

## **Emerging contaminants in marine wildlife**

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It has been reported that Pearl River Delta (PRD) was significantly contaminated by various toxic chemicals such as persistent organic pollutants (POPs) and personal care products. Recently, several groups of new POPs such as polybrominated diphenyl ethers (PBDEs) and perfluoroalkyl substances (PFASs) have been identified in various abiotic environmental matrices and even detected in the wildlife. It is noteworthy that levels of these persistent toxic pollutants were significantly elevating in the blubber samples of marine mammals over the past decade. In addition to these new POPs, other emerging contaminants, which were regarded as the potential replacements for the banned chemicals, have also been determined in the local environmental samples such as sediment and seawater. In view of the rich marine biodiversity in Hong Kong waters, it is important to elucidate the occurrence of these emerging contaminants in the coastal environment of southern China, especially the vulnerable species such as marine mammals and coral communities. The present study will therefore examine and discuss the occurrence and spatial variations of several emerging contaminants including halogenated flame retardants, perfluoroalkyl substances and organic UV filters in different environmental matrices.

## **Mechanistic insights into induction of the molluscan vitellogenin gene by environmental estrogens**

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Marine molluscs, such as oysters, respond to estrogenic compounds with the induction of the egg yolk protein precursor, vitellogenin (Vtg), availing a biomarker for estrogenic pollution. Despite this application, the precise molecular mechanism through which estrogens exert their action to induce molluscan vitellogenesis is unknown. In vertebrates, Vtg gene transcription is primarily regulated by the estrogen receptors (ERs). Although the existence of an ER gene ortholog in the molluscan genome has been known for some time, its role in estrogen signalling has yet to be deciphered. As a first step to address this question, we isolated the Vtg and ER gene sequences from the Sydney rock oyster, *Saccostrea glomerata* and characterised their interaction with a series of functional analyses. Using luciferase reporter assays, we demonstrated that sgER activated the *sgVtg* promoter through binding to estrogen-responsive element half sites ( $\frac{1}{2}$ EREs) in an estrogen-independent manner. Ligand binding assays using fluorescent-labelled  $17\beta$ -estradiol (E2) and purified sgER protein confirmed that sgER is devoid of estrogen binding. Notably, the transcriptional activation of both *sgER* and *sgVtg* by E2 was abolished by the specific ER antagonist ICI 162,780. To determine whether transcription of *sgER* and/or *sgVtg* is epigenetically regulated, the DNA methylation status of their gene promoters was assessed following exposure of *S. glomerata* to E2. The bisulfite sequencing results showed that both gene promoters were predominantly hypomethylated, regardless of their transcriptional levels. Overall, our findings suggest that the estrogen responsiveness of *sgVtg* is regulated by a novel ligand-dependent receptor, presumably via a non-genomic pathway(s) of estrogen signalling.

## **Toxicity of tributyltin and triclosan in tributyltin-binding protein 2 knocked-out mutant strain of Japanese medaka, *Oryzias latipes***

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A concentration of free-form drug in blood is critical to exert its toxicity. Binding protein in blood, i.e., acid alpha acid glycoprotein (AAGP) or albumin bind with drug and might play an important role in detoxification of them. However, few studies were performed on this topic. Tributyltin-binding protein type 2 (TBT-bp2) is the homolog of AAGP which bind to small hydrophobic molecules and suspect to play an important role in transportation, detoxification, and excretion of xenobiotic compounds. In this study, we established a homo strain of TBT-bp2 knockout (KO) (-/-) medaka, *Oryzias latipes*, by using CRISPR/Cas9 system in F2 generation. To analyze the function of TBT-bp2, tributyltin and triclosan (TCS) was exposed to TBT-bp2 KO (-/-) medaka for 94 hours in a semi-static system and evaluate their toxicity on survival and alternation of behavior. Using mRNA-seq, no expression of TBT-bp2 was confirmed in the liver of TBT-bp2 KO (-/-) medaka. Compared with wild type medaka, the survival, swimming speed and number of vocal sound were decreased in the TBT-bp2 KO (-/-) medaka exposed to TBT or TCS ( $p < 0.05$ ). These results indicated that TBT-bp2 play important roles in detoxification of TBT and triclosan. The difference of gene expression of TBT-bp2 KO (-/-) medaka exposed to TBT and TCS will be discussed in more detail.

**Endocrine disruption of sulfamethazine on marine medaka (*Oryzias melastigma*) via the hypothalamus-pituitary-gonad-liver axis**

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In this study, the endocrine disrupting effects of sulfamethazine (SM<sub>2</sub>) on marine medaka (*Oryzias melastigma*) were assessed for the first time. Marine medaka were exposed to SM<sub>2</sub> (0.01 mg/L and 0.1 mg/L) for 28 days, and the reproduction behaviour, steroidogenesis, liver vitellogenin (VTG) and the genes expression involved in hypothalamus-pituitary-gonad-liver (HPLG) axis were investigated to understand the underlying mechanisms. The alteration pattern of genes in 7<sup>th</sup> day showed that SM<sub>2</sub> had the positive regulatory effects mainly in males than in females with a dose-dependent behaviour. The alteration of cytochrome P450 genes in gonad could further affect steroidogenesis and resulted in hormones disorder. The T/E2 ratio in both sex was used to reflect endocrine abnormality with the corresponding regulation of CYP19 genes. The up regulation of vtg1 and vtg2 in males could lead to the increases VTG level in liver, and the reproduction behaviour such as egg production and fertility rate were significant increased before the 14<sup>th</sup> day, but at the end of the exposure it showed no significant difference. The result would need to be further confirmed with HPGL genes expression in 28<sup>th</sup> day. Overall, the results indicated that SM<sub>2</sub> had deleterious, sex-specific effects on endocrine system in marine medaka exposed by environmental related concentrations, with certain insidious mechanisms.

**Endocrine disruptive effects of flutamide in male marine medaka (*Oryzias melastigma*)**

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Myriad of man-made chemicals in commercial and industrial use leak into the marine environment and have been shown to exert negative effects on the endocrine systems of many aquatic organisms. These endocrine disrupting chemicals (EDCs) can impair sexual differentiation, maturation, and reproductive functions in vertebrates. A class of EDCs, the anti-androgens, inhibits the androgen receptor and disrupts androgen signalling, leading to adverse effects such as feminization of male fish. Using the marine medaka (*Oryzias melastigma*) as a fish model, the anti-androgenic effects of flutamide on reproductive functions in male fish of two different age groups (3- and 4-month-old) were examined by treating the fish with 3.2 mg/L flutamide for 2 weeks. Sperm motility was significantly reduced in flutamide-treated 4-month-old male fish (but not for 3-month-old) compared to the respective untreated controls. Histological examination of the testes revealed a significant reduction in testes lobule width in flutamide-treated 4-month old male fish, indicating impaired spermatogenesis. Quantitative RT-PCR examination of testes of flutamide-treated and control fish showed that mRNAs of several steroidogenesis-related genes – 17 $\beta$ -HSD1, 17 $\beta$ -HSD7, 3 $\beta$ -HSD, CYP11A, AR $\alpha$  and ER $\beta$  – were upregulated in 3-month-old fish, while 17 $\beta$ -HSD3 and ER $\beta$  were downregulated in 4-month-old fish. Interestingly, mRNAs of Foxl2 and VASA (genes associated with spermatogenesis) were also downregulated in 4-month-old fish, suggesting disrupted steroidogenesis and spermatogenic activities by flutamide in 4-month-old male fish. Overall, flutamide appears to exert more severe anti-androgenic effects on older male fish (4-month-old) than younger fish, and studies to better understand the molecular mechanisms underlying such differential effects are currently underway.

## **Occurrence and ecological risks of phenyltin compounds in the marine environment of Hong Kong**

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Organotin compounds, especially triphenyltin (TPT), are amongst the most prevalent and toxic endocrine disrupting chemicals occurring in urbanized coastal regions of China because of their extensive use in antifouling paints, agricultural, and various industrial activities. Even though the International Maritime Organization (IMO) of the United Nations prohibited the use of organotin compounds in antifouling paints on ship hulls of sea-going vessels in 2008 due to their notorious effects on various marine organisms, Hong Kong has only imposed a new legislation to regulate their use on ship hulls since January 2017. There is a dearth of information regarding the occurrence of TPT in the marine environment of Hong Kong, particularly in seawater, sediments, and marine organisms. This study, therefore, aimed to assess the contamination statuses of TPT and its degradants in various marine environmental compartments, and evaluate the ecological and human health risks associated with these chemicals. TPT and its degradation products (mono- and diphenyltin; MPT and DPT) were quantified in seawater, marine sediments, and 12 species of marine organisms using a gas-chromatography coupled to a mass-spectrometer (GC-MS). High concentrations of TPT and its degradation products were detected in all analyzed compartments, indicating that Hong Kong is seriously contaminated by these compounds. Samples from the western waters of Hong Kong generally contained higher concentrations of TPT than that of southern waters, suggesting that the contaminated freshwater discharges from the Pearl River as well as heavy shipping activities in the west could be potential sources of these contaminants. Ecological risk assessments based on environmental concentrations of TPT showed that marine organisms inhabiting Hong Kong's waters are at high risks due to exposure to TPT. In particular, benthic organisms are at higher risks because these compounds strongly adsorb onto marine sediment and the benthic organisms may uptake these chemicals through contact with the contaminated sediment and consumption of the sediment. Moreover, the calculated hazard quotients of TPT exceeded 1, suggesting that human consumption of benthic fishes, including the bartail flathead, the rabbitfish, and the burrowing goby could cause potential health risks.

## **Occurrence of four algae toxins in the marine water of Hong Kong**

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Algal toxins are natural organic compounds produced by toxic algae in the water and they have caused lots of harms for marine ecology and human health over the past decades. In this study, 24 marine water samples were collected in the marine of Hong Kong. Four algae toxins, including saxitoxin (STX), domoic acid (DA), okadaic acid (OA) and azaspiracid-1 (AZA-1) were analysed by Ultra-performance liquid chromatography (UPLC) – mass spectrometry/mass spectrometry (MS/MS). STX was not existed in all samples while AZA-1 was detected in all samples (range from 5.14 ng/L to 11.9 ng/L). Lower concentrations of DA and OA were detected in the open marine area than in the narrow marine area. The concentrations of DA, OA and AZA-1 in Eastern marine area were higher than in the Western and Southern marine area. The concentration differences of DA were higher in these samples (range from 0 ng/L to 10.3 ng/L) than other three toxins. The concentrations of the detected algae toxins were much less than the WHO recommended level (in µg level). The risk quotients (RQs) of ecological risk assessment and human health assessment were all far less than 1. There was no evidence showing that these four algae toxins had the significant harms for marine organisms and humans in Hong Kong. Three groups were conducted from these 24 samples by the principal components analysis (PCA), suggesting that agriculture, untreated domestic waste water and coastal fisheries contributed to eutrophication, which caused high levels of DA, OA and AZA-1. Further study of the risk of these four algae toxins is necessary in Hong Kong.

## **Transgenerational effects of polystyrene nanoplastics on freshwater crustacean daphnia**

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Microplastics (MPs; 0.1  $\mu\text{m}$  to 5 mm in size) have been found to be widespread in marine environments and their potential environmental impact has become a prominent question in aquatic toxicology research. Moreover, weathering of a single MP particle can yield billions of nanoplastics (NPs;  $< 0.1 \mu\text{m}$ ), and the smaller NPs are expected to be a bigger threat than MPs because they potentially have much higher concentrations in the environment and can cross biological membranes. To date, little is known about the long-term impact of NPs in the freshwater systems. Thus, in this experiment, we employed a model freshwater invertebrate *Daphnia magna* to investigate the transgenerational impacts of polystyrene nanoplastics (PS-NPs). Parent *Daphnia* (F0) was exposed to 0, 1, and 50 ppm of 20 nm PS-NPs from birth until the production of the first neonates (F1). The F1 generation was then moved to a clean medium for a two-generation recovery period. The effects on the organism's mortality, physiological and behavioral changes, reproduction as well as the uptake of PS-NPs were quantified for each generation.

The results showed that PS-NPs were internalized in the gastrointestinal tract, appendices, and brood chamber in F0 *Daphnia* in a concentration-dependent manner. Interestingly, PS-NPs were detected in F2 *Daphnia* even after a long two-generation recovery in the clean medium. Low mortality was observed in F0 and F1 generations at 50 ppm PS-NP exposure but no mortality in F2 generation. Exposure to PS-NPs also caused a concentration-dependent decrease in heartbeat rate as well as curling rate of appendages in F0, and 50 ppm PS-NPs significantly reduced the heartbeat rate and curling rate of appendages compared to the control treatment. The movement of tentacle and claws was not affected by PS-NP exposures over all three generations. Furthermore, the swimming behavior was tracked and quantified, revealing a concentration-dependent increase in the total swimming distance in both F0 and F1 but recovered to normal in F2. In general, PS-NP-exposed *Daphnia* produced fewer eggs and neonates than controls, and the largest difference ( $\sim 20\%$  fewer eggs and neonates than control) was observed in F2. The current study demonstrates that PS-NPs can be internalized by *Daphnia* and transferred down to the next generations that show abnormal hyperactivity and reduced reproduction. These changes could be associated with increased risk of predation and population decline of *Daphnia*. Given the determinant role that *Daphnia* plays in controlling the phytoplankton as well as providing food to upper trophic levels, PS-NPs may have impacts on the food web dynamics in the freshwater systems.

## **Leaching of endocrine disrupting chemicals from marine microplastics under common life stresses**

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Microplastics are able to sorb harmful substances or contain additives that can cause adverse effects to organisms, among which are endocrine disrupting chemicals (EDCs). The present study aims to determine EDC concentrations and their endocrine activity in leachates of field-collected marine plastics under stress conditions that are known to occur during the plastic life cycle. Estrogens were the dominant EDCs on plastic particles, which were either concentrated from surrounding water or be leftovers from plastic manufacturing. Bisphenol A had the highest detection frequency (75%) with an average concentration of  $475 \pm 882 \mu\text{g/kg}$ , and followed by bisphenol S, octylphenol and nonylphenol. Moreover, smaller marine plastics leached greater quantities of EDCs, which was due to the sorption from surrounding seawater being more efficient for smaller particles. It is found that normal life stresses such as microwaving (MW) and autoclaving (AC) can decrease the EDC concentrations, but solar irradiation (solar) can increase EDC concentrations in leachates. Even though organisms with higher metabolic ability for EDCs exhibited higher estrogenic effects, the comprehensive toxicity of plastic leachates after common-life treatments were still limited (below EC10) if adopting 0.1% as plastic contribution for EDCs uptake. In future studies, the accurate contribution of plastic bound EDCs through cooking need to be further explored, and monitoring of microplastics in human diet remains important because their concentrations may change in the future.

**Bioaccumulation and multiple toxic effects of different shapes of microplastics in zebrafish**

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Different shapes of microplastics are widely detected in the environment and organisms and microplastics mainly reside in the gut. However, the influences of shapes on the bioaccumulation and toxicity of microplastics in the gut are largely unknown. Three shapes (bead, fragment, and fiber) of fluorescent microplastics of comparable size in one dimension were used to quantify the accumulation of microplastics in zebrafish gut. At the meanwhile, zebrafish were exposed to the three shapes of pristine microplastics for 21 d to evaluate multiple intestinal toxicities. The accumulation of microplastics in the gut was fiber (8.0 µg/mg) > fragment (1.7 µg/mg) > bead (0.5 µg/mg). All shapes of microplastics induced inflammation and permeability increase in the gut, and caused the down-regulation of genes related to lipid metabolism, hormone metabolism, and protein secretion. Compared with spherical microplastics, non-spherical microplastics suppressed intestinal epithelial cell regeneration and attenuated the intestinal response to the stimulus. Microplastic fibers also exacerbated oxidative stress and intestinal lesions and decreased the relative abundance of *Pseudomonas* and *Gordonia* in the gut microbiota. The shape significantly affected the accumulation and toxicity of microplastics in zebrafish gut. Non-spherical microplastics have higher accumulation and toxicity than the spherical microplastics. Shape-dependent effects should not be ignored in the health risk assessment of microplastics.

**A regional approach for understanding toxicity of microplastics to marine biota**

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Plastics are known to be ingested by a range of marine species but there remains limited information on the potential impacts this causes particularly at environmentally relevant concentrations over prolonged exposures and to multiple polymers at a time. Our project seeks to address this by examining the toxicity of a selection of polymers both singularly and in combination over a part life cycle exposure. Also, by using common species or genera found across the three participating countries, Australia, Singapore and Hong Kong, and running tests at locally reported exposure levels it is hoped that comparative evidence of impacts will be gathered across the Asia-Pacific region. By working collectively across the region, we stand a better chance at addressing the plastic pollution problem. This paper will describe the methods used and discuss results found to date with the view to standardizing the approach for assessing toxicological impacts of microplastics.

**Microplastics pollution in the Greater Bay Area: Distribution and impact**

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Plastic pollution of coastal environment is one of the most pressing issue of our modern society as its linked to our lifestyle and our capacity to develop efficient waste management. Based on the population and waste management practices around the Greater Bay Area (GBA), researchers recognised the Pearl River has a key location for plastic release into the Ocean<sup>1</sup>. Using data on microplastic abundance around the Pearl River Delta collected during the last three years we investigated the regional distribution and its impact on the marine environment. We estimated macro and microplastics abundance and distribution in Hong Kong waters and beaches in order to understand the parameters responsible for their presence. We observed a higher abundance of microplastic after rainfall events, however no specific types of plastics were preferentially accumulated suggesting that plastic waste mismanaged are washed up into the river and/or oceans. When looking at the plastic abundance in nearshore waters, we observed a significant accumulation of both macro and microplastics at the breaking wave area. More particularly we observed that highly buoyant plastics as expanded polymer preferentially accumulated at breaking wave area, indicating the importance of plastic polymers in microplastic pieces behaviour and distribution in the marine environment. Finally, we look at the ingestion of microplastics by marine organisms as benthic fish and mangrove crabs and we highlighted that more than half of the individual collected had ingested microplastics. The GBA is under an increasing pressure in term of plastic pollution and marine organisms are already affected by it, therefore it is crucial to develop efficient waste management strategies to limit microplastics input into the GBA coastal waters.

## **Geological and biological factors on marine debris ingestion of fish in the waters of Hengchun Peninsula, Taiwan**

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Many studies have indicated that marine creatures are at risk of plastic ingestion. In Taiwan, relevant studies are still lacking. In this study, we quantitatively analyze plastic debris ingestion of coral reef and pelagic fish in coastal Hengchun Peninsula, including Kenting National park, located in southern Taiwan. In the 117 fish specimens we examined, 94.87% of them had ingested marine debris, and all of the debris was microplastics (<5mm). The average number of ingested microplastics was  $5.62 \pm 5.07$  pieces per fish (ranged 0-32 pieces per fish). The major type of microplastics was fiber (96%) and the major color was blue (43%). The quantity of ingested microplastics was not significantly different among fish from different locations (west, south, and east coast of the peninsula) or habitats (coral reef and open ocean). Also, the sizes, families, and trophic levels of the fish were not significantly associated with the quantity of ingested microplastics. Our results show a high prevalence of microplastic ingestion but no biomagnification of microplastics in fish. This research is the first to study microplastic ingestion in marine fish of Taiwan. More research is much needed to better characterize the biological and ecological impacts of marine debris on fish.

**Microplastics in the estuary environment and its impacts to aquatic organisms**

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Microplastics have raised much concern as an emerging and persistent environmental pollutant. In this study, the composition of microplastics in the estuary environment was examined. The seasonal distribution pattern and settlement characteristics of microplastics in estuarine sediment were further investigated. The results showed that the concentration of microplastics in rainy season was significantly lower than that of dry season, suggesting rain washing, tides and salinity could affect the accumulation of microplastics in the estuarine sediments. The settlement experiment results showed that microplastics tended to stay in top sediment layer which has a smaller particle size. The biological uptake and bioaccumulation of microplastics in the marine organisms were studied. Microplastics in the form of fibers were mainly distributed in the gills and mantle of the studied oysters, and the detected microplastics in the oysters were mostly cellophane. Swimming performance was studied in the fish and the results showed that exposure to microplastics significantly decreased fish larvae swimming competence, which may have significant impacts on its population fitness in the aquatic environment and further ecological consequences.

**The genome of rotifer, copepod, and marine medaka: A potential use for molecular ecotoxicology and mechanistic study**

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To date, many genome information is available from diverse marine and freshwater aquatic organisms. In my lab, we have successfully assembled 12 genomes from the rotifers *Brachionus* spp., the copepods *Tigriopus japonicus* and *Paracyclops nana*, the water flea *Daphnia magna*, and the small fishes including marine medaka *Oryzias melastigma*, *Kryptolebias marmoratus*, *Kryptolebias hermaphroditus*.

This information accelerated the examination of mechanistic toxicities in these aquatic organisms in response to environmental stressors. Also, new technologies, such as CRISPR/Cas9 gene targeting, can be applied based on this genome information. In this presentation, I will introduce some examples of how I can use these genome data to obtain the extensive mechanistic toxicity data in response to several environmental stressors.

## **Application of metagenomics and metatranscriptomics in marine ecology research**

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Metagenomics and metatranscriptomics has been widely used in the ecological research to analyze the microbial composition and function in the environment. Such as Tara Ocean project hosted by EMBL, which had published a special issue on *Science* in 2015. And the global topsoil microbiome project funded by the Estonian Research Council, etc., which had published on *Nature* in 2018. In marine pollution and ecotoxicology, metagenomics is mainly used in petroleum degradation, microbial pollution caused by sewage discharge, and excessive nutrient pollution (such as N and P). Here we present three recent projects related to marine ecological pollution. The first project, we used eukaryotic metatranscriptomics to compare the gene expression profiles of seven phytoplankton groups in the formation of a dinoflagellate *P. donghaiense* bloom. Genes related to organic P, resisting environmental stress and pathogenic microorganisms were highly expressed during the formation of *P. donghaiense* bloom compared with other phytoplanktons. *P. donghaiense* was more competitive than other phytoplankton in organic nutrient utilization, especially for organic P, resisting environmental stress and pathogenic microorganisms and genetic characteristics, these allowed it to form bloom under low DIP condition. The second project, we used eukaryotic metatranscriptomics to compare the gene expression profiles of zooplankton and protists during the diurnal variation of a dinoflagellate *P. donghaiense* bloom. We found Arthropoda - the dominant group of zooplankton, was most active in the early morning, and significantly enriched the functions of Regulation of actin cytoskeleton, Oxidative phosphorylation, Phagosome, Apoptosis and Cellular senescence. Correspondingly, Dinophyceae and Diatom Bacillariophyta, which were the two most critical group of *P. donghaiense* bloom, expressed less in the early morning due to lack of light. This reveals the variation of Arthropoda growth and reproduction during the *P. donghaiense* bloom. The last project, we used metagenomics to compare the distribution of microbial antibiotic resistance genes in mangrove rhizosphere soils in Donggangzhai, Shenzhen, and Beihai, Fangchenggang, which belong to Hainan, Guangdong and Guangxi Provinces respectively. Antibiotic resistance genes were detected in all regions, and the *adeF* gene was the most detected, which belongs to *Tenderia electrophaga*, *Singulisphaera acidiphila*, *Paraburkholderia phymatum* and *Chromobacterium violaceum*. It can lead to fluoroquinolone antibiotic and tetracycline antibiotic resistance. The number of *adeF* genes is significantly different in each region, Shenzhen has the most, followed by Donggangzhai, and Guangxi provinces was the least. It is directly related to the extent of human activity.

**A proteomic analysis of skeletal tissue anomaly in the brain coral *Platygyra carnosa***

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Although skeletal growth anomaly (GA) is a common coral disease, little is known about its molecular pathology. This study aims to characterize the molecular pathology of GA in the brain coral *Platygyra carnosa* by comparing the proteomes of normal and GA polyps using iTRAQ-coupled LC-MS/MS. A total of 818 proteins were identified and quantified, among which 76 were differentially expressed between the normal and GA samples. Around 33% of these differentially expressed proteins (DEPs) were also differentially expressed at the transcript level. The KEGG and Reactome analyses indicated that these DEPs were mainly enriched in pathways related to viral infection and carcinogenesis, providing new evidence on the involvement of pathogens in coral GA.

**Assessing biodiversity in Hong Kong western waters using environmental DNA approach**

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The freshwater outflow of Pearl River renders the seawater in the western Hong Kong low in salinity, turbid and fast flowing, compared to their counterpart in eastern waters. The poor visibility in the western waters hinder the biodiversity survey by typical visual census method. In the present study, the biodiversity of Hong Kong western waters was examined using molecular technique - Environmental DNA (eDNA). Five sites in western waters were selected, from which seawater and sediment samples were collected for eDNA analyses. Polymerase Chain Reaction (PCR) amplification of genetic marker cytochrome *c* oxidase subunit I (COI) for all samples were conducted and sequenced by next generation sequencing (Illumina MiSeq). The results of blast-searching sequences obtained against online database showed that many major groups of marine organisms such as decapoda, porifera, mollusca, and scleractinia, could be detected by eDNA approach. However, a large quantity of DNA remained unknown in identity. Further study will be sought to test if eDNA is effective in revealing biodiversity in marine environment.

**The protective role of the freshwater and marine rotifer glutathione S-transferase sigma- and omega proteins in the heavy metal-exposed transformed *Escherichia coli***

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Glutathione S-transferases (GSTs) play important role in the phase II of detoxification to protect cells against oxidative stress generated by exogenous toxicants. Previous studies of GSTs in invertebrates have mainly focused on the identification and characterization. Here, we isolated omega and sigma class of GSTs from the freshwater rotifer *Brachionus calyciflorus* and the marine rotifer *Brachionus koreanus* and explored their antioxidant function in response to metal-induced oxidative stress. The recombinant Bc- and Bk-GSTs proteins were successfully expressed in the transformed *Escherichia coli* and its antioxidant potential was characterized by measuring kinetic properties and enzymatic activities in response to different pH, temperature, and chemical inhibitors. In addition, a disk diffusion assay, reactive oxygen species (ROS) assay, and morphological analysis revealed that the GSTs-transformed *E. coli* protect cells from oxidative stress induced by H<sub>2</sub>O<sub>2</sub> and metals (Hg, Cd, Cu, and Zn) at a significant level. Stronger antioxidant activity was exhibited by GST-S proteins compared to GST-O in both rotifers, suggesting that GST-S play a prominent function as an antioxidant defense mechanism in *Brachionus* spp. Overall, our study clearly showed the antioxidant role of Bk- and Bc-GSTs in the transformed *E. coli* and provides more in-depth understandings of the GST class-specific and interspecific detoxification in the rotifer *Brachionus* spp.

**Effect of PBDEs on prostaglandin pathway in pantropical spotted dolphin fibroblast cell line (PSP-LWHT): An *in vitro* study**

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As a persistent organic pollutant, PBDEs have been detected not only in environmental medium but also in Marine mammals. Due to its similar structure to hormones, PBDEs have been shown to have strong hormone-disrupting effect which may lead to disruption of immune system and reproductive system of mammals. As the advanced predators in the Marine environment, cetaceans are extremely vulnerable to PBDEs. Since prostaglandins are associated with immune regulation, it is necessary to evaluate the potential threat of PBDEs to dolphins by *in vitro* experiments using dolphin skin fibroblasts as models. Our current *in vitro* studies indicate that PBDEs could trigger the prostaglandin pathway by increasing expression level of prostaglandin synthase 2 in a Pantropical spotted dolphin skin fibroblast cell line model. After treatment with 250ng/ml BDE-47, 250 ng/ml BDE-100 and 1000ng/ml BDE-209, prostaglandin synthase 2 in dolphin skin fibroblast cell line was found to be increased significantly at 12 and 24h, but decreased at 48 and 72h. We further measured the concentrations of prostaglandin E2 and cAMP, which response to the prostaglandin synthase 2 and prostaglandin E synthetase. However, the concentration of prostaglandin E2 and cAMP did not increase, which was speculated to be time and concentration dependence. In addition, the EP1-4 receptors of prostaglandin E2 was further detected by molecular and protein levels. EP1-4 receptor changed with the time goes on. Our results showed that BDE-47, -100 and -209 may interfere prostaglandin E2 synthesis by increase / decrease the mRNA expression and protein expression of prostaglandin synthase 2 and prostaglandin E receptors in Pantropical spotted dolphin skin fibroblast cell line.

**Transcriptomic analysis of the effects of PBDEs on pygmy killer whales (*Feresa attenuate*) dermal fibroblasts**

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Polybrominated biphenyl ether (PBDEs) is a ubiquitous pollutant, and its presence had become a major problem in the aquatic environment. Its potential toxic effect on aquatic animals, especially cetaceans, has attracted worldwide attention. As cetaceans are top-predators and sentinel species that could predict the impact on human health, it is important to assess the potential threat of PBDEs to cetaceans. BDE-47, BDE-100 and BDE-209 are the predominant PBDE congeners had detected in environmental samples and biological tissues. In order to better understand the mechanism of their potential toxic effect, we have established cell lines of pygmy killer whales (*Feresa attenuate*) dermal fibroblasts as an *in vitro* tools for molecular assessments. In this study, high-throughput sequencing was performed with the cells which were exposed to different concentrations of BDE-47, BDE-100 and BDE-209 for 24 hours. By gene function annotation classification and weighted gene correlation network analysis, gene expression changes were associated with common cell functions to elucidate the transcriptome response and its toxicological mechanism of PBDE congeners. Then the mRNA expression levels of the differentially expressed genes were validated by quantitative real-time PCR. These results provide a good foundation for further study of toxicity mechanism and molecular biomarkers of PBDEs pollution using dolphin cell *in vitro* models. In the future, the regulation and interaction of differentially expressed genes will be further studied to elucidate the toxicological mechanism of PBDEs.

## Marine biotoxins: From poison to potion

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Ciguatera fish poisoning (CFP) is a circumtropical disease resulting from the consumption of fish and marine products naturally contaminated with a suite of toxins named ciguatoxins (CTXs). CTXs are bioaccumulated and biotransformed along food chains in coral ecosystems. CTXs present in coral reef fishes are potent sodium channel toxins that activate voltage-sensitive sodium channels, causing cell membrane excitability and instability. A range of gastrointestinal (e.g. vomiting, diarrhea, abdominal pain, nausea), neurological (paresthesias in the extremities and oral region, itching, muscle and joint pain and fatigue) and cardiovascular (hypotension and bradycardia) disorders can result after the consumption of ciguatoxic fishes. This illness is recognized as a major threat to food sustainability and food security at a global scale and has been ranked by worldwide public health institutions as one of the most common food-borne illnesses, adversely affecting an estimated 50,000 – 500,000 people globally on an annual basis, but could be largely under-reported and under-estimated owing to a lack of CFP diagnosis.

Over the past 10 years, significant progress has been achieved in the development of a variety of analytical tools. However, the multiplicity of CTXs, the difficulties in synthesizing these complex molecules and the notorious lack of commercially available reference materials (standards) necessary for the calibration of detection tests, still constitute major impediments towards the implementation of effective mitigation and prevention programs. In this context, many laboratories have dedicated extensive effort in obtaining CTXs standards purified from either toxic cultures of *Gambierdiscus* spp. or toxic fish material.

Here, we report the characterization of a set of six CTX standards purified from mass-cultures of a French Polynesian strain of *Gambierdiscus polynesiensis* (TB-92) and a novel strain of Kiribati *Gambierdiscus* sp. (1D001), as well as from toxic samples of the moray-eel *Gymnothorax javanicus*. Toxic fractions were obtained using bioactivity-guided fractionation following standard extraction and purification protocols developed at the Institut Louis Malardé and the National Research Institute of Chinese Medicine. The identification and degree of purity of these standards were further evaluated via ultra-high-performance liquid chromatography coupled to a high-resolution mass spectrometry analysis performed in two distinct laboratories for validation purpose. The identity of each CTX analogue was confirmed by its exact mass, isotopic pattern and fragment ions obtained from full-scan and PRM modes analysis in Thermo Quadrupole-Orbitrap, Thermo LCQ Fleet and TripleTOF 5600, AB Sciex mass spectrometers. Quantification of these fractions was also approached using LC-MS/MS calibration curves established with standards widely used in research laboratories and using qNMR experiments. Plans to make these new CTXs standards commercially available as reference materials for seafood product certification and safety are currently under consideration by Institut Louis Malardé and the National Research Institute of Chinese Medicine respectively, an outcome that is particularly promising in the context of the multiple international initiatives recently implemented for a better control of ciguatera and its adverse effects on global populations.

Marine biotoxins have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favourable as lead structures for drug discovery. Marine benthic dinoflagellates are ideal potential producers of many useful substances, such as bioactive compounds for pharmaceutical use, enzymes, nutraceuticals and cosmetics. Therefore, marine benthic dinoflagellates represent a largely underexplored source for exploring novel bioactive compounds of microbial origin. Bioactive substances produced by marine benthic dinoflagellates could be used as a culturable and dependable source for bioactive compounds which could be developed as low cost commercial products for routine screening as well as for various pharmacological experiments and drug discovery.

**Coastal land-water-biodiversity nexus and resilience-based management in Guangdong-Hong Kong-Macao Greater Bay Area of China**

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Resilience thinking explores the sustainability discourse and transformation of coastal complex system. Our group proposed an innovative framework of resilience-based management which includes the coastal land-water-biodiversity (LWB) nexus to emphasize the resilience of Guangdong-Hong Kong-Macao Greater Bay Area (GBA) of China, associated with implementation of resilient coastal spatial planning, which was suitable for the development of the social-ecological system in GBA and requirements of integrated land-sea management. We had applied the coastal resilience framework in terms of the LWB nexus into many coastal megacities and regions, e.g., Xiamen, Shenzhen, Ningbo, Haikou, Lianyungang and Southern Fujian Coastal region (Xiamen, Zhangzhou and Quanzhou). In the GBA case, we are involving with the method of resilient spatial planning and the techniques of eco-engineering to provide a systematic spatial planning framework for resilience assessment, which could set up critical ecosystem restoration demonstration in GBA. The decision-supporting system of resilient coastal space was built to associate with implementation of a constructive scientific decision for making the land-sea connection and enhancing the resilience of GBA and the coastal zone of China.

## **Health effects and mechanisms of exposure to environmental contaminants in marine cetaceans from the Pearl River Estuary, China**

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The Indo-Pacific humpback dolphins (*Sousa chinensis*) from the Pearl River Estuary (PRE) have suffered catastrophic declines in recent decades, primarily due to degradation of habitat quantity and quality. Marine pollutants that concentrate in the blubber of humpback dolphins could potentially disrupt immune and reproduction systems, making them vulnerable to disease and causing dwindling birth rates in exposed populations. The levels and spatiotemporal trends of heavy metals, legacy and emerging persistent organic pollutants (POPs) in humpback dolphins and finless porpoises from the PRE during 2004–2017 were assessed to examine the health effects due to pollutant exposure. The hepatic levels of Cr, As, and Cu in the PRE dolphins were among the highest reported for cetaceans globally, and the levels of Zn, Cu, and Hg in some of the animals were sufficiently high to cause toxicological effects. PFOS levels in 46% of dolphin liver samples exceeded the hepatic toxicity threshold in cetaceans. Many contaminants had the peaked levels in recent years, especially for Per- and polyfluoroalkyl substances (PFASs) and heavy metals, reflecting an elevated exposure of the PRE dolphins to toxic pollutants. Apparent geographic differences in hepatic levels (adjusted for age) of heavy metals and PFASs were found, with dolphins stranded near the river mouths in Lingdingyang (LDY) showing significantly higher levels than those from the West-four region (WFR) (t-test,  $p < 0.05$ ). This fine-scale spatial distribution pattern in the PRE dolphins is likely to be driven by the environment, since the nearshore area in LDY was known as contamination “hotspot” in the PRE due to its proximity to the riverine outlets, i.e., land-based source of contaminants. Epidemiological evidence showed that infectious disease-caused deaths in finless porpoises stranded in the PRE are linked to their high body burden of DDTs, suggesting these chemicals make them more vulnerable to disease. The cytotoxicity effects of Cr(VI) were analyzed on fibroblasts isolated from the skin of *S. chinensis* (ScSF). Cr(VI) markedly inhibited the viability of ScSF cells via induction of apoptosis accompanied by an increase in the production of reactive oxygen species and the population of G2/M arrest or apoptotic sub-G1 phase cells, up-regulation of p53, and activation of caspase-3. Cr(VI) induced depletion of mitochondrial membrane potential in cells through regulating the expression of anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) proteins, resulting in decrease of the ATP level, cytochrome c release from mitochondria into cytosol, and the activation of caspase-9. The intensive human activities due to the rapid development of the Guangdong-Hong Kong-Macao Greater Bay Area will inevitably cause large amount of contaminants to be released into the Pearl River Estuary (PRE). Regulation in chemical use and release is urgently needed to reduce dolphin exposure to environmental contaminants.

**Impacts of *Deepwater Horizon* oil and dispersants on various life stages of oysters *Crassostrea virginica***

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The 2010 *Deepwater Horizon* Oil Spill is the largest oil spill in the U. S. history and released millions of liters of oil into the Gulf of Mexico. Many of the polycyclic aromatic hydrocarbons and other oil constituents found in oil are toxic. The oil spill, which continued for three months and which resulted in extensive exposure of nearshore habitats well beyond the period when oil was being discharged from the wellhead, coincided with the spawning season of the Eastern oyster (*Crassostrea virginica*), an environmentally and commercially important shellfish species in the Gulf of Mexico. The long planktonic nature (14 to 21 days) of oyster larvae, sedentary nature of adult oysters, high filtration rates and their micro algal/particulate diet make them vulnerable to acute exposure to contaminants both in solution and bound to suspended sediment, and adsorbed onto algal and other particles.

We conducted a series of experiments to examine the effects of the *Deepwater Horizon* (DWH) oil and dispersants on various life stages of oysters ranging from gametes to adults. Oysters were exposed to water accommodated fractions of oil (with and without dispersant), sediment elutriates, contaminated sediment, and contaminated algae. Fertilization success, morphological development, growth, survival, and settlement success after these exposures were measured. Fertilization success decreased, developmental abnormalities increased, and larval growth, survival, and settlement, and reproductive development of adults decreased in a dose-dependent manner relative to oil exposure and exposure duration.

Results suggest that exposure of various life stages of oysters to oil and dispersants will have a negative impact on overall growth and survival and may have implications on the planktonic community, food chain and population structure of oysters.

**A stochastic modeling of the impacts of spill location on the fate and transport of oil spills in the Vancouver Harbour**

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The potential risk of oil spill in Vancouver Harbour, as well as in the English Bay, is expected to increase due to the raise cargo handling in Port of Metro Vancouver (PMV) and proposed Trans Mountain Expansion Project (TMEP) in the BC coast. In the present study, the transport and fate of the oil spills have been stochastically modelled by using the three-dimensional and particle- based Oil Spill Contingency and Response (OSCAR) model. The model was forced by hydrodynamic forcing modeled by the Finite-Volume Community Ocean Model (FVCOM). Twenty anchorages, which belong to PMV, in the English Bay were selected to study the impacts of spill locations on the fate and transport of oil spill in the English Bay and Vancouver Harbour. The tidal excursion length in the English Bay and Vancouver Harbour that calculated based on the hydrodynamic variables from FVCOM model was also studied and compared with the probability of oil contamination. The results indicated that the probability and areas of contamination in water column, on water surface, and on the shoreline were very sensitive to the oil discharge locations in the English Bay. Moreover, the transport of the spilled oil was mainly controlled by the tidal currents, which were strong spatial variation in the English Bay and Vancouver Harbour.

## **Pollution and distribution of PAHs in south areas of Japan after oil spill from Sanchi Tanker**

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On January 2018, the Sanchi tanker sank off the coast of Okinawa, Japan. Most of condensate for 136,000 ton put in the tanker spilled, and could reach and diffuse around Japanese coast with the huge area. At first, because the spilled condensate was remarkable large amount, the effects to the ecological system at the polluted area were concerned. However, the components of condensate possibly could become low concentrations in short period owing to their volatility, solubility, and low molecular weights. Alternatively, the heavy oil used as the fuel followed to spill from Sanchi, and had been drifted around the south areas of Japan for a few months. Large amounts of heavy oil had continued to reach widely the shorelines of Amami and Takara Islands from the middle of February, and then also Okinawa Island from the first of March. The reached oils were large ball shapes and high viscosities, and involved much amount of sea water. Many citizens and volunteers actively removed the oil on the shoreline for one month.

We collected shellfish, sediments and the residue of oil ball in some sites of Amami and Takara Islands mainly polluted by the spilled heavy oil on the first of March 2018, and investigated the polluted conditions with PAHs and their derivatives.

The individual PAHs in the drifted oil were relatively low concentrations compared with those observed in the previous oil spill accidents such as the spill at the Guimaras island, Philippines, where we had been investigating the variations of PAHs for 5 years after the spills. This result suggest that the spilled heavy oil was weathered during drifting in the ocean, and large part of PAHs degraded.

Some kinds of shellfish such as snails and bivalves were collected in Amami and Takara Islands. Total concentrations of 16 EPA PAHs in the most of shellfish were under 100 ng/g dry weight. However, a species of oyster accumulated high concentration of PAHs, and, for example, the concentration of 456 ng/g dry weight was detected in the oyster collected in the north site of Amami. Additionally, 1673 ng/g dry weight of total PAHs in oyster collected at the north site of Takara Island. Oyster was observed high accumulations of PAHs in the polluted sites by the previous oil spill, and this bivalve could possibly much accumulate PAHs than other shellfish.

Total concentrations of PAHs were roughly similar at all sites of Amami, and this tendency suggest that the spilled oil wrapped the coastal area of the Island widely and evenly. Also the PAHs in shellfish collected Amami and Takara Islands were much lower concentrations than those at the research at Guimaras Island just after one month from spill. This result means that the oil pollutions at Amami and Takara Islands held to a minimum.

**Distribution and potential sources of persistent toxic substances in sediment across coastal areas of the Yellow Sea and Bohai Sea in Korea and China**

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The pollution of the Yellow Sea and Bohai Sea with persistent toxic substances (PTSs) due to the rapid economic growth for decades has been a subject of intensive research. However, despite significant amount of research efforts devoted, inconsistency in study regions, sampling periods, and methodologies makes the evaluation of pollution degree in these seas as a whole difficult. In order to address this issue, we aim to comprehensively investigate 1) the distribution of PTSs, such as polycyclic aromatic hydrocarbons (PAHs), alkylphenols (APs), and styrene oligomers (SOs), and 2) the sources of PTSs and sediment organic matter in the Yellow Sea and Bohai Sea. The surface sediments were collected at a total of 125 sampling sites covering the coastal areas of the Yellow Sea and Bohai Sea in July 2018. The great concentrations of target PTSs exceeding corresponding guidelines were detected at sites near the industrial and urban areas, reflecting the main sources of PTSs. Fresh inputs of APs and SOs were also confirmed at these sites. In addition, the PAHs were found to be mainly originated from pyrogenic sources. The results suggested terrestrial input to the sediment organic matter as the source of PTSs in the Yellow Sea and Bohai Sea. Overall, we could specify the hotspot areas more precisely from systematic sampling over larger area across the Yellow Sea and Bohai Sea. Furthermore, our study suggest that reliable and effective pollution management of coastal regions requires coherent monitoring at a large scale through national and international collaboration.

## Identification of novel AhR-active chemicals in sediments of an industrial area using effect-directed analysis

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In this study, effect-directed analysis was applied to identify the major aryl hydrocarbon receptor (AhR)-active chemicals in sediments collected from a highly industrialized area (Ulsan Bay, South Korea). The specific objectives were: i) to investigate highly potent fractions of sediment organic extracts by use of H4IIE-luc in vitro bioassay; ii) to determine concentrations of known AhR-agonists such as polycyclic aromatic hydrocarbons (PAHs) and styrene oligomers (SOs); iii) to identify unknown AhR agonists in the highly potent fractions by use of GC-QTOFMS; and finally iv) to evaluate contributions of traditional and novel AhR agonists to total induced potencies. Results showed that great AhR-mediated potencies were found in fractions of organic extracts containing aromatics with log Kow values between 5 and 8. The great concentrations of PAHs and SOs were also found in those fractions, but those chemicals could explain only a small portion of the total induced AhR potencies. Results of the GC-QTOFMS analysis indicated that 405 and 463 compounds were detected in the highly potent fractions such as F2.6 and F2.7, respectively. We selected 16 candidates as tentative novel AhR agonists considering 1) peak abundance, 2) library matching factors, 3) aromatic ring numbers, and 4) commercial availability of standard materials. Among 16 candidates, 7 chemicals such as 1-methylchrysene, benzo[j]fluoranthene, 3-methylchrysene, 5-methylbenzo[a]anthracene, 11H-benzo[b]fluorene, benzo[b]naphtho[2,3-d]furan, and benzo[b]naphtho[2,1-d]thiophene exhibited significant AhR-activity. Relative potency values (RePs) of 7 novel AhR agonists were newly obtained, which were greater or comparable to that of benzo[a]pyrene (BaP). Results of potency balance analysis suggested that instrument-derived BaP equivalent (BEQ) concentrations (sum of PAHs, SOs, and 7 novel AhR agonists) could explain 2.5 to 300% of bioassay-derived BaP-EQ. Novel AhR agonists were widely distributed in the industrial sediments, thus studies on sources, distributions, and ecotoxicological effects of these chemicals are strongly needed in the future.

**Anthanthrene and 3-hydroxychrysene as novel AHR active compounds:  
Predicting potential toxicity of PAHs by bio-physical approach**

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Polycyclic aromatic hydrocarbons (PAHs) which refer to a group of compounds with two or more fused benzene rings are ubiquitous environmental pollutants generated primarily from anthropogenic materials (e.g. coal, oil, petrol, and wood). Despite myriad kinds of PAHs present in the marine ecosystem, conventional risk assessment has considered only a selected number of the PAHs. Potential toxicity identification of new PAHs has garnered significant interest in ecotoxicology community. In the present study, the aryl hydrocarbon receptor (AhR)-mediated potencies as the biological activity of chrysene-based PAHs were investigated using the H4IIE-*luc in vitro* bioassay. Among target PAHs, two compounds, dibenzo[def,mno]chrysene (*a.k.a.*, anthanthrene) and 3-hydroxychrysene showed novel AhR-mediated potencies. In addition, relative potency values of dibenzo[def,mno]chrysene (0.2) and 3-hydroxychrysene (0.88) compared to that of benzo[*a*]pyrene (1.0) were newly obtained from the dose-response test. Considering such great potency of these novel AhR-active compounds, their presence in the marine environment should be carefully monitored in the future. On the other hand, a mechanism behind such material-oriented activation of toxicity cannot be fully understood with current statistics-based toxicity prediction models. That is why we are also developing a bio-physical communication model relating the material's bioactivity and physical properties using first principle calculations and synchrotron-radiation X-ray spectroscopy. This model would serve as an absolute toxicity assessment tool for determining the potential toxicity of yet-to-known chemicals.

## **Deregulation of miRNA/mRNA in transgenerational bone deformity in medaka induced by ancestral exposure to BaP**

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Benzo[a]pyrene (BaP), a toxic polycyclic aromatic hydrocarbon, is ubiquitous in the environment. Our previous studies showed that environmental level of BaP (1 µg/L) can induce vertebral compression in medaka (*Oryzias latipes*) F1-F3 larvae descended from ancestrally exposed F0 parents. A reduced osteoblast (OB) abundance and impaired notochord sheath in F3 larvae was persistent to adulthood, indicated by decreased bone thickness and increased microcracks in ancestral BaP-exposed F3 male. Moreover, aberrant expression of *osterix (osx)*, *coll10a1 (coll10)* and regulating miRNAs (including *miR-214*) were found in the F3 offspring ancestrally exposed to BaP, which could potentially link to a reduced OB activity. However, the precise mechanisms of bone mRNA/miRNA deregulation underlying BaP induced transgenerational vertebral deformities remain unclear. It is hypothesized that ancestral exposure to BaP can deregulate bone mRNA/miRNA (including the *osx/miR-214* pair), perturb OB differentiation and/or maturation, impair OB abundance and/or activity in developing F1-F3 vertebrae, resulting in vertebral compression in F1-F3 offspring. To test the hypothesis, the double transgenic *coll10:nGFP/osx:mcherry* medaka were exposed to 1 µg/L BaP for 21 days. F1-F3 embryos were collected for studying *in vivo* and *in situ* expression of *coll10* (pre-mature OB marker) and *osx* (mature OB marker) along the vertebral column using fluorescence and confocal microscopy. Bone mineralization (OB activity) was examined with alizarin complexon staining using 0 and 17 days post hatching (dph) larvae. Histopathological analysis of vertebral malformations (alizarin red staining) were investigated using 17 dph larvae. The results show that ancestral BaP exposure attenuated OB differentiation and maturation, reduced bone mineralization on the developing vertebrae, and significantly induced vertebral compression in the F1-F3 ancestral BaP-exposed larvae (vs the respective control). Micro-architecture of adult vertebrae was evaluated by micro-CT. Molecular pathways involved in deregulation of mRNAs and miRNAs were identified using bone transcriptomes of F3 ancestral BaP-exposed male fish (vs F3 control male) by Next Generation Sequencing. The results will be presented and discussed.

**Microplastics in mussels and the indication for coastal microplastic pollution**

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Microplastic has been confirmed as an emerging pollutant in marine environments. One of the primary environmental risks of microplastics is their bioavailability for aquatic organisms. Bivalves are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the water column.

We conducted large scale investigations on microplastic pollution in mussels along the coastlines of China from 2015 to 2017. Our results suggested that microplastic was widespread in mussels. Our results also supported positive and quantitative correlations of microplastics in mussels and in their surrounding waters and that mussels were more likely to ingest smaller microplastics. We further conducted exposure experiments to find out the novel ways beyond ingestion for mussels to take microplastics from the environments. Our results suggested that microplastics could adhere to the surface of soft tissue or fuse into the byssus of the mussels. These new findings make us rethink about the bioavailability, accumulation and toxicity of microplastics to aquatic animals.

Mussels are suitable sentinel organisms for microplastic pollution because of their wide distribution, vital ecological niches, susceptibility to microplastic uptake and close connection with marine predators and human health. We propose the use of mussels as target species to monitor microplastics and call for a uniform, efficient and economical approach that is suitable for a future large-scale monitoring program.

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## **Microplastic contamination in the commercial fish from the Pearl River Estuary, China**

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Microplastics are ubiquitous in aquatic environments and can be ingested by a wide variety of marine organisms. To date, microplastic contamination in estuarine fish has been poorly understood, despite their ecological and economic importance. In this study, microplastics were detected in 26 species of commercial fish collected from the Pearl River Estuary. Microplastic abundance in each species ranged from 0.17 to 1.33 items individual<sup>-1</sup>, with an average of  $0.57 \pm 0.32$  items individual<sup>-1</sup>. Microplastics were found in all of the gills, 92.3% of the stomach and 73.1% of the intestines. The filtration areas of gill varied from 0.088 to 862.174 mm<sup>2</sup> in different species and had a strong positive relationship with microplastic abundance in gills. Feeding habits and living habitats of fish showed little influence on microplastic abundance. Nevertheless, the microplastic concentration was positively correlated to the trophic levels of fish samples. Fibres were the dominant form of microplastics, accounting for 93.4% of the collected plastic particles. Majority of microplastics (90.4%) were in the size ranges less than 3 mm. The most common identified polymers were polyethylene terephthalate (38.2%), polypropylene-polyethylene copolymer (27.3%), and cellophane (25.5%), together accounting for 91.0% in the examined items. Prevalence of microplastics in the gill parts suggests that the previous studies might underestimate microplastic accumulation and their biological impacts on fish, especially for those from the highly polluted estuaries.

**Microplastic ingestion among: I. Marine fishes from different feeding guilds, and II. Fishes from the marine and freshwater environs in eastern Visayas, Philippines**

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Microplastics are garnering wide global concern, and yet, none had been reported from Eastern Visayas, Philippines. This study has two sections, investigating microplastic (MPs) ingestion among (I) the detrital feeder mullet, *Valamugil speigleri* and the herbivorous white-spotted spinefoot, *Siganus canaliculatus*, and (II) between the freshwater rockmountain bass, *Kuhlia rupestris*, and the marine *S. canaliculatus*. Part I aimed to determine and compare the amount of microplastics in the guts of fish differing in feeding guilds, individual sizes, and body weights. The second part compared MPs amount in fishes and their surrounding waters. Both sections made use of the fish's entire gastrointestinal tract (gut) dissolved in 10% KOH incubated for 60°C from 12-24 h. Section I data revealed more rabbitfishes (58.57%,  $n=70$ ) were ingesting MPs than their mullet counterparts (30.00%). Pearson correlation averaging 0.06 suggests a weak correlation between fish weight and MPs amount for both species. Higher number of MPs were shown by the herbivores ( $1.21 \pm 1.61$ ) than by the detritivores ( $0.47 \pm 0.90$ ). Similar findings were observed for Section II, wherein more *S. canaliculatus* individuals (66.0%,  $n=100$ ) ingested MPs than its freshwater counterpart, *K. rupestris* (45.0%), as well as a very weak correlation between fish weight and number of MPs, and also, more MPs recovered from rabbitfish ( $n=101$ ) than in *K. rupestris* ( $n=70$ ). More MPs were ingested by *S. canaliculatus* near the estuary compared to the other fish samples from the other stations, although there was no significant difference ( $p=0.23$ ) in the number of MPs between the water samples from the sampling stations.

**The ingestion of microplastics and micro-particles by mangroves crabs is related to their feeding habits**

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Mangroves are increasingly impacted by multiple sources of pollution, and plastic is not an exception. Crabs are a dominant component of the mangrove food web and play a critical role to maintain ecosystem functioning and health. Understanding the potential transfer of micro debris within these key organisms will help assessing the overall impact of plastic pollution at ecosystem scale. We quantified and characterized the micro particles found in the cardiac stomach and gill chambers of four species of crabs from three mangrove forests of Hong Kong. We observed a significant variability in abundance and types of anthropogenic micro-particles across sites and species. Interspecific differences proved to be strongly related to the role of each species in the food web and could also be explained by their peculiar feeding habits, with less selective species ingesting more particles. Interestingly, this trend seems inversely related to the trophic level of the crabs.

## **Sorption kinetics of benzo[a]pyrene, PAH mixture and PCB-126 by virgin polyethylene of different sizes**

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Considering the co-existence of microplastics and organic pollutants in Deep Bay oysters, we specifically examined the sorption kinetics of virgin polyethylene (PE) of different sizes (1 - 10  $\mu\text{m}$ , 90 - 106  $\mu\text{m}$  and 212 - 250  $\mu\text{m}$ ) towards benzo[a]pyrene, PCB 126 and a mixture of PAH compounds, at different contact duration (0, 0.25, 0.5, 1, 2, 4 and 8 hours) and salinity (0, 20 and 35 ppt) under laboratory conditions.

Adsorption of benzo[a]pyrene (B(a)P) on larger plastic pellets 90 - 106  $\mu\text{m}$  and 212-250  $\mu\text{m}$  PE quickly reached to an equilibrium after 30-min mixing, while smaller plastics 1-10  $\mu\text{m}$  PE took 1 hour to reach maximum sorption. The B(a)P sorption capability between plastics of different sizes were, however, no different after 8-hour mixing (1.1 to 1.3 mg B(a)P adsorbed per gram). In contrast, the amount of PCB 126 adsorbed by virgin PE was much fewer (0.1 mg PCB 126 adsorbed per gram after 8-hour mixing) than benzo[a]pyrene adsorption, and the PCB 126 sorption equilibrium took 1 hour to reach. Extremely small PE particles (1-10  $\mu\text{m}$ ) with larger surface area showed a 6.6 to 7.9-fold PCB 126 adsorption capacity of larger PE particles (90-106  $\mu\text{m}$  and 212-250  $\mu\text{m}$ ). This illustrated that extremely small microplastics would have a higher sorption capacity towards certain pollutants and pose a higher risk deserving our greater attention. Salinity (0, 20 and 35 ppt), however, did not affect the adsorption of B(a)P and PCB 126 by virgin PE.

On the other hand, microplastics of all sizes showed a higher sorption capability towards hydrophobic PAHs composed of 5 to 6 aromatic rings (B(a)A, B(b)F, B(k)F, B(a)P and B(g,h,i)P) than less hydrophobic PAHs with 2 to 4 aromatic rings (ACE, FLN, ANT, PHE and PYR). The adsorption results of ACE, FLN and PYR showed that smaller plastics (1 - 10  $\mu\text{m}$ ) with greater surface area had a significantly higher adsorption capability than larger microplastics (90 - 106  $\mu\text{m}$  and 212 - 250  $\mu\text{m}$ ). Comparing the adsorption of benzo[a]pyrene on plastics in a single and multi-solute system, competitive sorption onto PE was not observed. Overall speaking, our findings confirm the capability and role of microplastics as a vector transferring organic pollutants to marine biota.

**Synergistic toxicity of microplastic and organic pollutants on the marine rotifer *Brachionus koreanus***

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Among the various materials found inside microplastic pollution, nano-sized microplastics are of particular concern due to difficulties in quantification and detection; moreover, they are predicted to be abundant in aquatic environments with stronger toxicity than micro-sized microplastics. Here, we demonstrated a stronger accumulation of nano-sized microbeads in the marine rotifer *Brachionus koreanus* compared to micro-sized ones, which was associated with oxidative stress-induced damages on lipid membranes. In addition, multixenobiotic resistance (MXR) conferred by P-glycoproteins (P-gps) and multidrug resistance proteins (MRPs), as a first line of membrane defense, was inhibited by nanoplastic pre-exposure, leading to enhanced toxicity of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and triclosan (TCS) in *B. koreanus*. Our study provides a molecular mechanistic insight into the toxicity of nano-sized microplastics towards aquatic invertebrates, and further implies the significance of synergetic effects of microplastics with other environmental persistent organic pollutants.

**Of mangroves and men: Anthropogenic marine debris in Hong Kong mangroves**

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In this era of single-use plastic, the issue of the prevalence of anthropogenic marine debris (AMD) in the marine environment is becoming increasingly apparent. Numerous studies have shown that plastic can represent over 90% of debris found on beaches, but there are few studies investigating it in other coastal habitats, for example - the mangroves.

This research project investigates the occurrence of anthropogenic marine debris in Hong Kong mangroves. Little research has been performed on mangrove rubbish in general, and understanding the distribution and type of rubbish in the mangroves is vital to continuing research in the area.

The mangroves are often lauded as a natural buffer against damaging weather events such as storms and typhoons, but the features of the mangroves that make them such good buffers (such as complex root systems and high density) may also mean that they potentially are great “catchers” of marine rubbish.

We designed and performed standardised surveys to collect and compare data about quantity, diversity, and possible sources of marine litter in different zones of the mangrove. Our results indicate that the sources and types of rubbish vary between the seaward zone and the landward zone of a mangrove. This information is vital for understanding the source of anthropogenic marine debris in mangroves, and how we can work to reduce its occurrence.

In general, anthropogenic marine debris impact is measured by abundance and weight, here we will also discuss the importance of considering the “area covered” by marine litter. The presence of marine litter in mangroves has implications not only for the mangrove plants themselves, but also for the animals (e.g. crabs) that inhabit them.

## **Marine plastics pollution: A geostatistical approach for joint prioritization of multiple stakeholder perspectives in Hong Kong**

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Pollution of the marine environment in Hong Kong by discarded plastics, polystyrenes and other items is a cumulative and accelerating problem that has yet to be fully realized or resolved. In particular, the regions beaches have much higher than average micro-plastic pollution with sources, transport and deposition all subject to uncertainty. This quintessential characteristic must be mapped to ensure stakeholders can achieve greater involvement in ongoing marine spatial planning and management. We employ geostatistical interpolation as the mapping approach, to enable a regional scale assessment of the spatial distribution of three quantities of interest, denoted by *Plastics Waste*, *Ports Shipping Intensity* and a notional measure we refer to as *Ecological Insecurity* to be determined. We generate map layer representations for each of these factors to characterize the perspectives held by corresponding stakeholders and then employ associated uncertainty maps to enable joint prioritization of key highlighted areas. Prioritized area mapping (PAM) is a geospatial approach that links perspectives of otherwise disparate stakeholders to ecosystem-based-management, thereby balancing ecological, socio-economic and governance principles across temporal and spatial scales. The PAM approach serves as a generic scoping tool to help assess any marine spatial planning problem.

## **Integrated use of ChemTHEATRE and AIST-MeRAM for rapid and cost-effective risk assessment of environmental contaminants**

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There is a general trend toward the growing importance of open data worldwide. It appears to be essential that development of scientific data repositories be accelerated. In the field of environmental chemistry and ecotoxicology, a huge number of monitoring data on chemicals in various environmental and biological specimens have been reported in scientific journals. However, comprehensive, public repositories to store such valuable data set of the chemicals do not exist; researchers are forced to spend lots of time and cost in collecting and utilizing the published data, when modelling environmental behavior and fate of, and performing the risk assessment for, the chemicals of interest. Therefore, it is desirable that various stakeholders in the field should work together to improve and promote secondary use of the data. To this end, we have created a platform to register and visualize the monitoring data of environmental contaminants, named 'ChemTHEATRE' (Chemicals in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement). To date, data described in more than 66 projects have been registered on the platform. Users can find e-archived chemical concentration data in the environmental and biological specimens each with associated metadata such as sampling date and location, species, and biometrics, in addition to the detailed description of experimental methods.

Bridging ChemTHEATRE to AIST-MeRAM (Multi-purpose Ecological Risk Assessment and Management Tool) storing chemical property and/or hazard/toxicity information provides us high accurate and transparent assessment of ecological risk of chemicals. Much effort is currently being devoted to visualizing e-archived data sets, and enhancing available data-model interfaces to simulate global dynamics of chemical pollution, with Finely-Advanced Transboundary Environmental model (FATE), and to promote a series of integrated exposure and effects analyses. It is thus expected that ChemTHEATRE will be not only a dedicated follow-up and forecasting tool of international regulations on pollution control in the light of traceability and responsible-care engagement of chemicals, but also a 'communication theatre' where a variety of stakeholders can improve their risk literacies and develop new projects through open data access.

**eDNA metabarcoding on zooplankton improves the ecological status assessment**

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Worldwide, aquatic ecosystems are monitored by using a range of biological quality elements that determined by morphological species identification which is time-consuming and expensive. Although eDNA based technology like metabarcoding has unprecedented capacity to species identification and has obvious advantages compared with traditional sampling and image recognition, most studies in this area just used it to estimate the biodiversity. How to utilize this approach to assess ecological status remains largely unexplored. Here, taken zooplankton as an example, we explored the possibility of eDNA metabarcoding approach on ecological status assessment in a freshwater ecosystem and developed an outline of eDNA based ecological status assessment. We studied the spatial and temporal variation of zooplankton community in three seasons using eDNA metabarcoding and found both species composition and bio-interaction have obvious difference among sampling seasons, which indicate that a seasonal dependent zooplankton index should be considered in ecological status assessment. A total of 60 different zooplankton indices were calculated based on the eDNA monitoring and most of them highly correlated with water quality in one or all three seasons. Both qualitative and quantitative biological indices play an important role in eDNA based assessment. Seasonally dependent zooplankton integrity index can well reflect the aquatic ecological status, and this method improves the timeliness of the bioassessment. Our results demonstrate that eDNA based biomonitoring can be used to assess the ecological assessment in the future.

**Dynamic analysis of an ecological security pattern relying on the relationship between ecosystem service supply and demand — A case study on the Xiamen-Zhangzhou-Quanzhou bay-type city cluster**

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Ecological security refers to the state in which human's benefits in the ecosystem are not threatened. This study diagnoses the spatial characteristics of regional ecological security from multiple perspectives and focus on zoning management that is an effective approach to reducing the spatial difference in city cluster ecological security. Based on the supply and demand theory, this study constructs a framework for a dynamic model of the relationship between ecosystem services supply and demand, and uses this relationship to characterize the ecological security pattern in the Xiamen-Zhangzhou-Quanzhou city cluster. Through multiple scales (Including inland and coastal zone), This article evaluates the status of the ecosystem service supply and demand of the city cluster and analyzes the zoning of the ecological security patterns using the InVEST model, landscape quality index, ecological resilience model, and value modification model, etc. This article also analyzes the factors and current status of the ecological security pattern by using the environmental Kuznets curve. Finally, we propose differentiated management measures for government and stakeholders according to different ecological security zoning. The four main findings are as follows: (1) in total, 50% of counties in the city cluster belong to the "high supply imbalance zone" of ecological security, and 70% of these counties are within Zhangzhou City; 32.14% of counties fall into the "high demand imbalance zone" of ecological security, and these counties include districts and coastal areas; 17.86% of counties belong to the zone where both the supply and demand for ecological security are imbalanced; no counties are located in a zone where both the supply and demand for ecological security are balanced. (2) The demand for ecosystem services in the Xiamen-Zhangzhou-Quanzhou city cluster is weak. There are still over 80% of counties below the average level. There is a notable spatial difference between some coastal counties of Xiamen and Quanzhou and surrounding counties. The overall ecosystem service supply of the city cluster is better, but the difference in ecosystem service supply between the coastal and inland counties is remarkable, and it presents an increasing trend from the coast to inland. Zhangzhou performs quite well, and all counties in Zhangzhou perform above the average level, but Xiamen shows an opposite pattern, and there is a distinct difference between the inland and coastal counties in Quanzhou. (3) From the environmental Kuznets curve of ecological security that we constructed, it can be suggested that the whole region is still in a "dilemma state", where there is great disharmony between the ecological environment and economic development. Meanwhile, the study helps to explain the dislocations in supply and demand, and the spatial difference in the ecological security patterns of the Xiamen-Zhangzhou-Quanzhou city cluster. (4) Trough the analysis of coastal ecological security in the Xiamen-Zhangzhou-Quanzhou city cluster, we found that there are obvious spatial differences in the marine environmental status under the jurisdiction of each county, and the marine environmental status under the jurisdiction of Xiamen City is not positive, while Yunxiao county, Hui'an county and Quangang district shows well. And an excellent marine environment situation has contributed to the promotion of the overall level of ecosystem service supply. Meanwhile, this paper believes that it is an urgent task to deal with the relationship between the coastal zone and inland.

## Seasonal and spatial river fluxes of the pollutants in response to adjacent coastal water quality in Hainan Island, China

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Changing human activities had accelerated global element cycling and appreciably altered marine ecosystems in the riverine-estuarine systems. This was evident by dramatic shifts in the supply of main chemical pollutants (COD, TN, TP). However, few of the studies were known about the concentration and flux and of these seasonal changes in the whole Hainan Island, China. We investigated the Hainan Island, the second largest island in China, to assess the spatiotemporal concentration and fluxes of pollutants using seasonal monitoring data (2017) based on sea and land integration. Seasonal and spatial patterns concentrations and flux were apparent and linked to hydrology feature. The results showed that the concentration of COD in estuarine water around Hainan Island was in the range of 4.20-10.44 mg/L, while TN and TP concentrations were in the range of 0.21-5.16 mg/L and 0-0.31 mg/L, respectively. The highest concentrations of COD, TN and TP were found in Wenjiao River, Changhua River and Luodai river estuaries. The estimated annual flux of pollutants from the main rivers of Hainan island discharged into coastal waters were  $1.5 \times 10^8$  kg for COD,  $1.8 \times 10^7$  kg for TN and  $1.1 \times 10^6$  kg for TP, respectively. The total land-based pollutants load were mainly from four rivers (Nandu River, Wanquan River, Jiuqu River, Yanzhou River), and which occupied 82.6% for COD, 91.0% for TN, 83.3% for TP. The largest contribution month to annual pollutants load was October, accounted for 15.3% of COD load, 13.7% of TN load and 17.8% of TP load. However, compared to the spatial rivers of pollutants fluxes, the largest flux contribution was Nandu River which contributed  $4.3 \times 10^7$  kg for COD,  $7.5 \times 10^6$  kg for TN,  $3.7 \times 10^5$  kg for TP. Monthly fluxes for COD, TN and TP were especially pronounced during the storm rainfall event in October. The annual average concentration pollutants in coastal surface water are ( $0.79 \text{ mg/L} \pm 1.10 \text{ mg/L}$ ) for COD, ( $0.06 \text{ mg/L} \pm 0.15 \text{ mg/L}$ ) mg/L for DIN and ( $0.01 \text{ mg/L} \pm 0.01 \text{ mg/L}$ ) for DIP, respectively. There was a significant negative correlation between pollutants (COD, DIN and DIP) and salinity ( $P < 0.01$ ) during wet and normal season, suggesting that pollutants in surface seawater mainly came from the land-based pollutants input in Hainan Island adjacent seawater. Overall main chemical pollutants level of Hainan Island adjacent seawater was relatively low, but the problem of water quality in some local coastal water areas were still outstanding. The coastal water quality management should consider the storm water influence and intensive monitoring estuary water quality should be introduced in practical monitoring scheme during storm rainfall events. The effective improvement of water quality should link coastal water quality monitoring with the land-based pollutant load input in Hainan Island.

## **Quantifying the relative importance of chemical versus noise pollution to marine mammal stranding/mortality events**

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Mass stranding/mortality events of marine mammals have been attributed to several causes. These include chemical pollution, shellfish poisoning, parasites, some pathologies associated with nutritional status, and military manoeuvres using sonar (noise pollution). A working hypothesis causally linking chemical pollution and mass stranding/mortality is that higher trophic-level marine mammals may have been particularly susceptible to infection because their immune systems were suppressed through the bioaccumulation of environmental contaminants such as polychlorinated biphenyls (PCBs), whilst the relative importance of chemical versus noise pollution (and other causes) has been poorly understood.

Numerical models such as Finely-Advanced Transboundary Environmental model (FATE) allow us to predict exposure of various environmental media (including the atmosphere, oceans, soils, and biota) to chemicals, on a variety of spatiotemporal scales. Using datasets of mass stranding/mortality events and hearing thresholds of marine mammal species, and FATE-predicted exposure of marine mammals to selected 22 PCB congeners, we quantify the degree to which mass stranding/mortality events are associated with noise pollution. To this end, we developed hypothetical noise pollution events in selected coastal areas and then perform a series of composite analyses to delineate the relative importance of chemical versus noise pollution. A re-sampling technique (Monte-Carlo simulations) was used to test statistical significance of the relative importance. Our discussion will highlight a new metric and its application to the past lawsuit cases in which navy exercises allegedly could have been responsible for mass stranding/mortality events of some Odontoceti species, thereby providing new insights into the chemical-versus-noise debate.

**A mesocosm study for evaluation of the purification capacity of pollutants in the tidal flat**

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As amount of pollutants transported from lands into the tidal flat increases, interest in the purification of pollutants by the tidal flat among marine ecosystem services has been increasing. However, it is difficult to accurately estimate the purification capacity of a tidal flat, and the literature on this matter is relatively scarce. In this study, we aim to develop a reliable estimation method under controlled environmental conditions. Two polluted groups (in polluted sediment vs. polluted water) that could occur in the marine environment were designed and the purification capacity was evaluated with respect to the type of tidal flat (salt marsh vs. bare tidal flat) and the concentration of pollutants such as chemical oxygen demand (COD) and total phosphorus (TP). We measured the amount of pollutants removed by the tidal flat daily for 14 days with well-controlled experimental system, which simulated an artificial tide for 12-hour period. The results showed that the removal rates of COD and TP reached at the highest values after about 7 days in both polluted groups, indicating a stabilization period for the tidal flats exposed to pollutants and change in the microbial community degrading the pollutants. At the beginning of exposure, the removal rate depended on the concentration of pollutants (the greater concentration resulted in larger removal rate), while the removal rate was eventually saturated to the same level for all concentrations. Furthermore, the salt marsh showed greater removal rate than bare tidal flat, indicating the effect of phytoremediation such as nutrient uptake. Overall, the pollutant concentration and the presence of halophyte were found to be the major factors governing the purification capacity of tidal flat and thus considered as important variables in quantitatively determining the total amount of removed pollutants by tidal flat.

**Disruption of thyroid hormone homeostasis and lipid metabolism by exposure to novel brominated flame retardants (NBFRs) in zebrafish**

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Novel brominated flame retardants (NBFRs), such as decabromodiphenyl ethane (DBDPE), bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH), are alternatives to legacy brominated flame retardants, e.g., deca-BDE (BDE209), penta-BDEs. The large-scale use of them has led to rapid increase in environmental contamination, as well as in wild life and human. By using zebrafish (*Danio rerio*) embryos (larvae) as model, we investigated potential toxicity of DBDPE and TBPH. Chemical analysis showed that both of them can bioconcentrate in zebrafish upon water-borne exposure. For DBDPE, increased thyroid hormone concentrations (T4 and T3) was observed, accompanied by altered certain gene and protein expressions along the hypothalamic–pituitary–thyroid (HPT) axis, indicating thyroid endocrine disruption, furthermore, we found that exposure to DBDPE shows stronger activity compared with BDE209. For TBPH, we investigated the potential impact on lipid metabolism of TBPH and its major metabolite, mono(2-ethylhexyl) tetrabromophthalate (TBMEHP). Molecular docking was performed to evaluate the affinity between the chemicals and PPAR  $\gamma$ , and the results show that both TBPH and TBMEHP can constitute hydrogen-bond with the active pocket of PPAR  $\gamma$ , while TBMEHP has stronger affinity with PPAR  $\gamma$  *in silico*. In zebrafish embryos assay, exposure of TBPH and TBMEHP, can significantly reduce the concentrations of the total triglyceride (TG) and cholesterol (TCH) in larvae. As an important gene involved in lipid metabolism under the regulation of PPAR  $\gamma$ , altered transcriptions of *fabp11a*, as well as reduced methylation its promoter was further observed.

## **Implication of tannin changes in two mangrove plants, *Kandelia obovata* and *Bruguiera gymnorhiza* under PBDE contamination**

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Mangrove plants are known to be tolerant to many types of pollutants, including heavy metals and persistent organic pollutants (POPs). Polybrominated diphenyl ethers (PBDEs), due to their strong hydrophobicity, persistence and toxicity, are a group of ubiquitous POPs in mangrove ecosystems. The tolerance of mangrove plants could be due to their antioxidant systems, including enzymatic and non-enzymatic antioxidants, to counterbalance the negative effect of reactive oxygen species (ROS) caused by these environmental stresses. Tannins, a group of phenolic compounds with a large number of phenol sub-structures and high redox potentials, are well-known of their antioxidant activity. The response of tannins in mangrove plants to PBDE contamination and their antioxidant potential for the tolerance are still unknown. The present study therefore aims to reveal the changes of tannins, both in concentration and chemical structure, in two mangrove plants, namely *Kandelia obovata* (Ko) and *Bruguiera gymnorhiza* (Bg) under PBDE stress, as well as evaluate the antioxidant potential of mangrove tannins.

An environmentally relevant contamination level of mixed PBDEs in mangrove sediment was prepared by adding a commercial penta-BDE mixture (TBDE-71X) and BDE-209 at a ratio of 1:4 (20 mg Kg<sup>-1</sup>). The sediments were planted with either Ko or Bg, and the effects of PBDEs on the growth and changes of tannins in these plants were investigated over twelve months. The mixture of TBDE-71X and BDE-209 showed significant adverse effects on the growth of Ko during the first three months, with significant decreases in dry biomass of leaf, stem and root; however, no significant difference in growth was found between Ko planted in PBDE contaminated sediments and control (without PBDEs) at the end of twelve months. Tannins in the leaf, stem and root of PBDE-treated Ko decreased significantly to counter-balance the toxic oxidative stress of PBDEs during the experiment, especially under the 12-months long-term treatment. Compared with Ko, changes of biomass and tannins in Bg were less significant, suggesting that Bg was a more tolerance mangrove species than Ko. The structural information of tannins obtained by HPLC-ESI-MS and MALDI-TOF-MS techniques revealed that procyanidins (PC) and prodelfinidins (PD) were the major types of condensed tannins for Ko and Bg, respectively. The numbers of hydroxyl groups (OH) on the B-ring of phenol sub-structures were two and three for PC from Ko and PD from Bg, respectively. Condensed tannins of Bg with more OH exhibited higher antioxidant activity than those of Ko, thus Bg was more tolerant to PBDE stress. This study firstly showed the direct evidence that both the concentration-level and structure-level of tannins play important roles in the tolerance of mangrove plants to PBDE contamination.

## **Air-sea gas exchange and deep sea fate of legacy POPs in the Arctic Ocean**

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The Arctic Ocean is known to be contaminated by various persistent organic pollutants (POPs). In order to determine the air-sea gas exchange and deep-sea fate of legacy polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), surface seawater and boundary-layer air samples were collected onboard the icebreaker *Xuelong* (Snow Dragon) from the North Pacific to the Western Arctic Ocean in 2010; Moreover, polyethylene passive samplers were deployed in vertical profiles in the Fram Strait, the only deep water passage to the Arctic Ocean, and in air and surface water in the Canadian Archipelago in 2015 and 2016. Results demonstrated the ubiquitous presence of PCBs and OCPs in air, surface seawater and throughout the water column in the Arctic Ocean. Levels of  $\Sigma$ hexachlorocyclohexanes ( $\Sigma$ HCHs, 5.5-30.6  $\text{pg m}^{-3}$  in the atmosphere, 38.6-758.9  $\text{pg L}^{-1}$  in seawater) increase with increasing northern latitude from North Pacific to the Western Arctic Ocean (35°N to 82°N), but no similar spatial gradients were identified for hexachlorobenzene (HCB, 1.0-63.8  $\text{pg m}^{-3}$  in the atmosphere, not detected-9.5  $\text{pg L}^{-1}$  in seawater). In addition to net volatilization of  $\beta$ -HCH, our data suggest that air-sea gas exchange is dominated by net deposition of all other HCHs and HCB in the North Pacific and the Western Arctic; While air-water fugacity gradients implied net volatilization to the atmosphere during the summer/sampling time for most investigated POPs in the Canadian Archipelago. In the Fram Strait, higher concentrations of  $\Sigma$ PCBs (1.3-3.6  $\text{pg L}^{-1}$ ), and dichlorodiphenyltrichloroethane ( $\Sigma$ DDTs, 5.2-9.1  $\text{pg L}^{-1}$ ) were observed in the deep water masses (below 1000 m), similar to nutrient-like vertical profiles. There was net southward transport of HCB and  $\Sigma$ HCHs of 0.70 and 14 Mg per annum, but a net northward transport of  $\Sigma$ PCBs at 0.16  $\text{Mg a}^{-1}$  through the Fram Strait.

## Effects of perfluoroalkyl acids on environmental microbial community

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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<sup>-1</sup>, 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.

## **Perfluorinated compounds in the aquatic food chains of two sub-tropical estuaries from the South African east coast**

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Persistent pollutants threaten ecosystems and human health. Man-made per- and polyfluoroalkyl substances (PFASs) are now ubiquitous in the environment, and remain in largely unknown concentrations and with unknown effects on aquatic life. The Umvoti Estuary is known to be severely degraded and stressed by human activities, while the Matigulu Estuary is considered to be in a fair condition and less impacted. This study aimed to assess PFASs present in different compartments of the Matigulu and Umvoti estuaries. Samples of water, sediment, invertebrates and fish were collected in the estuary mouths and an upstream site in each estuary. This study was the first known study to assess PFASs in these South African estuaries. A number of PFASs were found in water, sediment and biotic samples from both estuaries; with perfluorooctanoic acid (PFOA) present in every sample. Perfluorooctyl sulfonate (PFOS) was measured in all fish tissue samples and concentrations were significantly higher than other PFASs. Average PFOA concentrations from Umvoti water samples were higher than any other PFOA concentrations recorded in South African systems. PFOA and PFOS concentrations in invertebrates and fish species that feed in the water column were higher than the species that feed on or near the sediments. Highest PFASs were recorded in the Slender glassy (*Ambasis natalensis*) followed by the Mozambique tilapia (*Oreochromis mossambicus*) and the Cape stumpnose (*Rhabdosargus holubi*). In terms of tissue distribution PFOA and PFOS concentrations were higher in liver than muscle samples. Overall the PFAs were highest in environmental samples from the Umvoti, compared to the Matigulu, confirming the higher pollution status of this system. Human risk assessment revealed that PFOA and PFOS concentrations in fish from the Matigulu and Umvoti were below the Minimum Risk Levels (MRLs) for safe human consumption. Further investigations are needed to make conclusive statements on the bioavailability, bioaccumulation and possible risk to human health from PFASs and metals in these estuarine systems.

## **Spatiotemporal variations of retinoic acids in the marine environment of Hong Kong**

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As main metabolites of vitamin A, retinoic acids (RAs) exhibit different isomers which are critical for embryonic development of animals. However, either deficiency or excessive intake of RAs can lead to abnormal development in animals. For instance, teratogenic effects have been observed in amphibians, fish embryos and mammals at elevated levels of RAs. Since RAs and their metabolites are present in the urine of human and other animals under normal physiological conditions, RAs are thus constantly detected in sewage effluents and rivers in China and Japan. Yet, harmful algal bloom incidents in coastal marine water are common in urbanized coastal cities like Hong Kong while cyanobacteria can generate RAs, leading to the potential threats to marine animals. Hong Kong is an urbanized coastal city and its coast suffers from eutrophication with frequent occurrence of harmful algal blooms. Therefore, this study aims to: (1) investigate spatiotemporal variations of concentrations of different RAs in the coastal waters of Hong Kong; and (2) explore the relationship between the concentrations of RAs and selected environmental parameters, such as total suspended solids and chlorophyll *a*. Our preliminary results showed that 13c-4-oxo-RA was the predominating RA among the 30 sampling locations. The extracellular and intracellular concentrations of 13c-4-oxo-RA ranged from not detectable to 4.3 ng/L and not detectable to 29.5 ng/L, respectively in the dry season of between 2017 and 2018. Spatially, the water control zone of Deep Bay exhibited higher concentrations of RAs than other water control zones, probably due to the influence of the contaminated discharge from the Shenzhen River. Our results provide exposure data for ecological risk assessments of RAs in Hong Kong, and serve as reference for other highly urbanized cities around the world.

## **Legacy and novel halogenated flame retardants in the seawater and atmosphere of Bohai Sea: Spatial trends, seasonal variations and influencing factors**

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Distribution characteristics and seasonal trends of 17 HFRs, including 8 polybrominated diphenyl ether (PBDE) congeners, 7 novel brominated flame retardants (nBFRs) and 2 dechlorane plus (DPs) isomers were concurrently analyzed in the surface seawater and low atmospheric samples in August and December of 2016, and February and June of 2017 during four research cruises in the Bohai Sea, China. The total concentrations of PBDEs, nBFRs and DPs were in the range of 1.77 - 29.9, 9.18 - 623.3 and 0.084 - 7.84 pg/L in seawater, and 0.197 - 46.2, 0.291 - 1997 and 0.032 - 2.61 pg/m<sup>3</sup> in atmosphere, respectively. HFRs were mostly presented in suspended particulate matter (SPM) in water and total suspended particulate (TSP) in air samples. DBDPE was the predominant compound both in air and seawater samples, and its concentrations were one order of magnitude higher than those of BDE 209. Relatively high concentrations in water were discovered in the Laizhou Bay, where the largest manufacturing base of BFRs located and weak water exchange capacity happened. A distinct seasonal variation was observed for HFRs in seawater, with significantly higher concentrations (both in mass and volumetric units) in winter (December 2016 and February 2017) and lower levels in summer (August 2016 and June 2017). The major controlling factors are the intense resuspension of sediment induced by large wind waves in winter and phytoplankton scavenging effects in spring and summer. No obvious seasonality was found for HFRs in atmosphere. Normally, for the air masses passed through the nearby industrial regions, high concentrations of DBDPE (up to 1780 pg m<sup>-3</sup>) were co-existed with high levels of TSP (up to 150 ug m<sup>-3</sup>), whilst for the air masses from the west pacific region, very low concentrations of DBDPE (low to MDL) together with low levels of TSP were found.

## **Acclimation and epigenetic adaptation of Chinese edible oysters to ocean acidification: Implications on aquaculture**

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Globally, edible oysters are facing serious threats from human-CO<sub>2</sub> induced coastal acidification, especially when they are developing gonads and larvae. This climate change associated stressor has already caused observable impacts on oyster seed production in the USA, even in controlled hatchery conditions. China – as the producer of over 80% of the world’s edible oysters – is seriously concerned about this emerging issue because oyster growers in China depend heavily on wild seeds, over which they have no control. Substantiating this fear, Chinese oyster growers have observed unprecedented high summer mortalities over the past few years, possibly as an effect of multiple stressors involving acidification. Therefore, we have initiated a project to look at how acidification affecting larval recruitment, calcification products in terms of shell structure and mechanical properties, and importantly metabolic pathways that are used for acclimation. To our surprise, that a significantly higher proportion of the *Magallana hongkongensis* (Hong Kong oyster) populations in China are resilient to near-future levels of acidification. However, their larvae failed to select an optimal habitat (i.e. substrate with biofilms) for recruitment and also showed significantly lower recruitment rate under acidification (pH 7.4). The pressing question is then how exposure to acidification affects the susceptibility of oysters to pathogens. Based on our data, oysters might be resilient to pathogens under acidification. Of particular interest, the rapid “non-genetic” adaptation potential of oysters to acidification was studied by analyzing acidification-induced epigenetic marks on DNA, i.e. DNA methylation. The availability of annotated genome, tissue-specific transcripts, and well described stress and immune response pathways of the oyster populations in China provided us with solid groundwork for examining the inheritance and memory mechanisms of the acquired stress-tolerating mechanisms. In this talk, we will summarize these findings. Knowledge generated from this project providing an important stepping stone for the development of climate change stress and disease tolerant strains for aquaculture in the era of unprecedented global environmental change.

## **The decadal response of sublittoral meiofauna community structure to multiple stress in the Bohai Sea**

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The region of the Bohai Sea (118~122°E, 37~41°N) is among the most densely populated of any marginal sea worldwide, and the sea is of great commercial importance as one of the major fishing areas in China. Over several decades, the Bohai Sea marine ecosystem was faced with multiple stress from environment and human beings. In this study, sublittoral meiofauna community data collected from the Bohai Sea in 2014 was integrated with historical data during 1980s-2000s, to detect decadal response of meiofauna especially free-living marine nematodes community structure to the multiple stress.

Meiofauna abundance increased gradually over the past three decades. Meiofauna abundance averaged in  $1761 \pm 659$  ind.10cm<sup>-2</sup> in 2014. It was higher than that of 2008 ( $975 \pm 819$  ind.10cm<sup>-2</sup>), 1990s ( $869 \pm 509$  ind.10cm<sup>-2</sup>) and 1980s ( $789 \pm 292$  ind.10cm<sup>-2</sup>). Nematode abundance showed the same trend over the past three decades: averaged in  $1598 \pm 579$  ind.10cm<sup>-2</sup> in 2014, which was higher than that of 2008 ( $883 \pm 750$  ind.10cm<sup>-2</sup>), 1990s ( $758 \pm 475$  ind.10cm<sup>-2</sup>) and 1980s ( $527 \pm 262$  ind.10cm<sup>-2</sup>). A total of 89 species of free-living marine nematodes were identified in 2014, belonging to 69 genera, 28 families, and 3 orders. Xyalidae (most of them were deposit feeders) was the most dominant family in 2014 accounting for 52.0% of total nematode abundance. We caught sight of continuous increase of Xyalidae's dominance over the past three decades, and the replacement of dominant families. Chromadoridae and Comesomatidae were the most dominant families in 1980s accounting for 40.6% of total nematode abundance. Most of them were assumed to feed on fresh organic material as diatoms and microalgae. The significant replacement of dominant families suggested the stressed environment and food resource constraint. Our results also revealed the nematodes for  $c-p = 2$ , which was characterized with a short life-cycle and high tolerance to disturbance, increased in abundance significantly over three decades, indicating more stressed or eutrophic conditions in 2014 than before. Furthermore, the significant decrease of Shannon-Wiener diversity index  $H'$  from  $4.2 \pm 0.6$  (1980s) to  $2.6 \pm 0.6$  (2014) suggesting the increase of environmental stress as well.

The development of coastal industry, fishing activities, mariculture, oil platform construction, and marine transportation increased environmental pressure in the Bohai Sea. As results, nematode diversity decreased, nematode community structure changed and was characterized by higher fraction of colonizers. At the same time, induced by global warming, surface sea temperature of the Bohai Sea increased approximately 0.4°C over last 30 years. Present results showed a significant increase in the proportion of small-sized nematode species in 2014. Our study provides another evidence that reduced body size is the universal ecological response to global warming in aquatic systems.

## **Influence of different temperature on the effect induced by selenium in silver pompano**

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Temperature is an important factor for influencing the toxicity of metals particularly trace metal element in marine environment. However, little is known about the outcome of exposure to metal in different water temperatures associated with climate change. Biochemical and physiological parameters are considered as environmental biomarkers, widely applied to assess multiple stressors in the marine environment. To get in depth knowledge on this topic, *Trachinotus blochii* were exposed to different concentrations of selenium (10 and 100 µg/L Se), at different temperature, control (26 °C), cold stress (18 °C) and heat stress (34 °C) for 96 hours under laboratory conditions. Survival rates, accumulation of selenium in gill and liver tissues were recorded with respect to the temperature. Biochemical parameters include Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), scavenging activity of Reduced glutathione (GSH) and oxidative stress effect of malondialdehyde (MDA) in the gill and liver tissues of *T. blochii* were investigated. Present results evidenced that different water temperature modulates the effects of selenium on biochemical responses on fish. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in gills and liver tissues of *T. blochii* were effectively increased after exposed to selenium under heat stress rather than cold stress. The scavenging activity of reduced glutathione (GSH) level is also significantly induced after the exposure of selenium under heat stress compare to cold stress. The increased oxidative stress effect of malondialdehyde (MDA) levels was observed in gills and liver tissues of *T. blochii* exposed to selenium under heat stress compared to cold stress. Increased integrated biomarker response (IBR) value was attained by the synergetic effect of selenium and heat stress after 96 h exposure period. The present findings implies that, simultaneous stress due to fluctuation of water temperature and selenium exposure can induce oxidative stress and alter the biochemical responses in silver pompano, which can be used as a suitable biomarkers to detect the effects of pollution on marine environments.

**Keywords:** Selenium, Temperature, *Trachinotus blochii*, Oxidative stress, IBR.

## **Influence of salinity on the biochemical response in *Trachinotus blochii* exposed to selenium**

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Salinity shifts in estuarine and coastal area are becoming a major topic of concern and are one of the main factors for affecting metal bioavailability and stress factors on marine organisms. Selenium (Se) is an essential trace metal element, but many aspects of its toxicity remain unclear, particularly in the marine environment. Physiological and biochemical related biomarkers are a great interest due to their responses to environmental stressor which provide valuable data for biological monitoring in marine pollution. Hence, we studied the effects of different salinity levels (5, 15 and 35 psu) on the response of the silver pompano *Trachinotus blochii* is exposed to different concentrations of selenium (0, 5-100 µg/L) for 96 hours. Survival rates, accumulation of Se in different tissue parts were recorded. Biochemical analysis was performed to assess the antioxidant enzymes of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), scavenging activity of Glutathione (GSH), oxidative stress effect of malondialdehyde (MDA), and Na<sup>+</sup>/K<sup>+</sup>-ATPase (NKA) activity in the gill and liver tissues were investigated. Selenium exposed fishes showed that the alteration in an antioxidant enzymes, NKA activity and induced oxidative stress effect. These results clearly showed that the higher salinity level has mitigated the harmful effects of selenium. Salinity acted as a strong protective factor, with an increased oxidative damage (lipid peroxidation) in low salinity (5 psu), and the least in 100% sea water (35 psu) respectively. We applied an overall index, an integrated biomarker response (IBR), which increased under high concentration of selenium condition but recovered to the normal levels under high salinity treatment. Our results confirmed that exposure of sublethal concentrations of waterborne selenium is an alteration of biochemical and physiological responses in fish, and highlight the important protective role of higher salinities ameliorating selenium toxicity associated with enhancing biochemical and physiological response in this model estuarine teleost.

**Keywords:** Selenium, Salinity, Silver pompano, Oxidative stress, IBR.

## **The coral health status of *Platygyra* spp. at thermal and salinity tolerance limits in Hong Kong waters**

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Dissolved oxygen and pH fluxes are considered drivers and indicators of coral energetics dynamics, including metabolic processes of respiration, photosynthesis and calcification. The metabolic function of shallow ecosystems is threatened by severe fluctuations in temperature and salinity. The ability to accurately measure physiological rates of benthic organisms in natural conditions as a response to these fluctuations gained attention in recent years. Although *Platygyra* spp. corals are considered resistant species and well-adapted to survive in the marginal reef, such as Hong Kong eastern waters, the knowledge of its physiological response to short term anomalies at high temperature and low salinity is still lacking.

In this study, we used an innovative underwater diver-portable respirometer called CISME (Coral In-Situ Metabolism) designed to make non-destructive measures of coral energetics to combine laboratory-controlled conditions and *in-situ* monitoring of coral health status. We selected the temperature of 30°C as physiological limit over which corals go under stress. Gross photosynthesis ( $P_g$ ,  $p < 0.01$ ) and calcification rates ( $G$ ,  $p < 0.05$ ) significantly decreased at high temperature (30-32°C). In few cases, photosynthetic efficiency ( $F_v/F_m$ ) was also reduced. As result, the coral energetics ( $P_g/R$ ) was seriously affected by increasing temperature ( $p < 0.01$ ). On the other hand, the decrease of salinity (up to 21 psu) caused a decrease of calcification and whiteness ( $p < 0.05$ ) but did not affect the overall energetic status. These experimental results are in line with *in-situ* observations. Although the sea surface temperature in Hong Kong is usually below 30°C and seawater salinity above 30 psu, the increase of acute temperature and heavy rainfall events in Hong Kong waters expected in the near future will seriously affect the survival of these corals.

The application of such a method promises benefits for fast and reliable field monitoring of healthy and stressed corals. These data provide useful information on metabolism of *Platygyra* spp. in order to improve the accuracy of forecasting their health status under the future climate change scenarios and extreme events.

**Interacting effect of temperature and salinity on physicochemical properties and toxicity of zinc oxide nanoparticles towards the marine copepod *Tigriopus japonicus***

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Given the diverse physicochemical characteristics of nanomaterials (NMs) and their complex interactions with different environmental conditions, such as temperature and salinity, no comprehensive regulation of nanomaterials (NMs) is currently available in any countries and these NMs are mostly regulated together with their bulk counterparts. Besides, few studies have focused on the evaluation of the toxicity of NMs under multiple stressors. In this study, we investigated the combined effects of temperature and salinity on physicochemical characteristics of zinc oxide nanoparticles (ZnO-NPs), which was the 5<sup>th</sup> mostly produced NMs and the 7<sup>th</sup> most prevalent NMs in commercial products. Its toxicity was also compared with its bulk counterpart and constituent ion. The results were then correlated to the toxicity of ZnO-NPs towards *Tigriopus japonicus*, which is an intertidal copepod species commonly occurring along the Western-Pacific region. Our results show that the aggregate size of ZnO-NPs generally decreases with decreasing temperature and salinity, while their ion dissolution rate decreases with increasing temperature and decreasing salinity. However, the surface response model (RSM) suggests that salinity has a minimal effect on the toxicity of ZnO-NP towards *T. japonicus* while they are most toxic at the highest test temperature. The RSM also predicts that ZnO-NPs at a zinc concentration meeting the Sea Water Quality Standard of China (0.5 ppm) could still pose potentially harmful effects to *T. japonicus* with a temperature higher than 30 °C and salinity lower than 12 PSU. These results suggest that current regulations possibly jeopardise the coastal marine ecosystem which is simultaneously facing the environmental contamination of the emerging NMs and the extreme weather events, such as heatwave and heavy rainfall, that are associated with global climate change.

## **How eutrophic water could shape the benthic food web in Hong Kong's coastal waters**

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It is a well-known fact that coastal eutrophication and associated harmful algal bloom are detrimental to the marine ecosystem as the decomposition of large quantity of dead microalgae often results in depletion of dissolved oxygen levels leading to hypoxia in the seabed and suffocation of sessile benthic organisms. On the contrary, phytoplankton is also regarded as the primary food resource for marine food webs. The additional supply of phytoplankton could theoretically be beneficial to the food web via the bottom-up effect such as lengthening the food-chain and broadening the food niche. However, empirical knowledges about the effect of eutrophication on the food web were scarce and were mostly restricted to freshwater systems. To better understand the effect of eutrophication, this study aims at elucidating how different eutrophication associated parameters (e.g., inorganic nitrogen and dissolved oxygen) and other stressors (temperature and pH) correlate with the changes of the benthic food web of coastal waters in Hong Kong. Hong Kong is an excellent place for studying this issue due to strong seasonal and spatial variation in phytoplankton concentration (0.775 - 8.975  $\mu\text{g/L}$ ), dissolved oxygen level (2.875 – 6.600  $\text{mg/L}$ ), and inorganic nitrogen level (0.045-0.993  $\text{mg/L}$ ). We collected marine benthic organisms by a shrimp-trawler in different times and different locations. The biota samples were subject to stable isotope analysis. Through the Bayesian stable isotope analysis, we estimate different food web indices. The results of multiple regression analysis indicated the importance of inorganic nitrogen in driving the increase of food-chain length and trophic diversity. The inorganic nitrogen concentration and species richness of benthos were found to have comparable effect size which collaboratively explained 74% and 81% of the variation in food-chain length and trophic diversity respectively. Conversely, other stressors such as dissolved oxygen, temperature, and pH had not observable effect in shaping the food web in this study. These results jointly implied that promotion of phytoplankton growth from eutrophication could be beneficial to the coastal food web, although further laboratory study and field-based manipulative study will be required to confirm our postulation.

**Marine biological invasions at high latitudes in the North Atlantic: Overview and case studies from Iceland**

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Marine biological invasions and consequent establishment of non-indigenous species (NIS) is a global environmental and economic problem. Coastal ecosystems around the world are being increasingly exposed to various NIS which may become invasive, challenging the biodiversity and natural resources of their new ecosystem. These effects can for example include modification and/or destruction of habitats, competition with native organisms for food or space, and NIS may enhance transmission of viruses and pathogens. Most marine biological invasions have resulted from commercial shipping, via extensive unintentional transport of marine organisms in ballast water and hull fouling.

With increasing shipping activities, and ongoing climate change which further exacerbates the problem, the number of marine species expanding their original range has increased and such changes have been observed at high latitudes in the North Atlantic Ocean. Current and future studies and perspectives on biological invasions at high latitudes (Arctic and sub-Arctic regions) will be viewed and ongoing studies in Iceland on the newly established rock crab (*Cancer irroratus*) and the vase tunicate (*Ciona intestinalis*) will be introduced and discussed.

## **Trade patterns and artificial structures determine occurrence and assemblage composition of non-native species**

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Loss of natural habitat from ocean sprawl coupled with non-native species (NNS) introductions are among the top threats to marine biodiversity globally. Rapid Assessment Surveys (RAS) conducted on intertidal natural rocky reef and artificial structures recorded occurrence of non-native mobile and sessile invertebrates and macroalgae within harbours along the south coast of England. NNS assemblage composition varied significantly between natural and artificial habitats, with artificial habitats supporting greater numbers of NNS. Overall, 24 NNS were found, with two species discovered in new localities (the carpet sea squirt, *Didemnum vexillum* (Kott, 2002) and the red alga, *Chrysomenia wrightii* (Harvey) Yamada, 1932). Assemblage composition varied among harbours and regions (West, Central, East), with the Central region supporting the greatest number of NNS. There was a positive relationship between average number of national and international vessels arriving and NNS richness within region. Thus, the presence of artificial structures and international transport play important synergistic roles in the introduction, persistence and spread of non-native species into and from harbours.

**Monitoring ecological fitness of wild Pacific oyster *Crassostrea gigas* in Gwangyang bay on the south coast of Korea using cellular biomarkers**

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Widely distributed in intertidal and subtidal ecosystem in the northwest Pacific region, the Pacific oyster *Crassostrea gigas* has been used as a sentinel species in the marine environmental monitoring programs. In the present study, we investigated effects of environmental stressors on fitness of the oysters in Gwangyang bay on the south coast of Korea, one of the most heavily industrialized coastal areas in Korea, using various cellular biomarkers. For the analysis, we collected oysters from inner and outer Gwangyang bay in October 2012 when most oysters completed their reproductive activities. The ecological fitness of oyster included condition index (CI, tissue weight/shell cavity volume), total carbohydrate and protein contents of each oysters, and the cellular biomarkers included the total hemocyte counts (THC), hemocyte mortality, DNA damage, and phagocytosis activities. Levels of lipofusin (LF), neutral lipid (NL) and the lysosomal membrane stability of the digestive tubular (DG) cells were assessed from fresh frozen sections. CI, the total carbohydrates and proteins in oysters collected from the inner bay were significantly lower ( $P<0.05$ ) than the levels measured from the outer bay. Flow cytometry demonstrated that the THC of oysters in the inner bay was significantly lower ( $P<0.05$ ), while the hemocyte DNA damage rate in the inner bay was significantly higher ( $P<0.05$ ) than the levels in the oysters in the outer bay. LP density in the DG cells in the oysters collected from the inner bay were also significantly higher ( $P<0.05$ ), whereas the DG lysosomal membrane stabilities were significantly lower ( $P<0.05$ ) than the levels measured from the outer bay. No significant difference was observed between the total PAHs and heavy metals in the tissues of oysters from the inner bay and the outer bay. The poor ecological fitness of oysters observed from the inner Gwangyang Bay was believed to be linked to the poor water circulation in the inner bay, which may affect negatively supply of foods to the oysters.

## **Larval behavior and biomineralization in oysters – A global change perspective**

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In the coastal environment, organisms are exposed to environmental changes and anthropogenic pressures (e.g. xenobiotics). To properly address acclimation and adaptation capacities of living organisms in a global change perspective, it is crucial to consider their responses to multiple stressors.

In the future ocean, the excess emission of CO<sub>2</sub> would lead to a reduction of 0.32 unit of pH by 2100. Ocean acidification (OA) disrupts normal shell formation of many calcifiers. Morphology and content of larval shell are factors that influence larval mobility and therefore, could impact normal swimming behavior. Apart from structural changes in the larval shells, ocean acidification could modify behavior and sensory capacities by interacting with the GABA<sub>A</sub> receptor.

Notably, behavioral disorders could also occur when animals are exposed to an emerging pharmaceutical pollutant called oxazepam (OXZP). OXZP has been regularly detected from 9-23 ng L<sup>-1</sup> in the Bay of Brest (France) which is an important oyster recruitment area. In recent reports from estuaries water, level of OXZP ranges from 50 – 2250 ng L<sup>-1</sup>. Substrate selection during the larval settlement of the pediveliger larvae involves a sophisticated neurophysiological response to environmental cues (e.g. oyster reef noise, biofilm presence) which can be disrupted in this changing environment.

Here we hypothesized that simultaneous exposure to OA and OXZP have an effect on behavioral and neurophysiological responses in the development oyster *Crassostrea gigas* in its early life stages. We exposed oyster larvae to -0.32 pH unit and environmental concentration of oxazepam (at ng L<sup>-1</sup> level) according to a 2\*2 full factorial experimental design from fertilization to post-larval stage (about 21 days). Biomineralization efficiency in terms of the overall energetic status, shell growth and chitin content, and behavioral consequences in terms of swimming pattern and pediveliger settlement are monitored. Our future work continues to characterize the gene expression related to biomineralization and the neurophysiology and we aim to visualize the neurological changes in larvae using microscopy.

## **Male fitness landscapes of different populations show distinct responses to ocean acidification and freshening**

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The response of species to climate change will reflect differences in physiological selection. While much research has focussed on considering male fitness landscapes under a single climate factor (such as ocean acidification), combined effects remain relatively understudied. Consequently, here we were interested to identify if multiple climate drivers (i.e. ocean acidification and freshening) would shift the male fitness landscape of a common marine species (the oyster *Crassostrea gigas*). Moreover, we were interested in investigating whether these effects would differ between individuals from populations that have experienced different environmental histories. Our results show that males from a population with a stable environmental history showed considerable potential for selection under acidification and freshening, due to a modified fitness landscape and increased trait variance. Intriguingly, however, the population-level effect of these two drivers in combination was small. In contrast, males from a recent invasive population and a more variable environment showed no shifts in male fitness landscape and reduced trait variance under acidification and freshening, and no overall response to acidification. These results demonstrate the need to consider and document variation between, and within, populations to forecast the effects of environmental change.

## **Effect of estuarine dike on benthic food web in Geum River Estuary, Korea**

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Stable isotopic method was utilized to investigate functional change(s) in benthic food web from the Geum River estuary, where natural river flow has long been interrupted by an artificial dike (completed in 1990). The carbon and nitrogen isotopic compositions ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of marine benthic organisms (> 1,000 samples for >10 species) and direct food sources, such as sediment organic matter and microphytobenthos (MPB), were analyzed, at seasonal basis, for three years (2015-2017). As for tracing potential food sources in altered lotic system, the waterborne particulate organic matter (POM) was simultaneously analyzed encompassing in- ( $\text{POM}_{\text{fw}}$ ) and out-side ( $\text{POM}_{\text{sw}}$ ) of the dike. First, the isotopic signatures of POM indicated significant differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between freshwater and seawater, indicating site-specific irregular discharge of terrestrial organic matter into the sea. The  $\delta^{13}\text{C}$  value of  $\text{POM}_{\text{fw}}$  was depleted from those in benthic organisms,  $\text{POM}_{\text{sw}}$ , and MPB, which indicated lesser influence (or contribution) of terrestrial organic matter to marine benthic organisms. Meantime, the dietary contribution for benthic organisms was primarily attributable to MPB (50 to ~100%), reflecting the growth (or biomass) dependent accumulation by selective feeding. Of note, seasonal isotopic signatures of freshwater POM from the Geum River reflected a eutrophic lake state (sometimes hypertrophication). Overall, the spatiotemporal variations evidenced from the isotopic signature in benthic food web from the Geum River estuary indicated altered processes in natural trophic pathways.

**Interspecific toxicity of arsenate, arsenite, and arsenic trioxide and its antioxidant response in marine rotifers and copepods**

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Arsenic is a well-known metal to cause a biological toxicity and is commonly used as biocides. The toxicities of arsenic have been reported to be different depending on their chemical forms (e.g., arsenate, arsenite, and arsenic trioxide). To date, only a few studies have been investigated on arsenic toxicity in marine invertebrates, even though arsenic is widely distributed in marine environment. Here, we assessed toxicities of three different forms of arsenic, namely, arsenate, arsenite, and arsenic trioxide with three marine rotifers (*Brachionus plicatilis*, *B. rotundiformis*, and *B. koreanus*) and two marine copepods (*Paracyclops nana* and *Tigriopus japonicus*). Of different forms of arsenics, arsenic trioxide was the most toxic in rotifers and copepods, followed by arsenite and arsenate. Interestingly, rotifers have exhibited stronger tolerance to arsenic, compared to copepods, although copepods are known to be more resistant to environmental stressors in general. Particularly, while the toxicity values in rotifers were comparable, copepods had a big difference between *T. japonicus* and *P. nana*, indicating that there is likely to have interspecific mechanism in defense in response to arsenic. Different antioxidant responses with defensome were also shown the difference between copepods and rotifers, depending on the chemical forms of arsenic, supporting our *in vivo* results. Our study provides a better understanding on toxicity and defense mechanism in response to arsenic in aquatic invertebrates.

**The mechanism underlying *Acropora tenuis* bleaching by a photosystem II herbicide, elucidated by measuring delayed fluorescence**

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Delayed fluorescence (DF) was used to investigate coral bleaching by Irgarol 1051, a photosystem II (PSII) herbicide used worldwide. The DF is a weak light emission, lasting milliseconds to several minutes, produced by photosynthetic biota placed in the dark after exposure to light. We examined color succession and the DFs of symbiotic dinoflagellates in the hard coral *Acropora tenuis*, which had been exposed to a solvent control or 1 µg Irgarol 1051/L (Irgarol regime) for 14 d. The Irgarol regime corresponds to the ambient levels recorded in international port regions. The coral colors were photographed, and the DFs were measured using a high sensitivity luminometer on a daily basis at the same time throughout the experiment. The coral color was estimated from the RGB values, which were based on the digital photograph images. The coral color and DFs under the solvent control regime were stable throughout the experiment, whereas the coral color under the Irgarol regime showed gradual bleaching. During the Irgarol regime, the slow decay DF component (S-DFI; 10.1–60.0 s) decreased after 1 d, and the fast decay component (F-DFI; 0.1–10.0 s) significantly decreased after 6 d. The latter was significantly related to changes in coral color succession, which indicates that if the electron accumulation functions of quinones Q<sub>A</sub> and Q<sub>B</sub> deteriorate, coral color will bleach.

**Effect of booster biocide on blood cockles, *Tegillarca granosa*, from major cultivation areas of peninsular Malaysia**

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Blood cockle (*Tegillarca granosa*) is an important protein source for human in many tropical, subtropical and warm temperate areas. This species has high economic value, thus has been one of main marine aquaculture products in Malaysia. Ironically, Malaysia's coastline is a fertile breeding ground for cockles. However, the production of cockles drastically reduces to about 12,500 tons in Year 2017 from its peak production in Year 2010 of about 78,000 tons. This situation may occur due to the pollutants released into marine ecosystem and inadequate management of wild cockle population, which result in high mortality of adult cockles and spats. The attention on emerging of marine pollutants such as booster biocide compounds in marine ecosystem become more concern nowadays. These compounds have been utilized to substitute organotin compounds in formulation of antifouling paints. More than 20 compounds have been used including Irgarol 1051, diuron, Sea-Nine 211, M1, dichlofluanid and chlorothalonil. The booster biocides are believed to cause less damage effect to aquatic lives. However, they also potential to produce significant risk effect on the marine environment. In upcoming days the higher concentrations of booster biocide are expected after its have been used as the ingredient in ship paints and also used as herbicides in the agriculture activities. To clearly understand the booster biocide contamination status in Malaysian marine environment, the present study is conducted to determine the relationship of current pollutant detected in major blood cockle cultivation areas and the total production of blood cockles.

## **Occurrence and distribution of phthalates and non-phthalate plasticizers in sediment from a semi-enclosed bay of Korea**

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Phthalates (PHTHs) have been widely used as plasticizers in commercial industrial products. The occurrence of PHTHs in marine environment is of great concern due to their adverse health effects to aquatic organisms and humans via seafood. Based on domestic and global regulations on PHTHs in certain products, these contaminants have been replaced by non-phthalates plasticizers (NPPs). However, limited data is available on the occurrence and environmental fate of PHTHs and NPPs in coastal environments. In this study, 16 phthalates and five NPPs were measured in sediments from 54 locations of Masan Bay in Korea, highly contaminated by the persistent organic pollutants (e.g., dioxins) and toxic metals. The sediment samples were analysed by a gas chromatography coupled to triple quadrupole mass spectrometer with optimized solid phase extraction method. The highly detected compounds in all sediment samples were dimethyl phthalate (DMP), di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), and diisodecyl phthalate (DiDP), di(2-ethylhexyl) adipate (DEHA), acetyl tri-n-butyl citrate (ATBC), and tris (2-ethylhexyl) trimellitate (TOTM), indicating widely consumed in Korea. The total concentrations of PHTH and NPPs in all sediment ranged from 91.3 to 24900 (mean: 1400) ng/g dry weight and 0.86 to 687 (78.5) ng/g dry weight, respectively. Higher-molecular-weight PHTHs such as DiNP, DEHP, and DIDP showed the highest concentrations due to their higher consumption and octanol-water partitioning coefficient ( $K_{ow}$ ). The highest concentration of NPPs was found for TOTM, indicating an emerging contaminant in coastal environment. The overall distribution of PHTHs and NPPs in sediment showed the decreasing gradient from inner to outer part of the bay, suggesting the influence by anthropogenic activities. A clear decreasing trends in concentrations of PHTHs and NPPs in sediment from the outfall of a wastewater treatment plant (WWTP), implying contamination sources of these contaminants from WWTP activities. Our findings highlight the necessity of ecotoxicological survey associated with the exposure of phthalates and their alternatives to living organisms in aquatic environment.

**Keywords:** DEHP; TOTM; WWTP; DEHA; DiNP; DIDP

**Adverse effects of two pharmaceuticals: Acetaminophen and oxytetracycline on life cycle parameters, oxidative stress, and defensome system in the marine rotifer *Brachionus rotundiformis***

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Accumulation of pharmaceuticals drugs and residues in the aquatic environment is of great concern, regardless of enhanced dilution and strict regulation. In addition, due to specific target mechanism of pharmaceuticals, their fate of outcome upon environment expose may be the same or comparable in vertebrates and/or invertebrates possessing identical/similar targets receptors. Yet, studies on the toxicity of various pharmaceuticals have been elucidated through strictly in-vivo experiments, demonstrating the adverse effects on the life-parameters such as life span and reproduction. Among various pharmaceuticals tested in aquatic organisms, paracetamol (N-acetyl-p-aminophenol, commonly known as acetaminophen [APAP]) is one of the most widely used drugs as antipyretic and analgesic drug in human therapeutics. To investigate the adverse effect of two widely used pharmaceuticals, paracetamol (acetaminophen [APAP]) and oxytetracycline (OTC) on the marine rotifer *Brachionus rotundiformis*, various environmentally-relevant concentrations were exposed. Up to date, acetaminophen and oxytetracycline have been considered as toxic, if used above threshold concentration or overdose. However, this study demonstrated these two pharmaceuticals even at low concentration (i.e., ug/L scale) elicited oxidative stress through the generation of reactive oxygen species (ROS) along with the increased glutathione S-transferase activity, despite no-observed effect in in-vivo population growth. To validate the generation of toxic effect, mRNA expression analysis were performed of the entire 26 cytochrome p450s (*CYP450s*) of phase I and 19 glutathione S-transferase (GSTs) of phase II, of *B. rotundiformis*. The mRNA expression analysis suggested specific genes, CYP3045A2 and GSTs1, s4, and o1, that are responsible in detoxification of APAP and OTC, resulting in no significant changes in the population growth and the undetermined No Observed Effect Concentration (NOEC) in the marine rotifer *Brachionus rotundiformis*.

**Differential *in vivo* hemocyte responses to nano titanium dioxide in mussels:  
Effects of particle size and concentration**

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Nanoparticles are ecologically hazardous to marine organisms. Flow cytometry was used to investigate the immune related indexes of haemocytes in the thick shell mussel, *Mytilus coruscus*. Mussels were exposed to 2 sizes of TiO<sub>2</sub> nanoparticles (NPs) (25 and 100 nm) at 3 concentrations (0.1, 1 and 10 mg/L) for 21 days, followed by 7-day recovery period. In general, size and concentration dependent toxicity was pronounced, 25nm-NP and highest concentration (10mg/L) being the most toxic. Total haemocyte counting (THC), phagocytosis activity (PA), esterase activity (EA), lysosomal content (LC), mitochondria number (MN) and mitochondrial membrane potential (MMP) were significantly decreased whereas haemocyte mortality (HM) and reactive oxygen species (ROS) were increased. Though a slight recovery from the TiO<sub>2</sub> exposure was observed, significant carry-over effects were still detected. These results highlight the importance of differential size and concentration effects of metal oxide NPs on toxicity mechanisms in aquatic animals.

**Levels and fate of retinoids and oestrogenic endocrine disrupting chemicals in traditional sewage treatment plants and a novel sewage treatment process**

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Discharge of partially treated effluent from sewage treatment plants (STPs) is a significant source of chemical contaminants, such as retinoids and oestrogenic endocrine disrupting chemicals (EDCs), which are continuously input into the marine environment of densely populated and urbanized coastal cities. In this study, we investigated the removal efficiencies of retinoids and EDCs in three sewage treatment plants (STPs) of Hong Kong and in a novel sewage treatment process that couples with chemically enhanced primary sedimentation (CEPS) of sewage and fermentation of sludge. Overall, the sewage treatment processes in the three STPs were not highly efficient in the removal of retinoids and EDCs from wastewater influents, with the range of removal efficiencies in the aqueous phase of 41–82% and 31–79%, respectively. The CEPS process showed the comparable removal efficiencies for retinoids and EDCs from wastewater with the three STPs of Hong Kong. During acidogenic fermentation of sludge, about half of retinoids and EDCs were transferred from supernatant and accumulated in sludge. Given that the CEPS process can produce a large amount of sludge which contains the valuable resources like organic and phosphorus (P), this novel sewage treatment process integrating CEPT-based wastewater treatment and resource recovery from sludge acidogenic fermentation offer additional advantages than the conventional STPs.

**A review of heavy metals in sediment and living organisms in Malaysia tropical lagoon ecosystem**

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A geochemical survey of the Setiu Lagoon sediments located in eastern Peninsula Malaysia was investigated in order to define the distribution and concentration patterns of selected heavy metals namely, Cu, Zn, Pb, Cr, As and Hg. The temporal and spatial distribution maps of the concentration of all the studied metals were produced as isopleth maps based on geography information system technology. Meanwhile, the correlation between these studied elements with types of sediment was also investigated. The level of pollution attributed to these heavy metals were evaluated using several pollution indicators to determine anthropogenically derived sediment contaminations. Enrichment factors, index of geoaccumulation index and pollution load index were used to assess and visualize using ArcGIS software. Association with adverse effects to aquatic organisms was determined, using the classification of the sediments according to the sediment quality guidelines. On the other hand, levels of these selected metals were also determined in shellfishes collected from the. Only fleshes (in-toto) were analyzed with the purpose of the degree accumulation of these heavy metals. To safeguard public health, heavy metals studied in the flesh were assessed for human consumption according to provisional tolerable weekly intake and provisional tolerable daily intake. Further to gather the status of contamination, the levels of each chemical element in sediments and shellfishes were compared with levels found by other investigators from Asian and other regions.

**Transgenerational reproductive impairments induced by 17 $\alpha$ -Ethinylestradiol on marine medaka (*Oryzias melastigma*)**

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17 $\alpha$ -ethinylestradiol (EE2), a well-known estrogenic endocrine disrupting chemical (EEDC), is widely used in oral contraceptives and hormone therapy. EE2 can be found in soil, waters and biota because it is lipophilic, chemically stable and relatively resistant to degradation. Growing studies in vertebrates indicate that parental EE2/EEDC exposure could induce reproductive impairments in F1-F2 offspring. However, there is no evidence on whether reproductive impairments could be induced by ancestral EE2/EEDC exposure on offspring ( $\geq$ F3), which have never been prior exposed to the EEDC. In this study, 6-month-old adult marine medaka (*Oryzias melastigma*, F0) was exposed to environmental levels of EE2 for 7 and 21 days, and F1 embryos were hatched in same concentration of EE2 water. F1 generation was generated by mating EE2 exposed F0 male fish with EE2 exposed F0 female fish, F2 was generated by mating F1 male fish and F2 female fish and so on for the F3 and subsequent generations. The multigenerational approach was used to assess phenotypic reproductive impacts of EE2 on adult marine medaka (F0) and their offspring (F1-F4), including fecundity, fertilization success, hatching success, hatching time (reproductive competence index, RCI) and sex ratio. The results indicate that EE2 not only impaired reproductive fitness (reduced RCI) on directly exposed F0 generation, but also induced reproductive impairments on the unexposed F4 generation. Changes of sex ratio were also detected in F1-F3 generations. Further analyses on F4 generation showed that ancestral EE2 exposure could (1) impair testes integrity, (2) alter sex hormones balance in male and female fish, (3) deregulate reproductive related gene expression, (4) change the global sperm DNA methylation level, and (5) modified histone proteins expression. Detailed findings will be presented and discussed. These results indicate that the potential mechanisms of reproductive impairments caused by EE2/EEDCs may involve epigenetic modifications via germline inheritance.

## **Advance of passive sampling for marine pollutants**

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Passive sampling has made enormous advancements over last few decades and has become popular as a tool to measure a wide range of contaminants. A number of passive samplers have been developed and available for both inorganic and organic chemicals. Compared with the conventional sampling methods, such as grab sampling, passive sampling offers a number of distinct advantages, i.e. in situ time-integrating and labor-saving continuous monitoring, lower detection limits, cleaner sample matrices and easier sample storage.

The marine environment is an important sink of anthropogenic pollutants. Many organic and inorganic pollutants (including persistent organic pollutants, endocrine disrupting chemicals, pharmaceuticals and personal care products, heavy metals, radionuclides and nutrients and etc.) are discharged into the marine environment and transported globally through the marine circulation. They can bio-accumulate in marine organisms, bio-magnify through the food-chains and could finally threaten the sustainable development of marine ecosystems and human health. Passive sampling has been extensively applied for monitoring the pollutants in the atmosphere, seawater and sediments of marine environment around the world from estuaries to open ocean even the deep Arctic. This approach can also be used to study biogeochemical processes/cycles and fates of pollutants in the marine environment and their effects (namely bioavailability) on marine organisms.

In this presentation, we plan to show the advances of passive sampling for marine pollutants with several examples of development and applications of these techniques for monitoring the pollutants including inorganics and organics in various matrices of marine environment, such as atmosphere, seawater and sediments, utilising different passive sampling devices, which demonstrate the advantages of the techniques.

## High-resolution mapping of pollutants across sediment/water interfaces using DGT passive sampling technique

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Large quantities of pollutants (e.g. excess phosphorus, trace metals and polar organic pollutants) have been released to rivers, lakes and oceans, due to human activities. These pollutants could be further transported to the sediments, where they may be immobilized or released to the overlying water bodies. At the sediment/water interface, many biotic and abiotic processes (e.g. adsorption-desorption, oxidation-reduction) drive the (re)mobilization of pollutants. As a result, the distribution of pollutants across sediment/water interface is highly heterogeneous at both spatial and temporal scales. Pollutant transport across the sediment/water interface usually occurs at the submillimeter or even smaller scales. The determination of these submillimeter-scale elemental distribution helps elucidate the fate of pollutants in marine and fresh-water systems. High-resolution diffusive gradients in thin-films (HR-DGT) is one of the most popular passive sampling techniques that have been used to evaluate the fine-scale distribution of pollutants in sediments and waters. In this study, we applied a newly developed HR-DGT to assess the spatial and temporal distribution of pollutants (e.g.  $P^{III}$ ,  $P^V$ ,  $Cr^{VI}$ , As, Mo, Sb and W) across the sediment/water interface. The submillimeter- to millimetre- multi-elemental distributions across the sediment/water interface in one or two dimensions were obtained by analyzing the HR-DGT binding layer using ICP-MS after gel slicing, laser ablation ICP-MS or colorimetric analysis. In addition, we combined HR-DGT with planar optode, a fluorescence-based imaging technique, to simultaneously capture anion mobilization and  $O_2$ /pH fluxes in sediment mesocosms. Our results demonstrated the coexistence of high DGT fluxes of different anions (e.g. P, As, Mo, and Sb), which suggests the presence of localized “hot” spots with high microbial activities in sediments. The net flux of the apparent diffusion calculated using a numerical model, indicated that the hot spots were still growing, i.e. element dispersion outwards. Data of Mo and Sb suggest that even at ultralow diffusional fluxes ( $\leq 0.04 \text{ pg cm}^{-2} \text{ s}^{-1}$ ), HR-DGT is able to acquire element distributions in water and sediment at high resolution. When four discrete hot spots ( $1200 \mu\text{m} \times 3000 \mu\text{m}$ ) were targeted for comparison, anion fluxes were statistically different (Kruskal–Wallis,  $p < 0.05$ ,  $n = 240$ ), with the four zones exhibiting clear separation in a principal component analysis. Interestingly, we observed that the increased anion fluxes were generally accompanied by depleted  $O_2$  concentration, implying that the mobilization of P and As in sediments was probably due to the reduction of redox-sensitive iron oxides. This in-situ DGT technique with minimum disturbance on samples and its flexibility in combination with other imaging techniques, such as planar optodes and zymography, is a powerful tool to evaluate the environmental behaviours in the sediment/water interfaces in fresh-water or marine systems.

**Trace metal body burdens of live mussels in offshore sites of port Phillip Bay in Victoria, Australia: Seasonal and site comparisons, and comparison with artificial mussels**

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Pollution of marine environments with trace metals from industries and human activities, and their effects on human activities has been one of the major public concern. In this study, the body burdens of eight trace metals: Cd, Pb, Cu, Zn, Cr, Se, Hg, and As, were measured in wild live blue mussels *Mytilus galloprovincialis* collected in 14 selected sites in Port Phillip Bay in Victoria, Australia. Seasonal variation in metal body burdens during winter and summer, between seasons and sites were compared.

The results illustrate significantly higher body burdens of Pb, Cu, Zn, and Hg, and lower body burden of Cr and Se, during summer, in the sites where industries and city centre are located. Geelong was the most contaminated site where elevated levels of six trace metals (Cd, Pb, Cu, Zn, Se, and Hg), including 3 toxic, non-essential metals were detected in mussels.

In addition, in the current study, we deployed “Artificial Mussels” (AMs) that have been tested worldwide and have shown good potential for trace metal monitoring in previous studies. AMs were deployed in summer, for 28 days, at the same 14 sites of Port Phillip Bay and retrieved when LMs were collected. The concentrations of the eight metals listed above, were compared between AMs and LMs in summer. The results indicate a significant correlation in the accumulation of Pb, Cu, Cr, and Hg between AMs and LMs, with a lower correlation in Zn, and irregular correlation in Se and As. The AM uptake of Cd was close to the lower limit of measuring method thus the data cannot be compared.

**Field test of beach litter monitoring by commercial aerial drone**

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The visual survey is the commonest method to quantify and characterize beach litter. However, it is very labour intensive and difficult to carry out on beaches which are remote or difficult to access. We herein report an alternative approach for monitoring beach litter using an unmanned aerial vehicle (UAV), or aerial drone, with automated image requisition and automatic image processing. Litter of different sizes and colours, and made from different materials were placed randomly on four beaches. Images of beaches with different substrates were obtained by the drone at different heights and light conditions. Litter on the beaches was identified from the photos by untrained personnel with the highest accuracy of >90%. The quantification of beach litter was three times faster using the drone than visual census. This study indicates the potential of using the drone as a cost-effective and an efficient sampling method in routine beach litter monitoring programs.

## **Contribution of nuclear applications to study the multiples stressors in marine organisms**

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Research on marine organisms has primarily focused on single stressors. Recently there has been a trend to broaden the scope to more complex studies; this is particularly true for global stressors such as climate-change drivers since there are many complex biogeochemical interactions involved and there still exist large uncertainties of their combined effects. Nuclear techniques allow for studying complex conditions that marine organisms will likely face in the future and constitute a complementary tool to assess potential effect of local and global threats. The use of radiotracers offers many advantages in comparison to more classical approaches. Among them, it is a unique tool to better understand the fate of contaminants on organisms in a changing environment, and can also help assess some physiological reactions to counteract such stressors. Such tools have been used for the past 3 decades to assess contamination (metals, organic compounds and radionuclides) but more recently, methodological advancement have been made to study the synergistic effects of multi-stressors on marine organisms at different organizational levels (from the cells to the individuals). This paper presents the perspectives of assessing the effects of multiples stressors on marine organisms by using radiotracers, especially in the context of the future changing environment and will be illustrated through a series of experiments.

## **Towards a standardized assessment of marine biodiversity in Hong Kong**

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In coastal cities with intense human activities, such as Hong Kong, measuring biodiversity is a valuable tool to support marine conservation and promote sustainable development. Biodiversity assessment and monitoring are crucial to provide a baseline of biodiversity that can be used for environmental impact assessment, conservation management, and comparison of spatial and temporal changes. Biodiversity measurements, however, can be highly affected by sampling methods and sampling efforts. If the sampling method is inappropriate and the sampling effort is low, then the results cannot truly reflect the actual biodiversity in the area of concern and would misguide the environmental authority to make incorrect conclusions that may jeopardise the biodiversity protection. In this study, we aimed to provide a biodiversity baseline and propose standardized biodiversity sampling methods for relevant intertidal habitats in Tolo Harbour and Channel (Tolo area) of Hong Kong, including intertidal rocky shore, intertidal soft shore and mangrove habitats. We analysed the data collected in the mega-project entitled “Assessing the Marine Biodiversity and Ecology of Tolo Harbour and Channel”, which was initiated by the Environment Bureau of the Hong Kong SAR Government with a view to assessing the current status of marine biodiversity and ecology in the Tolo area. We also reviewed relevant biodiversity studies in the same area. For each habitat type, we combined all available data and construct the relationship between the cumulative sampling effort and the cumulative species number (i.e., species accumulation curve) from which the environmental authority can recommend a desirable sampling effort for uncovering different portions of species occurring in the area (e.g., 50%, 70% and 90%). These habitat-specific species accumulation curves will serve as essential references and provide guidance for future biodiversity surveys and ecological impact assessment of coastal development projects. Our results provide the foundation to standardize the sampling methods and sampling efforts in assessing marine biodiversity in Hong Kong and beyond. With more accurate biodiversity assessments, better informed decision making and improved environmental protection can be achieved.

## **Metabolic and digestive capacity decrease in the blue mussel exposed to hypersaline desalination brine**

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Impact of SWRO desalination wastewater release to receiving marine environments has most often focused on the concentration and dispersion of hypersaline plumes. Understanding the biological response in *in situ* organisms directly exposed to such plumes, increases the precision with which to evaluate ecological risk and effective *in situ* monitoring of the discharge. *Mytilus edulis* was exposed to a gradient of 34 psu to 54 psu SWRO desalination brine over 7 days. Metabolic rate measured via oxygen consumption decreased at salinities above 35 psu, culminating in a 63% drop by 45 psu, followed by a single rapid spike in oxygen uptake at 54 psu. Effective concentration point estimates for metabolic rate decline at sustained brine exposures were EC10 36 psu and EC50 38 psu. Histological alteration of the digestive gland, showed a positive correlation between elevated brine concentration and digestive gland atrophy. Digestive tubule thickness decreased by 10% at 36.5 psu, through to 50% at 45 psu brine salinity exposure. Onset of mortality occurred at 40 psu, increasing to 58% at 54 psu. A pilot *in situ* field evaluation of blue mussels exposed to continuous desalination discharge over three years, revealed an average digestive gland atrophy equivalent to that of experimental mussels exposed to 35 psu - 37 psu SWRO brine concentration. The histological biomarker response in this opportunistic population signifies water quality conditions adjacent to the outfall plume conducive to growth, and indicates the discharged 70 psu brine concentrate dilutes to a mixed seawater salinity of less than 40 psu.

## **Assessment of accumulation and biotransformation of arsenite and arsenate in zebrafish in the presence of humic acid**

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Arsenic toxicity is related to the chemical speciation, and inorganic arsenic could be transformed to MMA, DMA or AsB organoarsenic compounds in aquatic organism. Natural organic matter in water environment could impact the arsenic accumulation and biotransformation in aquatic organism. At present, researches mainly focus on arsenic accumulation and toxicity in the presence of dissolved organic matter which have different types, contents and molecular weights, but the accumulated total arsenic in organism cannot fully reflect arsenic toxicity. So we should pay more attention to arsenic biotransformation which has environmental significance. In this paper, we investigated the accumulation and biotransformation of arsenite (As(III)) and arsenate (As(V)) to zebrafish in the presence or absence of different concentrations of humic acid (HA) by ICP-MS and HPLC instruments. The results showed that after the 96h exposure, the arsenite and arsenate could be accumulated and transformed in zebrafish. In the presence of HA, the total As accumulation of arsenite in zebrafish is significantly reduced, and the As(III) distribution is decreased, the AsB distribution is increased compared to the only arsenite treatment. But the effect is not significant on As(V) to zebrafish in the presence of HA compared to the only arsenate treatment. Reasons for the different effects of humic acid on As(III) and As(V) is that due to different uptake pattern of As(III) and As(V) in zebrafish, transport and internalization of As(III) is hindered by HA. And the complexation of arsenite and HA impact the bioaccumulation and transformation of arsenite in zebrafish. This study has environmental significance and provide a new perspective for understanding the effect of humic acid on As(III) and As(V) in organisms for water environment.

## **Organic phosphorus chemical structure and enzymatic hydrolysis characteristics of algae in eutrophic lakes**

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Cyanobacterial blooming is a severe problem in eutrophic lakes globally. Generally speaking, degradation of cyanobacteria and release of their nutrients (e.g. organic and inorganic phosphorus) from the dead bodies provides the major nutrient source for repeated algae blooming. Research on transformation of the structures, influencing factors and biodegradation characteristics of organic phosphorus (P) in algae is a crucial step in understanding the mechanism of algae blooming and biogeochemical cycle of P in eutrophic lakes. In this study, algae from Lakes Taihu and Chaohu in China were taken as examples for investigation of the biogeochemical cycle of organic P in eutrophic lakes. Based on the laboratory simulation experiments and analysis of samples in the field, by combining with the new technologies such as solution  $^{31}\text{P}$ -nuclear magnetic resonance (NMR), high resolution 2D  $^1\text{H}$ - $^{31}\text{P}$  NMR and enzyme hydrolysis, the component characteristics, conversion process and bioavailability of organic P (e.g., labile monoester P, diester P and phytate) in different stages of algae blooming (e.g. early, late and during the process of algae blooming) were studied. The biogeochemical cycle of organic P from algae and its relationship with algae blooming were also analyzed. This project will provide important theoretical information for control of the eutrophication levels in eutrophic lakes.

**Monoamine metabolism and behavioral responses to *Chattonella* sp. in yellowtail (*Seriola quinqueradiata*) fed with L-tryptophan supplemented dietary**

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Mass mortality of cultured yellowtail (*Seriola quinqueradiata*) was caused by a *Chattonella* blooms in Japan and Korea. In this study, we investigated the monoamine metabolism and behavioural responses to *Chattonella* exposure in yellowtail (5.0 ± 0.3 cm) fed with L-tryptophan (L-TRP) supplemented dietary. The L-tryptophan is the immediate precursor of the serotonin (5-HT). Yellowtails were given normal feed (TRP-) or feed containing 1.5% TRP (TRP+). *Chattonella* exposure experiments were conducted after 7-d and 30-d supply of L-TRP. The results showed that 7-d supply of TRP significantly increased the spontaneous swimming speed of fish. At the density of 250 cells/ml, *Chattonella* exposure significantly increased the swimming speed of fish (TRP-), but did not affect that of fish (TRP+). At the densities of 500 and 1000 cells/ml, *Chattonella* exposures (at 500 and 1000 cell/ml) tended to first increase (immediately after exposure) and then decrease (3 h after exposure) the swimming speed of fish in both groups. 30-d supply of TRP elevated the brain concentrations of 5-HT, 3-Methoxy-4-hydroxyphenylglycol (MHPG), and epinephrine (E), and decreased that of homovanillic acid (HVA) in fish (TRP+). *Chattonella* exposure (at 1000 cells/ml) significantly elevated the ratio of serotonin and dopamine turnover in fish (TRP-), but did not affect the serotonin turnover in fish (TRP-). In addition, 30-d supply of 1.5% L-TRP in feed cannot prolong the survival time of yellowtail exposed to *Chattonella* sp. at 1000 cells/ml, but can increase their body weight. This project was partly supported by fund from Fisheries Agency, Japan.

## Hemolytic toxicity of benthic dinoflagellates isolated from Hong Kong waters

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Benthic dinoflagellates have been found in Hong Kong waters; most of them are producers of algal toxins and pose threats to marine ecosystems. One of the toxicities of these algal toxins is to cause hemolysis in fish. For example, maitotoxin (MTX) is one of the potent toxins that can induce hemolysis. In this study, we aimed to assess the hemolytic toxicity of six species of locally collected toxic benthic dinoflagellates, namely *Coolia tropicalis*, *C. maleyensis*, *Fukuya retzuleri*, *Fukuya* sp. HK Type I, *Amphidinium carterae* and *Prorocentrum* cf. *lima*. Each species of algal extract will be examined by hemolytic assay using the freshly collected fish blood of local reef fishes including *Cephalopholis boenak* and *Acanthopagrus schlegelli*. More than 50% erythrocytes were lysed in *C. boenak* and *A. schlegelli* exposed to extracts from *C. tropicalis*, *F. retzuleri* and *A. carterae* at a concentration of 0.1 mg ml<sup>-1</sup>. The hemolytic activities were significantly lower in the other three algal species. Results from chemical analysis showed that a novel algal toxin 44-methylgambierone (formerly known as MTX-3) has been detected in *C. tropicalis* and *F. retzuleri*. The hemolytic and toxic effects of these two algal species are currently being elucidated using the marine medaka larvae which are treated with microinjection of the different concentrations of the toxic algal extracts. Some of our preliminary results showed that hemorrhage in marine medaka larvae occurred when microinjected with water-soluble extract of *C. tropicalis* at doses over 340 ng/fish. Our coming investigations will focus on endpoints including the blood flow rate and morphology of red blood cells. The findings of this study showed that some of the benthic dinoflagellates from Hong Kong are ichthyotoxic and 44-methylgambierone may be one of the compounds that is responsible for the hemolysis in fish.

**Intra-specific phylogenetic diversity and toxicity of benthic dinoflagellates from Hong Kong waters: A case study of two toxic species *Amphidinium carterae* and *Ostreopsis cf. ovata***

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*Amphidinium carterae* and *Ostreopsis cf. ovata* are abundant and cosmopolitan benthic dinoflagellates that are known to be toxin producers. In temperate regions including the North Atlantic Ocean and the Mediterranean Sea, blooming events of these two species often associate with the fish poisoning and human intoxication. The pattern of occurrence and toxicity of the strains found in the seasonal, tropical regions (e.g. the South China Sea) is however not clearly known. In recent years, based on our benthic dinoflagellate survey in Hong Kong waters, these two species are isolated from both coral communities and rocky reefs, and may potentially influence the benthic community structure if there is any blooming event. Our preliminary toxicity study showed that different strains of the same species would vary in toxicity, which may be related to intra-specific variation between the genotypes of the same species. In this study, we investigate the intra-specific phylogenetic diversity (using gene region ITS1-5.8S-ITS2) and toxicity (using invertebrate bioassay) of different genotypes of *Amphidinium carterae* (10 strains) and *Ostreopsis cf. ovata* (21 strains) isolated from Hong Kong waters.

## **Effect of eutrophication on coral fatty acid profiles in Hong Kong**

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In zooxanthellate corals, *Symbiodinium* supplies the host tissue with photosynthate from dissolved inorganic carbon (autotrophy). Simultaneously, corals ingest food, such as plankton, and inorganic and organic matter (heterotrophy). These different energy sources have distinct fatty acid profiles, which thus differentiate corals utilizing either feeding mode. Thus, fatty acid profiles in coral tissues are being used as biomarker to evaluate the quality of nutrition to corals in the midst of environmental change. However, responses of coral species-specific fatty acid profiles under cultural eutrophication has not been investigated. To test the impact of eutrophication on fatty acid profiles, we collected 2 different genera (*Acropora* sp., *Platygyra* sp.) in different sites, with each site showing distinctly different water quality. Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detector (GC-FID) were performed to identify and quantify fatty acids. For the baseline, the 5 different genera of corals showed different concentrations of total lipid and fatty acid profiles due to their different diet. The key fatty acids were C18:3n3 ( $\alpha$ -Linolenic acid), C18:3n6 ( $\gamma$ -Linolenic acid), C20:4n6 (Arachidonic acid), C20:5n3 (Eicosapentaenoic acid), C22:6n3 (Docosahexaenoic acid) and concentrations of these fatty acids were significantly lower in the most polluted site. A biomarker for autotrophy (C20:5n3/ C22:6n3) showed a tendency to increase with water quality. Additionally, we measured oxidized lipid by Liquid Chromatography-Mass Spectrometry (LC-MS) to evaluate how eutrophication induced hypoxia degraded fatty acid metabolism. These results suggest that we can apply fatty acid profiles as biomarkers to determine the level of eutrophication. It further provides information about suitable target species for successful restoration and conservation purpose.

## **Identification coastal zone vulnerable to phytoplankton growth in the Seto Inland Sea, Japan**

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Water quality had been improved in most areas of the Seto Inland Sea, harmful algal blooms were still frequently observed in some regions of the sea. Based on a nonlinear perspective and an empirical approach with several non-nutrient environmental factors, a novel indicator, vulnerable index, was established to estimate surface chlorophyll *a* concentration in the Seto Inland Sea with long-term monitoring records during the period 2003–2012. Results suggested that models that included both salinity and water clarity were more predictive than that did not. The inclusion of distance to coast or water stability resulted in further improvement of model performance, whereas the improvements were limited. Highest Vulnerable Index were observed in the coastal regions of Osaka bay, Harima Nada, Hiroshima Bay and lowest Vulnerable Index in Aki Nada, Iyo Nada, offshore area of Suo Nada and two channels connecting the Pacific Ocean. We also found that the coastal areas with highest Vulnerable Index coincide with the areas adjacent to highly populated watersheds, indicating that high natural potential for phytoplankton growth as well as high anthropogenic nutrient input from neighboring residences combined to result in the frequent red tide occurrence in the areas mentioned above. Vulnerable Index provides a simple and clearly defined way to identify vulnerable coastal zone in nature to phytoplankton growth. We suggest that vulnerable index be incorporated in future decision-making process and different management measures be implemented according to the property of vulnerable index in different water bodies of the Seto Inland Sea.

## **Spatial and temporal distribution of cyanobacteria toxin (microcystins) in Geum River Estuary**

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Global warming and climate change are one of the major causes of frequent occurrence of cyanobacterial blooms in domestic and overseas lakes. Microcystins (MCs) produced by cyanobacteria persist in aquatic ecosystems for a long time and cause hepatotoxicity and bioaccumulation in aquatic organisms. In recent decades, cyanobacterial blooms have been found with high concentrations of MCs in freshwater environments, and there have been reports of damage to coastal organisms. Marine environmental samples (surface water, suspended particles, and surface sediments) were collected from 5 stations (8 stations at 29 June) at the inner and outer sea dike once a day for five days from 26 June 2018. Concentrations of MCs (MC-LR, MC-RR, MC-YR, MC-LA, MC-LF, MC-LY and MC-LW) were analyzed qualitatively and quantitatively using UPLC-MS/MS to investigate spatial and temporal distribution of MCs entering into the coastal area through the Geum River Estuary. Particulate MCs showing relatively high concentrations ( $6.6 \mu\text{g L}^{-1}$  of total MCs) in the inner part of the dike were moved out to coastal area after large discharge ( $2.8 \times 10^7 - 1.0 \times 10^8 \text{ ton day}^{-1}$ ). And then it gradually decreased from the third survey when there was little discharge ( $0 - 2.5 \times 10^7 \text{ ton day}^{-1}$ ). The dissolved MCs maintained a constant level ( $0.5 - 3.2 \mu\text{g L}^{-1}$ ) during the sampling period (for 5 days). Meanwhile, sedimentary MCs showed a tendency to increase after third survey. This seems that the particulate MCs entering into the coastal area were destroyed by osmotic pressure or precipitated several days later. The MCs originated from freshwater did not disappear but exist in various marine media for several days. Therefore, it is considered that the MCs can cause acute toxicity in coastal organisms during the summer bloom season, demonstrating an evidence of the persistence and potential biological impacts of MCs in coastal ecosystems.

**Comparative toxicities of algal extracts from benthic dinoflagellates *Coolia malayensis* and *Coolia tropicalis* to marine medaka *Oryzias melastigma***

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*Coolia* are a group of benthic and epiphytic toxic algae (BETA) which can produce bio-toxins. They are globally distributed and have been reported in Hong Kong waters. The emerging threat of different species of *Coolia* to marine organisms is not well documented. In this study, we aimed to elucidate the toxic effects of microinjecting algal extracts of *Coolia malayensis* and *C. tropicalis* on embryonic and larval development of marine medaka *Oryzias melastigma*. Endpoints including lethality and effects on development and behaviours were recorded in the 72 h after the administration of microinjection. It is found that only the water-soluble extract of *C. tropicalis* caused lethality in larvae when microinjected with a dose single over 340 ng/fish and induced a concentration-dependent increase in mortality. The highest mortality rate (85%) was observed at 72 h after the microinjection (6800 ng/fish). Other toxic effects including hyperkinetic twitching, spinal curvature and internal haemorrhaging were observed as early as 24 h after exposure. However, the algal extract showed no significant difference in heartbeat rate of medaka larvae between different treatments. *C. malayensis* showed no lethal toxicity to marine medaka embryo at 8 dpf after the microinjection. With the upcoming results on the embryonic toxicity of *C. tropicalis*, gene expression analysis and immunohistochemistry staining, the differential toxicities of the two *Coolia* species to marine medaka will be further illustrated. The findings of this study can aid future risk assessment of BETAs in Hong Kong waters.