS. Fish were collected at 0, 24, and 96 hr.

Metabolites with significantly different levels between exposed and control groups were screened by one-way of variance. Principal component analysis (PCA) followed to be applied to the screened metabolites data, and principal component (PC) scores for each sample obtained. Each individual exposure group of phenanthrene and pyrene clustered at similar place on two dimensional PCA score plots, being separated from control group along the PC1 axis. This result suggests that the effects of individual exposure of phenanthrene or pyrene produced similar metabolite profiles in medaka. In contrast, the group simultaneously exposed to phenanthrene and pyrene clustered away from control group along the PC2 axis. This suggests that the combined exposure to both PAHs induced different effects to medaka from those induced by single exposure of both PAHs. The differences in metabolite profiles between single and combined exposure groups were mainly found the variations of amino acids and glucose. All essential amino acids in the single exposure groups increased from 0 to 24 hr, while those in control were decreased during that period. These increases were considered to be caused by degradation of the proteins in medaka muscle, because of generating essential amino acids. Perhaps the excess energy and proteins to correspond to the exposure of phenanthrene and pyrene. On the other hand, the variations of essential amino acids in the group exposed to both of phenanthrene and pyrene behaved similarly to the control group from 0 to 24 hr, and seemingly unaffected by the exposure. However, all essential amino acids and glucose in the combined exposure group didn't change even between 24 to 96 hr, while those in the control significantly increased during the period. These results suggest that the combined exposure of phenanthrene and pyrene inhibited the degradations of protein, glycogen, and gluconeogenesis. As a result, the effects by combined exposure of phenanthrene and pyrene were unpredictable from the single exposure.

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OMICS: P-55

Effects of aquaculture effluent on benthic ecosystems using eDNA in Rongjian River, China

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In 2016, China contributed to 61.5% of the global aquaculture production due to a high demand edible protein that are sustainable and a vast expanse of freshwater and marine water aquaculture land. However, discharge of chemical pollutants (e.g. antibiotics, heavy metals, inorganic nutrients) from aquaculture may threaten aquatic ecosystem health. Several studies detected organic pollutants in aquaculture effluent and the adjacent rivers, sediments, and lakes. Organisms in sediment are responsive to pollutants and can be used as alternative ecological indicators of pollutants of aquaculture. The purpose of this study was to evaluate the effects of aquaculture on aquatic ecosystems using eDNA. Water and sediment samples (n = 12) were collected upstream and downstream of discharge points of aquaculture fields along Rongjian River in Eastern Guangdong, China. Effect of inorganic nutrients on aquatic organisms was determined by characterizing samples using DNA metabarcoding approaches and then correlated to inorganic nutrients (total nitrogen and total phosphorus) and dissolved oxygen. The results of this study will help establish the applicability of eDNA in determining effects of human activities on aquatic environments.

Establishment and characterization of finless porpoise *(Neophocaena phocaenoides)* dermal fibroblast cell line

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Finless Porpoise **(***Neophocaena phocaenoides***)** are inhabit shallow coastal waters of the Indian and Western Pacific Oceans, and in the large river systems. In the present study, we established and characterized pygmy killer whale cell line to develop *in vitro* studies. Skin tissues were cultured in a six-well plate in a complete medium containing (DMEM and Ham's F12 nutrient mixture, fetal bovine serum 10%, antibiotic 1% and essential amino acids). Primary cells (FP-LWH) growth appeared on the tissues ages after six days of culturing and first culture in 16 days. The fibroblast cells identification was performed by vimentin fluorescence and vimentin protein expression. Plasmids which encode the SV40T antigen gene was used to transfect the primary cells and further passages expended the expended for. The stability of transfected cells was verified by immunofluorescence, RT-PCR, and western blotting methods at (5, 10, 20 and 30th) generation levels. In conclusion, we have established Finless porpoise (*Neophocaena phocaenoides*) cell line (FP-LWHT) for the first time and it provides opportunity to conduct environmental, Pathological and molecular research *in vitro* to improve the conservation research.

Keywords: Finless porpoise; Dermal fibroblast; Immortalization; Cell line characterization; Conservation biology

Alteromonas flava sp. nov. and *Alteromonas facilis* sp. nov., two novel copper tolerating bacteria isolated from a sea cucumber culture pond in China

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Copper is prevalent in aquatic systems due to its use as an algaecide and as an antifouling (AF) agent. Two bacterial strains, P0211^T and P0213^T, were isolated from a sea cucumber culture pond in China. The strains were able to resist high copper levels. Phylogenetic analysis based on the sequences of the 16S rRNA gene and five housekeeping genes (*dnaK*, *sucC*, *rpoB*, *gyrB*, and *rpoD*) supported the inclusion of these strains within the genus *Alteromonas*. Genomic analyses, including average nucleotide identity (ANIb and ANIm), DNA-DNA hybridization (DDH), and the percentage of conserved proteins (POCP), clearly separated strains P0211^T and P0213^T from the other species within the genus *Alteromonas* with values below the thresholds for species delineation. *Alteromonas* spp. are reportedly early colonizers of copper-based AF paints, although little is known about their mechanisms of resistance and attachment. In this study, two isolates were phenotypical, chemotaxonomical, and genetically characterized to determine their taxonomic position. In addition, the strains were able to resist high copper levels, and we explored the existence of genes related to copper tolerance by analyzing their genomes.

Corallincola holothuriorum sp. nov., a new facultative anaerobe isolated from sea cucumber intestine

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A novel Gram-stain-negative, facultatively anaerobic, amphitrichous, short rod and creamy-white bacterial strain, designated strain C4^T, was isolated from sea cucumber intestine in Weihai, Shandong, China. Optimal growth of the strain was observed at 28-30 °C, pH 6.5-7.0 and at a concentration of 3% NaCl. The G+C content of the genomic DNA was 49.0 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence indicated that strain C4^T is a member of the genus *Corallincola* and had most similarity with *Corallincola platygyrae* JLT2006^T. The major cellular fatty acids of strain C4^T were $C_{16:1}$ ω7c/iso-$C_{15:0}$ 2-OH, $C_{16:0}$ and $C_{18:1}$ ω7c. The sole respiratory quinone was Q-8. The predominant polar lipids in strain C4^T were phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified phospholipid (PL1). Based on morphology and physiological characteristics, strain C4^T should be classified as a novel species in genus *Corallincola*, for which *Corallincola holothuriorum* is proposed. The type strain is C4^T (=ATCC BAA-2611^T = CICC 10839^T).

OMICS: P-59

High-resolution RAD-seq based linkage map, reference genome, and QTL mapping of the sex chromosome in the marine medaka *Oryzias melastigma*

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Marine medaka (*Oryzias melastigma*) is an important fish species in marine ecotoxicology and considered a model species because its biological features include certain advantages such as small body size and short generation time. A high-density genetic linkage map is a very useful resource in genomic research, including comparative genomic analysis and verification of *de novo* genome assembly. In this study, we developed a high-density genetic linkage map for *O. melastigma* using restriction-site associated DNA sequencing (RAD-seq). The genetic map consisted of 24 linkage groups with 2,354 RAD-tag markers. The total map length was 1690.8 cM with an average marker space of 0.72 cM. The genetic map was integrated with the reference-assisted chromosome assembly of *O. melastigma*, which mapped 706.6 Mb (90.7%) of genome sequence onto the linkage map. The values of Benchmarking Universal Single-Copy Orthologs (BUSCO) and N50 (total genome length 779.4 Mb) were 97.1% and 29.1 Mb, respectively, suggesting an integrated assembly with improved quality. Using MapQTL analysis with RAD-tag markers, we identified a major quantitative trait locus for sex traits of around 9.5 and 9.7 Mb on the Om10 scaffold. The integration of the genetic map with the reference genome of marine medaka will serve as a good resource for studies in molecular toxicology, genomics, CRISPR-cas9, and epigenetics.

Generation of albino via *SLC45a2* gene targeting by CRISPR/Cas9 in the marine medaka *Oryzias melastigma*

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To produce albino in the marine medaka *Oryzias melastigma*, we disrupted the solute carrier family 45 (*SLC45a2*) gene by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 with a single guide RNA (sgRNA). Selected sgRNAs was able to target a *SLC45a2* gene. Of the survived embryos (21 individuals) after injection, 11 embryos (52.4%) were shown $SLC45a2^{+/-}$ or $SLC45a2^{-/-}$ genotypes, indicating that this approach efficiently produced the knock-out mutants. Of 11 mutants, three individuals showed homozygous pattern, having albino phenotype, and also observed different insertion and deletion (indel) mutation profiles near the DNA cleavage sites using targeted deep sequencing. Albino with $SLC45a2^{-/-}$ genotypes can transfer its phenotypes to offsprings, providing a beneficial effect to examine the inner organs more easily. This albino marine medaka will be a good model for marine molecular ecotoxicology in examining diverse *in vivo* endpoints.

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Development of immune-stimulating antimicrobial peptide feeding regime for the hybrid grouper

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Poor water quality is one of the major factors underlying a weakened immune system in fish, resulting an increased susceptibility of fish to pathogenic microbes and parasites. To prevent high economic losses due to fish diseases, "prophylactic" oral administration of antibiotics is a common practice used in aquaculture industry for protection of farmed fish from microbial infection. This has contributed to a considerable source of environmental antibiotic pollution, which not only disturbs the aquatic microbiota community structure, but also entails the development of antibiotic-resistance microbes. Most importantly, from the public health perspective, consumption of antibiotic residuals in fish tissues is a major threat for human health. It is thus imperative to reduce the use of antibiotics in aquaculture. In fish, the antimicrobial peptides (AMPs), found in mucus and plasma, play an important role in pathogen defense, preventing pathogens infiltration and spreading of pathogens in vivo. Due to their broad antimicrobial capacities, environmental safety, non-toxicity to host cells and their demonstrated potential as therapeutic agents, AMPs are a promising group of endogenous immune proteins to be considered for antibiotic replacement in fish, The hybrid grouper, *Epinephelus* sp. x sp. is one of the most important aquaculture fish in Hong Kong, China and Southeast Asia, due to its high economical value and a growing consumer demand. In grouper aquaculture, bacterial and viral infections are a major problem during larval and juvenile stages (< 3 months), while losses in the adult population are mainly induced by parasites. The long term goal of this study is to reduce antibiotic use in grouper aquaculture and enhance juvenile survival by using immune stimulating AMPs (e.g. beta-defensin and piscidin-3). Recombinant AMP protein production was performed using the BL21 cells (*Escherichia coli*), which is necessary to generate large quantity of small immune peptides for subsequent AMP feeding experiments. Results on optimization of beta-defensin and piscidin-3 protein expression in BL21 cells, pathogenic bacteria *Edwardsiella tarda* (edwardselliosis) host resistance assay in juvenile grouper, anti-bacterial infection efficiency of AMP as well as problems encountered will be presented and discussed.

Comparison of six RNA extraction methods on freshwater benthic biofilms

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Biofilms in freshwater ecosystems are ubiquitous and play an important role in the maintenance and monitoring of freshwater health. With the advent of accessible high throughput sequencing technologies, extensive studies of freshwater biofilms have led to significant breakthroughs in the exploration of biofilm phenome, with regard to community structure, functional attributes and physiological response to divers environmental stressors. However, the effective nucleic acid recovery from benthic freshwater biofilm samples remains an intricate endeavour, especially in relation to the isolation of easily degradable RNA. To date, there are no standardized protocols for effective RNA isolation from freshwater benthic biofilms. In this paper, we compared six RNA extraction methods on freshwater biofilms, from column-based commercial kit isolation to non-silicon based chemical reagent extraction regarding RNA integrity, purity and yields. Our results revealed that column-based kit isolation led to ideal RNA extraction outcomes qualified for downstream applications, compared to all other tested chemical extraction methods.

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Could benthic biofilm analyses be used as a reliable proxy for water environmental health?

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The quality of water undoubtedly reflects the health of our surrounding environment, society, and economy, as these are supported by various freshwater ecosystems. Monitoring efforts have therefore been considered a vital means of ensuring the ecological health of freshwater environments. Nevertheless, most aquatic environmental monitoring strategies largely focus on bulk water sampling for analysis of physicochemical and key biological indicators, which for the most part do not consider pollution events that occur at any time between sampling events. Because benthic biofilms are ubiquitous in aquatic environments, pollution released during sporadic events may be absorbed by these biofilms, which can act as repositories of pollutants. The aim of this study was to assess whether benthic biofilm monitoring could provide an efficient way of properly characterizing the extent of pollution in aquatic environments. Here, bulk water and benthic biofilms were sampled from three Hong Kong streams having various pollution profiles, and subsequently compared via high-resolution microscopy, metagenomic analysis, and analytical chemistry. The results indicated that biofilms were, indeed, reservoirs of environmental pollutants, having different profiles compared with that of the corresponding bulk water samples. Moreover, the results also suggested that biofilms sampled in polluted areas were characterized by a higher species richness. While the analytical testing of benthic biofilms still needs further development, the integration of chemical-pollutant profiles and biofilm sequencing data in future studies may provide unique perspectives for understanding and identifying pollution-related biofilm biomarkers.

Bacteria in the phycosphere of macroalgae

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In 1972 Bell and Mitchell proposed the concept of the phycosphere - a region around algae where close algae-bacteria interactions occur. For example, one gram dry weight of the algae *Ulva lactuca* has been shown to be associated with around 10^{10} bacteria. Phycosphere bacteria predominantly belong to the *Alpha-*, *Gamma-* and *Deltaproteobacteria*, the *Bacteroidetes* and the *Actinobacteria*, as has been shown for example for speciemen of the brown algae *Fucus vesiculosus*, *Cystoseira compressa*, and *Macrocystis pyrifera* in the North and Baltic Seas. Some bacterial clades are widely distributed in macroalgae, such as *Loktanella* spp. (order *Rhodobacterales*, class *Alphaproteobacteria*), which haven been identified on various macroalgal species, including *Fucus vesiculosus*, *Ulva australis* and *Cystoseira compressa*. *Planctomycetes* are also known to be abundant on macroalgae, a phylum of bacteria known for their ability to remineralize organic compounds and thereby providing nutrients for their macroalgal hosts. *Rhodobacterales* have been previously isolated from *Thalassia hemprichii* and *Zostera marina* and are primary surface colonizers with known nitrogen fixing ability. The surfaces of healthy macroalgae host well structured bacterial communities that promote algae growth and fitness. In contrast, certain members in the family *Rhodobacteraceae* are known to cause infections and disease (with the potential of getting more severe at increased temperatures) in *Fucus vesiculosus* and *Delisea pulchra*. Martin T. Croft *et al.* have demonstrated that microalgae can acquire vitamin B_{12} through direct interaction with bacteria and proposed that the nature of this interaction is symbiotic, with the algae supplying fixed carbon in exchange for vitamin B_{12}. Murakami *et al*. have shown that macroalgae also have such associated bacteria that might provide them with vitamin B_{12}.

The composition of macroalgae-associated microbiota is known to undergo changes with changing conditions of the host, as well as over space and time. For example, healthy vs. stressed *Ecklonia radiata* exhibit different microbial communities. Host traits may therefore be critical in determining the community composition of associated microbiota or *vice versa*. Other studies have documented spatial variations in colonization patterns. For instance, high within-species variability was observed among microbial communities associated with specimens of *Ulva australis* from different rock pools. Likewise, seasonal changes in bacterial colonization patterns have been observed. For instance, bacterial communities associated with *Fucus vesiculosus* showed persistent seasonal phylum-level variation over two consecutive years. Recent research conducted on the Mediterranean macroalgae *Cystoseira compressa* also revealed rather dynamic associated bacterial communities. Apart from the seasonality, host-associated microbiota are known to be tissue-specific. Despite all this variability, the composition of algae-colonizing microbial communities is still host-specific. For example, bacterial community fingerprinting by DGGE (denaturing gradient gel electrophoresis) of various macroalgae at different locations showed that community patterns were more similar for algae of the same species from different sites than between algae of distinct species from the same site. Furthermore, *Laminaria saccharina* had specific bacteria within its young and undisturbed tissues regardless of seasonality or geographic location.

The taxonomic composition and functional biology of bacteria that associate with algae is diverse and complex. Bacteria can produce unique bioactive compounds, such as antibiotics, toxins, or phytohormones, which they released to promote algal fitness. Studies show that there are abundant antimicrobial and antineoplastic resources in algae-colonizing microorganisms. At present, many bioactive compounds have been obtained from microorganisms that associated with microalgae.

The ability to recognize and respond to microbes is an essential survival strategy for macroalgae, but our understanding of macroalgae-microbe interactions at the molecular level interactions is still very limited. We therefore aim to investigate bacteria that associate with macroalgae using representative species of all three major lineages (red, green and brown algae), by both cultivation-independent and cultivation-based analysis. In the first approach, we will analyze algae-associated microbial communities with 16S rRNA gene tag sequencing and in the second approach we will sequence a large number of novel cultivated algae-associated bacteria. The combined results will substantially enhance our understanding of algae-bacteria interactions and pave the way for screening approaches for useful bioactive compounds.

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Microbiomes in digestive tracts of Hong Kong's marine fishes

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The fish digestive tract houses plenty of diverse microorganisms including bacteria, archaea and fungi, which jointly create a complex microbial ecosystem. These microbial communities are capable of enhancing the host metabolic homeostasis with beneficial effects on digestion, nutrition uptake, growth, and immune defence against disease and invasive pathogens. Fish gut microbial communities are affected by a variety of factors, among which host phylogeny, feeding habits and trophic levels play crucial roles in shaping the gut microbiomes. Nowadays, DNA sequencing and bioinformatic analysis greatly facilitate the exploration of complex microbial communities such as fish gut microbiomes.

This study aimed to uncover the general profile of the abundance and diversity of gut microbiomes from wildly-caught marine fish in Hong Kong, compare the diversity variation affected by fish phylogeny and feeding habit, and reveal the clustering pattern of host-microbiome and feeding habit-microbiome. Twenty different marine fish species were sampled from coastal area and market of Hong Kong. Their gut microbiome patterns shaped by host phylogeny and feeding habit were studied with 16S rRNA gene amplicon sequencing, bioinformatics analysis and statistical analysis.

The result showed that fish gut microbial community is a simple system with relatively low diversity and there is a large proportion of unknown fish gut microorganisms at the genus level. The dominant phyla of fish gut microorganisms are Proteobacteria and Firmicutes, while the dominant genera include *Clostridium*, *Photobacterium*, *Ralstonia*, *Acinetobacter*. Carnivorous fishes tend to harbour less diverse gut microorganisms than omnivorous and herbivorous fishes. Bacteria that are able to produce potential digestive enzymes (e.g. cellulase, chitinase and lipase) are found based on their discriminative abundances corresponding to different feeding habits.

Responses of two intertidal benthic copepod communities to seawater acidification

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Ocean acidification is posing an ongoing threat to marine organisms and ecosystems. In this study the responses of two intertidal benthic copepod communities to seawater acidification were examined using microcosm experiment. Microcosms were incubated for 56 days in natural seawater (as control) and acidified seawater (pH 7.3, CO_2 concentration: 1900ppm) which simulating the predicted ocean acidification level in 2300 in a carbon dioxide climate incubator. In both types of sediment, total abundance of benthic copepods was significantly suppressed at pH 7.3 on 56d, however, the increasing of opportunistic species resulted in a less reduction of benthic copepod's total abundance in coarser sediment. Community shift occurred on 56d for both sediment types. The community-based microcosm study indicated that seawater acidification could lead to changes of benthic copepod communities in intertidal sediments under extreme acidification scenario, but the communities inhibited in different sediments might response divergently.

International Conference on Marine Pollution and Ecotoxicology

Climate change: P-67

The transgenerational effect in *P. annandalei* on ocean acidification and mercury toxicity

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Ocean acidification (OA), a continuous decrease in pH resulting from the absorption of increased anthropogenic CO_2, has become a major global threat to the fitness of marine ecosystem. The atmospheric CO_2 level is projected to reach 1000 μatm by the end of 2100, leading to a decline of 0.3-0.5 units in seawater surface pH(7.6-7.9). In addition to increasing global atmospheric CO_2 levels, human activities have also led to a mass of mercury (Hg) emission into the atmosphere, which is eventually deposited in marine environments. Thus, OA and Hg pollution may co-occur after a long time low pCO_2 conditions in ocean. In this condition, transgenerational effect of ocean acidification may be induced for marine organism. Transgenerational effect, in which the experience of the parental generation can shape the phenotype of their offspring, may serve as such a mechanism. In my study, marine copepod *P. annandalei* was conditioned to low pH for three consecutive generations and then exposed to ocean acidification and mercury pollution (alone and combined) for one generation. Three important life history traits (development time to maturation, number of nauplii/clutch, and total fecundity) were examined for each generation and mercury accumulation for last generation. Specifically, DIA quantification proteomics was performed to study the critical functional proteins and biological processes. The preliminary results indicate that the transgenerational effect may aggravate toxicity of Hg, and under the combined exposure (OA and Hg), ocean acidification significantly reduced mercury accumulation. The molecular mechanism needs further analysis of the protein data.

Predator prey interaction between predatory gastropod *Thais clavigera*, barnacle *Amphibalanus amphitrite amphitrite* and mussel *Brachidontes variabilis* under ocean acidification

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Since responses to ocean acidification (OA) are species-specific, it is impossible to extrapolate responses at the species level to community or ecosystem levels unless changes in species interactions under OA are known. We investigated predator-prey interactions between a predatory muricid gastropod *Thais clavigera* and its prey, the barnacle *Balanus amphitrite amphitrite* and mussel *Brachidontes variabilis* under three pCO_2 levels, 380, 950, and 1250 μatm. *T. clavigera* increased its searching time for *B. amphitrite amphitrite* at 1250 μatm pCO_2. At 950 and 1250 μatm pCO_2 levels, the prey consumption rate was dependent on the exposure time of *T. clavigera* and the longer the exposure, the lower was the prey consumption rate. The prey preference between *B. variabilis* and *B. amphitrite amphitrite*, however, was independent of pCO_2. The findings suggest that changes in predator-prey interactions under OA will result in long term changes of population dynamics of interacting species.

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Climate change: P-69

The impact of seagrass bed on microbenthic algae under increased CO_2 and temperature in coral reef ecosystem

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The atmospheric CO_2 concentration is expected to reach 800~1000 ppm by the end of the century. Increase in atmospheric CO_2 will elevate the dissolved CO_2 in the ocean and lead to a decrease in pH, and warming of the ocean water. Marine ecosystems consist of millions of species of creatures, and a lot of seemingly unrelated biota are actually affecting each other. For example, a study showed that seagrass in coral reef ecosystem can remove bacterial pathogens that are harmful to fishes and invertebrates, and even humans. Since seagrass and algae compete for the same resources, the phytoplankton and benthic algae in a coral reef ecosystem might be negatively affected when seagrass is present. However, other studies showed that benthic algae can sometimes grow so thick on macroalgae or seagrass that they eventually decimate the hosts. Thus, it is far from conclusive as of how seagrass will affect phytoplankton and benthic algae. In this study, we used mesocosms to simulate a future (year 2100) tropical coral reef area by setting the CO_2 concentration at 800 ppm, and investigated the main and interactive effects of elevated temperature (25 ^oC and 28 ^oC) and CO_2, with and without the presence of seagrass meadows, on phytoplankton and microscopic benthic algae. The results indicated that at either temperature, there was no significant difference in phytoplankton biomass with or without seagrass. However, the benthic microalgal biomass was significantly enhanced at both temperatures in the seagrass-treated group as compare to the control. The benthic algal communities and their lipid contents were not significantly different between seagrass and control groups. We conclude that under high CO_2 concentration, the growth of microbenthic algae will be enhanced in coral reef ecosystem when seagrass meadow is present, which might enhance some primary consumers and possibly alleviate the impact of global warming and acidification on higher trophic levels.

Effects of ocean acidification on life parameters and antioxidant system in the marine copepod *Tigriopus japonicus*

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Ocean acidification (OA) is caused by alteration of global ocean carbon chemistry due to the increased pCO_2 in the atmosphere and led deleterious impacts on the marine ecosystem. Although detrimental effects of OA were reported in marine organisms, the potential impact of OA on aquatic invertebrates still largely remained unknown. Here, we examined changes on life parameters and antioxidant system in response to lower pH (7.5 and 7) in the marine copepod *Tigriopus japonicus*. Exposure of OA to copepod led the reduced growth rate with decreased fecundity and body length. Also they showed the increased reactive oxygen species with the enhanced glutathione S-transferase and glutathione reductase activities but showed the decreased glutathione peroxidase and superoxide dismutase activities in pH-dependent manner, indicating that OA exposure caused to interrupt the redox system of *T. japonicus*. Of several oxidative stress-related genes, *GSTs2b* was significantly up-regulated in response to OA. These findings will be helpful for a better understanding on the potential impact of OA on life parameters and antioxidant system in the marine copepod *T. japonicus*.

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Emerging chemicals of concern (2): P-71

Sex-specific immunomodulatory action of the environmental estrogen 17α-ethynylestradiol alongside with reproductive impairment in fish

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Estrogenic endocrine disrupting chemicals (EEDCs) occurred widely in coastal waters and marine sediments all over the world. It has been recognized that EEDCs can mimic the normal functions of estrogen and impair fish reproduction. Growing evidence shows that EEDCs can also suppress fish immune systems. However, there is lacking of study in fish considering concomitantly EEDC-induced impacts on immune and reproductive function, which is essential for holistic assessment of the risk of sublethal levels of EEDCs in aquatic environments. In this study, 17α-Ethynylestradiol (EE2), a representative EEDC, was used for parallel evaluation of EEDC-induced immune suppression (immune marker gene expression, leukocyte numbers, host resistance assay, and immune competence index) and reproductive impairments (estrogen responsive gene expression, fecundity, fertilization success, hatching success, and reproductive competence index) in the established marine fish model, *Oryzias melastigma*, considering sex-specific induction, adaption and recovery responses under different EE2 exposure scenarios. The findings in marine medaka clearly demonstrate that the impacts of EE2 are sex-dependent. Male fish were more sensitive to the presence of low levels of exogenous EE2 and exhibited a clear positive relationship between EE2 doses and impairments of immune competence as well as reproduction. It is also evident in male fish that direct contact of EE2 is essential to sustain impairments of immune competence and reproductive output. However, for females, low EE2 (33 ng/L) could enhance fish immune competence (hormesis), restore reproductive fitness (adaptation) and even increase fish resistance to pathogenic bacterial infection upon abatement of EE2. However, prolonged exposure to high EE2 (113 ng/L) not only reduced female fish survival upon pathogen challenge, but also perturbed fish recovering from reproductive impairment, resulting a persistent impact on the F1 output. The immunomodulatory pathways altered by EE2 were deciphered for male and female fish, separately. Immune gene expression analyses showed that EE2 exposure could compromise tlr3, tlr5 and c3 in male fish, and tlr3 and c3 in female fish, which may be indicative of EEDCs-induced impairments of immune competence. In conclusion, immune impairments in the EE2-exposed fish poses an immediate thread on the survival of F0 population, whereas impaired reproduction in the EE2-exposed fish can directly affect F1 output. Parallel evaluation of sex specific immunomodulatory impacts and reproductive impairments are necessary when assessing the risk of EE2/EEDCs on survival fitness and sustainability of fish population (F0 and F1 generations).

Occurrence and removal efficiency of retinoic acids in sewage by chemically-enhanced primary treatment and secondary treatment plants

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Sewage treatment plants (STPs) are important in a densely populated cities like Hong Kong to control the release of unwanted harmful pollutants from households and industries into the water receiving body (i.e., coastal marine waters) and hence maintain the water quality for supporting all beneficial uses. Yet, studies showed that retinoic acids (RAs) and their metabolites can be continuously detected in both influent and effluent of STPs due to their presence in urine of human and animals. It was reported that the concentration of 13c-4-oxo-RA could reach 104.9 ng/L in influent of one of the STPs in Osaka, Japan suggesting that sewage treatment facilities are one of the major sources of RAs. Since RAs are teratogens to animals, the quantities of them and the performance of STPs are critical to protect the marine environment. In Hong Kong, 75% of sewage is currently treated by Chemically Enhanced Primary Treatment by applying flocculates and coagulates to augment the removal of suspended solids from the water column and then the settled sludge can be eventually separated from the sewage. About 20% of sewage in Hong Kong is treated by secondary treatment which is a biological treatment with provision of activated sludge in which microorganisms can utilize the organic matter and some nutrients in the sewage for their growth, and thereby effectively remove organic matters and pathogens. The higher the treatment level is, the better the effluent quality can be achieved. Yet, the water quality objectives of Hong Kong only cover conventional water quality parameters, but they do not encapsulate emerging chemicals including RAs. Therefore, this study aims to investigate: (1) the concentration of RAs in both influent and effluent of selected STPs in Hong Kong; and (2) the removal efficiency of RAs from influent and the quantities that are retained in sludge. Our preliminary results showed that the STPs in Hong Kong, depending on the treatment type, can remove about 40-80% of RAs suggesting that the STPs play an important role in removing RAs from sewage and reducing their potential risks to the coastal marine ecosystem.

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Emerging chemicals of concern (2): P-73

In vivo toxicities of nine engineered nano metal oxides to the diatom *Skeltonema costatum* and the rotifer *Brachionus* sp.

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Various nano forms of metal oxides have been designed and produced for industrial and commercial uses over the past two decades. Following use, they may be released into natural aquatic environments as manufacturing and household wastes. There were three main objectives in this study. First, this study compared the *in vivo* toxicities of nine engineered nano metal oxides including nano α-alumina (α-nAl_2O_3), nano γ-nAl_2O_3 (γ-nAl_2O_3), nano indium oxide (nIn_2O_3), nano magnesia (nMgO), annealed nMgO, nano tin oxide (nSnO_2), nano titanium dioxide (nTiO_2 in anatase and rutile form) and nZnO towards the marine diatom *Skeletonema costatum* and the marine rotifer *Brachionus* sp. Second, an attempt was made to identify genetic biomarkers that may be utilized for assessing sub-lethal toxic effects of these nano metal oxides to the rotifer, and providing insights into their toxic mechanisms. Third, this study further evaluated the levels of *in vivo* reactive oxygen species (ROS) generated by three selected nano metal oxides (i.e., nMgO, nSnO_2 and nZnO) in the rotifer which were chosen based on the results of the gene expression study.

According to the results of this study, among the nine nano metal oxides, nZnO was the most toxic one towards the diatom *Skeletonema costatum* while nSnO_2 was the least toxic. nZnO was also the most toxic towards the rotifer *Brachionus* sp., while the other nano metal oxides were found to be non-lethal. nMgO and nZnO were confirmed to trigger ROS-mediated toxicity to the two marine organisms, while nTiO_2 (anatase and rutile) might induce oxidative stress as shown by their acellular ROS production in seawater. nZnO may also wreak havoc in the endocrine system of organisms, as indicated by the increased *RXR* transcription. Annealing nMgO can help to reduce its toxicity via removal of O_2^- and impurities from its surface.

This study provides a useful dataset for the construction of an aquatic toxicity database for nanomaterials (NMs). Such a database can provide information for scientists, manufacturers and regulators across the world, facilitating the development of more environmentally friendly products for commercialization, while reducing the ecological impacts of NMs by imposing restriction on the releases of NMs with high toxicity.

Fata And Toxicity of UV-Filters in Marine Environment

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UV filters have been widely used in sunscreen products, and they have partially ended up into the marine environment via human recreational activities and sewage treatment plant drainage, forming one of the emerging marine pollutants. As UV filters have many characteristics such as extensive use, continuous emissions and stability, its potential risks to the environment and ecology have become a hot topic in the field of environmental research all over the world. This paper analysed the environmental behaviors of UV filters in the ocean, such as migration, transformation and volatilization, and then summarized the toxic effects (i.e., growth inhibition, reproductive inhibition, death and malformation) of the inorganic UV filters (mainly nano-TiO_2 and nano-ZnO) and the organic ones (mainly benzophenones, camphor derivatives and cinnamic acids) on the marine organisms (i.e., algae, seashell, fish, coral, sea urchin). The research also analysed the inherent toxicity mechanism from the perspective of oxidative damage, neurotoxicity and endocrine disability. The prospect and future directions of this field were also discussed. This review, thus, can provide reference for the scientific research and pollution control.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (2): P-75

Effect of TiO_2 on photoreaction system of *Phaeodactylum tricornutum*

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Chlorophyll a content, Fv/Fm and expression of photosynthetic related genes are chosen as the index to assess the toxic effect and toxicity variation with time of different nano-titanium dioxide ($nTiO_2$) treatments (0, 5, 10, 20, 50, 100 mg/L) to *Phaeodactylum tricornutum*. Measurement of chlorophyll a content showed low toxicity of nano-titanium dioxide to *P. tricornutum*. For example, chlorophyll a content of 10 mg/L $nTiO_2$ treatment is 76.35% of that of control group at 48 hour and 94% of control at 120 hour. Besides, the result of chlorophyll *a* contents and Fv/Fm values both demonstrated that the toxic effect grew weak over time, which also indicated the resistance of *P. tricornutum* to $nTiO_2$. And the result of expression of photosynthetic related genes indicated that the resistance of *P. tricornutum* to $nTiO_2$ might be correlated to the rbcS and LcyB down regulated during the beginning of the exposure and up regulated during the late period of the exposure.

Physicochemical properties and toxicities of surface-modified zinc oxide nanoparticles towards freshwater and marine microalgae

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Zinc oxide nanoparticles (ZnO-NPs) are effective blockers of ultraviolet radiation but they can cause growth inhibition and developmental malformation in various aquatic micro-organisms due to the release of zinc ions and the interaction between the nanoparticles and the cells. Concerns have been raised over the potential environmental impacts of silane-coated ZnO-NPs because of their wide applications in commercial sunscreens and their easiness of being washed into the aquatic environment. This study aimed to compare the physicochemical properties between silane-coated and uncoated ZnO-NPs, and elucidate their toxicities towards aquatic microalgae. The surface of ZnO-NPs (20 nm) was modified by 3-aminopropyltrimethoxysilane (A-ZnO-NPs), and dodecyltrichlorosilane (D-ZnO-NPs). These two coated-nanoparticles, uncoated ZnO-NPs and bulk ZnO were characterized in terms of particle size, zeta potential, aggregate size, dissolution and surface chemistry. Three freshwater algae and three marine algae species were exposed for 96 h to ZnO, uncoated ZnO-NPs, the two coated ZnO-NPs and $ZnSO_4$ at 10 concentrations ranging from 0.1 to 100 mg/L. The results showed that uncoated ZnO-NPs formed larger aggregates and released more zinc ions than the two coated ZnO-NPs. Although the sensitivity towards the test chemicals among the test algal species varied, A-ZnO-NPs and uncoated ZnO-NPs were consistently more toxic than D-ZnO-NPs in terms of algal growth inhibition. The marine diatom *Thalassiosira pseudonana* exposed to ZnO-NPs, A-ZnO-NPs and D-ZnO-NPs resulted in different gene expression profiles, suggesting that they exhibited different toxic mechanisms to this algal species. The results of the study provide some useful insights for development of eco-friendly nanoparticles for sunscreen products in the future.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (2): P-77

The influence of organic matter on the biological uptake of nanoparticles: Role of adsorption ability and endocytosis capacity

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The interactions between organic matter and nanoparticles (NPs) are always inevitable in the aquatic environment. Combined with their high specific surface area and chemical reactivity, NPs would highly affect the distribution and transformation of pollutants in the organism. Even though, it is still hard to predict the influence of organic matter on the biological uptake of NPs as the resulting influence is always complicated. Accordingly, a quantitative model based on the two-step internalization process was adopted to study that how would the coating of organic matter affect the adsorption ability of nanoparticles on the cell membrane and influence the nano-bio interaction. In the present study, FITC-SiO_2 and Au@SiO_2 were prepared to verify the model validation and study the influence mechanism of organic matter, respectively. Using *Tetrahymena thermophila* as the testing organism, it was found that all the organic matter promoted the concentration of internalized Au@SiO_2 but also reduced the adsorption ability. Moreover, the proteins greatly increased the internalization capacity of Au@SiO_2, while humic acid only slightly increased the value. Such increments finally compensated for the loss of adsorption ability, especially for the protein groups. As a consequence, more than 90% of Au@SiO_2 was internalized in the presence of proteins, while the internalization ratio was only 79% for HA group and 54% for control group. Overall, the membrane adsorption-endocytosis model could serve as a valuable tool to quantify the effect of organic matter on the adsorption ability and endocytosis process of nanoparticles.

The toxic effect of graphene oxide to five freshwater algae

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The increassing application of graphene oxide (GO) has attracted much attention on its toxicity to aquatic organisms. Studies on the adverse effect of GO to various algae have shown different results, which is particularly significant because of the crucial role of algae in maintaining the ecological balance of the aquatic environment. However, the effect of GO on the differences of multi-species algae and the general rule of toxic mechanism remains largely unknown. In this study, we exposed five freshwater algae (*Chlorella vulgaris, Scenedesmus obliquus, Microcystis aeruginosa, Chlamydomonas reinhardtii, Cyclotella sp*) to GO with the aim of increasing our understanding on differences in the toxicity and the rules in toxic mechanism. Results showed that GO significantly inhibited the growth of cells at 10 mg/L. Meanwhile, average cell area, dividing cell ratio and the content of chlorophyll a were affected to varying degrees. The scanning electron microscopy (SEM) results show that GO interacted with algae through envelopment and penetration (Figure 1). Specially, *Scenedesmus obliquus* and *Cyclotella sp* were severely damaged and other three algae were more likely be wrapped by GO. However, the differences in physiological feature observed by SEM and transmission electron microscope (TEM) resulted in the differences in growth inhibition, cell division, oxidative stress and membrane permeability through interfering shading effect and mechanical damage. Especially, there was no significant difference in the permeability of *Microcystis aeruginosa*, which was different from other four algae in 10 mg/L ranging from (120.1 ± 6.33) % to (240.9 ± 47.4) %.Correlation analysis showed that oxidative stress and mechanical damage were the common rules of toxic mechanism of GO to multi-species algae that may apply to all algae in the experiment. Accordingly, the potential influence of graphene-family on the toxic effect of multi-species aquatic organisms deserves more attention.

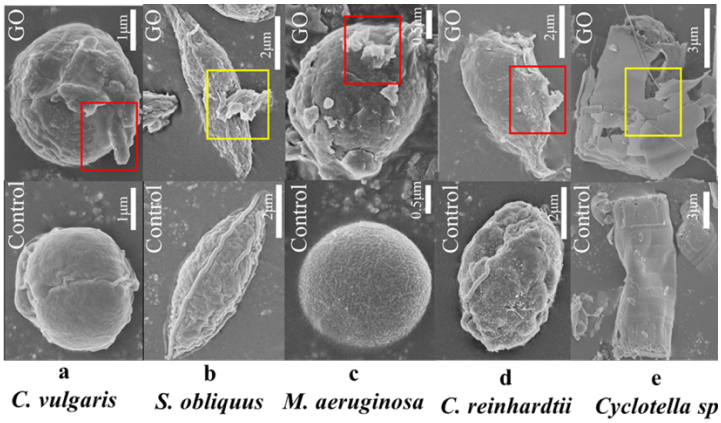

Figure 4. SEM images of algae cells in control groups and interations between cells and material; Red pane donate envelopment of material, yellow pane denote the damage of cell.

International Conference on Marine Pollution and Ecotoxicology

Emerging chemicals of concern (2): P-79

Metatranscriptomics reveals detoxification of gut symbiont enhances zooplankton Daphnia magna fitness to silver nanoparticles

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The release of antibacterial silver nanoparticles (AgNPs) is rising every year with plenty of negative effects on aquatic organisms. In spite of many reports about toxic test of AgNPs on different aquatic organisms, the influence of AgNPs on bacteria involved holobiont system is never studied. Since the AgNPs will significantly enrich in the gut of zooplankton, we conducted a study combining electronic microscopy monitoring, metatranscriptome of extracted gut section and gut microbiota translates, under environmentally relevant concentrations of AgNPs (0.43 μg/L for surface water and 4.3 μg/L for sediment), to investigate the response and possible detoxification mechanism of the gut microbiota to AgNPs in *Daphnia magna*. The results of AgNPs exposure revealed that the intestinal cells showed down regulated proteasome, ATP synthesis, and fatty acid biosynthesis genes accompanying with decreased digestion, body length, and number of neonates when comparing with control. However, in the third brood of AgNPs exposed (0.43 μg/L) *D. magna*, these toxic inhibitions were relieved with the up regulated dissimilatory sulfate reduction, flagellin, and short-chain fatty acids synthesis genes in its' gut microbiota. The similar symptomatic palliation was also found when *Daphnia* are made germ-free and inoculated with the AgNPs adapted microbial inoculum. Together, these results show that the gut microbiota may precipitate Ag^+ with sulfate reduction product (sulfide) and aggregate nanoparticles with flagellin to help host relieve the toxic effects. However, the holobiont system will not survive with a higher concentration of AgNPs (4.3 μg/L).

Two antidepressants fluoxetine and sertraline cause growth retardation and oxidative stress mediated modulation of defensome in the marine rotifer *Brachionus koreanus*

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To understand the effects of two widely used antidepressants, fluoxetine and sertraline, we examined mortality, population growth, reactive oxygen species (ROS) levels, glutathione *S*-transferase (GST) enzymatic activity, and transcriptional regulation of defensomes (e.g., cytochrome P450s [CYPs] and GSTs) in the marine rotifer *Brachionus koreanus* (*B. koreanus*). The no observed effect concentration (NOEC)-24 h and median lethal concentration (LC50)-24 h of fluoxetine were determined as 1000 μg/L and 1560 μg/L, respectively, while NOEC-24 h and LC50-24 h of sertraline were 450 μg/L and 507 μg/L, respectively. Moreover, growth retardation was observed in response to fluoxetine and sertraline, suggesting that fluoxetine and sertraline have a potentially detrimental effect on life cycle parameters of *B. koreanus*. ROS level and GST enzymatic activity was significantly (P<0.05) increased in response to fluoxetine and sertraline, but no change in GST enzymatic activity was observed in fluoxetine. Furthermore, transcript levels of defensoms (e.g., cytochrome P450s [CYPs] and glutathione *S*-transferases [GSTs]) were significantly (P<0.05) modulated (up and/or down) in response to fluoxetine and sertraline, respectively. Our results indicate that fluoxetine and sertraline can induce oxidative stress with transcriptional modulation of defensomes, resulting in growth retardation in the marine rotifer *B. koreanus*.

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Emerging chemicals of concern (2): P-81

Preliminary survey of antibiotics contents in the Dongjiang River

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Dongjiang River is the most important source of drinking water in the Southern China region, occurrence of antibiotics in the river will pose a severe risk to the population in the region. Monitoring of the occurrence and distribution of antibiotics in the river is essential for understanding and controlling the risk posed.

In this study, we investigated the concentration of five antibiotics including tetracycline, norfloxacin, sulfamethoxazole, sulfadiazine, and sulfamethazine in the upstream, midstream, and downstream of the Dongjiang River. Our data indicated that the total concentration of antibiotics decreased from 54.26ng/L at the upstream to 29.32 ng/L at the midstream. However, the total concentration of antibiotic increased again to 41.62 ng/L at the downstream. A more detail investigation of the concentration of each antibiotic indicated that the distribution of individual antibiotic varied along the river. While concentration of tetracycline and norfloxacin in the river decreased from the upstream (4.71 ng/L and 32.76 ng/L respectively) to the downstream (3.05 ng/L and 18.40 ng/L respectively). A reverse trend was observed for sulfamethazine (from undetectable to 4.46 ng/L) and sulfamethoxazole (from 4.24 ng/L to 13.13 ng/L). Interestingly, concentration of sulfadiazine decreased from 12.55 ng/L at the upstream to 0.38 ng/L at the midstream but increased again at the downstream (2.58 ng/L).

These suggested that the increase of antibiotic concentration at the downstream of the river was largely contributed by sulfadiazine, sulfamethazine, and sulfamethoxazole and antibiotics in the river may be originated from different sources in the upstream and downstream of the river.

Sources and distributions of organic matters in coastal sediment from tidal flats and estuary, Korea

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The coastal sediments have been highlighted as globally significant carbon and nitrogen sinks, while the dynamics and conditions relating to sedimentary organic matters have been poorly studied, particularly in tidal flats. This present study aimed to investigate the regional and temporal distributions of surface sedimentary organic matters in five representative tidal flats in Korea; Ganghwa, Garolim bay, Jeungdo, Suncheon bay, and Nakdong estuary. The mud contents, chlorophyll-*a*, total organic carbon and nitrogen (TOC and TN), carbon and nitrogen stable isotopes ratio (δ^{13}C and δ^{15}N) in surface sediments were monthly analyzed from January to December, 2018. Results indicated that Suncheon bay had greatest TOC and TN (1.27 % and 0.18 %, respectively), followed by Ganghwa (1.09 % and 0.11%), Jeungdo (0.44% and 0.06%), Garolim bay (0.36% and 0.04%), and Nakdong estuary (0.19 % and 0.03%). In general, mud content was found to be a key factor controlling the sedimentary organic matters, with a positive correlation to TOC ($r=0.66$, $p<0.001$) and TN ($r=0.44$, $p<0.001$), respectively, cross the five study areas. Of note, the typical tidal flats such as Garolim bay, Jeungdo, and Suncheon bay showed winter to early spring bloom of microphytobenthos (MPB), with elevated values for TOC, TN, and δ^{13}C, which indicated the sources of organic matters are derived from MPB (marine-derived) in the corresponding season. On the other hand, δ^{13}C and δ^{15}N of Nakdong estuary sediments (terrestrial-derived) had decreased when barrage gates were open for discharging freshwater during the flood season (Sep-Oct). Altogether, the spatiotemporal patterns of organic matters indicated the combined influences of sediment grain size, MPB blooming, and terrestrial inputs in tidal flats of Korea. Overall, the tidal flats played an important role as an organic carbon and nitrogen reservoir, and it could be affected by anthropogenic activities, further studies and long-term monitoring would be necessary.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-83

The influence of temperature on seed germination and seedling growth of an exotic mangrove species *Laguncularia racemosa*

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Laguncularia racemosa (Combretaceae, Lr), one of the fast-growing exotic mangrove species, was introduced for afforestation along the southeast coast of China in the last two decades. Compared with another widely used exotic mangrove species, *Sonneratia apetala*, information about Lr is still limited. Due to the lack of research on the adaptive mechanism and potential invasion of Lr, whether this exotic species will cause biological invasion in mangrove wetland is still controversial. The present study aims to evaluate the effect of low temperature on seed germination and early growth of Lr as these two factors have been considered as important indicators for the adaptive and invasive abilities of an exotic plant species. Results showed that Lr seeds did not germinate under 5°C, and the time achieved 70% germination percentage was related to temperature, which was 34, 26, 20 and 10 days at 10, 15, 20 and 25°C, respectively. The healthy seedlings germinated under 25°C were selected, their growth and physiological responses, including photosynthetic parameters, activity of peroxidase (POD) and content of MDA, were monitored under three different temperature, namely, 25, 10 and 5°C at 6, 12, 24, 72 and 120 hours. Both POD activity and MDA content were higher under lower temperature and increased with treatment time. Among photosynthetic parameters, net photosynthetic rate, transpiration rate, stomata conductivity and water use efficiency were positively correlated with temperature but relationships with treatment time were negative. The seedlings under low temperature stresses (5 and 10°C) after 24, 72 and 120 hours were then transported to 25°C for recovery in the next 48 hours. Photosynthetic data showed that previous exposure of seedlings at low temperature was good for seedling growth. These findings indicated that an exotic mangrove species (Lr) could germinate and grow at low temperature (10°C), it could also recover after exposure to even lower temperature like 5°C, suggesting that Lr could spread to high latitudes, thus the invasive potential of Lr and related mechanisms need further research.

Effect of simulated ocean acidification and heavy metals of copper and cadmium on development, reproduction and SOD enzyme activity of *Tigriopus japonicus*

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Heavy metals pollution in marine environment has caused great harm to marine organisms. The ongoing ocean acidification will seriously threaten marine ecosystem health and function. Copepods play an important role in marine ecosystem. In present study we evaluated the toxicological effect of seawater acidification and heavy metals of Cu and Cd on the development, reproduction and SOD activity of *Tigriopus japonicus*. Except for development retardation, there was no significantly negative synergistic effect observed on survival rate, female/male ratio, offspring number and egg-sac number for both Cu and Cd under acidification conditions, which indicating that copepod larvae might be more vulnerable in a polluted and acidifying marine environment. SOD enzyme activity at high Cd concentration was suppressed at pH 7.7 but enhanced at pH 7.3 and 6.5 but the effect was not significant for Cu. This result was in accordance with the acute toxicity results which showing that the negative synergistic effect of Cu and acidity was more obvious than Cd and acidity.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-85

Responses of toxic algae to environmental variables in the Pearl River Estuary and Hong Kong waters

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The emerging threat of toxic algae to marine ecosystems has attracted worldwide attention. They are capable of producing toxins that can cause fish kills or even cause severe intoxication in humans. In previous study, toxic algae that can produce lipophilic algal toxins were found in Pearl River Estuary (PRE) and Hong Kong (HK) waters, but the toxin producing mechanisms remain unrevealed. Besides, the information on the toxin levels in environmental samples of this area, is largely unknown. The present study aims to investigate the distribution of typical lipophilic algal toxins (LATs) in seawater of PRE and HK waters and to verify the relationship between toxin levels and environmental variables. Five types of lipophilic algal toxins (OA, DTX1, YTX, PTX2, GYMA) were detected in seawater samples with different distribution patterns. The effect of environmental variables on toxin distribution was studied by multivariate analysis. Among all physical, chemical, biological factors, salinity plays a most important role in the toxin distribution of PRE waters. The effects of temperature and salinity on the growth, toxicity, and toxin production of toxic algae were also investigated under laboratory conditions. Specific growth rates, Fv/Fm, phaeo-pigments, brine shrimps bioassay, LATs in algal extracts were used to evaluate the responses of *Coolia malayensis* to temperature and salinity. *Coolia malayensis* showed positive correlation with temperature in terms of the log-phase photosynthesis efficiency, pigments amount, toxicity, and contents of putative LATs (OA analogue and AZA analogue), and its toxicity increased with longer acclimation time. More upcoming results of the laboratory experiments on salinity will be discussed as well.

Bleaching in subtropical corals: Differential susceptibility to environmental stressors and molecular mechanisms

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Recent studies suggest that subtropical coral communities may serve as refuges for global environmental change, but the relative susceptibility of subtropical coral species to stressors and the underlying mechanisms of stress responses are largely unknown. We propose to study the effect of high temperature and low salinity of corals in subtropical Hong Kong where these two environmental stressors have been known to cause bleaching in recent years. We have constructed a laboratory exposure system for determining the bleaching thresholds for nine species representing several growth forms. We will expose them to a combination of thermal and salinity treatments. For the two most sensitive and two most resistant species, we will compare their physiological and transcriptomic responses to temperature and salinity stressors, followed by gene co-expression network and positive selection analyses to identify the underlying mechanisms of stress responses. Overall, our study will determine the potential coral "winners and losers" of global environmental change in Hong Kong for the first time, uncover molecular mechanisms of coral bleaching considering coral as a holobiont of coral host and its symbionts, and develop a set of diagnostic biomarkers of coral thermal/salinity stress.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-87

Exploration of factors affecting changes in megabenthic community structure in Tokyo Bay, Japan: (I) Long-term community-level study

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Tokyo Bay is a heavily eutrophicated area in Japan and has experienced great changes in both environment and biota in response to consequences of human activities in metropolitan areas and/or changes in meteorological and oceanographic conditions. To assess effects of these factors on the ecosystem, monitoring surveys for investigating changes in the biocoenosis are essential. We have conducted fisheries-independent trawl surveys at 20 sampling sites covering whole bay areas twice a year from 1977 to 1995 and from 2003 to 2018. Fishes, crustaceans, mollusks and echinoderms were identified to species level, and abundance (number of individuals/km^2) and biomass (kg/km^2) were calculated for each species. We found substantial changes in the community structure during the sampling period. The total abundance and biomass of the megabenthic community increased from the late 1970s and peaked in 1987, followed by drastic declines in the late 1980s. In the 1990s, the total abundance and biomass remained low, at less than half the 1987 level. These changes between the late 1970s and mid 1990s were mainly attributed to drastic changes in the small-medium sized dominant species (e.g., mantis shrimp *Oratosquilla oratoria*, whipfin dragonet *Repomucenus valenciennei*, and marbled sole *Pseudopleuronectes yokohamae*). In the early-mid 2000s, the total abundance remained low as that of 1990s, meanwhile the total biomass increased substantially, which was caused by increase in large-sized fish species (e.g., Japanese sea bass *Lateolabrax japonicus* and elasmobranchs). In the late 2000s to early 2010s, the total abundance and biomass of the megabenthic community showed marked increase, which was caused by occurrence of the strong year class of a single bivalve species, Kobelt's *ark Arca boucardi* in 2009. The abundance and biomass of Kobelt's ark showed gradual decline in the mid-late 2010s, while large-sized fish species remained abundant during these periods. During 2000s-2010s, there were no sign of recovery in the population of the small-medium sized species that were abundant in 1970-1980s. To investigate potential effects of environmental factors on the changes in the megabenthic community, we performed distance-based redundancy analysis (dbRDA). The dbRDA showed that increase in water temperature and decrease in dissolved inorganic nitrogen and copepod density are significantly correlated to changes in the community structure from 1980-1990s to 2000-2010s. In addition, the major component of the bottom sediment in the bay shifted from coarse sand/gravel to fine clay/silt around the late 1990s, which might have resulted in increase in organic matter in the sediment, and also could have enhanced the formation, expanded spatial distribution and delayed disappearance of bottom hypoxia. These results imply that changes in environmental conditions in the bay might have affected changes in the abundance, biomass and species composition of megabenthic community in Tokyo Bay, Japan.

Exploration of factors affecting changes in megabenthic community structure in Tokyo Bay, Japan: (II) Population-level studies in four dominant species

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Substantial changes in the megabenthic community has been observed in Tokyo Bay during the past 40 years. Here we show results of population-level studies for selected four dominant species in Tokyo Bay, Japan that could help better understand the mechanisms of changes in the community structure.

1) Mantis shrimp *Oratosquilla oratoria*; Abundance peaked in mid 1980s, followed by marked decline in late 1980s and remained low since then. Although spawning season lasts from spring to summer, the population is maintained predominately by summer cohort, probably due to disturbance of juvenile settlement of spring cohort by the presence of bottom hypoxia prevailing the wide areas in the bay in summer. There were interannual changes in the abundance of settled juveniles from summer cohort. Delayed disappearance of hypoxia in autumn, during which the majority of summer cohort settled to the bottom, could have resulted in decrease in the survival rate as well as contracted spatial distribution of juveniles. These findings imply hypoxia is one of the major factors impeding recovery of the population.

2) Whipfin dragonet *Repomucenus valenciennei*; Changes in the growth and reproductive traits were evident between 1990s and 2000s. Abundance in 2000s decreased to 14% of that in 1990s. Body length at which dragonet attain gonadal maturation decreased by 20% in 2000s compared to that in 1990s. In addition, timing of the onset of the first spawning became earlier in 2000s (starting from spring) than that in 1990s (from summer). We also found a significant decrease in growth for both sexes from 1990s to 2000s. Changes in these life history traits may reflect trade-off for allocating available energy resource to reproduction rather than to somatic growth under limited prey abundance to enhance a chance for recovery of the population.

3) Marbled sole *Pseudopleuronectes yokohamae*; Substantial decrease in abundance from 1970-1980s to 1990-2000s was evident, where water temperature showed increasing trend throughout these periods. Field surveys during the reproductive season in winter showed a significant negative correlation between water temperature and larval density in Tokyo Bay. Controlled laboratory experiments showed decrease in the survival rate of larvae at >10°C. These evidence suggests increase in water temperature might have affected mortalities during the early life stages, and resulted in decrease in recruitment of settled juveniles during 2000s.

4) Starspotted smooth-hound *Mustelus manazo*; Abundance increased substantially in 2000s compared to 1970-1990s. Decrease in fecundity and the amount of prey consumed were evident during 2000s. In addition, undeveloped eggs were found in the uteri during 2000s, but they were totally absent during 1990s. These changes may be associated with density-dependent effects; e.g., an increase in predatory elasmobranchs species, as well as a decline in their prey organisms, which could have led to increased inter/intra-specific competition for prey.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-89

Shifting diets for benthic deposit feeders in invasive halophyte environment: Case study in Ganghwa tidal flat, Korea

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The influence of invasive halophyte *Spartina alterniflora* on the dietary contribution to benthic invertebrates was investigated in Ganghwa tidal flat, Korea. The three benthic deposit feeders (*Cerithidea ornate*, *Perinereis aibuhitensi*, and *Macrophtahlmus japonicus*) and potential food sources (particulate organic matter, sediment organic matter, invasive halophyte *S. alterniflora*, native halophytes *Suaeda japonica* and *Phragmites australis*, microphytobenthos, and meiofauna) were seasonally analyzed for stable carbon isotopes ($\delta^{13}C$) at two contrasting sites; one for *Spartina* habitat (DM) and the other for native halophytes habitat (DG). The invasive halophyte *S. alterniflora* showed significantly enriched $\delta^{13}C$ (-13.7 ± 0.2 ‰) compared to native *S. japonica* (-25.6 ± 0.6 ‰) and *P. australis* (-25.9 ± 0.5 ‰). By the influence of the *S. alterniflora*, the $\delta^{13}C$ of the sediment and deposit feeders (-21.2 ± 0.2 and -13.9 ± 0.8 ‰) were significantly enriched in DM, compared to those in DG (-22.9 ± 0.2 and -15.2 ± 1.1 ‰). A two-source mixing model revealed that the invasive *S. alterniflora* served as a main food source for the three deposit feeders in DM, and the contribution has gradually increased in the growth season of *S. alterniflora*. On the other hand, meiofauna was utilized as a significant food source to them in the native halophytes habitat, DG. The smaller dietary contribution of native halophytes back supported that the three deposit feeders might have utilized more actively the invasive *S. alterniflora* in Ganghwa. Overall, the present study indicates that habitat changes in tidal flats by invasion of new halophyte species might influence the trophic shift of some benthic invertebrates, primarily for deposit feeders.

Trophic transfer of trace elements in a euryhaline teleost, the turbot *Scophthalmus maximus*: Contrasting effects of salinity on Mn and Zn

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Diet is often shown as the main pathway of trace elements incorporation in aquatic organisms such as fish. Abiotic parameters may play a role on the trophic transfer of trace elements. While the influence of salinity on dissolved trace element uptake has been extensively studied in various aquatic taxa, effects of this environmental variable on trace element incorporation through the food pathway remain poorly known. In this context, we investigated the potential influence of salinity on the trophic transfer of two essential elements (Mn and Zn) in a euryhaline fish, the turbot *Scophthalmus maximus*, using radiotracer techniques. Although euryhaline organisms are able to deal with large variations in salinity, trophic transfer of trace elements in can potentially be affected. After a gradual acclimation of three weeks to three salinities (10, 25 and 38), three batches of juvenile turbots were fed with radiolabelled pellets (^{54}Mn and ^{65}Zn). Kinetic parameters (such as assimilation efficiency and efflux rate) of these elements were determined after a 21-d depuration period. Trophic transfer of Mn at the highest salinity was significantly lower than for the other conditions ($p<0.05$) whereas salinity did not significantly influence Zn trophic transfer ($p>0.05$). We demonstrated that salinity has contrasting effects on the trophic transfer of trace elements in euryhaline fish. The effects of salinity are interpreted through a mechanistic approach mainly based on the membrane transport mechanisms (active sor passive) of trace elements. Implications of these findings on the transfer of trace elements in aquatic environments are then discussed.

Biological responses to multiple stressors: P-91

Interactive effect of freshwater acidification and selenium pollution on the antioxidant response and biochemical changes in *Oreochromis mossambicus*

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Freshwater animals have been used to assess impact of selenium contamination and the effect of freshwater acidification when both factors are acting alone, but few reports are available on the impacts of both factors acting in combined. In the present study, laboratory experiments were conducted to investigate the effect of antioxidant enzymes of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), scavengers activities of Metallothionein (MTs) and Glutathione (GSH), oxidative stress effect of malondialdehyde (MDA) and Na+/K+-ATPase (NKA) activity in the gill and liver tissues of Mozambique tilapia, *Oreochromis mossambicus* were exposed to combined effects of different pH levels (7.5, control; 5.5, low pH) and Se concentrations (10 and 100 μg/L). The obtained results clearly showed that under low pH condition the toxic effect of Se was similar to the control pH. In both cases, the biochemical responses in fish were altered under stressful conditions. In both stressors response in fish used scavengers activity (GSH and MT) is enhanced against the over production of ROS. Our findings is further demonstrated that *O. mossambicus* exposed to these combined stressors for induced differential responses of antioxidant defence mechanisms of decreased SOD and CAT and an increased GPx. However, organisms were not able to prevent cellular damage (MDA) is produced for oxidative stress when exposed to Se under different pH levels. Furthermore, inhibition of Na+/K+-ATPase activity was showed *O. mossambicus* is exposed combined effects of freshwater acidification and Se.

Keywords: Trace metal; Freshwater acidification; Freshwater fish; Oxidative stress; Neurotoxicity

The establishment of trophic state index in seawater: A case study of Dapeng Bay

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Algal blooms over years have caused considerable worldwide impacts on biological ecology, aquaculture, recreational activities and human health. The causes of algal bloom are mainly derived from the eutrophication of ambient water. Therefore, to develop indices on evaluating the nutritional status of the waters for predicting algal blooms becomes an important technique. At present, most of the indices are focus on freshwater environment (lakes, reservoirs and rivers), including Carlson trophic state index (CTSI) for nutritional status, River Pollution Index (RPI) and Water Quality Index (WQI) for contaminating state. Very few indicators are developed for seawaters. For many years, Dapeng Bay is a lagoon prone to algal blooms. A long-term water monitoring data from 2015 to 2016 indicated that the dissolved oxygen and pH values in the seawater were significantly correlated with the occurrence of algal blooms. In this study, we develop a Real-time Trophic State Index (RTSI) by referring to the nutrient grading system of waters defined by Carlson trophic index. TRSI was calculated with a formula containing the factors of dissolved oxygen saturation and pH value to define the waters eutrophication status. With this new RTSI, we can define the seawaters in oligotrophic at RTSI<2, mesotrophic at 2≤RTSI<4, eutrophic at 4≤RTSI<6, and hyper-eutrophic at RTSI≥6. RTSI is superior to contemporary indices with its simplicity, no complicate nutrient content measurement required, and a real time situation manifesting when the whole system is built under computer control. Using RTSI, we will be able to get alerting on the changes in water quality as earlier as possible, and refer to management control.

Keyword：water quality, algal bloom**,** trophic state index, Dapeng Bay

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Biological responses to multiple stressors: P-93

Study on algae outbreak in Yundang Lake based on deep learning

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Located in the western part of Xiamen Island, Yundang lake is a typical saltwater lagoon, so it's water quality and hydrological conditions have particularity and complexity. Due to the serious eutrophication of water bodies, algal blooms have been frequent for many years. The purpose of the study was to find the key factors for the algae outbreak in Yundang lake, and to select the appropriate machine learning method to propose a model for predicting and warning the algae outbreak, and to provide suggestions for the treatment of algae outbreaks in Yundang lake. By using significant analysis I chose transparency, pH, COD, TP, active phosphate, suspended solids, anionic surfactants and Hg as the key factors affecting algae outbreaks. Using a deep learning algorithm and taking the chlorophyll a concentration as the output to propose a model of algae outbreaks in Yundang lake. The results showed that the prediction results were more accurate when the chlorophyll a concentration was higher. In the next step, we will find a more appropriate method based on this study to propose a model for predicting and warning the algae outbreak and promote them to other areas.

Exploring the determinant for the dominance of cyanotoxins and odorous compound producing microalgae in Hong Kong drinking water sources

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Dongjiang River, the major source of Hong Kong drinking water, has faced severe water pollution stresses in recent years due to the rapid urban and industrial development along its hinterland in Guangdong Province. Microalgae are deeply linked to water quality and serve as a biological indicator. The water quality, especially the nutrient levels, determines the variety and abundance of microalgae. Moreover, the dominance of certain toxin/odour producing microalgae not only affects the quality of the raw water, but also creates severe problems for the subsequent water treatment. In this study, the seasonal succession of microalgal community together with the major environmental variables along the Dongjiang River and in three reservoirs (two mainly contain Dongjiang water and the other mainly contains rainwater) was investigated. Results showed that two blue-green microalgal species, cyanotoxin producing *Microcystis* sp. and odorous compound producing *Anabaena* sp., were among the dominant microalgal species found in two local reservoirs which both mainly contained Dongjiang water; but they were not among the dominant ones along the Dongjiang River, which had higher nitrogen and phosphorous nutrient levels than the two reservoirs. Therefore, except from the nitrogen and phosphorous, there should be other decisive environmental factors which contribute to the massive growth of these two blue-green microalgal species. Thus, the probable physical/chemical conditions and triggering factors for the occurrence and blooming of cyanotoxins and odorous compound producing microalgae is discussed.

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Biological responses to multiple stressors: P-95

A review on bivalves' biofiltration rate: limitations and recommendations for valid comparison

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Bivalves provide important ecosystem functions and services, including habitat provision, water filtration and biodeposition. However, ecosystem functions provided by marine bivalves have been disrupted in recent decades due to multiple anthropogenic drivers such as global climate change, over exploitation and pollution. The biofiltration rate of bivalves is particularly sensitive to various environmental factors, such as temperature, salinity, dissolved oxygen and clarity of water, making them susceptible to stresses. Over the years, a number of studies estimated the biofiltration rate of bivalves under different environmental conditions. But, these studies used different protocols making it difficult to compare the results between studies. Through using the effect of temperature on the biofiltration rate of marine bivalves as an example and applying metadata analysis, this study primarily aimed to examine and identify factors that limit a valid comparison of the biofiltration rate among various studies.

Factors limiting valid comparison of the biofiltration rate of bivalves can be divided into two major categories: experimental conditions and experimental protocol related factors. Since the biofiltration rate of bivalves is sensitive to various environmental factors, the standardization of experimental conditions is critically essential to allow valid comparison of results between different studies. Though the control of experimental conditions is relatively easy to implement, different experimenters often adopted different experimental conditions. Such discrepancies present an obstacle that hinders the generalisation of the results across different species from different studies.

Other experimental protocol related factors include size of animals (e.g., shell height, dry weight), acclimation conditions (i.e., environmental condition, feeding frequency and quantity), acclimation duration, type of the tracer for accessing the biofiltration rate (e.g., seston, suspended algae, neutral red solution, chlorophyll a), recovery time from the last handling of the bivalves, initial concentration of the tracer for accessing the biofiltration rate, and experiment duration. For some of these factors, there have been methods developed to standardize the results from different studies. For example, an allometric equation for describing the relationship between size of bivalves and their biofiltration rates has been developed and used for prediction of the biofiltration rate of various bivalve species based on their size. Although acclimation condition and duration have substantial effect on filtration rate, standardization methods for these factors are currently not available. Therefore, standardization and harmonisation of the experimental protocol are prerequisite for enabling comprehensive and integrative analysis of the data produced from different studies. In view of this, we proposed a guideline to standardize the experimental protocol for measuring the biofiltration rate of marine bivalves.

The size-based bioavailability of land-based don and contribution to Jiaozhou Bay eutrophication

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In recent years, the proportion of DON in the total nitrogen of the Jiaozhou Bay is increasing, and the content of DON in the sea water is high, and there is potential eutrophication pressure. For a long time, the influence of terrestrial DON input on eutrophication is difficult to be determined. In this study, Jiaozhou Bay was taken as the research object, Dagu River (DR), Qingdao Brewery (TB), Haibo River Waste Water Treatment Plant (HWWTP) and Licun River (LR) were selected to represent agricultural source, industrial source, urban living source and urban non-point source respectively, and DON bioavailability and its influencing factors were studied through DON cultivation experiment. By clarifying the relationship between the source, molecular size and bioavailability of terrestrial DON, the bioavailability of terrestrial DON can be further understood from the aspects of the bioavailability proportion (BDON%) and kinetics with the help of ultraviolet means. The bioavailability of terrestrial DON with different molecular sizes was different ($p<0.05$).The bioavailability proportion of high molecular size DON (HDON, >1000Da) is higher than that of low molecular size DON (LDON, <1000Da), with values of 58.04%±2.5%, 54.73%±7.3%, 44.70%±3.8%, and 35.10%±1.9% for HDON from industrial source, urban living source, urban non-point source and agricultural source respectively, and values of 47.2% ± 8.9%, 41.92% ± 1.99%, 38.09% ±9.1%, and 29.5% ± 2.2% for LDON, respectively. There were significant differences in the degradation rate constants ($p<0.05$), with degradation rate constants of 0.3 to 0.67d^{-1} for HDON, whilst 0.13 to 0.75 d^{-1} for LDON. The ultraviolet absorption spectrum of water body can reflect the structure and molecular size of DON in the sample to some extent. The results showed that the values of $SUVA_{254}$ and DOC/DON were significantly and negatively correlated with the bioavailability of DON, which was consistent with the experimental results of DON degradation. In order to study the influence of different terrestrial DON inputs on the water quality of Jiaozhou Bay, a modified 3d coupling process water quality model of Jiaozhou Bay was used to conduct a numerical simulation scenario analysis on the basis of the land source survey data in 2012. Haibo River (industrial source, urban living source and urban non-point source) and Dagu River (agricultural source) are selected for scenario analysis. The DON input of Haibo River contribute more eutrophication on the eastern to the northeastern waters of the bay, approximately 50% converted to DIN in Jiaozhou Bay. While, DON input of Dagu River contribute more eutrophication on the northwestern to the center waters of the bay, approximately 30% converted to DIN in Jiaozhou Bay.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-97

Land-based nutrient load driving the initial outbreak of green tide (*Enteromorpha prolifera*) in the yellow sea by hydro-biogeochemistry modeling

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The green tide formed by large green algae (*Enteromorpha prolifera*) has been outbreaking in the South Yellow Sea for 11 years. The outbreak of large-scale green tide will have a serious impact on the marine environment, ecological functions, landscape and coastal social economy. Eutrophication is generally considered to be the most important driving cause of the green tide in the Yellow Sea. However, it is difficult to obtain the concentration of nutrient in time under realistic conditions, so the prediction and evaluation of eutrophication cannot be performed well. The hydro-biogeochemistry model can better simulate the migration and transformation of pollutants in the sea area. On the basis of establishing the three-dimensional water quality diffusion equation, the other main migration-transformation equations of chemical pollutants are discretized separately, and the corresponding calculation modules are compiled. It is coupled into the water diffusion module to obtain the concentration changes of chemical pollutants such as nitrogen and phosphorus nutrients. The hydrodynamic model is verified by the same-process tide proof-test and whole-process tide proof-test. The results are basically consistent with the satellite monitoring data and the field survey data. According to the input of pollutants in Jiangsu near shore in 2015, the concentration changes of nitrogen and phosphorus nutrients are simulated by model. The results show that the cosine similarity coefficient (SI) of DIN is about 0.71±0.03, with average RSD of 34% ± 4%; whilst the SI of PO4-P averages about 0.58 ± 0.10, with average RSD of 43% ± 2%. Based on the hydro-biogeochemistry model, the influence of the land-based nutrient load on the occurrence of green tide is discussed. The results showed that the nutrient load of the Jiangsu was more contributed to the initial outbreak of the *E. prolifera,* where lies in the vicinity of the northern Jiangsu shoal. While, the nutrient load of the Yangtze River might be the reason of the green tide moving to the eastern part of Nantong.

Nutrients and phytoplankton assemblage responses to anthropogenic sources in Shenzhen coastal water, China: Results of mesocosm studies

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The ongoing increase anthropogenic sources inputs to coastal water has changed the seawater quality and increasing nutrient discharge has contributed to a shift in nutrients and phytoplankton in coastal water. Shenzhen Bay is a semi-enclosed shallow bay in the Greater Bay Area of China, which was seriously polluted during the past 40 years. However, few studies were known about anthropogenic sources input on phytoplankton community change in winter by filed mesocosm method. This project assessed nutrient dynamics and variability in phytoplankton community composition in response to anthropogenic nutrient sources by filed mesocosm culture method, each with distinct sources from aquaculture pond, urban drainage channel, industrial sewage outlets. Specific objectives were to (1) investigate dynamic change of dissolved nutrients and composition in mesocosm, and (2) quantify phytoplankton biomass and growth rate responses to various anthropogenic nutrient sources. The results showed that the nutrients concentration decreased after anthropogenic source water addition. The TDN and TDP increased obviously in winter with the addition of land-based pollutants. In addition, due to atherogenic wastewater addition. The proportion of organic and inorganic nutrients had also changed in mesocosm. Compared with control mesocosm experiment, the biomass of phytoplankton with addition of anthropogenic source water was significantly high($p<0.05$). Originally, dominant species of control mesocosm were *Gloeotilopsis planctonica* and *Klebsormidium silva*, which account for 21.3%, and 62.1% of the total cells abundance. However, dominant species of experiment mesocosms were *Klebsormidium silva*, *Stichococcus bacillaris* and *Synechocystis* sp. in S2, S3 and S4, experiments mesocosm, respectively, which account for 82.3%, 42.7% and 42.9%. Phytoplankton community had changed induced by anthropogenic source input. Different anthropogenic source had various effect on phytoplankton community change. The anthropogenic source water input promoted the breeding of Bacillariophyta, Cyanophyta and Chlorophyta. The correlation between sewage nutrient and abundance of phytoplankton cells indicated that negative correlation appeared in Chlorophyta with nutrients content in winter. The phytoplankton cells was the lowest density with only 1.65×10^7 cells/L in winter culture period, because water temperature, light and other environmental factors of winter limited the phytoplankton to grow in a certain degree. This study highlights the importance of anthropogenic sources inputs on nutrients and phytoplankton assemblage, particularly nutrients in the forms of DON and DOP if harmful algal blooms were to be controlled. This suggests that effective controlling the land-based nutrients discharge of inorganic and organic forms, such as unused feed and organic excreta from urban rivers and mariculture ponds, may improve water quality and reduce the annual occurrence of harmful algal blooms in the adjacent the Greater Bay Area of China.

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Biological responses to multiple stressors: P-99

Algicidal activity of two bacterial strains isolated from *Karenia* blooming water against *K. Mikimotoi*

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The potential use of algicidal bacteria as one of the biological controls to suppress harmful algal blooms has been extensively studied in the past decades. Both Hong Kong and mainland China have suffered greatly from blooms of *K. mikimotoi*. In 2016, icthyotoxic *Karenia* bloom spread across Tolo habour in Hong Kong. Massive fish kills were observed in several fish farming zones during the outbreaks of the harmful algal blooms, which were estimated to have killed 200 tons of fish. However, the bloom naturally demised after a month. We hypothesized that algicidal bacteria may play a role in the depletion of the algal bloom.

In this study, two bacterial strains were isolated from the waters during the *Karenia* bloom in Tolo habour (one particle associated bacterial strain (P4) was isolated via filtration of the blooming water and another free-living bacterium (F5) was isolated from the filtrate of blooming water). The algicidal effect of these two bacterial strains on *K. mikimotoi* was investigated. Log phase cultures of *K. mikimotoi* were exposed to the coressponding bacterial cultures for 14 days. The results showed that the algal cell concentration was significantly reduced ($P<0.0001$) 78% and 80% after exposure to each bacterial culture P4 and F5 respectively. Interestingly, similar results were observed in exposure with coressponding bacterial supernatant (i.e the fermented bacterial broth). These results indicated that these two bacterial strain were potential algicidal bacteria for biological control of *K. mikimotoi*. Algicidal activity found in the exposure with bacterial supernatant implicated that the release of algicidal compounds are involved in the killing action of the *K. mikimotoi*.

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Growth and toxicity of *Karenia mikimotoi* strain isolated from Hong Kong waters

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Karenia mikimotoi was well-known ichthyotoxic dinoflagellate species which blooms dominated by this species are often leading to massive kills of fish and shellfish around the world. *K. mikimotoi* has been regularly found in Hong Kong waters since 1980s. Massive fish kills were reported in a number of fish farming zones in Tolo Harbour and Long Harbour during the outbreaks of algal blooms caused by *K. mikimotoi* in 2016. More than 200 tons of fish were estimated to be killed in the algal blooms. Although there have been many studies on this ichthyotoxic species over the past decades, it remains unclear how this species is killing fish and shellfish. Moreover, study on local strain of *K. mikimotoi* is extremely limited.

In this study, growth and ichthyotoxicity of a *K. mikimotoi* strain isolated from *the* corresponding algal blooms in Yim Tin Tsai during the period of December 2015 to February 2016 were characterized. Growth rate and maximum cell density of the local strain growing in three different culture media (f/2-Si, Li and GSe) were determined and compared. Ichthyotoxicity of the strain was evaluated by exposing the fish model marine Medaka (*Oryzias latipes*) to log phase *K. mikimotoi* cells at $2.5X10^5$ cells mL^{-1}. Our results indicated that the local *K. mikimotoi* strain achieved the highest growth rate (0.249 day^{-1}) and maximum cell density ($2.5X\ 10^5$ cells/mL) when the cells were cultivated with L1 medium. Specific growth rate of other *K. mikimotoi* strains that have been reported generally range from 0.15 to 1 day^{-1}. Therefore, the local strain of the present study demonstrated a moderate growth rate when compared to other *K. mikimotoi* strains. For the toxicity test, 100% mortality of medaka fish was recorded after 60 minutes of exposure. The medial lethal time (LT_{50}) was around 30 minutes. No mortality was found in seawater control throughout the experiment. Upon the exposure to the *K. mikimotoi* cells, the medaka fish swam fast and tilted up and down at the beginning stage. Then medaka fish was struggled to swim and losing of balance with spasm was observed. The medaka fish would eventually die with stop moving of fish operculum. No blockage of mucus and algal cells was observed by naked eyes in the gill of the exposed medaka fish which indicated that the killing effect may not be attributed to the blockage of the fish gill.

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Biological responses to multiple stressors: P-101

Reproductive toxicities of P-CTX-1 in marine medaka (*Oryzias melastigma*)

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Ciguatera Fish Poisoning is the most frequently reported seafood-toxin illness in the world caused by consumption of seafood containing Ciguatoxins. Among the Pacific Ciguatoxins, P-CTX-1, has been regarded to be the most toxic member. The objective of this study is to investigate the reproductive toxicities of P-CTX-1 in marine medaka. Five pairs of four-month adult medaka fish were exposed to P-CTX-1 for 4 weeks at five doses of P-CTX-1 by feeding with alive marine medaka larvae microinjected with P-CTX-1. There was a negative correlation between egg-productivity of female medaka and feeding dosage. A single feeding dosage at 1665 ppb per week was selected for evaluating parent-of-origin effect of P-CTX-1 in the developing medaka embryos. The results indicated that the average hatching time of the eggs from medaka parents fed by P-CTX-1 was 17.50±4.89 days which was longer than that of normal offspring at 12.67±2.07 days. Hatching rate of the offspring from medaka parents after feeding by P-CTX-1 at 1665 ppb per week was 4.76±0.02% which was much lower than that of normal offspring at 87.92±0.04%. Moreover, abnormal swimming, weight lost and diarrhea were observed in adult medaka after exposure to P-CTX-1. With more results on the quantification of P-CTX-1 in the tissues of ovary and testis in adult marine medaka and their offspring, we can provide more information on how the P-CTX-1 being transferred from parents to offspring. This study advanced the understanding of P-CTX-1 mediated reproductive toxicity in fish, and thus contributed to the toxicity assessment of CTXs in marine ecosystems.

Bioaccumulation of microcystins and its antioxidant responses in brackish water flea: evidence for the possibilities of accumulation and impact on marine environments

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Microcystins (MCs), toxins produced by cyanobacteria (e.g. Microcystis, Anabaena, Oscillatoria and Nostoc genera), is one of the great issues which are concerned in fresh water environments. Recent studies have reported that major harmful algal blooms affect lakes, rivers, wetlands, even estuaries and nearshore marine waters. In Korea, particularly, the estuarine environment is likely to have high concentration of MC outflow due to the occurrence of harmful algae on the inner side of the dam in Summer season and the sudden discharge. Furthermore, many studies already have reported that microcystis have tolerance to salinity. However, effects of microcystins in marine organisms are still poorly understood in estuarine. In this study, lab-based experiments firstly carried out using MC-LR to find the effect of MC on brackish water flea *Diaphanosoma celebensis* as oxidative stress inducer. Then, comparative studies were conducted using the field sample (microcystis bloom occured Nakdong River water). The experiments using MC-LR (62.5, 125, 250 and 500 μg/L) showed significant transcriptional modulations of antioxidant enzymes (superoxide dismutase (*Mn-SOD* and *Cu/Zn-SOD*), catalase (*CAT*), and *glutathione S-transferase* (*GST* kappa, mu, and theta)) and increasing of SOD activity and lipid peroxidation (LPO). The water collected from the Nakdong River was measured for dissolved and particulate MC concentrations after different treatments of salinity, and bioaccumulation was measured in *D. celebensis* exposed to water of 15psu after 48 hours. In water sample from Nakdong river, significant increase in particulate MCs with increasing salinity demonstrates the need for further studies on the production of MC in microcystis according to salinity that are resistant. Furthermore, the biological accumulation was observed in the water collected from the Nakdong River which was exposed to brackish water flea *D. celebensis* although the concentration of MCs was significantly low (less than tens of □g/L, sum of dissolved and particulate forms) compared to lab-based experiments (hundreds of □g/L levels, 62.5, 125, 250 and 500 μg/L). The antioxidant response measured by the mRNA expression of several antioxidant genes (*CAT*, *GSTmu*, *GSTtheta*, *Cu/Zn-SOD*, and *Mn-SOD*) of *D. celebensis* were also significantly modulated according to the dilution of Nakdong River water. The findings observed during MC test in Nakdong River water suggest that MCs would act as possible contributor to oxidative stress on *D. celebensis* through regulation of antioxidant mechanisms and affect to estuarine ecosystem by accumulation in organisms even in low concentrations.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-103

Quantification of ammonia removal process and ammonia oxidizing archaea and bacteria in Pearl River Estuary in summer

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Among the various nutrient, nitrogen has received particular attention because of the magnitude of the associated environmental concerns and complexity of nitrogen cycling after discharged into the aquatic environment. The Pearl River Estuary is one of the most complex estuarine systems connecting to the South China Sea through three sub estuaries, Lingdingyang, Modaomen and Huangmaohai. The rapid economic development in this region has led to excessive discharge of nutrients and pollutants in the past 30 years. The transformation of the ammonia is a critical step in the inorganic nitrogen cycling. Nitrification is one of the oxygen consuming process in bottom hypoxia formation in Pearl river estuary. In this study, we investigated the nitrification rates and ammonia uptake rates through stable isotope and quantitative PCR of ammonia oxidizing archaea and bacteria to reveal the ammonia removal processes in this dynamic estuary. Ammonia were assimilated by phytoplankton at the surface layer even in the dark condition while nitrification was the dominant process in the bottom water. In general, AOA outnumber beta-AOB in most of the stations showing ammonia oxidizing archaea is the dominant nitrifier in the estuary. AOA is the dominant nitrifer and mostly in the free-living form while AOB peaks at the upper part of estuary in the particle-attached fraction. Differential distributions of AOA and beta-AOB showed the niche partitioning of these two nitrifers in the dynamic estuary.

The partitioning and distribution of different forms of phosphorus in a eutrophic estuary in the northern Taiwan

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The partitioning and distribution of different forms, including dissolved and particulate inorganic and organic P (DIP, DOP, PIP and POP), of phosphorus in an eutrophic estuary in northern Taiwan were studied by means of measuring their concentrations in the estuarine waters. In addition, estuarine sedimentary phosphorus (SP) were also determined by the sequential extraction method which divides the SP into five fractions. The total dissolved and particulate P concentrations within the estuary ranged within 0.42-12.42 μM and 482-6190 mg kg^{-1}, respectively, and the distributions generally exhibited the removal behavior. DIP generally accounted for more than 80% of the total dissolved P (TDP) pool, within the middle estuary. However, this contribution decreased with increasing salinity and dropped to less than 20% of TDP. In contrast, DOP became dominant in the lower estuary because its concentration was slighter higher than DIP. PIP dominated the total particulate P (TPP) concentration and accounted for 43-86% of the TPP pool within the estuary. The partitioning coefficient of P ($\log(K_D)$) fell in the range of 4.03-4.59 and the value seemed to slightly increase at the lower estuary. The total concentrations of SP within the estuary ranged within 398-1780 mg kg^{-1}, and the concentrations positively correlated with total organic carbon and negatively correlated with sediment grain size. It is worth noting that SP was chiefly dominated by the Fe-bound fraction, accounting for 30-79% of the total SP pool. The detrital P played the second important fraction and accounted for 10-62% of the total SP pool.

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Biological responses to multiple stressors: P-105

Distribution and partitioning of nitrogen species in a eutrophic estuary in the northern Taiwan

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The Danshuei estuary in the northern Taiwan is an eutrophic estuary which is continuously input of the domestic effluent. Nitrogen speciation, including ammonium, nitrite, nitrate, dissolved organic N (DON), particulate inorganic N (PIN) and organic N (PON), in the estuarine water were analysed and their partitioning and distributions were studied. In addition, the estuarine sediments were also analysed for the total N and total organic C (TOC). An extremely high concentration, up to 450 μM, of total dissolved N (TDN) was determined at the upper estuary and the value decreased to about 100 μM at the lower estuary, suggesting that Danshuei estuary is a poorly eutrophic estuary. The contribution of DIN (ammonium + nitrite + nitrate) could exceed more than 80% of total dissolved N (TDN) within estuary. Ammonium was the major speciation of DIN and a relatively high concentration, up to 370 μM, was determined at the middle estuary. The concentrations of total particulate N (TPN) ranged within 1100 - 21800 mg kg^{-1} within the estuary and PON accounted for 39-91% of the TPN pool. The distributions of these speciation of N in the Danshuei estuary generally decreased with the increasing salinity. The value of distribution coefficient, log (K_D), between TPN and TDN ranged from 2.75 to 3.65 within the estuary and the value variation with salinity was not significant. The concentrations of total N and TOC in the estuarine sediment ranged within 0.36-1.03% and 0.11-2.00%, respectively. A relatively lower ratio, 0.13-2.32, between TOC and TN in the sediment of the Danshuei estuary, suggesting that this estuary is a nitrogen enriched environment.

Application of anaerobic bacterial ammonification pre-treatment to microalgal foodwaste leachate cultivation and biofuel production

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Foodwaste constitutes the largest component of municipal solid waste in many urbanized societies. Direct discharge of nutrient-rich foodwaste leachate (FWL) impacts the environment by eutrophication of body of water. Our previous study demonstrated the use of microalgal technology in treating FWL and for biofuel production. Two robust species, *Dunaliella tertiolecta* and *Cyanobacterium aponinum* were selected as they can tolerate under diluted FWL up to 10%. However, suppression of growth observed in higher concentration of FWL that hindered the application.

In this study, we alleviated this issue by introducing an in-situ anaerobic bacterial ammonification pre-treatment prior to microalgal cultivation. Anaerobic bacterial digestions at pH 7 would not only transform organic nitrogen for microalgal growth but also produce methane as another source of biofuel. Upon anaerobic bacterial pretreatment of FWL, the concentration of resulting digestates for optimum growth of *C.aponinum* and *D.tertiolecta* were increased to 30% and 50% respectively, where over 80% of nutrients from the digestate were removed by this anaerobic bacterial-microalgal hybrid system. The hybrid system can also operate in continuous mode, where the algal strains grew steadily at 6 hour hydraulic retention time (HRT). The fatty acid methyl ester (FAME) profile of the resulting biofuel were analysed, where C_{18} stearic and $C_{20\text{-}22}$ unsaturated fatty acids were the major components. The biodiesel produced from the anaerobic bacterial-microalgal hybrid system was similar to other existing products and can be used as a feedstock for alternative source of energy.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-107

Estimation of nitrogen and phosphorus losses from agricultural system in China based on big data

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Agricultural pollution is a major contributor to water pollution, especially to the high concentrations of nitrogen and phosphorus in surface water. Clear evidences have shown that the continued water contamination of nitrogen and phosphorus is dominated by the increased chemical fertilizer consumption in food production. Understanding the distribution of nitrogen and phosphorus losses from the agricultural system in China will help stakeholders become active in making proper decisions for ecologically sustainable development. Over the years, many studies have been carried out to investigate the non-point-source nitrogen and phosphorus losses over different regions within China. In this study, we examined a large sample of the related scientific literature covering most provinces except for southwestern China, published over the period spanning 1993 to 2019. Several variables, such as nitrogen and phosphorus loss, crop types, slope and geographical information, were extracted from these studies, with the aim of determining the contents of total nitrogen (TN) and total phosphorus (TP) in soils. Finally, combined with CMADS precipitation and literature information, the amount of nitrogen and phosphorus losses in China was estimated. Results show that there is a large spatial variability of the total amount of nitrogen and phosphorus loss in China, which is closely related to the diversity in terrestrial environments and agroecosystems. The loss of TN ranges from 0.00125kg/hm^2 to 2993kg/hm^2, and the loss of TP varies between 0.00018kg/hm^2 and 786.3kg/hm^2.

Hypoxia in autumn of the East China Sea

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Hypoxia in coastal oceans has expanded globally since last half century. It has also been frequently observed in the inner shelf of the East China Sea (ECS) since the 1950s, especially outside the Changjiang River estuary. The area of hypoxia could even reach 15,400 km^2 in the ECS. It should be noticed that this and nearby area is also an important fishing ground of the ECS. The hypoxia has been mostly pronounced in summer of the ECS. Surprisingly, this phenomenon was observed in autumn, at the end of October, in this study. The DO value could lower to 1.32 mg l^{-1}. Temporally, this might be one of the latest hypoxia persisted throughout the year in the ECS. Accompany with hypoxia, high concentrations of nitrate and dissolved inorganic carbon but with low pH values were also observed in this area. This finding makes us to re-consider and re-evaluate the impact, both temporal and spatial, of hypoxia in the ECS.

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Biological responses to multiple stressors: P-109

Upwelling impact on phytoplankton blooms and hypoxia along the China coast in the East China Sea

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Phytoplankton blooms and hypoxia has frequently occurred along the coast off the Changjiang estuary and the Zhejiang in the East China Sea. Surprisingly, upwelling has also been observed in the similar regions. These phenomena are mostly pronounced during summer period. In this study, we intend to explore how upwelling impact on algal blooms and hypoxia concurrently. Results showed that hypoxic water, DO conc. ≤ 2.0 mg l^{-1}, was upwelled to 5-10m below surface water. Accompany with hypoxic event, high dissolved inorganic nutrients, e.g., nitrate and phosphate, of bottom water was also upwelled to surface water. Phytoplankton blooms, Chl *a* conc. > 6.0 μg l^{-1}, were observed in the surface water, and its growth was mainly stimulated by enriched nutrient of upwelled water. This study suggests that both positive and negative effects of upwelling on ecosystem should be evaluted simultaneously in the future.

Effects of hypoxia on field distributions and laboratory physiological changes of mesozooplankton

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Spreading hypoxia is an increasing threat to the marine ecosystem, but the effects of hypoxia on mesozooplankton have not been fully understood. In this study, we investigated the distributions of mesozooplankton in relation to hypoxia in the field and further examined their physiological response to hypoxia from aspects of survival, respiration, and ingestion in the laboratory. Field survey was conducted in the PRE, a seasonally hypoxic estuary in China. Our results showed that copepods were the predominant group in mesozooplankton both in surface and bottom layers at all the stations. Mesozooplankton exhibit strong diel vertical migration (DVM), and most of mesozooplankton could reside in the hypoxic waters during daytime due to the DVM. The presence of bottom hypoxia seemed less influenced the mesozooplankton distribution than DVM in surface and bottom waters. In the lab, three representative mesozooplankton (copepods: *Paracalanus parvus*, *Acartia erythraea* and *Temora turbinate*) species were isolated and examined at the simulated hypoxic conditions (hypoxic: 2 mg L^{-1}, control: 7 mg L^{-1}). We found that *P. parvus* and *A. erythraea* showed no decline in survival at hypoxic conditions, while *T. turbinate* was more sensitive to hypoxia, with a survival rate of 79 % at hypoxic conditions. Feeding of *P. parvus* was also not influenced by hypoxia, while the feeding rate of *A. erythraea* and *T. turbinate* dropped significantly to 68.1% and 33.5%, respectively, under hypoxia condition as compared with those in the controls. A decrease of respiration rates was observed in all three species at hypoxic conditions, but the small copepod *P. parvus* seemed less been affected by hypoxia than the other species. Our laboratory results indicated that copepods tolerance to hypoxia is species-specific, and their metabolism may reduce at the hypoxic condition. Coupling with our field data that quantifying copepod biomass in the PRE, hypoxia could cause a decrease of 19.0 ± 6.46 % utilized carbon by respiration, equivalent to 72.2 ± 69.7 mg C m^2 day^{-1}.

International Conference on Marine Pollution and Ecotoxicology

Biological responses to multiple stressors: P-111

The seasonal and spatial variation of diatom-dinoflagellate in the hypoxia nearshore area of northern Yellow Sea, Yantai, China

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The nearshore area of northern Yellow Sea in Yantai, China is typically affected by anthropogenic activities, which can be divided into three parts, Sishili Bay, the Muping ocean ranching area (MR) and off-Muping ocean ranching stations (off-MR). Summer hypoxia was recorded in this area, so the seasonal and spatial variation in phytoplankton were studied in 34 stations from May to September in 2016. Seasonal variation in dissolved oxygen (DO) showed that averaged bottom-DO (2m above the benthos, B-DO) decreased from July and reached to the lowest at 3 mg L^{-1} in August, and then increased to 5 mg L^{-1} in September. The seasonal variation in the abundances of diatoms and dinoflagellates were both correlated with B-DO though in different ways; diatom abundance decreased with decreasing B-DO, whereas dinoflagellates increased with decreasing B-DO. The seasonal variation in dominant species showed that diatom was the absolute dominant species except for August. *Paralia sulcate* was the dominant diatom species except for August when the *Ceratium macroceros* absolutely dominated as a dinoflagellate species. Spatial variation in B-DO showed that it decreasing from Sishili Bay from May and then intensified and extended to MR and Off-MR. B-DO of Sishili Bay was significant lower than that of Off-MR in June and July, same with spatial variation in diatom, but dinoflagellate had opposite spatial distribution in June and July. No spatial difference was found for B-DO in May August and September, same with both diatom and dinoflagellate except for a significant higher abundance in Off-MR in September. As an absolute dominant species in August, *C. macroceros* had no significant spatial difference, same with the B-DO. *P. sulcate* had significant higher abundance in Off-MR than Sishili Bay in each month and even disappeared in August, the same trend with spatial variation in B-DO. Both the seasonal and spatial variations in diatom-dinoflagellate abundance and dominant species showed the negative effects of DO decreasing on the diatoms and the relative tolerance in dinoflagellates. Results in variation of dominant species also indicated *P. sulcate* and *C. macroceros* can be used as potential diatom-dinoflagellate indicators.

Spatiotemporal variation of meiofauna assemblages in relation to natural and anthropogenic factors in two contrasting coastal habitats

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Our study aimed to investigate the spatio-temporal distributions of meiofaunal assemblages and their associated key environmental variables in two distinct nearshore habitats. The two habitats were a semi-enclosed bay on the south coast of Korea (Jinhae Bay) and an open coastal region in the East Sea, Korea (Samcheok coast), respectively. Biotic and abiotic data were collected from six sites in the two habitats over 12 consecutive seasons (i.e., three years, from 2013 to 2016). First, the results generally indicated that most of environmental parameters, such as dissolved oxygen, sedimentary organic content, and sediment facies were significantly different between the two target areas. Similarly, the significant differences in meiofauna assemblages were found cross two study areas, among seasons, and among sites within each area. Of note, the estimated variation of meiofauna assemblages among sites were greater compared to the variation detected among seasons or between areas. The site-specific variations in meiofauna assemblages were attributable to various local factors, including sediment grain size, sediment organic content, sea-water temperature and salinity. Indeed, sediment properties were found to be the predominant environmental factors driving the spatial and temporal variations of meiofauna assemblages in both areas. However, it should be noted that the anthropogenic stresses, such as hypoxia etc., were superimposed on the natural environmental factors at Jinhae Bay. The present study confirmed both natural and anthropogenic factors collectively influenced the site- or region-specific variabilities in meiofauna assemblages.

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-113

Modelling fate and transport of polychlorinated biphenyls in the abyssopelagic ecosystems

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It is widely accepted that marine pollution by persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) is systemic and is a serious threat to the ecosystems. Whilst exposure of various trophic-level consumers to PCBs in epipelagic zones can be inferred from monitoring datasets, PCBs concentrations of, and fluxes to, meso-to-abyssopelagic zones are largely unknown. A few studies reported bioaccumulation of PCBs in the hadalpelagic zones, such as the Kermadec and Mariana Trenches. However, fate and transport of PCBs in such deep-sea ecosystems have been poorly understood.

In this study, using Finely-Advanced Transboundary Environmental model (FATE), we examine PCBs concentrations of, and fluxes to, abyssopelagic zone. FATE is the most recently developed 3-D dynamic multi-media model and is capable of predicting the global fate and transport of PCBs in and across the atmosphere, oceans, soil, vegetation, and cryosphere. The modelled ocean compartment includes much of the abyssopelagic zone and reproduces dynamical processes (advection and diffusion) with spatial resolutions of 1.0° × 1.0° × 50 layers (0 - 5,500 m). The model also reproduces bioconcentrations in lower trophic-level organisms (particulate organic carbon (POC) as a proxy for phytoplankton and zooplankton) and bioaccumulations in global fish communities whatever the trophic levels.

Measured PCBs concentrations data for deep-sea organisms and sediments was compiled from open literature. FATE was forced by emission inventories, climate data, land cover and marine phytoplankton data, to facilitate evaluation of global PCB dynamics for the period 1931–2018 for selected PCBs congeners (including PCB#28 and #153). Results of FATE simulations showed relatively higher PCBs concentrations in POC at the bottom of the abyssopelagic zone over Mariana, Japan, Kuril-Kamchatka, Puerto Rico, Java Trenches than over Kermadec and Tonga Trenches. Our analysis of dynamical and bioaccumulative processes through model-data comparisons, pointed to the importance of downward fluxes of PCBs, and helped identify pollution 'hotspots' in the abyssopelagic ecosystems over the world.

Changes in microbial community response during natural purification of pollutants in the tidal flat system; a mesocosm study

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While microbial communities naturally vary from freshwater to saline aquatic system, their compositional shift would be one potential index in natural purification process in given environment. The present study employed a laboratory controlled mesocosm system to estimate the purification capacity of a tidal flat by monitoring of microbial community changes for 7 days. Microbial community changes were analysed by use of metagenomic approaches; DNA extraction, followed by Illumina Miseq and analyses of alpha and beta diversity based on the 16s rRNA gene amplicons using the softwares QIIME and R. In the mesocosm types mimicking marine environment (bare tidal flat, vegetated flat, and polluted sediment), total nitrogen (TN) mitigation was observed as the fertilizer increased to 1% and 5%. Meanwhile, the total organic carbon (TOC) concentrations did not show certain trend(s) during the periods of mesocosms. With the results of the high-throughput sequencing analyses, a total of 7,450 operational taxonomy units (OUT) were observed. Two phyla (Proteobacteria, Bacteroidetes) dominantly accounted for >78.1% of the total phyla, based on metagenomic analysis, followed by the six phyla (Verrucomicrobia, Chloroflexi, Cyanobacteria, Actinobacteria, Acidobacteria, Firmicutes, Gemmatimonadetes, and Planctomycetes), with >17.4% out of the total phyla. In the mesocosm with vegetated flat, alpha diversity (Chao1) timely increased, indicating the corresponding microbial communities responded to the TN mitigation. PCoA plot showed the similarity in microbial community between the bared and vegetated flat, and dissimilarly was observed between non-polluted and polluted sediment. Overall, the results collectively suggested that the compositional changes in microbial communities are strongly associated with the purification process of pollutants in contaminated environments. The future study addressing taxa dependent mechanism would be necessary.

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-115

Sensitive response of nematode community to organic pollution: A laboratory microcosm experiment

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Meiofauna can offer many advantages as biological indicators since they are ubiquitously distributed and highly diverse in marine sediments. As the dominant meiofaunal group, free-living marine nematodes have a direct development phase, with a short life-cycle of one to three months. Hence, changes in nematode community can be readily observed in short-term studies than many macrofaunal groups. In the present study, a two-month laboratory microcosm experiment was conducted to assess the response of nematode communities to organic pollution, i.e., to compare nematode communities in sediments with different organic contents, which were manipulated by mixing defaunated sediment formed by defrosting a frozen sediment sample collected in Victoria Harbour, Hong Kong with the same sediment, after the removal of organic matter through combustion. Three treatments with high (1.24% of TOC), medium (1.14% of TOC) and low (1.01% of TOC) organic contents were set up. At the start of the investigation, fresh sediments containing the same nematode community collected from Victoria Harbour were added to each of the three treatments. The nematode communities in these treatments at the end of the experiment were analysed. Results showed that nematode abundance was the highest in the high organic content treatment. No significant differences were found for species number, Shannon-Wiener Diversity index and species richness among the treatments. However, there were significant differences for feeding types of nematode community, which showed that nematode communities in the medium and low organic content treatments had higher trophic index values than that in the high organic content treatment. Biological trait analysis also showed that nematode community in the low organic content treatment had higher proportions of nematodes with longer adult length than those of other two treatments. The present findings suggested that nematodes could reproduce and proliferate quickly in sediments with high organic content, therefore increasing their abundance substantially within the two-month study. In addition, feeding types combined with biological trait analysis could be more sensitive indicators to reflect the effects of organic pollution than traditional diversity indices even under small organic content differences.

Toxicity evaluations of sediments collected in Tokyo Bay using embryos of Java medaka (*Oryzias javanicus*)

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Because of the low water solubility, hydrophobic chemicals widely spread in the aquatic environment, and continuously settle on sediments. Especially, we already know that sediments in the coastal area near the city with high density of populations and industrial area are polluted by chemicals discharged by the human activities. Sediments are also an important habitat not only for the benthic animals but for even eggs spawned by fish. However, chemicals polluted sediments have been possibly threaten to the benthic organisms in aquatic environment especially closed to the big cities, and most of their present conditions have been almost unknown. Therefore, the explorations for sediment toxicities to the aquatic organisms are strongly required.

The Ariake sea is located in the Kyushu Island, western Japan, and has the hugest tidal flat in Japan. The sea is surrounded by Fukuoka, Kumamoto, Nagasaki and Saga prefactures, and has only one bay mouth. Some rivers such as the Chikugo and Omuta Rivers flow in the sea. While this is one of the major areas with many fishery resources in Japan, the resources and biodiversity have been reduced and lost, respectively, in the sea by some kinds of pollutants.

Fish embryos are particularly suitable for chemical testing as early life stage assay, because they are particularly sensitive to chemicals, easily observed their abnormalities, and no-require the large scale facility. Java medaka (*Oryzias javanicus*) can be easily kept similar with freshwater medaka, except for using sea water, and obtained embryos the year round. Therefore, this fish has been increasing to use as the marine indicator in the several ecotoxicological fields. In this study, we investigated and evaluated the toxicities in tidal flat sediments collected in the Ariake Sea using the embryos spawned by Java medaka. We prepared the only sediments with the slight pore water and without upper water, and exposed embryos to pollutants in sediments.

While only low mortality was observed for embryos control group (3.3 ± 4.7%), 96% mortalities of embryos kept on sediment collected at the north part of the Ariake sea was observed. This site closes to the river mouth, and the contribution of chemicals achieved there with river was suspected. Additionally, the delay or shortening of the period from embryo to hatch, unhatching, and abnormalities in embryo or on hatching larvae, and were observed. Although we explore the relationship between the toxicities in sediments and the residue levels of PAHs and heavy metals such as Zn, Cu, Pb, and Cd, we couldn't the remarkable correlation. The toxicities caused in embryos were different and depended on each site, perhaps there might be some the pollutants affected to organisms.

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-117

Toxicity evaluations of sediments collected in Ariake sea using embyos of Java medaka (*Oryzias javanicus*)

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Tokyo Bay closes to the populated areas as Tokyo metropolitan area, and Yokohama and Chiba cities and also has the large industrial areas as Keihin and Keiyo Industrial Areas along the shoreline. As a result, many kinds of pollutants directly and indirectly discharged to Tokyo Bay. Because the bay has only a narrow mouth and the water exchange is limited, the pollutants result to be residue in the bay for long period. Polycyclic aromatic hydrocarbons (PAHs) are derived from petrogenic sources such as oil spill or pyrogenic sources associated with the smoke from industrial operations and the exhaust gas from vehicles with gasoline or diesel fuel. In the present study, the distributions of parent and mathylated PAHs in sediments were investigated at Tokyo Bay. The sediments were collected by Ekman-Berge grave sampler along with shoreline (25 sites). PAHs were measured by GC/MS.

The high concentration of total PAHs including dibenzothiophene were detected on the site near Kawasaki city (12,252 to 34,391 ng/g dry weight). At this site, PAHs with molecular weights with more than 178 were detected with especially high concentrations, for example, 3639 ng/g of fluoranthene, 3944 ng/g of pyrene, and 4740 ng/g of benzo(a)pyrene. On the other hands, the total concentrations of sand area especially widely spread in east side of the bay were detected below 300 ng/g dry weight. PAHs with high concentrations tended mainly to distribute on the shoreline along with the west side, and this tendencies might be deeply related to the circumstances around the sites and the qualities of sediments.

Positive changes in trophodynamics and population dynamics of a benthic predatory fish following the trawl ban in a heavily disturbed marine environment

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Hong Kong's coastal waters were known to be ecologically diverse due to the interplay of hydrography, habitat heterogeneity and anthropogenic disturbances. However, unrestricted fishing activities and pollution have led the Tolo area of Hong Kong from a productive ecosystem to an impoverished one over the past decades. This study aimed to investigate the trophodynamics and population dynamics of a predatory benthic fish, *Platycephalus indicus*, in the Tolo area after a permanent territory-wide trawl ban being implemented on 31st December 2012. We hypothesized that the trawl ban would reduce anthropogenic disturbances on the benthic ecosystem and thereby promote the recovery of the population of *P. indicus*. Specifically, the fish population was expected to have a broader trophic niche, and show increases in trophic position, abundance, biomass and size after the trawl ban. We collected *P. indicus* from inner and outer Tolo areas before (2012) and after (2015) the trawl ban, while the samples were subject to stable isotope analysis. The results of stable isotope analysis indicated that trophic niches and trophic positions of *P. indicus* collected from both inner and outer Tolo areas significantly increased after the trawl ban. Concurrently, their abundance and biomass increased significantly in outer Tolo area, while size of the fish in inner Tolo area became significantly larger after the trawl ban. These are positive signs of recovery of the population associated with the reduction of fishing pressure. By examining the available water quality data of the seabed, we found that there was a significant decrease in turbidity in both inner and outer Tolo area after the trawl ban. This study provided evidences on the initial recovery of the population of *P. indicus* in Tolo areas, indicating that the management intervention with the trawl ban was effective to facilitate ecosystem recovery and fisheries resource enhancement. However, declining trends in dissolved oxygen and pH were observed in inner Tolo area, triggering a concern. Further management actions will be required to investigate the root of these problems and improve the water quality in inner Tolo area.

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-119

Impact of antibiotic contamination on benthic microbial community in Rongjiang River

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Antibiotics are widely used in aquaculture, agriculture, and medicine to prevent control and control human and animal diseases. It is estimated that in 2013 more than 53,800 tons of antibiotics were discharged into rivers and streams in China. However, previous studies demonstrated that antibiotics may cause adverse effects on microbial communities that are essential for recycling nutrients, mineralization of organic matter, and degradation of organic pollutants. The goal of this study was to establish the effect of aquaculture on estuarine ecosystems. We investigated the characteristics of microbial communities in sediments collected from Rongjiang River using high throughput sequencing to offer new insights on the impact of anthropogenic activities in freshwater and estuarine ecosystems. The concentration of inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^+) and antibiotics (tetracyclines, quinolones, sulfonamides, and macrolides) were determined in river and estuarine sediment.

Changes in soil microbial biomass and community structure of a constructed mangrove wetland for municipal wastewater treatment during 10-years operation periods

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Constructed wetlands (CWs) have been used for wastewater treatment in many countries, however, their long-term performance and changes in microbial community have seldom been reported. The present study investigated the changes of soil characteristics, microbial biomass carbon (MBC) and microbial community structure in a pilot-scale horizontal subsurface-flow mangrove constructed wetland, which has been used for treating municipal wastewater since 2005. These changes were monitored at different operation periods during 10-years operation. The constructed wetland consisted of two independent belts planted with *Aegiceras corniculatum* (Ac) and *Kandelia obovata* (Ko), two dominant mangrove species in South China. Surface soils (0-10 cm) were collected from the two belts in the 2nd, 3rd, 9th and 10th year of operation. The results showed that the contents of soil organic matter and total nitrogen, as well as microbial structure, differed significantly among operation years in both Ac and Ko belts. At the beginning of the operation, MBC was significantly higher than that in the 9th and 10th year of operation; however, microbial respiration in the soil substrate was stable during 10-years operation. Microbial communities based on phospholipid fatty acid analyses (PFLAs) revealed that the ratios of Gram-positive to Gram-negative bacteria, fungi to bacteria, monounsaturated bacteria to saturated bacteria were significantly higher in the 10th year of operation than those at the beginning in both Ac and Ko belts. The highest correlations between the soil microbial community and organic matter, and the soil microbial community and total nitrogen were found in the 10th year of operation, implying that the soil microbial community in this constructed mangrove wetland was more sensitive to changes in soil characteristics after long-term operation, and could be used as an indicator on the performance of CWs.

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-121

Soil microbial community and physicochemical properties of two different succession phase mangrove forests in Futian, Shenzhen

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Coastal habitats across the world are under heavy population and development pressures, and also subjected to frequent natural disasters. Mangrove forests which distribute in inter-tidal region between the sea and the land are important barriers for preventing soil erosion, protecting inhabitants from storms, and reducing pollution from upland runoff. However, more than half of the world's original mangrove has been disappeared during past decades duo to various anthropogenic activities. Mangrove restoration is increasingly becoming the focus of researchers and governments. Many restoration projects have been attempted, with mixed results, throughout the world in recent years. The experience tells that continuous monitoring and assessment in those conducted or on-going restoration projects are important for successful restoration or making further effective measures.

In this study, we aim to investigate the soil microbial community structure, microbial diversity and soil physicochemical properties of two different succession phase mangrove forests in Futian, Shenzhen. One is a seventy three –year-old primary forest, the other is an adjacent seventeen- year-old secondary forest (a natural restoration forest after clear-cutting). Phospholipid fatty acid (PLFA) profiles of both primary and secondary forest showed obvious advantages in bacterial fatty acid (53% and 51% in winter samples, while 54% and 56% in summer samples). Fungal/bacterial and monounsat/sat value indicated that the difference in community structure between primary and secondary forests was not significant ($p>0.05$). Results of Biolog ECO plates, which were used for investigating the soil microbial functional diversity, also showed no distinct ($p>0.05$) difference between primary and secondary forests. Different from the microbe, some soil physicochemical properties varied significantly between two studied forests, i.e., total nitrogen ($p<0.001$) and available phosphorus ($p<0.001$). The above results plausibly indicated that even the soil microbial community has rebuilt successfully, integrated ecosystem restoration of the degraded mangrove still need long time to complete.

Microbiota shift during leaf decomposition in different simulated mangrove sediments

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The decomposition of mangrove litter is important for environment nutrient cycling. The dynamic changes of mangrove plant polyphenols during the process of leaf decomposition and their association with sediments and microbial diversity were studied in simulated indoor tidal systems. Soluble polyphenols could be released at the early stage of leaf decomposition, leading to higher content of tannin and decreasing content of ammonia nitrogen. Soil characteristics fluctuated significantly at the early stage of leaf decomposition, while it became stable as the process goes on. Microorganisms were of higher capacity to metabolize carbon in the early stage of leaf decomposition than the latter one. And microorganisms in *Avicennia marina* and *Sonneratia apetala* sediments were able to metabolize carbon with significantly higher capacity than that in *Kandelia obovata*, all of which were higher than the blank control one. Microorganisms in *S. apetala* sediment could use more varieties of sugars as carbon sources. Microbial diversities in all the sediments increased at the early stage of leaf decomposition, corresponding to the development of carbon metabolism capacities. Studies here provide important information of correlationships between carbon utilization and microbial diversity during leaf decomposition in different simulated mangrove sediments. Results will be useful for further research on nutrient cycling in mangrove forests.

Keywords: mangrove, leaf decomposition, polyphenol, tannin, microbial diversity

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-123

Does *Laguncularia racemosa* have more advantages on leaf energetic cost than *Sonneratia apetala* in Southern China?

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Laguncularia racemosa (Lr) is the most widely used exotic mangrove species in afforestation during the last 20 years in addition to *Sonneratia apetala* (Sa), another exotic species, in Southern China. Sa has been considered as an invasive species although its invasiveness is still debatable. The distribution and aggressiveness of Lr were even more serious than that of Sa in the North of Fujian province of China, therefore, it is essential to understand the biological characteristics and the invasive potential of Lr. Construction cost (CC) is a quantifiable measure of energy demand for biomass production, and low CC is hypothesized to give an exotic plant growth advantages and increase its potential to be an invader. In this study, we compared CC and other growth traits between Lr and Sa, as well as with two native mangrove species, namely *Kandelia obovate* and *Aegiceras corniculatum*. Results showed that CC (1.30 g glucose/g), caloric values (16.96 KJ/g) and carbon concentration (43.19 g/g) of Lr not only lower than those of Sa (1.37 g glucose/g, 17.84 KJ/g and 43.49 g/g) but also were significantly lower than those of native mangrove species (1.47 g glucose/g, 19.13 KJ/g and 45.87 g/g), while specific leaf area (SLA) was just the opposite (SLA of Lr VS Sa VS native: 8.28 VS 8.21 VS 5.72 m^2/kg). Lower CC and higher SLA make Lr grow fast and result in higher height (4.83 m) and wider chest circumference (40.03 cm) than those of Sa (4.43 m and 35.63 cm) and the two native mangrove species (2.42 m and 26.78 cm). These findings indicated that the invasive potential of Lr could be higher than that of Sa, explaining why Lr has wider distribution and is more aggressive than Sa in North Fujian, China.

Are the distribution and expansion of exotic invasive Asteraceae plants in overseas Chinese town wetland related to their leaf construction cost?

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Overseas Chinese town wetland (OCT wetland) located in Shenzhen Bay has been considered as part of the important coast wetlands of Guangdong-Hong Kong-Macau Greater Bay Area. Many exotic species especially Asteraceae plants were found in OCT wetland, became invasive species and caused serious ecological problems in last decades. However, the reasons causing such expansion are not clear but leaf construction cost (CC) was found to be related to the growth rates and expansion of an exotic species. The present study investigated the distribution and expansion of exotic invasive Asteraceae plants in OCT wetland, and the relationships between distribution and leaf construction cost (CC) of these plants, aiming to identify the reasons that causing the invasions. Results showed that there were nearly 40% of the total plant species (27 species) in OCT wetland were exotic invasive species and belonged to 13 families and 23 genera. Six of all invasive species were Asteraceae plants and their distribution area was larger than the other invasive plants. Among these six species, the CC of *Wedelia trilobata* was the lowest but its distribution and expansion index (coverage, frequency and importance value) were the largest, while the opposite was found in *Ageratum conyzoides*. The CC of all six invasive Asteraceae plants declined in the order of *W. trilobata* < *Mikania micrantha* < *Aster subulatus* < *Bidens pilosa* < *Praxelis clematidea* < *A. conyzoides*, but orders of coverage, frequency and importance value of these plants were just the opposite. The distribution and expansion index of all invasive Asteraceae plants were positively correlated with their CC. These findings indicated that lower CC might be one important reason causing the invasion of these exotic species in OCT wetland.

International Conference on Marine Pollution and Ecotoxicology

Ecological responses to pollution: P-125

Carbon isotope ration of leaf litter correlates with litter production in a mangrove ecosystem in South China

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As an important ecosystem component, litter production in mangrove ecosystems is recognized as directly relevant to ecosystem net primary productivity (NPP) and carbon storage. In the present study, we analysed the litter production of five mangrove forests collected between 2010 and 2016 along the coastline of Shenzhen, China At the intra-annual level, all species displayed distinct seasonality in litter production, with its peak occurring in the summer season when productivity is also the highest. Carbon isotope ration ($\delta^{13}C$) of the collected leaf litter revealed an interesting, strongly negative relationship between δ^{13} C and litter production across all the species/site combinations. Although it has long been known that δ^{13} C as a measure of water-use efficiency contains productivity as its primary controlling component, the observed $\delta^{13}C$-litter production linkage is novel. Our results provide a suggestion that $\delta^{13}C$ of leaf litter can be used as a convenient indicator of litter production and potentially *NPP* in mangrove ecosystems.

Marine biodiversity and ecology of Tolo Harbour and its Channel, Hong Kong

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In tandem with the development of the local Biodiversity Strategy and Action Plan under the international Convention on Biological Diversity, a mega-project was initiated by the Environment Bureau of the Hong Kong SAR Government to assess the current status of marine coastal communities in the Tolo Harbour and Channel, an area that is known to support highly diverse flora and fauna but has been affected by intense human activities such as coastal development, pollution and fishing. A multidisciplinary team consisting of 23 scientists and environmentalists from different local institutions jointly conducted a comprehensive ecological study between October 2015 and September 2018, covering a wide range of habitats (e.g., rocky and soft shores, mangroves, and subtidal rocky reef) and taxonomic groups (e.g., waterbirds, juvenile fish, plankton and coral). A total of 1,473 marine species and 124 bird species (including 45 waterbirds) were recorded, adding 294 new records of marine taxa (recorded as species, genus or family level) to the Hong Kong Register of Marine Species (HKRMS). As a highlight in this study, two new species were discovered and described, while one species was re-described. The team also documented the spatiotemporal variations of species diversity and the occurrence of 14 species of conservation priority. The integration of these data will contribute to the production of a GIS-based species database for scientific, conservation and educational purposes. More importantly, the results of this mega-project establish a blueprint of standardised, scientific practice for future marine biodiversity surveys and ecological impact assessments in various habitats in the marine environment of Hong Kong and beyond.

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Ecological responses to pollution: P-127

Baseline of Hong Kong coral communities: Substrate composition and growth form

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Despite their ecological and economic importance, there are no detailed substrate composition data for coral communities in Hong Kong. Under the support of the Agriculture, Fisheries and Conservation Department, we conducted comprehensive field surveys in 2017 to quantify coral community composition across 33 sites in Hong Kong. At each site, we quantified coral community composition by identifying corals down to the genus level. In addition, we deployed sediment traps to quantify sedimentation rate as well as several nutrient parameters (i.e. nitrogen, phosphorus and organic matter deposition rates) that are known to affect coral community development elsewhere. Our study showed that coral cover varied greatly between 11% and 79% across the study sites, with 15 sites having high coral cover (> 50%). Among the 33 sites, 30 were dominated by only a few genera, especially the *Platygyra carnosa* and *Porites* spp., and *Pavona decussata*. Only 2 sites were dominated by the branching corals *Acropora* spp. The substrate composition and growth form as well as the correlation between coral community structure and sedimentation and nutrient deposition rates will be presented.

Growth patterns of a massive coral in Hong Kong

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Skeletal extension and calcification of *Porites* corals have been used as proxies of environmental conditions of coral reefs. This study examined the skeletal linear extension rate and calcification rate of *Porites lutea* across 10 sites in subtropical Hong Kong, and measured eight environmental factors to determine the environmental drivers of coral growth. It was found that both the linear extension and calcification rates were much lower when compared with the corresponding values of the same species collected in tropical areas. The linear extension rate and calcification rate of *P. lutea* were both negatively correlated with sedimentation rate in local waters. In addition, *P. lutea* displayed reducing growth rate in six of the ten study sites over the past decades. These results indicate that Hong Kong is a marginal environment for *P. lutea*, and the temporal trend of reduced calcification rates may indicate increasing stressful conditions they experienced over recent decades.

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Underwater technique for coral *in-situ* monitoring

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Increasing environmental disturbances, multiple stressors, and pollution are the challenges for coral reef communities in Anthropocene. The current coral monitoring programmes and laboratory experiments are unable to provide sufficient knowledge for understanding how stressors affect the physiology at community and colony level, while underwater monitoring and research provide new insight for coral physiological change under different environmental scenarios. Although laboratory experiments allow factor manipulation in controlled conditions, the innovation of *in-situ* techniques, such as the benthic chamber incubations with a set of newly developed sensors, made the quantification of the productivity of coral community more accurate and precise in order to create baseline studies under natural environmental fluctuations and to investigate the effect of potential stressors.

Here we describe the current monitoring methods to discuss *in-situ* approaches in order to provide more valuable information to stakeholders and management departments. The real-time information and empirical data of coral metabolism and the physiological state will improve the knowledge about the current coral health status and the prediction of future scenarios of coral ecology.

Modifying the surface of Fe_3O_4/SiO_2 magnetic nanoparticles with amphiphilic polymer material to get an efficient sorbent for determination of 96 pesticides in seawater sample

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Magnetic Fe_3O_4@SiO_2@DVB-NVP NPs with moderate hydrophobicity/hydrophilicity were prepared by copolymerization of Divinyl benzene (DVB) and N-vinyl pyrrolidone (NVP) on the surface of Fe_3O_4@SiO_2 magnetic nanoparticles, which were used for the extraction of 96 pesticide residues from large volume of seawater sample prior to determination by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The predominant factors which affect the recoveries of 96 pesticides in the sample preparation were researched. By means of optimal conditions, good linear relationships were obtained in the range of 0.5 to 250 μg L^{-1} with determination coefficients (R^2) ranged from 0.9830 to 0.9999. The results displayed that the recoveries of the pesticides in seawater samples at spiked levels (0.001, 0.01, 0.1, 1.0 μg L^{-1}) ranged from 61.6% to 112% with the RSD less than 20.8%. The limits of detection (LODs) of 96 pesticides were from 0.009 to 0.200 ng L^{-1}. The present method can also satisfy the requirement for the pesticide residues analysis, display good application prospect and provide a novel method for rapid determination of trace level of multi-pesticide residues in real seawater samples. Compared to the other previously reported SPE based on HLB for analysis multi-pesticide residues in seawater, the present method, which is more economical and can save at least 50% time for sample pretreatment, gives a new approach for high-throughput sample pretreatment technique.

Keywords: Fe_3O_4@SiO_2 nanoparticles modified with N-vinyl pyrrolidone and divinyl benzene (Fe_3O_4@SiO_2@DVB-NVP); liquid chromatography-tandem mass spectrometry (LC-MS/MS); pesticides; seawater

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Observations on coral bleaching at Sesoko Island, Okinawa, Japan during the 2016 summer using aerial surveys

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Record high temperatures from 2015 led to a third mass coral bleaching event on a global-scale since first documented in the 1980s. Along the coasts of the Ryukyu Archipelago, southwest Japan, mass coral bleaching was reported in the Sekisei Lagoon during the summer of 2016. We conducted an aerial survey using a commercial drone over the coral reef on the southeast coast of Sesoko Island, located northwest of Okinawa Island. The survey was undertaken five times at low tide between June and October 2016. Aerial surveys were conducted under slightly cloudy to cloudy weather conditions to avoid the sun's reflection onto the images. A panorama image of the coral reefs covering the survey site was constructed using the photographs obtained from each aerial survey. Most of the large tabular *Acropora* were bleached between August and the beginning of September, while most of the massive type *Porites* were not bleached even at the beginning of September. The average seawater temperature between the end of July to the beginning of September was ca. 30 °C. The present study suggests that bleaching develops differently among a group of hermatypic corals at around 30 °C.

Novel noninvasive electroantennography method on a crab to evaluate the effects of chemical pollutants to olfaction

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Decapod crustaceans such as crabs and shrimps are increasingly used as a model animal in toxicity testing. However, information on the effects of pollutants to their physiological functions of sensory modality and behavior is still limited. Chemoreception is known as one of the most important sensory system in crustaceans for food searching and various social behaviors. Crabs and shrimps possess chemoreceptors (sensory sensilla) over entire body surface. Among the chemoreception in crabs and shrimps, the 1st antennule, where chemoreceptors are densely arrayed at the tip, plays important role to perceive low concentration odorant and is defined as olfactory organ. Since those sensory receptors are directly exposed to the ambient water, chemoreception can be disrupted by the water quality where they habitat. In this study, to establish a generic electrophysiological method to evaluate the physiological effects of pollutants in crabs, we attempted to develop a novel noninvasive method by which records an electroantennogram (EAG) from 1st antennule of crabs *Hemigrapsus* sp.

In the conventional methods, EAGs were usually recorded from exposed nerve fiver/bundle through incision on carapace or from nerve cut end in removed antennule. The method developed in this study enables to record EAGs without incisions of antennule. Crab was restrained by a clamp and about half of body was kept in seawater in a small tank so as to expose eyes and antennules in the air. One of the antennular peduncle of 1st antennule was hold by the laboratory made holding electrode (modified IC clip, used as recording electrode). A silver wire was used as a reference electrode and touched to the body carapace. Signals from the recording electrode were magnified by an amplifier and stored in PC through a AD converter. Chemical stimulus was given through a silicon tube placed nearby the tip of 1st antennule. In this study, we recorded EAGs of olfactory organ to amino acids to see basic chemosensory function, and also recorded responses to same stimuli while exposing antennule to different ambient pH water to see the effect of pH on sensitivity.

Electrical potentials of nerve fibers innervating to the olfactory organ were constantly recorded for a long period by the holding electrode. The 1st antennule responded to both mechanical and chemical stimuli, indicated that unimodal chemosensory sensilla (aesthetascs) and bimodal mechano- and chemo- receptive sensilla distributed in the 1st antennule. Recorded EAGs were sufficient to perform quantitative analysis on response intensities to different kinds of chemical stimuli while antennule was exposed to experimentally polluted water. As well as recently developed noninvasive method of electroantennography for marine shrimps (Machon *et al*. 2016), this novel recording technique for crabs will be useful to study the effects of pollutants in crustaceans at sensory physiology level. External morphology of 1st antennule, sniffing (antennular flicking) behavior of crab, and the effect of water pH reduction on sensitivity of chemoreception will be introduced.

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Ecological responses to pollution: P-133

Hemocyte characterization of three species of Arcidae, *Anadara broughtonii* (Schrenck, 1867), *Anadara kagoshimensis* (Lischke, 1869), and *Tegillarca granosa* (Linnaeus, 1758) as a biomarker for coastal environmental monitoring

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Marine bivalves are often used as a sentinel species in coastal environmental monitoring since changes in the environmental quality are often well preserved in their cells and tissues. *Anadara* and *Tegillarca* species of Arcidae, the blood cockles, are considered to be good sentinel species in monitoring coastal pollution and ecosystem health, because they are distributed widely in the subsurface of intertidal mudflats. Internal cellular defence of the blood cockles to physical and biological stresses are mediated by the circulating hemocytes, while their hemocyte types and functions are poorly studied. In this study, we first characterized morphology and immune-related activities of hemocytes of three common blood cockles *Anadara broughtonii*, *A. kagoshimensis*, and *Tegillarca granosa* using microscopy and flow cytometry. Microscopy and flow cytometry revealed that hemocytes of the 3 blood cockles could be classified into four types; erythrocytes, granulocytes, hyalinocytes, and blast-like cells. Erythrocyte was the most abundant and the largest round cells containing haemoglobin with numerous granules in the cytoplasm. Granulocyte was intermediated-sized hemocyte characterized with granules in the cytoplasm and long pseudopodia on the cell surface. Hyalinocytes represented the cytoplasm containing none or a few granules and many short pseudopodia covered the cell surface. Blast-like cells were the smallest of the four types of the hemocyte. Flow cytometry confirmed that the granulocytes were mainly engaged in the cellular defence exhibiting the most active phagocytosis activity and the largest lysosome content. Flow cytometry also indicated that the erythrocytes generated more reactive oxygen species (ROS) and reactive nitrogen species (RNS) than those of the granulocytes. Blast-like cells exhibited an absence of phagocytosis and extremely low oxidative capacity, suggesting that this population is not directly involved in the cell-mediated immune activities. In conclusion, microscopy and flow cytometry indicated that the blood cockles had four types of hemocytes, and the erythrocytes and granulocytes are mainly involved in the immunological activities.

Effects of reproductive activities on the hemocyte functions of the intertidal oyster *Saccostrea kegaki* (Torigoe & Inaba, 1981) in Jeju island off the south coast of Korea assessed by flow cytometry

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In bivalve molluscs, the circulating hemocytes are primarily responsible for the defence against to pathogenic agents, as well as to mitigate external and/or internal stresses. Accordingly, hemocyte parameters have been analysed to monitor health condition of marine bivalves. Hemocyte functions of marine bivalves are often depressed during post-spawning period, due to the spawning stresses and the energy depletion. In an effort to understand impacts of reproductive activity on the cellular defence system, we monitored the hemocyte parameters and annual gametogenesis of the oyster *Saccostrea kegaki* occurring on lower intertidal area from March 2011 to February 2012 in Jeju Island off the south coast of Korea. Hemocyte parameters including total hemocyte count (THC), percentage of hemocyte types, hemocyte mortality, and phagocytosis capacity were analysed using a flow cytometry, and the reproductive condition was examined using histology. *S. kegaki* spawned during June and August and most of the oysters were in spent and resting phases during September and January. The THC dropped dramatically from September to October then the count fluctuated with a wide range during October and February, when most of the oysters were in sexually resting. Hemocyte mortality showed its annual peak in October, when the oysters were actively absorbing the residual gametes in the follicles by phagocytosing the eggs and sperms. Phagocytosis capacities of the granulocytes also dropped dramatically from September to February. The level of energy reserve (glycogen) in the tissue during post-spawning was much lower than the level prior to spawn. The depressed immune capacities of *S. kegaki* during post-spawning period could be explained as active hemocyte migration from the haemolymph to the gonads to digest and absorb the residual gametes and the follicles. It was also believed that the observed low level of energy reserve in the oysters during post-spawning period was responsible for the low level of THC and the impaired immune capacities, as the oysters suffered from insufficient food acquisition from the ambient environment.

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Ecological responses to pollution: P-135

A method for screening of ecdysteroid agonist and antagonist activity using shrimp ecdysteroid receptor and human cell lines

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Crustaceans and insects are phylogenetically related under Arthropoda, and both undergo the vital process of ecdysis (or molting), which is tightly regulated by ecdysteroid hormones. In addition to molting and development, ecdysteroids are also suggested to regulate central nerve system and reproduction throughout the lifespan. Disruption of ecdysteroid signal is deleterious or even fatal. Ecdysteroids exert their functions through the binding to ecdysteroid receptor (EcR). Upon binding, the activated EcR migrates from cytosol to nucleus, then pairs up with another receptor RxR, followed by anchoring onto a specific nucleotide sequence namely ecdysteroid responsive element (EcRE) located in the promotor regions of ecdysteroid responsive genes.

Insecticides are designed as EcR agonists for pest control through interfering ecdysteroid signal. Diacylhydrazine (DAH) is a class of EcR agonist insecticide that is claimed to be highly selective against insect species under the orders of Coleoptera and Lepidoptera, and thought to be safe to other animals including crustaceans, based on the results of animal studies. However, long-term or physiological effects at sub-lethal level, such as growth defects and reproduction abnormalities are not taken into account.

Thus, EcR agonists/antagonists are potential endocrine-disrupting chemicals (EDC) that may exert adverse effects, whether acute or chronic, on crustaceans. Here we report a protocol developed for screening of EcR agonists/antagonists. This protocol combines *in silico* molecular modelling/docking method with *in vitro* reporter gene assay using the cloned cherry shrimp EcR and RxR. Human cell lines were used as hosts for expressing the shrimp receptors and harbor the responsive reporter gene construct. Several chemical compounds were tested including DAHs and some others that draw environmental concerns.

The results of molecular docking suggested that most of the tested compounds, DAHs or non-DAHs, can be accommodated in the ligand binding pocket of EcR with fairly good binding affinity as estimated. However, only the DAHs can induce weak to moderate signals of receptor mediated gene transactivation. On the other hand, only DAHs can noticeably antagonize the signal induced by ecdysteroids.

In conclusion, DAHs are weak agonists and moderate antagonists to cherry shrimp EcR *in vitro*, suggesting that they are potential threats to crustaceans as EDCs. Computerized molecular modelling/ docking method provides tool for high-throughput primary screening of candidate compounds, although accomplishment in docking dose not confer actual bioactivity. Development of *in vitro* reporter gene assay using cloned receptors provides customized method for accessing ecdysteroid activities to any specific species, and allows data accumulation for phylum-wise comparison and pesticide design/monitoring.

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Degradation of BDE-47 in mangrove sediment with amendment of extra carbon sources

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Polybrominated diphenyl ethers (PBDEs) have been widely detected in coastal wetland areas, but the removal of these toxic and persistent organic pollutants from contaminated sediments is still difficult and the process is often slow. How to enhance their removal becomes an important urgent issue, and co-metabolism with the supply of extra carbon might be useful because they can serve as electron donors during PBDE degradation anaerobically. In this study, different forms of carbon sources, namely formate, acetate, pyruvate, lactate, methanol and ethanol, were added to mangrove sediments contaminated with BDE-47, a dominant congener of PBDEs other than BDE-209, as additional carbon sources to stimulate the degradation of BDE-47. After 5-month incubation under an anaerobic condition, high removal of BDE-47 (around 70%) was found in all sediments. There were no significant differences between sediments with and without extra carbon, suggesting that the removal percentage might be limited by other factors that should be explored in future research. The profiles of the degradation products were also similar, with BDE-17, BDE-8 and BDE-7 as dominant debromination products, indicating extra carbon sources did not change the degradation pathway of BDE-47. However, methanol and acetate significantly improved the degradation rate Pearson's correlations showed that the degradation rates in all sediments, irrespective to carbon sources, were positively correlated with the abundance of *Dehalococcoides* spp. The higher removal rates in sediments with methanol or acetate amendments could be due to the higher abundance of *Dehalococcoides* spp. This study indicated that extra carbon sources such as methanol and acetate could stimulate the growth of *Dehalococcoides* spp. thus improve the degradation rate of BDE-47 in contaminated mangrove sediments.

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Capability of edible marcoalgae farming for coastal water cleaning

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Edible macro algae species are rich in nutrient and mineral content, which become very popular as healthy food worldwide. The coastal macroalgae farming has increased exponentially during the last 50 years. Seaweed cultivation provides massive biomass for the marine ecosystem as primary production, and it is valuable ecologically and economically. Nan'ao Island is in the east of Guangdong Province, it is the largest seaweed farming site in Guangdong Province. Two macroalgae species from Nan'ao Island was studied in the present work, *Porphyra haitanensis* (popular commercial species in Guangdong and Fujian Province) and Pyropia dentata (cultivated from wildlife species at Marine Biology Institute Marine Station, Shantou University). The capability of two different seaweed species for various metals uptake and accumulation from the coastal water was investigated during the growth season from the 1st harvest to the 3rd harvest. The metal content in the sediment was also measured in order to analysis the bioaccumulation factor.

Transcriptomic mode of action study of the natural source anti-fouling di(1H-indol-3-yl)methane (DIM) warn over the environmental consequences of its application

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Di(1H-indol-3-yl)methane (DIM), a derivative of Indolyl-3-carbinol (I3C) produced by cruciferous vegetables, was suggested to be an environmentally friendly anti-fouling compound. But exposure experiment using marine medaka highlighted the endocrine disrupting properties of DIM to marine life, especially vertebrates. Using the bryozoan *Bugula neritina* as the target fouling species, we find that short term 3-hours DIM treatments at the concentration of 4ppm or higher, or long term 12-hours DIM treatments at 2ppm or higher induce significant larval mortality and metamorphic abnormality. The bioassay results correlate with the concentration dependent up-regulation of HSP family proteins, pro-apoptotic proteins, ubiquitination protein, and the concentration dependent down-regulation of anti-apoptotic proteins and developmental genes. Unexpectedly, genes involved in fatty acid biosynthesis and protein synthesis are up-regulated, but, in general, the effect of DIM treatment on *B. neritina* larvae is comparable to that reported in human cancer cell lines. As DIM also changes the expression of sterol hormone biosynthesis genes, our results rise the concern that prolonged exposure to DIM may have a long-term negative impact to marine life. We suggest that DIM can be applied as a potent antifoulant, but with precise control on the releasing rate and concentration of the compound.

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AUSMAP: Understanding microplastic loads through a robust citizen science approach

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Plastics are a major pollutant of our waterways causing significant harm to wildlife as well as causing social and economic impacts. Microplastics are a significant component of these, however there is limited information on their sources and sinks in Australia. Thus, it is imperative to identify how much is out there and where the hotspots are, so targeted abatement strategies can be implemented. The Australian Microplastic Assessment Project (AUSMAP) is addressing this through the creation of a national citizen science program for mapping the types and amounts of microplastics on freshwater and marine shores. Through standardised collection and analysis techniques with rigorous quality control procedures this world leading program is assisting in management and policy development while raising community awareness on the issue.