

Abstract  
of  
Oral Presentation

**BIOACCUMULATION OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) AND HEAVY METALS IN THE BENTHIC POLYCHAETE, *NEREIS SUCCINEA* FROM A TYPICAL MARICULTURE ZONE IN SOUTH CHINA**

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Strong evidence has indicated that dichlorodiphenyltrichloroethane and its metabolites (DDTs) and heavy metals are ubiquitous contaminants in the sediments from mariculture zones in South China, yet bioaccumulation potential and toxicological effects of those contaminants to benthic organisms were scarcely studied in this area. The current study investigated the bioaccumulation potential of sediment-bound DDTs and heavy metals in benthic polychaete, *Nereis succinea* from the sediments collected from an aquafarming zone in Hailing Bay, a typical mariculture zone along the southern coastal of China. The concentrations of DDT and its metabolites in sediment ranged from 120 to 4882 ng/g dry weight (dw), with the parent DDT accounting for 40-68%. The estuary sediments were co-polluted by heavy metals, and Cu (2.16-3.53 mg/kg dw) was the most abundant metal, followed by Zn (1.41-3.02 mg/kg dw) and Cr (1.56-2.58 mg/kg dw). The bioaccumulation potential of DDTs and heavy metals to benthic organisms were assessed by exposing *N. succinea* to the estuary sediments for 28 d. Body residues of DDTs in *N. succinea* were 371-1898 ng/g dw, and the percentages of parent DDT were 11-26%, which was about three times smaller than that in the sediments. The biota-sediment accumulation factors (BSAFs) of the parent DDT and its metabolites were all lower than 1, except for DDE which had BSAFs higher than 1 and reached 4 for one sediment. This suggested that the bioavailability of DDTs in these sediments were low and the biotransformation of DDT to DDE occurred in the organisms. For heavy metals, body residues of Zn (10.3-66.5 mg/kg dw) was significantly higher than that of Cu (5.37-10.7 mg/kg dw), followed by Cr (0.20-3.2 mg/kg dw). The different order of Cu and Zn concentrations in sediment and organism implied that sediment-bound Zn possibly had higher bioavailability to *N. succinea* than Cu, while the low BSAFs suggested low bioavailability of the heavy metals in the estuary sediments.

## **BIOACCUMULATION OF POLY AROMATIC HYDROCARBONS (PAHS) BY DIFFERENT BENTHIC ORGANISMS IN THE CENTRAL ARABIAN GULF**

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Spatial and temporal variability of levels of Poly Aromatic Hydrocarbons (PAHs) bioaccumulation by dominant benthic invertebrates were examined during the period of 2014-2015. Dominant benthic organisms samples were collected seasonally from thirteen locations in the coastal water of Qatar starting in the winter of 2014 and for four consequent seasons. Ten abundant benthic invertebrate species representing different trophic levels were selected to assess the spatial and temporal variability of PAHs bioaccumulations in the Qatar coastal water. The species included gastropods, bivalves, and crustaceans with different trophic positions including carnivores, omnivores, herbivores and filter feeders. Samples were analyzed for 16 parent PAHs including low molecular weight parent PAHs (LPAHs) and high molecular weight parent PAHs (HPAHs), 18 alkyl homologs and dibenzothiophenes.

Levels of PAHs in tissue residues are found to vary significantly with species, locations, seasons and also with distance from shore ( $P < 0.05$ ). PAHs concentrations in tissues were significantly higher than those in sediments ( $P < 0.05$ ), ( $30 < \text{PAHs} < 4000 \text{ ng}\cdot\text{gm}^{-1}$  dry wt) indicating tendency of these persistent contaminants to bioaccumulate in the organisms. Bioaccumulation rate normalized to lipid content varied with location, species and also with size of organisms. The Highest levels of PAHs were associated with point sources with very few reports of levels higher than 1000 ng/gm. The trophic position of the organisms used in the measurements of tissue residues were determined using  $\delta^{15}\text{N}$  signatures. Significant correlations ( $P < 0.05$ ) were found between PAHs tissue residues concentrations and signatures of carbon and nitrogen stable isotopes emphasizing the roles of trophic pathways on the uptake and bioaccumulation levels of individual PAHs in marine invertebrates. The knowledge from this study intended to assist PAHs monitoring and identification of potential sources to guide management decisions. The outcome of the study is expected to help the regulatory agency (Qatar Ministry of Environment) as well as Gulf organizations such as ROPME to improve environmental laws and set standards based on these studies.

## **CHANGES IN SEDIMENT QUALITY AND MACROBENTHIC COMMUNITIES IN HONG KONG WATERS: A COMPARISON BETWEEN 2001 AND 2012**

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We will compare the sediment quality and macrobenthic community structure in Hong Kong waters from 2001 to 2012. This period is particularly interesting because the Phase I of the Harbour Treatment Scheme, a large scale engineering project aiming to improve the water quality in Hong Kong, was completed at the end of 2001, and it is expected that the improvement in sediment quality and recovery of macrobenthic community would take several years to accomplish. We will analyse data sets obtained from a study commissioned by the Agriculture, Fisheries and Conservation Department in 2001, and a study commissioned by the Environmental Protection Department in 2012. The data sets encompassed 35 sediment and benthic sampling stations across all of the seven Water Control Zones in Hong Kong, allowing us to distinguish background shifts from changes due to the implementation of pollution control measures. We will compare several parameters that are related to sediment quality, including sediment particle size distribution, organic matter, total Kjeldahl nitrogen and total phosphorus content, as well as the macrobenthic communities using univariate techniques and cluster analysis and multi-dimensional scaling ordination. We expect no substantial changes in a majority of the Water Control Zones that were not directly affected by the Harbour Treatment Scheme, significant recovery in Tolo Harbour and the eastern side of Victoria Harbour where sewage collection had reduced the direct discharge, and worsening of sediment and macrobenthic structure in the western side of Victoria Harbour where the treated effluent was discharged through a short submarine outfall. Overall, our analyses will provide a new baseline of sediment and macrobenthic community structure in Hong Kong, and establish an excellent example of faunal recovery from eutrophication in subtropical waters.

**TRACE METAL CONCENTRATIONS IN SEDIMENTS AND BIOTA OF THE SOUTH-EAST COAST OF NSW, AUSTRALIA WITH AN EMPHASIS ON METALS, Sn, Cu AND Zn USED AS ANTIFOULING AGENTS. “HOW PRISTINE IS THE PRISTINE SOUTH COAST?”**

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Tin, copper, zinc, cadmium, lead, selenium, cobalt, chromium, nickel and mercury concentrations were measured in sediments, and three organisms, the oyster, *Saccostrea glomerata*, a sediment dwelling gastropod, *Batillaria australis* and a rocky intertidal gastropod, *Austrocochlea porcata*, at 12 locations along the south coast of NSW Australia from Batemans Bay to Twofold Bay during 2009. Mean concentration in sediments were tin 0.05-1.8 µg/g; copper 0.05-1,626 µg/g, zinc 2.6-4,958 µg/g, cadmium 0.05-5.2 µg/g, lead 0.05-21.8 µg/g, selenium 0.05-10.4 µg/g, cobalt 0.05-5.92 µg/g, chromium 0.05-13.6 µg/g, nickel 0.91-22.4 µg/g and mercury 0.05-0.17 µg/g. Several locations exceeded the ANZECC/ARMCANZ (2000) interim sediment quality guidelines levels for copper, zinc, cadmium, nickel and mercury. Some sites had elevated tin concentrations that may potentially exceed guideline levels for Tributyltin (TBT). *S. glomerata*, *B. australis* and *A. porcata* generally had low mean concentrations of tin 0.05-1.42 µg/g; copper 0.05-550 µg/g, zinc 5-2363 µg/g, cadmium 0.05-4.6 µg/g, lead 0.05- 10.4 µg/g, selenium 0.05-4.5 µg/g, cobalt 0.05-5.5 µg/g, chromium 0.05-9 µg/g, nickel 0.05-18.8 µg/g and mercury < 0.05 µg/g respectively approaching background concentrations. Oyster have cadmium and lead concentrations below Australian food standards code. *S. glomerata*, and *A. porcata* mean tin concentrations were significantly correlated across locations indicating similar metal uptake processes. There were no significant correlations between trace metal concentrations in sediment and in organisms within locations, and no relationship with levels of boating activity and suspected antifouling contamination. Although not pristine, the low levels of trace metal contamination in gastropods and oysters indicate that this region is suitable for mariculture.

**APPLICATION OF RESPONSE SURFACE METHODOLOGY FOR OPTIMIZATION OF BIOSTIMULANT BALL FOR STABILISATION OF CHROMIUM AND ZINC IN CONTAMINATED COASTAL SEDIMENT**

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Numerous contaminants introduced to coastal sediment in urban area are accumulated in marine sediment and it significantly affect marine aquatic environment. The aim of present study is to optimize the bioremediation of slow releasing biostimulant ball (BSB) for stabilisation of the heavy metals (Cr, Zn) present in the sediment using Response Surface Methodology (RSM). The effective variables such as BSB size (1-5cm), distance (1-10cm) and month (1-4 months) on Cr and Zn stabilisation were investigated. The analyses of variance (ANOVA) and coefficient determination ( $R^2$ ) of Cr and Zn were 0.9650 and 0.9666. The maximum stabilization percentages of Cr and Zn were 15.8% and 79.5%, were observed at a 3 cm ball size, 5.5 cm distance, and a period of 4 months; these values are the optimum conditions for effective remediation of contaminated coastal sediment. The determination coefficient of the  $R^2$  value suggests that more than 96.50%, and 96.66% of the variance is attributable to the variables of Cr and Zn. This result revealed that the BSB is effective for Cr and Zn stabilisation in coastal sediment.

Key words: Coastal sediment, Biostimulant, Response surface Methodology, Cr and Zn stabilisation, bioremediation.

## **SELECTION OF DIURON-DEGRADING BACTERIA ISOLATED FROM MARINE SEDIMENTS OF MALAYSIA**

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Diuron is one of booster biocides suggested to replace organotin in formulation of antifouling coating paints. However, it is potentially to harm the environment due to its persistence and toxic properties. Thus, present study aimed to isolate, screen and identify the potential diuron-degrading bacteria collected from marine sediments of Port Klang, Malaysia. The screening was conducted by monitoring their ability to grow on the media plates containing 430 ng/L diuron (safe limit concentration) and further screened with higher concentrations of 600 ng/L and 1000 ng/L diuron. Results showed 16 strains survived at safe limit concentration. However, only three strains have high tolerance to survive up to 1000 mg/L. These three strains were characterized and identified by phenotypic tests and standard 16S rRNA molecular work. The strains were identified as *Aeromonas caviae* SZZ 01, *Acinetobactor tandoii* SZZ 05 and *Bacillus aerius* SZZ 19 (Genebank accession numbers: KU942476, KU942477 and KU942480, respectively). These strains have the potential to be a future diuron degraders.

## **APPLICATION OF THE MARINE MEDAKA *ORYZIAS MELASTIGMA* FOR MARINE ENVIRONMENTAL RESEARCH – A REVIEW**

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The Indian medaka *Oryzias melastigma* (McClelland) (synonyms: *O. dancena*, *O. melastigmus*, *O. melanostigma*) is widely distributed in brackish water and seawater in South Asia. Most environmental scientists did not know this medaka species until the Centre Marine Environmental Research and Innovative Technology (MERIT, PI, Rudolf Wu) initiated the development of *O. melastigma* as a small sized fish model for marine ecotoxicology in mid 2000. Since then, the common name “marine medaka” has been widely used to represent the seawater *O. melastigma*. Over the past decade, the marine medaka was adopted by many research teams and widely applied for monitoring and assessing impacts arising from anthropogenic activities in the marine environment. It has been used to investigate the impact of ubiquitous marine stresses (including hypoxia, endocrine disrupting compounds, antifouling compounds, harmful algal toxins, persistent organic pollutants, crude oil and emerging chemicals of environmental concern) on multiple endpoints, ranging from early embryonic development and growth to reproduction, immune function and survival of adults in a single to multi-generational scale. The marine medaka has contributed significantly to research on marine pollution and risk assessment. This presentation will review the major achievements made by marine medaka and provide an overview on the establishment of innovative technologies developed for *O. melastigma*. The strength and limitations using this small sized fish for molecular toxicology and risk assessment will be evaluated. The way forward and future directions using marine medaka for marine ecotoxicology will be discussed.

## **DISTURBANCES TO METABOLIC PROFILES IN JAVA MEDAKA JUVENILES EXPOSED OxyPAHs**

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Oxygenated polycyclic hydrocarbons (oxyPAHs) distribute widely in environment, because they have been found in atmospheric particulate matter, sediments, river and coastal water. OxyPAHs discharged directly into environment with the automobile exhaust emission and incomplete combustion. Moreover, they also generated through photo-oxidation of parent polycyclic aromatic hydrocarbons (PAHs). OxyPAHs are more persistent compared with the parent PAHs and their water solubilities is higher than those of PAHs. Their studies for toxicities to aquatic organisms are limited yet. Therefore, present study investigated the toxicity of oxyPAHs such as 1,4-naphthoquinone (NAQ) and 9,10-phenanthrenequinone (PHQ) to the juveniles of marinefish Java medaka (*Oryzias javanicus*) using GC/MS-based metabolomics approach.

Fish were exposed to 0.3 µg/L (NAQ-L group) and 30 µg/L (NAQ-H group) of NAQ, and 0.6 µg/L (PHQ-L group) and 60 µg/L (PHQ-H group) of PHQ in the sterilized seawater for 72 hours (h). Solvent control group, which fish were reared in oxyPAHs free seawater with DMSO, was established at the same time. Each 6 individuals were collected every 24 h. Fish were measured body length and weight, immediately frozen in liquid nitrogen, and stored at -80°C until analysis. Metabolites in whole fish were extracted, derivatized with trimethyl silylation, and analysed by GC/MS. The metabolites with significant differences one-way analysis of variance were analysed by principal component analysis (PCA).

In NAQ, groups of NAQ-L, NAQ-H and DMSO were not separated clearly from each other with PCA analysis. Most of metabolites couldn't find the significant differences between DMSO and both groups of NAQ. These results suggested that the effects with NAQ exposure couldn't show by metabolite profiles in medaka.

In PHQ, the PCA analysis showed that two exposure groups were separated along PC1 each other and also these separated from DMSO group at 24 h and 48 h. In the PCA analysis, PC1 meant effects from PHQ. At 72 h, DMSO, PHQ-L, and PHQ-H were also clearly clustered along PC1. The individual variation of metabolites showed the exposed groups with PHQ affects depending on increasing exposure concentrations. The variations of metabolites with exposures of PHQ showed that PHQ could affect to TCA cycle composed to the organic acids such as citrate, succinate, malate and fumarate. Those metabolites work on generate energy in the body, and the effects to those metabolites could lead to decrease the energy generations in both group of PHQ-L and PHQ-H at 48 h and 72 h. In addition, the amounts of some amino acids such as aspartate, asparagine, glutamate, and glutamine, in PHQ-L and PHQ-H groups increased compared with DMSO group at 24 h, 48 h and 72 h, and increased depending on the increase of the concentrations. These results suggest that the exposed fish may be newly in conduct protein synthesis and cause the further energy generation, because PHQ as xenobiotics eliminated from the body.

**THE SEX STORY OF THE MARINE MEDAKA *ORYZIAS MELASTIGMA*: LOOKS CAN BE DECEIVING****D R Peterson<sup>1</sup>, Y Takehana<sup>2</sup>, K Naruse<sup>2</sup>, J S Lee<sup>3</sup> and D W T Au<sup>1</sup>****<sup>1</sup>State Key Lab on Marine Pollution, Department of Biology and Chemistry, City University of Hong Kong****<sup>2</sup>Laboratory of Bioresources, National Institute for Basic Biology, Okazaki 444-8585, Japan**

The marine medaka, *Oryzias melastigma* (also known as *O. dancena*), has been well established as a small marine fish model for ecotoxicology. It has a clearly definable phenotypic sex difference between males and females as early as 2 months of age. Male fish can be visually distinguished from females by their long, jagged anal and dorsal fins. The marine medaka also shares similar gametogenesis and gonadal histology as the freshwater counterpart, *Oryzias latipes*, in which the sex-determining gene *DMY* has been identified on the Y chromosome. Recently, the sex determination mechanism has been identified in *O. dancena*, potentially enabling identification of genotypic sex of individuals. The sex determining locus on the Y chromosome in *O. dancena* is a cis-regulatory element which upregulates the expression of the *Sox3* gene. Although the region containing the cis-regulatory element has been sequenced, the coding region of this element yet to be identified. However, two single nucleotide differences between the *Sox3* genes on the X and Y chromosomes were sufficient to distinguish sex via PCR and a restriction digest. Due to the possibility of single nucleotide polymorphisms among the two strains of marine medaka *O. melastigma* and *O. dancena*, at this locus, we test the applicability of the *O. dancena* genotyping method for *O. melastigma*, using the whole genomic sequencing data of *O. melastigma* from the region surrounding and including the *Sox3* gene on the X and Y chromosomes.

17 $\alpha$ -Ethinylestradiol (EE2), an estrogenic endocrine disrupting compounds (EDCs) found ubiquitously in the marine environment, has been known to dysregulate the steroidogenesis pathway in fish, leading to partial (ova-testis) or complete transition from male to female and skewing the sex ratio of F1 offspring. Genetic XY male *O. melastigma* chemically sex changed to XY females, via embryonic EE2 exposure, will be used to determined disparity in phenotypic and gonadal sex with genetic sex in an individual. Furthermore, the likelihood of YY male offspring, resulting from the cross of the sex changed XY females with XY males, will be assessed to determine the usefulness of this technique in multigenerational studies. The feasibility to detect genotypic, phenotypic and gonadal sex, in rapid and cost effective ways, offers the marine medaka *O. melastigma* major advantages for risk assessment of estrogenic EDCs and sex-related toxicological studies.

**TOXIC EFFECTS OF TRICLOSAN ON ZEBRAFISH (*DANIO RERIO*) EMBRYO/LARVAE AND ZEBRAFISH LIVER CELL LINE****Z Zhou, J Yang and K M Chan****School of Life Sciences, The Chinese University of Hong Kong, Hong Kong S.A.R., China**

Triclosan (TCS, 5-chloro-2-(2,4-dichlorophenoxy) phenol) is an antimicrobial agent that has been widely used in personal care, household and industrial products. TCS and its by-products have been detected in surface water, soils, aquatic species and even humans. Although previous studies suggest that TCS is not acutely toxic, mutagenic or carcinogenic to mammals, increasing concern has been raised over its potential for endocrine disruption and toxicity to aquatic organisms. In this study, we first examined the 50% lethal concentrations (LC50) of TCS in zebrafish (*Danio rerio*) embryo/larvae and zebrafish liver cell line (ZFL). An *in vitro* time-course based study was then performed using quantitative polymerase chain reaction (qPCR) to investigate the effect of TCS on the mRNA expression level of genes involved in hypothalamus-pituitary-thyroid axis and liver metabolism, including cytochrome P450 family (CYP's), deiodinase family (*dio*'s), thyroid hormone receptor (*trβ*) and transthyretin (*ttr*). As a result, the 24 h and 96 h *in vitro* LC50 were measured at 9.28 μM and 8.75 μM, respectively. At mRNA level, short-term exposure (4 h, 12 h and 24 h) to TCS did not affect target genes, while long-term exposure (96 h) showed significant inhibitory effect on *trβ*, *dio 2*, *CYP1C1* and *CYP1C2* at 2.5 μM. Moreover, the inhibition of *CYP1C2* followed a dose-dependent pattern (up to 1.6-fold at 2.5 μM). The data *in vitro* suggested that TCS has a chronic effect on mRNA expression level.

The LC50 of TCS exposure from zebrafish embryos were determined as 9.26 μM [24 h post fertilization (hpf)], 4.08 μM (48 hpf) and 1.20 μM (72 hpf and 96 hpf), respectively. Major deformity was the delayed hatching by 86.5% from 1.13 μM (327 ppb). Upon exposure on larvae, the LC50 values were comparable and ranging from 1.26-1.46 μM throughout 96 h post hatching (hph). The lethal concentrations are environmental relevant; zebrafish embryo and larvae are hence testified to be a suitable model for TCS toxicity evaluation. On-going study is (1) to validate the mRNA expression differentiation in 2 hph zebrafish larvae exposed to TCS of 50 nM and 500 nM; (2) to perform *in vitro* receptor reporter gene systems, including aryl hydrocarbon receptor, thyroid hormone receptor, pregnane X receptor and retinoid X receptors, to study receptor-mediated gene regulation.

## **CHARACTERIZATION OF COPPER TRANSPORTERS IN ZEBRAFISH: PCR-CLONING OF ATP7A AND 7B cDNAs FROM ZEBRAFISH LARVAE**

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Copper is essential for human body needs and it serves as cofactor of many enzymes activity. However, copper homeostasis has to be maintained in the body and at cellular level. Inadequate or excess copper cause Menkes disease (MND) and Wilson's disease (WND) respectively. The major copper transporters Ctr1, ATOX1, ATP7A and ATP7B are identified in cellular copper regulation so far. ATP7A and ATP7B transfer  $\text{Cu}^+$  to nascent proteins synthesized in the Golgi apparatus and eliminate  $\text{Cu}^+$  from the cell. In human or mammals, ATP7B is highly expressed in liver, kidney and placenta, and ATP7A is expressed in the majority of tissues except liver. Mutations in human ATP7A or ATP7B cause MND or WND. Recently, Cd accumulation and uptake is also believed to be related to copper transporters.

Zebrafish (*Danio rerio*) is a powerful vertebrate model organism, as it shares a high degree of sequence and functional homology with mammals and its genome sequence is nearly completed. ZFL is the cell line of zebrafish liver used in this project. The movement of ATP7B in ZFL after copper challenge was found not as expected. However, ATP7A of that has not been conducted yet. Our gene expression profiles showed that ATP7A is expressed in high level in zebrafish liver and ATP7B expressed in the intestine with higher levels, unlike what's found in human. Hence, ATP7A is suspected to be the transporter that exports copper from the ZFL cell. The cDNAs of ATP7A and ATP7B of zebrafish were successfully obtained from zebrafish larvae and cloned in the expression plasmid for expression fusion protein with fluorescent proteins. Sequencing data has confirmed the identities of these two genes. The localization of ATP7A and 7B will be examined in ZFL cell-line after copper and cadmium challenges.

**TRANSGENERATIONAL SKELETAL TOXICITY OF BENZO(A)PYRENE IN FISH****F Seemann<sup>1</sup>, C B Jeong<sup>2</sup>, D R Peterson<sup>1</sup>, J S Lee<sup>2</sup>, A H Shanthanagouda<sup>1</sup>, C Winkler<sup>3</sup> and D W T Au<sup>1,4</sup>****<sup>1</sup>State Key Laboratory in Marine Pollution, Department of Biology and Chemistry City University of Hong Kong****<sup>2</sup>Department of Biological Science, Sungkyunwan University****<sup>3</sup>Department of Biological Sciences, National University of Singapore****<sup>4</sup>Shenzhen Key Laboratory for the Sustainable Use of Marine Biodiversity, Research Centre for the Oceans and Human Health, City University of Hong Kong, Shenzhen Research Institute**

Benzo(a)pyrene (BaP), an ubiquitous environmental pollutant, has been shown to affect bone metabolism in vertebrates subject to direct exposure. Our recent results indicate that the risk of BaP exposure in fish is far more severe than previously thought. Using the Japanese medaka, *Oryzias latipes*, as an aquatic vertebrate model, ancestral exposure to environmentally relevant concentrations, as low as 1µg/L BaP, has been shown to significantly increase the number of 17 dph larvae with compressed vertebrate segments. This was persistently found in the ancestrally exposed F1 to F4 larvae ( $p < 0.05$ ). Abnormal loss of notochord sheath, a lack of notochord epithelial integrity, reduced bone tissue, decreased osteoblast abundance and changed expression of key osteogenic genes, notably *osterix*, *alkaline phosphatase*, *BMP2* and *Sox9a/b*, were detected in the ancestrally exposed larvae. The findings suggest that ancestral BaP-exposure most likely perturbed osteoblast differentiation, resulting in defective notochord sheath repair and rendering the vertebral column more vulnerable to compression. To substantiate a causal relationship between molecular deregulation and the pathological vertebral segment compression, the double transgenic medaka lines (*osx:col10*; *twist:col10*) were used to repeat the BaP exposure experiment. Temporal and spatial expression of osteoblast marker genes (*osterix*, *collagen 10*, *twist*) in ancestrally exposed offspring were identified, localized and quantified using confocal microscopy. The epigenetic mechanisms leading to the transgenerational inheritance will be discussed. BaP intake was able to affect miRNA profile in paternally exposed F1 mouse blastocysts. Here, we will present unique knowledge of bone-related miRNA expression in medaka in response to ancestral BaP exposure in the F3 and F4 generation.

This study provides novel molecular and cellular insights into BaP-induced transgenerational bone impairment in the ancestrally exposed offspring. From the ecological risk assessment perspective, BaP needs to be regarded as a transgenerational skeletal toxicant, which exerts a far-reaching impact on fish survival and fitness. Given that basic mechanisms of cartilage/bone formation are conserved between medaka and mammals, the results may also shed light on the potential transgenerational effect of BaP on skeletal disorders in humans.

## COMPLEX IMPACTS OF C<sub>60</sub> FULLERENES AND/OR BENZO(A)PYRENE IN MARINE MUSSELS: APPLICATION OF INTEGRATED BIOLOGICAL APPROACH IN MARINE ENVIRONMENT MONITORING

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Whilst there is growing concern over the potential detrimental impact of engineered nanoparticles (ENPs), little is known about their potential toxic impact either alone or in combination with other environmental contaminations. Our work used marine bivalve: *Mytilus sp.*, a classic bio-indicator species to assess the biological responses following the exposure to C<sub>60</sub> fullerene and/or benzo(α)pyrene for 1 and 3 days. A 3 days post-exposure in fresh seawater was also applied to assess the recovery ability of *Mytilus sp.*. An integrated approach, which included determination of clearance rates (at individual level), histopathological alterations (at tissue level), DNA strand breaks using comet assay (at cellular level) and transcriptional alterations of *p53* (anti-oncogene) and *ras* (oncogene) determined by real-time quantitative PCR (at molecular level) was applied for the assessment of impacts following C<sub>60</sub> fullerene and/or B(α)P exposures. A significantly increased DNA strand breaks were found after 3 days exposure. A significant induction for *p53* and *ras* expression was found after exposure and the transcriptional alterations of both genes showed tissue and chemical-specific pattern. Furthermore, all biological responses at different levels were returned back to control level after the recovery period, suggesting the ability of *Mytilus sp.* to cope with both nanogenotoxicity and ecogenotoxicity induced by C<sub>60</sub> fullerene and/or B(α)P under experimental conditions. Overall the integrated approach appears to be a useful tool to assess and monitoring potential impact of ENPs either alone or in combination in a representative marine invertebrate which could be translated for other species and toxicants.

## ADSORPTION OF NANOPARTICLES ON AIR-BUBBLE AND SURFACTANT SUPPORTED POROUS MEDIA

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Nanoparticles released from nanomaterial products, enter soil and surface water and eventually the sea. Nanoparticles may flow through porous media having several environmental conditions such as air bubble-trapped and surfactant-coated. This study used a 2-dimensional porous medium, a glass bead column and porous filters to study the interaction of nanoparticles with air bubbles, surfactant foam and surfactant coated media. Fullerene, copper oxide (CuO) and zinc oxide nanoparticles were used for this study. Aqueous suspensions of nC<sub>60</sub> and metal oxide nanoparticles were prepared by the tetrahydrofuran saturation method and sonification, respectively. The nanoparticle suspensions were injected into the air bubble and surfactant foam trapped porous medium, and also into surfactant-coated porous filters. Effluent concentrations of nanoparticles were measured. The transparent 2-dimensional porous medium clearly showed that the air bubbles in pores acted as fullerene collectors. Fullerene aggregates were easily found on the bubbles. As water flowed through flow channels, air displacement occurred and fullerene aggregates also moved. Therefore, air movement resulted in abrupt increase of effluent fullerene concentration. Surfactant-adsorbed porous filters and surfactant foam removed some of the fullerene nanoparticles, while CuO nanoparticles were not adsorbed at all. The interaction potential energy curves implied that the nanoparticles were very stable in water and natural deposition of nanoparticles on the solid medium would not occur. Electrical bonding and hydrophobic interactions were the dominant forces for fullerene adsorption.

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## **ENVIRONMENTAL RISK OF NANOMATERIAL-INCORPORATED CONSUMER PRODUCTS AND THEIR REGULATIONS**

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Engineered nanomaterials with at least one dimension less than 100 nm present unique physicochemical characteristics when compared to their bulk forms because of their larger surface-to-volume ratio and/or their customized shape. Owing to their unique properties, nanomaterials are broadly applied in various commercial products, ranging from textile and electronics, to cosmetic, pharmaceutical and food products. In 2014, more than 1800 nano-based consumer products are available in the global market. Such wide applications of nanomaterials create a multi-trillion business globally. However, the extensive applications of nano-based products will inevitably result in an increased release of nanomaterials into the environment, triggering a new challenge to environmental regulators. Recent toxicological studies on nanomaterials have revealed their potential risks to the aquatic organisms and humans. Meanwhile, uncertainties remain because environmental factors such as the presence of humic acids, temperature and salinity can alter their toxicity while field studies on the interactions are scarce. And in reality, it is very difficult to detect and trace the nanomaterials released into the environment while it is a prerequisite for accessing their environmental and health risks. Such a deficiency of knowledge has hindered the effectiveness on regulations of nano-based products for protection of environmental and human health. This study aimed to: (1) prioritize the nanomaterials for immediate investigation based on their estimated global production and prevalence in the consumer products; (2) review available toxicity data of the prioritized nanomaterials and their environmental concentrations; (3) summarize current regulations in different regions and countries on the release of nanomaterials from the consumer product; and (4) discuss the challenges in regulation, risk assessment and management of nanomaterials. This study will be essential and beneficial for developing guidelines to manage the potential hazards of nanomaterials to the environment and humans.

**PERSISTENT ORGANIC POLLUTANTS IN BREAST MILK IN WOMEN POPULATION OF MERIDA, YUCATAN, MEXICO**

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Many social and environmental factors in daily life, such as exposure to persistent organic pollutants, can have deleterious effects on human health. Nowadays the human population is exposed to many pollutants through food, inhalation, direct contact through the skin, etc. To evaluate the possible factors that affect the health of women in the city of Mérida, Yucatán Mexico, with the help of the Hospital MaternoInfantil 100 samples of breast milk were collected for the analysis of COPs, and a survey was applied to evaluate the possible factors that promote exposure and bioaccumulation in the female population in Merida. Chlorobenzenes, Hexachlorocyclohexanes, Cyclopentadienes, DDTs, PCBs and Chlordanes were detected in almost all the samples of breast milk, suggesting a direct exposure to POPs in women, and a risk factor for newborns. However, concentrations were in general lower than those previously found in women in a coastal town very close to Merida. An analysis of the concentrations with the survey results shows significant positive correlations of PCBs and complications during pregnancy; and DDTs concentrations with complications inducing caesarean sections, among others. Exposure to persistent organic pollutants in this population is mainly related with daily routine activities that can be prevented with proper education and awareness of the population. It is necessary to promote awareness about the pollutants being bioaccumulated and their risks, and how to prevent this exposure

**INFLUENCES OF TEMPERATURE AND SALINITY ON PHYSICOCHEMICAL PROPERTIES AND TOXICITY OF ZINC OXIDE NANOPARTICLES TO MARINE DIATOM *THALASSIOSIRA PSEUDONANA*****M M N Yung<sup>1</sup>, K W H Kwok<sup>2</sup>, A B Djurišić<sup>3</sup> and K M Y Leung<sup>1</sup>****<sup>1</sup>The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong, China****<sup>2</sup>Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China****<sup>3</sup>Department of Physics, The University of Hong Kong, Hong Kong, China**

Climate change can result in rising average seawater temperature with more extreme thermal events, and frequent heavy rainfalls in some coastal regions. It is imperative to understand how naturally mediated changes in temperature and salinity can modulate the toxicity of chemical contaminants to marine life. Since zinc oxide nanoparticles (ZnO-NPs) are widely used in commercial sunscreens and commonly released into coastal marine environments, they are selected as a model pollutant in this study. Here, we experimentally investigated how temperature and salinity could influence the physicochemical properties and toxicity of ZnO-NPs to a marine diatom *Thalassiosira pseudonana* using a  $5 \times 5 \times 6$  factorial design (i.e., 5 temperatures  $\times$  5 salinities  $\times$  6 concentrations of ZnO-NPs). Our results showed that an increase in both temperature and salinity led to (1) larger aggregates of bulk ZnO and ZnO-NPs, and (2) a reduction in zinc ion concentration being released from ZnO and ZnO-NPs; these changes, in turn, lowered the concentration of bioavailable zinc ions and reduced the toxicity of ZnO-NPs to the diatom from 10°C to 25°C in terms of median inhibition concentration on the algal growth. However, a significant increase in the toxicity of ZnO-NPs was observed at 30°C, possibly due to a synergistic effect of the chemical toxicity and thermal stress. Consistently, ZnO-NPs were more toxic than ZnO and ZnSO<sub>4</sub> to *T. pseudonana*. The diatoms exposed to ZnO, ZnO-NPs and ZnSO<sub>4</sub> displayed substantially different gene expression profiles, suggesting that these chemicals have different modes of toxic action against the diatoms. Moreover, temperature had a stronger influence on the gene expression profiles of the diatoms than salinity did. The highest toxicity of ZnO-NPs was observed at thermal extremes (10 or 30°C) with low salinity (12 psu), implying higher risk of ZnO-NPs in estuaries in particular during seasonally extreme temperature events. The current results enable us to forecast the toxicity of ZnO-NPs to the diatoms at various combinations of salinity and temperature under different global climate change scenarios.

**RETINOIC ACIDS PRODUCED BY MARINE CYANOBACTERIA TRIGGER SEX CHANGE IN FEMALE GASTROPOD *REISHIA CLAVIGERA***

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Conventionally, the development of imposex, i.e., superimposition of male sexual characteristics on females, in marine neogastropods has been solely linked to the exposure of anthropogenic organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), in the marine environment. Here, we reported that marine cyanobacteria are able to produce retinoic acids (RAs), which can also induce imposex development in female whelks *Reishia clavigera*. We also demonstrated that the combined toxic effect of RAs and TPT to *R. clavigera* is synergistic. These findings have profound implications on revising our thinking about causes and magnitudes of imposex in marine neogastropods, and opening up a new and exciting research arena on ecotoxicology of cyanobacterial RAs.

## MICROPLASTICS IN BIVALVES FROM CHINA

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Microplastic has been confirmed as an emerging pollutant in marine and freshwater environments [1-3]. One of the primary environmental risks of microplastics is their bioavailability for aquatic organisms [4]. Bivalves are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the water column. We found the number of total microplastics range from 2.1 to 10.5 items/g in 9 commercial bivalves from a fishery market in China in 2014 [5]. We further investigated microplastics pollution in mussels (*Mytilus edulis*) from 22 sites along 12,400 mile coastlines of China in 2015. The number of total microplastics varied from 0.9 to 4.6 items/g and from 1.5 to 7.6 items/individual. *M. edulis* contained more microplastics (2.7 items/g) in wild groups than that (1.6 items/g) in farmed groups. The highest abundance of microplastic was found in intestine, followed by foot and stomach. We also found high microplastic pollution (0.2-12.5 items/g) in Asian clams (*Corbicula fluminea*) from Taihu Lake, China in 2015. The ratio of the microplastics in clams and sediments ranged from 38 to 3810 and was negatively correlated to microplastic numbers in sediments. The most common microplastic were fibers in morphotype and less than 1000  $\mu\text{m}$  in size in bivalves. Microplastics were successfully confirmed using micro-Fourier Transformed Infrared Spectroscopy and Scanning Electron Microscope. Our study suggested that microplastics were widespread in wild and farmed bivalves from marine and freshwater environments. Our study also indicated that binding was a novel way for organisms to transfer microplastics in the food chain. We proposed that bivalves (e.g. blue mussel and Asian clam) could be used as indicators of microplastic pollution in aquatic environments.

**MICROPLASTIC SIZE-DEPENDENT TOXICITY, OXIDATIVE STRESS INDUCTION, AND P-JNK AND P-P38 ACTIVATION IN THE MONOGONONT ROTIFER (*BRACHIONUS KOREANUS*)**

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Size-dependent toxic effects of microplastics were investigated using 0.05, 0.5, and 6  $\mu\text{m}$  diameters of polystyrene microbeads in the monogonont rotifer *Brachionus koreanus*. To reveal the toxicity and defense mechanism in response to microplastic exposure, the ingestion and egestion of fluorescently labeled microbeads, activities of several antioxidant-related enzymes, and phosphorylation of mitogen-activated protein kinases (MAPKs) were examined. The exposure of three different sizes of polystyrene microbeads led significant size-dependent retardation of growth rate as well as the reduced fecundity, lifespan, reproduction time, and body size in rotifer. The fluorescence of 6  $\mu\text{m}$  diameter of fluorescently labeled microbeads was mostly disappeared within 24 h, while fluorescence in 0.05 and 0.5  $\mu\text{m}$  of fluorescently labeled microbeads-exposed rotifers was still remained until 48 h, suggesting 6  $\mu\text{m}$  diameter of fluorescently labeled microbeads is more effectively egested from *B. koreanus* compared to 0.05 and 0.5  $\mu\text{m}$ . This observation possibly explains that smaller microbeads have more toxicity in size-dependent manner. Antioxidant-related enzymes and MAPK signaling pathways were activated size-dependently in response to the microplastic exposure, as shown in in vivo toxicity tests. This is the first report of size-dependency of the microplastic toxicity on rotifers with the biochemical analyses. Our results are the first step for a better understanding on size-dependent mechanistic toxicities of microplastics of rotifer.

## **MICROPLASTICS IN THE SEDIMENTS OF THE CHANGJIANG ESTUARY, CHINA**

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In order to improve knowledge of distribution of microplastics in China, we investigated into samples from 53 seafloor sediment locations of the Changjiang Estuary, which is largely affected by human activities. Microplastics (<5mm) were extracted from sediments by density separation, after which they were counted under microscope and categorized according to shape, color and length. Identification was carried out using Microscopes Fourier transform infrared spectrometer ( $\mu$ -FT-IR). Concentration of microplastics were mapped. The mean concentration in the Changjiang Estuary was  $12.1 \pm 0.9$  items per 100g dry weight. Among all the microplastics collected, fibrous (93%), transparent (42%) and small microplastics (<1mm) (55%) were the most abundant types. No clear correlation between microplastics and finer sediment fraction was found. Rayon, polyester, and acrylic were the most abundant types of microplastics identified, indicating the main source of microplastics in the study area was from washing clothes.

Key Words: microplastics, sediment, the Changjiang Estuary,  $\mu$ -FT-IR, rayon

**BIOMARKERS IN TWO FISH SPECIES FROM THE SOUTHERN GULF OF MEXICO. DIFFERENTIAL RESPONSES TO POLLUTANTS AND BIOMETRY**

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The Southern Gulf of Mexico is the main oil-producing area in Mexico, accounting for over 95% of domestic production. Oil industry represents over 35% of fiscal income in Mexico, and it has been the subject of many controversies, including its environmental impact. A series of oceanographic cruises have been undertaken in this region by Mexico?? Cinvestav?? to determine the pollutant concentrations in water, sediments and organisms potentially related to the oil industry, as well as their possible biological impact. In this context, we have evaluated the use of fish as sentinels of pollution. Two fish species have been our main targets: the catfish (*Ariopsis felis*) and the flatfish *Scyacium gunteri*. During a oceanographic cruise conducted in 2011, 30 catfish (*Ariopsis felis*) were obtained from 10 near shore stations, and 84 flatfish (*Scyacium gunteri*) from 28 deeper stations. Gene expression of CYP-450 1A, Metallothionein, Glutathion-*s*-Transferase, Catalase and Vitellogenin were evaluated in fish liver. Hydrocarbons (low and high molecular weight PAHs, and total hydrocarbons) were also measured in fish liver, and metals (Cr, Cd, Pb, Hg, Ni and V) were measured in muscle. Histological damage for both fish species (gill hypertrophia, granulomas and melanomacrophage centers in liver, and kidney granulomas) was evaluated. PAH metabolites (phenanthrene, b(a)pyrene, pyrene and naphthalene) were measured in bile. Fish size (cm) and weight (g) were recorded. Each one of the variables mentioned above were measured for each one of the 114 fish individuals. Differential biomarker responses by species, fish length and pollutants were approached using multivariate statistical analyses. Redundancy Analysis (RDA) was used for gene expression and Canonical Correspondence Analysis (CCA) for histological lesions, using CANOCO version 5. Significance was assessed using 999 unrestricted permutations and calculating a pseudo-F value. There were species differences for gene expression (pseudo-F=65.2; P=0.0001) and histology (pseudo-F=2.9; P=0.022). There were significant differences for the effect of fish size on gene expression and histology, with the exception of size effects on histology in catfish; then, fish size was used as a covariable in further analyses. There was a significant effect of metals on gene expression in flatfish (pseudo-F=1.9; P=0.009), but not for metabolites or hydrocarbons. For catfish there was a significant effect for metabolites (pseudo-F=4.5; P=0.001) but not for metals or hydrocarbons. There was no significant effect of any pollutant measured on flatfish histology, with exception of the metabolites, which had a significant association with catfish histology (pseudo-F=2.6; P=0.026). The present results showed that these two fish species respond differentially to pollutants. It is likely that these differential responses are related to differences in the biology of the fish species studied.

## **MICROPLASTIC POLLUTION IN MARINE WATERS AND SEDIMENTS OF HONG KONG**

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The presence of plastic waste with a diameter of less than 5 mm (“microplastics”) in marine environments has prompted increasing concern in recent years, both locally and globally. We conducted seasonal surveys of microplastic pollution in surface waters and sediments from Deep Bay (2 sampling sites), Tolo Harbour (2 sampling sites), Rambler Channel (2 sampling sites) and Victoria Harbour (3 sampling sites) in Hong Kong from June 2015 to March 2016. Suspected microplastics of different shapes and types (mainly fragment, line, fiber and pellet) were successfully recovered by density separation and filtration. From the batch of samples analysed some were identified as polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE) and a blend of polypropylene and ethylene propylene (PP+EPDM) by using Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR).

From June to July 2015, the amount of suspected microplastics (0.1 to 8.8 mm in length) found in Hong Kong’s coastal waters ranged from 31 to 1,187 microplastics per 100 cubic metres of surface water, with Rambler Channel being the most polluted region. In the meantime, a sediment sample from Victoria Harbour (West Kowloon side) was found to contain the highest amount of suspected microplastics with a total of 416 microplastics per kg of sediment. From the samples collected in November 2015, Rambler Channel was once again the most polluted region with 1637 suspected microplastics per 100 cubic metres of surface water. However, the greatest abundance of microplastics was observed in samples collected in March 2016 with surface water samples containing from 131 to 35,649 suspected microplastics, with Victoria Harbour (west Kowloon side) being the most polluted region. This project will further assess spatial and temporal trends of microplastic abundance and composition in collected sediments. In addition, organic pollutants and heavy metals adhered to or associated with the suspected microplastics — if any — shall be identified and quantified. We believe this project will deliver a more comprehensive picture of microplastic pollution in Hong Kong’s coastal regions and its potential ecological impact.

**TISSUE-SPECIFIC ACCUMULATION OF TRIPHENYLTIN COMPOUNDS IN MARINE FISHES IN HONG KONG****R C T Sham<sup>1</sup>, K K Y Ho<sup>1</sup> and K M Y Leung<sup>1,2</sup>****<sup>1</sup>The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong****<sup>2</sup>State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong**

Organotin (OT) compounds, in particular tributyltin (TBT) and triphenyltin (TPT), are ubiquitous in the marine environment due to their wide applications in antifouling paints and other industrial uses (e.g. as fungicides, wood preservatives and antibacterial textiles). These compounds are endocrine disruptive, and have threatened marine lives for more than 40 years. The International Maritime Organization (IMO) of the United Nations enacted a global prohibition on the usage of OT-based antifouling agents on hulls of sea-going vessels in 2008, yet Mainland China and Hong Kong have not adopted any policy to restrict the production, usage and release of these compounds. Thus high concentrations of these compounds, especially TPT, are still detected in marine organisms in South China Sea areas like Hong Kong and Shenzhen. Previous studies have demonstrated that marine organisms can accumulate TPT compounds from their surroundings and through the food chain; however, the distribution pattern of this group of compounds in fishes is not well-known. Here, we study the tissue-specific accumulation of TPT compounds with respect to their concentration in the whole organism. Four piscivorous fish species were sampled in the western waters of Hong Kong, and concentrations of TPT and its degradation products (i.e., diphenyltin and monophenyltin) in fifteen different tissue types (including bones, brain, dorsal muscles, fins, gills, gonad, head-remains, heart, liver, scale, skin, stomach, swim bladder, ventral muscles, and whole fish) were quantified using gas chromatography mass-spectrometry. Our results showed that the bioaccumulation of TPT was highly tissue-dependent. Liver consistently contained the highest concentration of TPT, whereas the lowest concentrations were found in skin and bones. The mass-balance model showed that dorsal muscles generally contributed to 30% of the total body burden of TPT compounds in fish. Currently, we are conducting statistical analyses (e.g. stepwise regression analysis) to investigate the relationships of TPT concentrations between the target tissues and the whole fish, and to identify the tissue(s) that has/have the most significant effect on TPT concentration of the whole fish. This would aid in predicting TPT concentration in the whole fish based on the level of TPT in a single tissue, and investigating the biomagnification of TPT in marine ecosystems.

## **USE OF MAMMALIAN ANTIBODIES ON MAPK SIGNALING PATHWAY IN ROTIFER AND COPEPOD ENVIRONMENTAL TOXICOLOGIC STUDIES**

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MAPKs (mitogen-activated protein kinases) mainly consist of extracellular signal-regulated kinase (ERK), stress-activated protein kinases (JNK), and p38 MAPK and are most well characterized as stress-related signaling pathway. MAPK pathways are also known to involve in many important processes including apoptosis, proliferation, and gene expression in response to extracellular stimuli. However, it is poorly understood their roles in rotifer and copepod, although they also may show similar roles in invertebrate as shown in mammal. In this study, we compared amino acid (AA) sequences of ERK, JNK, and p38 in the copepods (*Tigriopus japonicus*, *Paracyclopsina nana*) and the rotifer *Brachionus koreanus* with mammalian MAPKs for checking the conservation of domains and epitopes. As a result, protein kinase domains, including antibody binding and phosphorylation sites in all MAPKs, were highly conserved compared to mammals. Based on these results, we have performed Western blotting using mammalian antibodies onto *T. japonicus*, *P. nana*, and *B. koreanus* proteins and verified their successful bindings into *T. japonicus*, *P. nana*, and *B. koreanus*. In addition, we also used specific inhibitors for ERK, JNK, and p38 to check as to whether each MAPK signaling pathway can be inhibited or not. Here, we showed that mammalian MAPKs antibodies and their inhibitors are worked properly in *T. japonicus*, *P. nana*, and *B. koreanus*. This study shows the possibility of in-depth studies in signaling pathways in rotifer and copepod using mammal-originated antibodies in response to environmental stimuli.

## RAPID AND SENSITIVE ASSESSMENT OF CHLORINE-INDUCED TOXICITY AND RECOVERY IN MARINE DIATOMS BY SYTOX® GREEN STAINING

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Phytoplankton entrained into cooling water systems of coastal power stations are subjected to acute chemical stress due to biocides used for biofouling control. Current methods on phytoplankton assessment rely on total cell counts, chlorophyll, fluorescence or FDA staining etc. However rapid, sensitive and simple assessment methods can easily determine the viability in entrained phytoplankton are of importance in determining the biocidal efficacy and in ecotoxicity assessments. Suitability of the method for rapid toxicity assessments at power plant discharges was assessed using a centric (*Chaetoceros lorenzianus*) and a pennate (*Navicula* sp.) diatom as models as they represent important members of phytoplankton and productivity in coastal waters. Effect of in-use levels of chlorine (0.1, 0.2, 0.5 mgL<sup>-1</sup>) practiced at power stations, along with a high residual of 1.0 mgL<sup>-1</sup> was examined. SYTOX® Green fluorescence measurement was carried out using fluorescence microscopy and fluorescence microplate reader. Results were also compared with conventional cell counting done using haemocytometry. Haemocytometer measurements yield total cell counts and do not differentiate between live and dead cells. Chlorine treatment resulted in a concentration-dependent reduction (28, 28, 43 and 56% at 0.1, 0.2, 0.5 and 1.0 mgL<sup>-1</sup>, respectively) in cell numbers with *C. lorenzianus*, whereas the pennate diatom (*Navicula* sp.) was comparatively more tolerant with less reduction in cell numbers observed for similar concentrations (10, 15, 18 and 22 % reduction at 0.1, 0.2, 0.5 and 1.0 mgL<sup>-1</sup>, respectively). The centric diatom was more susceptible to chlorination compared to the pennate diatom, even at the lowest concentration (0.1 mgL<sup>-1</sup>) tested. Results showed that using SYTOX® green dye and microscopic counts were 16, 23, 43 and 51% cells with compromised membrane were observed for *C. lorenzianus* and with *Navicula* sp. it was 12, 20, 30 and 35% for concentration of 0.1, 0.2, 0.5 and 1.0 mgL<sup>-1</sup>, respectively. Results from both the methods showed similar trends; they were found to be correlated ( $P < 0.0380$ ). Substantive quantitative assays using fluorescence microplate reader in the case of *C. lorenzianus* showed increase in SYTOX® green fluorescence (indicating membrane-compromised cells) with increasing chlorine concentration. The percentage increase in SYTOX® Green fluorescence compared to control was 176, 304, 392 and 476%, at chlorine concentrations of 0.1, 0.2, 0.5 and 1.0 mgL<sup>-1</sup>, respectively, whereas with *Navicula* sp. the corresponding increase was 202, 342, 429 and 550%. Similarly chlorophyll-a measurements showed dose-dependent reduction with increasing chlorine concentrations for *C. lorenzianus* ( $P < 0.01$ ), while in the case of *Navicula* sp. the difference was not significant ( $P > 0.05$ ). Treated diatoms were observed for recovery after transfer to healthy medium (filtered sea water). Cells of *C. lorenzianus* treated with 0.1, 0.2 and 0.5 mgL<sup>-1</sup> of chlorine for 3-10 min were able to recover; however, at 1 mgL<sup>-1</sup> such recovery was not observed. In the case of *Navicula* sp., the cells were able to recover and grow even at the highest chlorine concentration and contact time (1 mgL<sup>-1</sup> for 3 and 10 min). Studies suggest that chlorine dose and contact time play a very important role in recovery of the phytoplankton. Moreover, the response was found to be species-specific. Recovery occurred when exposures times were shorter. Results show that SYTOX® Green staining can be used as a rapid method for assessing diatom mortality following biocide insult.

## **A NOVEL APPROACH TO ESTABLISHING BIOMARKERS OF CHEMICAL EXPOSURE IN MARINE MEGAFUNA**

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Chemical pollution in the marine environment is arguably one of the biggest threats to marine wildlife. Understanding the exposure and effects of anthropogenic pollutants on wildlife is therefore an important element of management and conservation efforts. Critical to this is the development of innovative and non-destructive methods for measuring the exposure of contaminants in wildlife. This is particularly important for protected or large animals, for which live animal manipulations have ethical and logistical constraints. Blood protein biomarkers are widely used in human health diagnostics, and hold great potential for assessing the exposure and effects of contaminants in endangered wildlife. Here, we present a novel and ethical approach to developing protein biomarkers of chemical exposure in marine wildlife. We focused on marine turtles, which are endangered marine megafauna that through strong fidelity to coastal foraging grounds are at high risk of chemical exposure. Primary marine turtle cell lines, established in our laboratory, were exposed to a range of contaminants known to accumulate in marine turtles at environmentally relevant concentrations. In addition, cells were exposed to blood extracts (containing the contaminant mixture of individuals) collected from turtles foraging adjacent to different pollution sources (e.g. urban, industrial and agricultural areas). Following exposure, the protein expression of these cells was quantified using gel electrophoresis and LC-MS/MS. Differential production of proteins was observed in exposed cells compared to the controls, indicating that these differentially expressed proteins may be promising biomarkers of contaminant exposure in marine turtles. In addition, the approach presented here provides a framework for developing protein biomarkers of chemical exposure in other marine megafauna.

## **ETHICAL AND HIGH THROUGHPUT ASSESSMENT OF THE EFFECTS OF CHEMICAL CONTAMINANTS IN MARINE MEGAFUNA**

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Chemical pollutants are accumulating in marine megafauna globally. However, due to the logistical and ethical constraints of conducting toxicological research on large, often endangered animals, very little is known about the effects of these contaminants in marine megafauna. *In vitro* (cell-based) toxicity bioassays provide an ethical and high throughput approach to studying the toxic effects of contaminants in animals, and can provide a proxy for the effects at the organism and population levels. Here, we present a cell-based approach to investigating the effects of chemical contaminants in marine turtles, and how this can be applied to other marine megafauna. Marine turtle primary cell lines were established under controlled laboratory conditions, and marine turtle specific cell-based assays that measure a variety of different endpoints, such as cell viability, endocrine disruption, oxidative stress and genotoxicity were developed. Results indicate that different metals and organic contaminants cause a wide range of effects on marine turtle cell function, and that heavy metals such as mercury may be having significant impacts on marine turtle health at concentrations currently being observed in these endangered animals. In addition, there appears to be some differences in toxicity between species, indicating that species-specific differences in toxicity must be considered. Further advancement of this line of toxicological research to include more contaminants, endpoints and species is necessary to gain a broader understanding of the effects of contaminants accumulating in marine megafauna. This is critical to better inform conservation and management strategies for these often threatened species.

**WHOLE GENOME ANALYSIS OF THE MARINE COPEPOD *PARACYCLOPINA NANA* AND IDENTIFICATION OF *HOX* GENES AND REARRANGEMENTS WITHIN THE SINGLE HOMEBOX CLUSTER**

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*De novo* assembly of non-model diploidy organism is highly difficult depending on their heterozygosities, as the genome assembly of highly heterozygous organisms can easily lead to poor results. In this study, we conducted whole genome assembly of the marine copepod *Paracyclopina nana*. The 1<sup>st</sup> genome assembly was conducted by Platanus assembler, while the 2<sup>nd</sup> genome assembly was conducted by Haplomerger2 for removing heterozygous contigs. As a results, we obtained total length was 85.8 Mb with 275 scaffolds (> 1 kb) and N50= 1.68 Mb. The results of Core Eukaryotic Genes Mapping Approach (CEGMA) were 95.97% of complete and 97.58% of partial core eukaryotic genes. Also, we reported the first identification of the entire complement of eight typical homeobox genes and the ftz gene in a 192.8 kb region in the assembly result of *P. nana*. A *Hox3* gene ortholog was not present in the *P. nana* *hox* gene cluster, while the *P. nana* *Dfd* gene were transcribed in the opposite direction to the *Daphnia pulex* *Dfd* gene, but in the same direction as the *Dfd* genes of the fruit fly *Drosophila melanogaster* and the red flour beetle *Tribolium castaneum*. The location of the *lab* and *pb* genes were switched in the *P. nana* *hox* cluster, while the order of the remaining *hox* genes was generally conserved with those of other arthropods.

## EVALUATION OF IMMUNOTOXICITY IN COMMON CARP (*CYPRINUS CARPIO*) BASED ON THE CHEMICAL EFFECTS ON A NATURAL HOST-PATHOGEN INTERACTION

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In order to assess chemically-induced immunosuppressive effects in fish, we have developed a test method for evaluation of the chemical effects on a natural host-pathogen interaction. As host, we selected common carp (*Cyprinus carpio*), a species well studied in fish immune system, and tested pathogenicity of three bacterial species, *Aeromonas hydrophila*, *A. salmonicida*, and *A. veronii* against carp. An experimental infection of *A. salmonicida* by immersion caused high mortality in carp without any additional stressor, while bath infection of others did not show pathogenicity; therefore we chose *A. salmonicida* as a natural pathogen. In a preliminary test, 90% mortality was observed after 9 days post-infection, when fish were exposed to  $1.0 \times 10^6$  colony forming unit (CFU)/mL *A. salmonicida* for 1 h. Based on these results, we decided to infect *A. salmonicida* at  $<1.0 \times 10^6$  CFU/mL, when analyzing the chemical effects on the host-pathogen interaction.

For validation of the test method, we used an immunosuppressive agent, dexamethasone as a positive control. Carp were exposed to 1 mg/L dexamethasone for whole experimental period, and fish in unexposed groups were received only 0.01% acetone. One week after the exposure test started, fish from each group were bath infected in 20 L water containing  $2.9 \times 10^3$  (low) or  $2.9 \times 10^4$  (high) CFU/mL *A. salmonicida* overnight. Unexposed and uninfected fish were set as control. After 7 days post infection, fish in the dexamethasone-exposed and high dose of *A. salmonicida* infected group started to die, and 40% mortality was observed in the group after 21 days post infection. The dexamethasone-exposed and low dose of *A. salmonicida* infected group showed 10% mortality after 13 days post infection. On the other hand, bacterial infection-associated mortality was not observed in *A. salmonicida* infected fish without dexamethasone exposure. These results clearly indicated that exposure to high concentration of dexamethasone suppressed the immune system of common carp, and caused subsequent mortality. Thus, the test method introduced in the present study is thought to be useful to evaluate chemically-induced immunomodulatory or immunosuppressive effects in fish.

**MONITORING PAHs AND ENDOCRINE DISRUPT CHEMICALS FROM YUNDANG AND XINGLIN WASTEWATER TREATMENT WORKS OF XIAMEN, FUJIAN BY ARTIFICIAL MUSSEL-SPMD**

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There are two wastewater treatment works: Yundang and Xinglin factories were monitored for Polycyclic Aromatic Hydrocarbons (PAHs) and Endocrine disrupt chemicals (EDCs): 4-Nonylphenol, Triclosan, Bisphenol –A, 17  $\beta$ -Estradiol and 17 $\alpha$ -ethynyl estradiol by Semi-Permeable Membrane Device (SPMD). Results showed PAHs could be reduced more than half left from influent 778.9 ppb to effluent water 173.6 ppb at Yundang WTW, 1086.5 ppb to 238.7 ppb at Xinglin WTW and EDCs have different reducing ratio at both wastewater treatment works. Results also showed SPMD could performance monitoring abilities well but biota such as mussel fish cannot survived at very polluted water such as wastewater. Also SPMD could integrate pollutants well in one month with easy chemical treatment and then after clean up and for Gas-Chromatography – Mass Spectrum determination.

**BROMINATED FLAME RETARDANT BDE-47 LEADS *DE NOVO* LIPOGENESIS IN THE COPEPOD (*TIGRIOPUS JAPONICUS*)**

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*De novo* lipogenesis (DNL) is a process of fatty acid synthesis, and several genes (e.g. sterol regulatory element binding protein [SREBP], ATP-citrate lyase [ACLY], and acetyl-CoA carboxylase [ACC]) are involved in this process. Up-regulation of DNL is linked with induction of fat mass through increasing fatty acid. To examine DNL in the intertidal copepod *Tigriopus japonicus*, we identified several genes by analyzing conserved domain structure of *T. japonicus* whole genome. To uncover the relationship of 2,2',4,4' tetrabrominated diphenyl ether (BDE-47) with the change of DNL, quantity of fatty acids, and lipid droplets, we exposed 2.5 µg/L BDE-47 to *T. japonicus* and measured transcription levels of DNL for 24 h. After exposure of 2.5 µg/L BDE-47 for 7 days, we found that palmitic acid and docosahexaenoic acid were significantly increased but arachidonic acid was decreased at days 1 and 4. After Nile red staining analysis for 24h in the BDE-47-exposed *T. japonicus*, we found the area of the lipid droplets were significantly increased in ovigerous female. This study provides a better understanding of the relationship of BDE-47 and DNL on lipogenesis in copepod in response to BDE-47.

**LEVELS, ISOMERIC PATTERNS, AND TEN-YEAR TEMPORAL TRENDS OF 1,2,5,6,9,10-HEXABROMOCYCLODODECANE (HBCD) AND 1,2-DIBROMO-4-(1,2-DIBROMOETHYL)CYCLOHEXANE (DBE-DBCH) IN CETACEANS FROM THE SOUTH CHINA SEA****Y F Ruan, X H Zhang, J C Lam, B Q Zhu and P K Lam****State Key Laboratory in Marine Pollution (SKLMP), Department of Biology and Chemistry, City University of Hong Kong, Hong Kong S.A.R., China**

Due to the worldwide restriction on polybrominated diphenyl ethers (PBDEs), several brominated flame retardants (BFRs), such as 1,2,5,6,9,10-hexabromocyclododecane (HBCD) and 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (DBE-DBCH), have been recommended as PBDE replacements used in polystyrene foam products. Information on the occurrence and fate of these emerging BFRs in the environment, especially DBE-DBCH, is quite limited. Little is known about levels of HBCD and DBE-DBCH in marine biota from China.

The present study aims to investigate the isomer-specific patterns and stereo-selective behaviours of HBCD and BDE-DBCH in two species of marine mammals, Indo-Pacific humpback dolphins (*Sousa chinensis*) and finless porpoises (*Neophocaena phocaenoides*), from the Pearl River Estuary (PRE) of the South China Sea. Ten-year trends of the levels of these stereoisomers in these cetaceans were also monitored. HBCD and DBE-DBCH were determined in blubber samples of these species. By use of specific chiral columns,  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -DBE-DBCH diastereomers, as well as  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCD enantiomers and two *meso* forms  $\delta$ - and  $\epsilon$ -HBCD, were successfully separated and quantified by GC-MS and UPLC-MS/MS, respectively. It was found that territorial waters in the PRE has been chronically contaminated by these PBDE replacements in cetaceans over the past ten years. Results also suggested that HBCD and DBE-DBCH have similar environmental fates, which warrants further investigation on their potential sources. The  $\alpha$ -isomers of both HBCD and DBE-DBCH were predominant in the samples, and the significant difference in the enantiomeric patterns of these BFRs were observed in these two species. Further studies have to be carried out to verify the mechanisms.

**THE FATE OF ANTIBIOTICS IN CLOSED LANDFILLS IN HONG KONG****S S Chung<sup>1</sup>, J S Zheng<sup>1</sup>, B Burket<sup>2</sup> and B W Brooks<sup>2</sup>****<sup>1</sup>The Hong Kong Baptist University, China****<sup>2</sup>Baylor University, USA**

Compared to many mainstream environmental fields, studies on the sources and fate of pharmaceuticals in the environment are in dearth, particularly in Hong Kong (HK). In a response to the Legislative Council, the current Secretary of Environment remarked that household waste medicine has “little effect of pollution to the environment or danger to public health” even if they are disposed together with normal household waste in landfills in HK. Is this true? Also, what is the fate of active pharmaceutical ingredients in landfills?

Informed by recent literature and taking into account the most commonly used human antibiotics in HK, 7 antibiotics (cephalexin [CLX], chloramphenicol [CAP negative], ciprofloxacin [CIP], erythromycin [ERY], roxithromycin [ROX], trimethoprim [TMP], and sulfamethoxazole [SMX]), other indicator drugs and wastewater tracers (e.g., caffeine, sucralose) have been chosen as key analyte groups in a study to examine wastewater and landfill leachate pharmaceutical residue concentrations in HK.

As part of the study, we collected and analyzed leachate from two closed landfills, Shuen Wan (SW) and Pillar Point Valley (PPV) landfills and one active landfill (WENT) in June 2015. SW was closed in 1995 and PPV 1996. Our preliminary findings are that even after 20 years, the concentrations of CIP (1077.6ppt) and ERY (2062.5ppt) in the raw leachate from SW are similar to or in some cases even higher than those in the current effluent from the 8 primary treatment works. On the other hand, a concentration of 147.2 ppt of CLX is found in SW raw leachate, much lower than that found in the current influent of the main wastewater treatment plant (973.6 ppt) in HK. Indeed, studies showed that the removal rate, transformation or degradability of antibiotics in an anaerobic environment varies with the type of antibiotics with some (e.g. tylosin & chlortetracycline [CTC]) reaching almost 100% and others (e.g. tetracycline and CIP) only around 50%. High removal rate of 92% for CLX in the anaerobic digestion process is also reported. Others found that the removal rate of oxytetracycline and CTC is not linear, with an initial removal of 94% in the first 7 days and continued removal assumes a much slower rate. Some studies conclude that sulfonamides are unresponsive in methanogenic conditions.

Seemingly conflicting results with published studies are also found. According to a recent study, CAP negative is below detection limit in the leachate samples of a landfill in Shanghai. However, a CAP concentration of 97.4ppt in treated leachate of the WENT landfill (currently in operation) is found in our study. What is the reason? The research team feels that there is a need to carefully examine the short and long-term fate of antibiotics in anaerobic environments to enhance human understanding and inform decision makers the actual level of environmental and public health risk from landfilling of unused pharmaceuticals in HK.

**PREDICTING ADVERSE IMPACT OF THE OXYTETRACYCLINE DISCHARGED FROM AQUACULTURE EFFLUENT IN THE COASTAL WATERS OF JANGHEUNG, KOREA: USING SITE-SPECIFIC MULTIMEDIA FATE MODEL**

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Nowadays the occurrence of pharmaceuticals in the marine system becomes emerging problem because of its propensity to induce microbial resistance and some risks to reduce the efficacy of current medication. There are various pathways to enter to marine ecosystem, but releasing pathway from aquaculture is considerable in Korea, because the usage is a lot and the effluent is emitted without any treatment. However, the Ministry of Environment in Korea still regulates the aquaculture effluent based on the value of BOD, COD, and SS. Therefore, the regulation standard for the pharmaceuticals usage in aquaculture should be introduced. In this study, multimedia mass balance model, which have proven to be useful in risk management for regulatory purposes, was used to predict the fate of chemicals to decide whether the pharmaceuticals from the effluent cause adverse impact on marine ecosystem and human.

Oxytetracycline (OTC) was chosen for target because of its amount of usage, toxicity, and side effects on human. The chemical behaviour of OTC was modelled with Fugacity model level IV, which was coupled with the jet-plume model JETLAG to implement the behaviour of the aquaculture effluent in the near-field. The flatfish farm in Jangheung was set for modelling region and horizontal meshes over the region consist of 8×12 grids, which are 500×500m in size, with three sub-compartments: air, seawater and sediment. The input of compartment properties were determined by the basic water quality measurements and monitoring which were conducted in Jangheung region to apply site-specific features. Instead of the reaction half-live value and chemical parameters from the chemical database, the equations of key removal processes for OTC, such as photolysis and hydrolysis, and salting-out effect constant were applied to the governing equations to be more accurate.

To validate the model, the modelling results were compared with surveyed data. We sampled the effluent and coastal water, and meteorological and hydrological data corresponding to that time were used for input of environmental conditions. Those samples were analysed by LC-MS/MS followed by extraction through HLB cartridge. Measured concentration OTC (28.1-381ng/L) in sampled points showed that considerable amount of OTC remained in seawater and caused a potential risk to marine ecosystem. Modelling results showed similar tendency with the surveyed data. The model outputs were analysed through propagating the uncertainty and sensitivity in input parameters using confidence factor. This results demonstrated the utility of this model to support the effective management of aquaculture effluent in the future.

**EFFECT OF 17 $\alpha$ -ETHINYLESTRADIOL (EE2) ON SOCIAL BEHAVIOR OF THE CLOWNFISH *AMPHIPRION OCELLARIS***

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The synthetic estrogen 17 $\alpha$ -ethinylestradiol (EE2) is extensively used in oral contraceptive pills, medication, cosmetics, and personal care products. It is also widely used in livestock and aquaculture. EE2 is commonly detected in wastewater effluents and surface waters including coastal water. Agonistic behavior is regulated by sex hormones and is crucial for maintaining social hierarchy in many coral reef fish. Endocrine disrupting chemicals such as EE2 may interfere fish social structure via disrupting their agonistic behavior. In this study, we aim to use the common clownfish (*Amphiprion ocellaris*) as an experimental model to characterize endocrine disrupting effects of EE2 in coral reef fish, with an emphasis on agonistic behavior and social structure. In the laboratory experiment, juvenile clownfish were randomly distributed to separated tanks to form small colonies of three individuals and were fed with EE2-dosed diet (100 ng/g food) or a control diet for 12 weeks (n=10 tanks/group). Once a week, each tank was videotaped and behavioral indicators of social status, including shelter utilization, agonistic behavior, and submissive response, were quantitatively analyzed from the videos. Mortality of the fish was also monitored to characterize social stress on subordinate individuals. Our results showed that social hierarchy may be less stable in the clownfish colonies exposed to EE2.

## IDENTIFICATION OF WHOLE CYTOCHROME P450 (CYP) GENES IN MARINE COPEPODS AND THE APPLICATION FOR ENVIRONMENTAL MONITORING OF MARINE POLLUTION

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The cytochrome P450 (*CYP*) enzymes are one of the multigene superfamily belong to phase I detoxification system. In this study, we identified 52 *CYP* and 46 *CYP* genes from two marine copepods, *Tigriopus japonicus* and *Paracyclopsina nana* after searching the gDNA- and RNA-seq databases and comparing them with other species *CYP* gene families. To annotate 52 *Tj-CYP* and 46 *Pn-CYP* genes, phylogenetic analyses of *CYP* genes were performed using a Bayesian method. We further analyzed specific expression patterns of 52 *Tj-CYP* transcripts in developmental and different sexual stages. Also, to examine the potency of *CYP* genes from two marine copepods *T. japonicus* and *P. nana* for environmental monitoring of oil pollution, we investigated effects of water-accommodated fraction (WAF) of crude oil on the development and reproduction with molecular and biochemical defense mechanisms of the intertidal copepod *T. japonicus*. We then examined expression patterns of these 52 *Tj-CYP* genes and 46 *Pn-CYP* genes in response to WAF and to the alkylated forms of two polycyclic aromatic hydrocarbons (PAHs; phenanthrene and fluorene). In addition, we provide information of *CYP* genes of a new species of *Tigriopus* was identified from the King George Island of Antarctica and was registered with phenotypic characters as *T. kingsejongensis*. This study will be helpful to better understand the function of *CYP* associated with detoxification mechanisms and its application for environmental monitoring to marine pollution in copepods.

**BIOAVAILABILITY OF POLYPROPYLENE TO GREEN SPOTTED PUFFER FISH  
(*TETRAODON NIGROVIRIDIS*)**

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The presence of plastic waste with a diameter of less than 5 mm (“microplastics”) in marine environments has prompted increasing concern in recent years. However, to date, there has only been limited research on microplastic pollution in Hong Kong’s coastal waters. Microplastic ingestion and translocation within organisms are identified globally. Yet, the exact toxicological effects of microplastics depend on the affinity of each chemical pollutants like phthalates and polycyclic aromatic hydrocarbons to different kinds of plastic. However, the data on toxicological effects from pure microplastic monomers are limited. This study attempts to use Green Spotted Puffer fish (*Tetraodon nigroviridis*) as a model fish to study the bioavailability of plastic materials in fish. Our preliminary field study showed that polypropylene (PP) is one of the major plastic materials found in local marine waters. We thus fed the fish in 10 ppt salinity for one week with blood worm (control), blood worm mixed with 5% PP, and blood worm mixed with 15% PP. The liver, intestine and gill ethoxyresorufin-O-deethylase (EROD) activities were used as a biomarker to measure the density of the exposure. Only slight EROD induction on liver samples (1.244-fold to control) from PP fed fish was observed. The suitability of puffer fish for this type of bioavailability study will be discussed.

**ASSESSING THE ECOLOGY AND BIODIVERSITY OF TOLO HARBOUR AND CHANNEL: FOR BETTER MONITORING AND MANAGEMENT OF MARINE ECOLOGICAL RESOURCES OF HONG KONG**

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In tandem the development of the local Biodiversity Strategy and Action Plan under the international Convention on Biological Diversity, a mega-project has been initiated by the Environment Bureau of the Hong Kong SAR Government to assess the current status of marine coastal communities in Tolo Harbour and Channel, an area that is known to support a highly diverse fauna but is threatened by intense human activities. A multidisciplinary team consisting of marine scientists and environmentalists from different institutions have joined to undertake a comprehensive ecological study, covering a wide range of habitats and taxonomic groups including rocky and soft shores, mangroves and juvenile fish assemblages. To cover both sheltered and exposed areas, the inner and outer parts of Tolo Harbour and Channel were stratified into four sections and biodiversity surveys were conducted in each section in the aforementioned habitats twice in the dry (December to March) and wet (June to September) seasons to encompass spatial and temporal comparisons. Prior to regular sampling, preliminary surveys of each habitat were conducted at three randomly selected sites to obtain species accumulation curves which were used to determine the number of replicates required at each site to ensure that the results were representative. This was compared with the sampling time available and man-power resources to generate the most cost-effective sampling strategy, and to provide estimates of potential error associated with this effort. Apart from regular, quantitative surveys which are conducted in both seasons, qualitative surveys of each habitat using systematic searches for organisms will be conducted in the wet season to produce a more complete species list of the study area. The current ecological status and economic value of marine resources in Tolo area will be evaluated through questionnaire surveys and economic modelling. The ultimate goals of this study are not only to document a species inventory in the study area for scientific, conservation and educational purposes, but also to establish a blueprint of standardised, scientific practice for future marine biodiversity assessments (e.g., environmental impact assessments and monitoring programmes) in Hong Kong waters.

## SPATIAL VARIATIONS IN DEMERSAL FISH ASSEMBLAGES IN THE DEGRADED COASTAL MARINE ENVIRONMENT OF HONG KONG

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Hong Kong's marine environment, which supports numerous marine organisms, has been subjected to various kinds of anthropogenic impacts over the past decades. Tolo Harbour and Tolo Channel located at the eastern waters (EW) of Hong Kong are a shallow semi-enclosed water body with limited water exchange and hence results in low dissolved oxygen level, particularly in summer months. This area also receives pollutant discharges through surface runoff and untreated sewage from surrounding residential areas. In addition to the local water pollution, the western waters (WW) of Hong Kong located at the Pearl River Estuary are considerably influenced by outflow of nutrients, sediments and pollutants from the Pearl River. During wet season, a large amount of freshwater, suspended solids and pollutants brought by the Pearl River enter into WW of Hong Kong. This study aimed to investigate spatial patterns in diversity and abundance of demersal fish assemblages in such degraded coastal marine waters of Hong Kong. Trawl surveys were conducted using a commercial shrimp trawler in EW (inner Tolo and outer Tolo) and WW (inner estuary and outer estuary) in 2004, 2007 and 2012. A total of at least 137 fish species from 69 families were recorded, among which the low valued ponyfish *Leiognathus brevis* and rabbitfish *Siganus canaliculatus* dominated the overall catches. These two species were small-sized, fast growing and in low trophic levels, which resulted in their high resilience to the environmental degradation in Hong Kong. Analyses of fish compositions in the levels of families revealed that the assemblages at the inner Tolo and outer Tolo of EW were significant different from each other. Five fish families (Leiognathidae, Signaidae, Platycephalidae, Callionymidae and Apogonidae) were typical at inner Tolo, while six families (including the five aforementioned families at inner Tolo and Gobiidae) were typical at outer Tolo. Fishes were generally more abundant at outer Tolo than inner Tolo. No significant differences in assemblage compositions were detected between the inner estuary and outer estuary of WW. The WW communities were commonly typified by Platycephalidae, Gobiidae, Sciaenidae and Cynoglossidae. From the surveys, the two communities of EW had significantly higher total abundance, but lower fish diversity than the inner estuary of WW. This was mainly attributed to the dominance of fishes from Siganidae and Leiognathidae in EW. As a territory-wide trawling ban has been imposed in Hong Kong's marine waters since 31 December 2012, the current results could serve as a baseline for evaluating the effectiveness of the trawl ban policy on the prospective recovery of demersal fish resources.

**A DECADAL STUDY OF SPATIAL AND TEMPORAL DYNAMICS OF COMMERCIALLY IMPORTANT CRUSTACEAN AND RELATED ENVIRONMENTAL FACTORS STRUCTURING CRUSTACEAN ASSEMBLAGES IN THE COASTAL MARINE WATERS OF HONG KONG**

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Hong Kong's coastal waters are known to be ecologically diverse due to the interplay of hydrography, habitat heterogeneity and anthropogenic disturbances. The abundance, biomass, species composition and diversity of marine crustaceans in Hong Kong are strongly affected. This study investigated the spatial and temporal dynamics of commercially important species of Decapoda and Stomatopoda during trawl surveys from 2003 to 2012. A total of 32 crustacean species from 7 families were recorded. Community structure differed significantly across eastern and western regions, with generally higher biomass and species richness of crustaceans in western waters. Although higher abundance and biomass of crustaceans were recorded in the wet season of 2012 in western waters by comparing with those of 2003, 2004 and 2007, the abundance and biomass of commercial crustaceans has generally remained at low levels over the past decade. Differences of community structure were also identified among 2003, 2004, 2007 and 2012 in wet and dry season in both waters. Community structure differences were significantly linked to a number of environmental water parameters: across eastern waters this included Unionised ammonia, turbidity, and related variables; while in western waters Unionised ammonia and pH were the two major environmental factors that drove the changes in the yearly crustacean community pattern. The results of this study provide important support for current and future crustacean community studies, and investigated the environmental factors which structured the crustacean community over years.

## RIVERINE NUTRIENTS FLUXES AND ENVIRONMENTAL EFFECTS ON CHINA'S ESTUARIES

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Estuary is freshwater and seawater intersection and mixed zone, and is the highest productivity ecosystem. The increases in estuarine nutrient fluxes significantly influenced the estuarine ecosystem. This study collected nutrients data in China's most of estuaries over years to estimate nutrient fluxes in major estuaries, and analyzed interannual variability of estuarine nutrient fluxes and estuarine environmental effects. The results showed that  $\text{NO}_3^-$ -N、DIN fluxes in the Yangtze River Estuary increased annually, the changes in nutrient fluxes ranged, from  $42.7 \times 10^4$  to  $143.8 \times 10^4$   $\text{t} \cdot \text{yr}^{-1}$  for  $\text{NO}_3^-$ -N, from  $31.5 \times 10^4$  to  $174 \times 10^4$   $\text{t} \cdot \text{yr}^{-1}$  for DIN; from  $2.471 \times 10^4$  to  $29.1 \times 10^4$   $\text{t} \cdot \text{yr}^{-1}$  for  $\text{NH}_4^+$ -N, and from  $0.569 \times 10^4$  to  $7.927 \times 10^4$   $\text{t} \cdot \text{yr}^{-1}$  for  $\text{PO}_4^{3-}$ -P, respectively. In the Yellow River Estuary, DIN fluxes increased annually, the annual average fluxes reached  $6.51 \times 10^4$   $\text{t} \cdot \text{yr}^{-1}$ ,  $\text{PO}_4^{3-}$ -P fluxes remained constant with annual average fluxes of  $211.4$   $\text{t} \cdot \text{yr}^{-1}$ . In Jiulong River Estuary, nutrient fluxes increased at first and then decreased. Runoff was the main influencing factors. Secondly, there was a clear correlation between nutrient fluxes, and human inputs within the catchment. Fertilizer, livestock and industrial wastes, damming, and other factors together exerted influences on the nutrient fluxes. Although the Jiulong River estuary has relatively small catchment, it had higher nutrient fluxes. This illustrated that Jiulong River Estuary is more affected by human activities. In addition, the study also found that the nutrient fluxes and near shore eutrophication were closely related. This showed that DIN and  $\text{PO}_4^{3-}$ -P fluxes had significant influences on near shore eutrophication.

Keywords: Estuaries; Nutrients; Nutrients fluxes; Environmental factors; Eutrophication

## **OCCURRENCE AND RISK ASSESSMENT OF ALGAL TOXINS IN MARINE WATER OF HONG KONG**

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Harmful algal blooms (HABs) is in frequent outbreak throughout the world in recent decades which resulted from rapid multiplication of algae cells when nitrogen and phosphorus pollution concentrated in near-coast waters, and brought great influence to marine water and human health. Over the past several decades, climate change and increasing environmental degradation provided good growth environment for certain phytoplankton species. Under favorable environmental conditions, proliferation and occasional dominance of these phytoplankton species can result in high densities of algae and the formation of algal blooms. Harmful algae can produce biotoxins to the aquatic system, which poison the aquatic organisms such as shellfish, crab and fish and destroy aquatic ecological balance, even endangers the human health through the biological enrichment. Toxins causing the PSP syndrome are of the saxitoxin (STX) group. *Alexandrium spp.* and *Gymnodinium spp.* are the main organisms which caused paralytic shellfish poisons (PSP). Hong Kong is located on the southern coast of China at the Pearl River Estuary and the South China Sea which is surrounded by the South China Sea on the east, south, and west. Hong Kong has suffered the damage of PSP for long history and two phytoplankton species, *Alexandrium spp.* and *Gymnodinium catenatum*. Therefore, studying the characteristics and the level of the algal toxins and assessing their risks in Hong Kong are of particular importance. In present study, 30 marine sites at the high incidence of HABs in the past of Hong Kong were sampled. *Alexandrium spp.* and *Gymnodinium catenatum* were quantified by Q-PCR, while PSTs were detected by HPLC-MS of 20 coastal water samples along Hong Kong. Risk assessment caused by marine toxins was measured. The present study can be useful to the local aquaculture development and provide effective suggestions and theoretical basis for present HAB monitoring and management.

**Keywords:** HABs, PSTs, bio-toxins; HPLC-MS, Q-PCR

## MARINE BENTHIC DINOFLAGELLATES IN SUBTIDAL HABITATS IN HONG KONG: SPATIAL AND TEMPORAL VARIATION AND PRELIMINARY TOXICITY ASSESSMENT

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Among the marine phytoplankton, dinoflagellates, which are often associated with outbreaks of biotoxins, fish kills and also anoxia/ hypoxia events in marine habitats, are the most important causative agents for harmful algal blooms (HABs) and seafood poisoning. Hong Kong is believed to be one of the worst HABs affected areas in the world, among the five major groups of biotoxins produced by marine phytoplankton, four groups are mainly produced by dinoflagellates. Dinoflagellate species that can cause these biotoxin-related food poisonings, including diarrhetic shellfish poisoning, neurological shellfish poisoning, paralytic shellfish poisoning and ciguatera fish poisoning, can all be found in Hong Kong waters. Therefore, it is important to monitor the occurrence of HABs, and biodiversity and distribution of dinoflagellates in local marine habitats. In Hong Kong, numerous research studies have focused on phytoplankton biodiversity and ecology, harmful algal blooms and food poisoning associated with dinoflagellates in the past decades. Most studies, however, mainly focus on pelagic forms of dinoflagellates, there is still a knowledge gap of benthic dinoflagellates in Hong Kong. Among the six common biotoxin-producing genera, i.e., *Coolia*, *Ostreopsis*, *Amphidinium*, *Prorocentrum*, *Gambierdiscus* and *Fukuyoa* (globular *Gambierdiscus*), which are epiphytic and live on macroalgae, dead corals, sand and rocks, five were found in Hong Kong. As biotoxins from benthic dinoflagellates can often be bioaccumulated via food chains and affect the reef ecosystems and human health. It is important to know whether there is any toxic benthic dinoflagellate in local marine habitats, and when/ where they distribute, so as to allow for assessing their potential risks to Hong Kong marine environment.

This study aimed to conduct baseline ecological studies and document the spatial and temporal variations, habitat (coral communities vs. rocky reefs) and substrate (macroalgae vs. rock) associations of marine benthic dinoflagellates in Hong Kong. In this study, over 200 strains of local benthic dinoflagellates have been isolated and cultured. A culture collection has been developed and maintained in State Key Laboratory in Marine Pollution (SKLMP). A series of marine invertebrate bioassays (such as copepod, rotifer, barnacle larvae and sea urchin larvae) will be conducted to assess the potential toxicity of these strains in local habitats. We anticipate that these findings would be useful baseline reference for future environmental monitoring, ecological investigations, environment risk assessment and trophic dynamic studies. This study is supported by Environment and Conservation Fund (ECF Project 12/2012).

**Keywords:** benthic dinoflagellate; epiphytic toxic algae; subtropical; subtidal reefs

**THE EUTROPHICATION STATES AND NUTRIENT-PHYTOPLANKTON DYNAMICS OF EUTROPHIED TROPICAL EMBAYMENT OF JAKARTA BAY, INDONESIA : TEMPORAL DYNAMICS FROM 2001 TO 2013**

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Eutrophication states of the most eutrophied tropical embayment in Indonesian waters was studied over the period of 2001-2013 in Jakarta Bay. There was a clear gradient of dissolved inorganic nutrient concentration, showing very high values in the river mouths and steeply decrease down the bay. This is followed by a steep gradient of Chl-a concentration, showing high phytoplankton biomass in the river mouths (31.4  $\mu\text{g Chl-a l}^{-1}$ ) and then decrease 2 folds in the near coastal waters (15.8  $\mu\text{g Chl-a l}^{-1}$ ) for then decreased 4 folds in the middle (7.8  $\mu\text{g Chl-a l}^{-1}$ ) and finally 14 folds in the outer part of the bay (2.2  $\mu\text{g Chl-a l}^{-1}$ ). There is no significant change in nutrient concentration (except nitrogen), phytoplankton biomass and thus eutrophication level during the last 12 years period (between 2001 to 2013), showing relatively stable but high nutrient pollution level in Jakarta Bay. Eutrophication level analysis resulted high level of eutrophication in the bay. Hyper-eutrophic level is always pronounced along the near-shore part of the bay, for then decreases to eutrophic level in the middle of the bay and mesotrophic class in the outer part of the bay. Algae bloom and hypoxia becomes a regular phenomenon which leads to mass mortality of fish in this bay.

**DETERMINING THE EFFECT OF CHANGES IN NITROGEN LOADS AND FORMS ON ALGAL COMMUNITY COMPOSITION AND PHYSIOLOGY IN LAKE TAIHU, CHINA****J J Yang<sup>1</sup>, H Gao<sup>1</sup>, M M Tong<sup>1</sup> and P M Glibert<sup>1,2</sup>****Zhejiang Universtiy**

Lake *Taihu*, the 3<sup>rd</sup> largest freshwater lake of China, has suffered an increasing number of harmful algal blooms over the past three decades, bringing about formidable ecological and economical losses. As a lake for drinking, fishing and entertainment, however, the ecosystem fates of different forms of nitrogen have rarely been paid much attention in the proceeding of the management of harmful algae and the improvement of water quality. To complement more understanding of blooms in the lake, therefore, the objective of this paper was to explore the role of nitrogen loads and forms on its algal community composition and physiology. In the experiment, different nutrient enrichment (ammounium, nitrate, urea and phosphate) and short-term incubation under natural light and temperature were carried out, during which phytoplankton community, biomass accumulation, and nutrient uptake efficiency were tracked every 24 hours. Pico-cyanobacteria represented the dominant fraction of species, while pico-eukaryotic cell density was 2 orders of magnitude lower in the phytoplankton community. A significant increase in growth was observed in the pico-cyanobacteria community with the addition of ammonium, nitrate and urea, in the opposite the response of biomass to the addition of phosphate, which suggested that N limitation had occurred in spite of enriching in phosphate. As for the nutrient uptake efficiency, maximum uptake rates achieved by phytoplankton on  $\text{NH}_4^+$  were significantly higher than those for  $\text{NO}_3^-$  and  $\text{CO}(\text{NH}_2)_2$  with or without nutrient pretreatment, indicating that phytoplankton preferred  $\text{NH}_4^+$  to  $\text{NO}_3^-$  or  $\text{CO}(\text{NH}_2)_2$  as a result the addition of  $\text{NH}_4^+$  inhibited the uptake of  $\text{NO}_3^-$  or  $\text{CO}(\text{NH}_2)_2$ .

**ONTOGENETIC CHANGES IN TOLERANCE TO HYPOXIA AND HYDROGEN SULFIDE DURING THE EARLY LIFE STAGES OF MANILA CLAM *RUDITAPES*****K Kodama<sup>1</sup>, T Akatsuka<sup>1</sup>, M Waku<sup>2</sup>, R Sone<sup>2</sup>, T Ishida<sup>2</sup> and T Horiguchi<sup>1</sup>**<sup>1</sup>National Institute for Environmental Studies, Japan<sup>2</sup>Aichi Fisheries Research Institute, Japan

Wind-induced upwelling of water hypoxic waters containing hydrogen sulfides (H<sub>2</sub>S) usually cause mass mortality of aquatic organisms inhabiting shallow and tidal flat areas. Despite relatively high tolerance of Manila clam *Ruditapes philippinarum* to severely hypoxic conditions, mass mortality of the clam also occur after this catastrophic upwelling event. H<sub>2</sub>S is highly toxic to aquatic animals because it interferes with reoxidation of cytochrome a in respiration, while other dissolved sulfides (HS<sup>-</sup> and S<sup>2-</sup>) are essentially non-toxic. The upwelling of H<sub>2</sub>S-rich hypoxic water may overlap in time and space of reproduction of Manila clam, thus it may result in failure of recruitment of the young-of-the-year individuals. However, little is known about effects of H<sub>2</sub>S on the survival of the early life stages of Manila clam due to difficulty in measurement of concentration of toxic H<sub>2</sub>S (excluding non-toxic HS<sup>-</sup> and S<sup>2-</sup>). Meanwhile, recent developments in microsensing technology have facilitated measurement of H<sub>2</sub>S concentration. In the present study, we examined tolerance of Manila clam during the early life stages to H<sub>2</sub>S under controlled laboratory conditions using the Unisense H<sub>2</sub>S microsensor. Larvae used in the experiment were obtained by artificial fertilization of eggs spawned by adult clams collected in Mikawa Bay, Japan in May 2015. Juveniles were collected from Rokujo tidal flat in Mikawa Bay, Japan in June and August 2015. Larvae and juvenile of Manila clam were exposed to normoxic (dissolved oxygen [DO] > 6 mg/L) and hypoxic (DO < 0.5 mg/L) water, and hypoxic water containing H<sub>2</sub>S. Several concentrations of H<sub>2</sub>S were set exploratory to determine fatal conditions for larvae and juveniles of Manila clam. To imitate changes of water mass from normoxic to hypoxic water with H<sub>2</sub>S under natural environment, c.a. 1.5 L of test water was supplied to 130-ml airtight containers including larvae or juveniles of Manila clam at the beginning of the experiment. Then 24-h exposure to the test water was conducted in static conditions. Water temperature and pH during the 24-h experiment ranged 22~24°C and 7.6~7.9, respectively. Survivability after the 24-h exposure in control and hypoxic conditions were high (90~100%) for all developmental stages, suggesting high tolerance of Manila clam at the early life stages to hypoxia. However, decrease in the survivability of larvae and juveniles was evident when H<sub>2</sub>S was added to hypoxic waters, and the extent of the decrease varied among the developmental stages as well as the concentration of hydrogen sulphide. Umbo larvae survived in low H<sub>2</sub>S concentrations (9 and 27 μM), whereas only 3.8% of umbo larvae survived in 193 μM of H<sub>2</sub>S. Tolerance to H<sub>2</sub>S substantially developed in the full-grown larvae, in which survivability increased to 98.5% and 58.2% in 652 μM and 1176 μM of H<sub>2</sub>S concentration, respectively. Meanwhile, survivability of juveniles after 24-h exposure to H<sub>2</sub>S were 70% in 750 μM of H<sub>2</sub>S for 4 mm of shell length (SL), 53% in 521 μM for 7 mm SL, and 10% in 443 μM for 12 mm SL. These results suggest that tolerance of Manila clam to H<sub>2</sub>S is highest in larvae at the full-grown stage, whereas the tolerance is gradually reduced as the clams develop to juvenile stage and grows in their body size.

## TRANSCRIPTOMIC RESPONSES OF MALE AND FEMALE MEDAKA GONADS TO HYPOXIC STRESS

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Hypoxia is a serious global problem affecting marine and freshwater environments worldwide where over 400 “Dead Zones” have been reported by the United Nations to date. Hypoxia is reported to cause severe reproductive impairments in fish causing heavy loss of fishery production over large areas. Laboratory and field studies have demonstrated that hypoxia can affect male and female reproductive systems at various levels. For example, hypoxia can suppress spermatogenesis and decrease sperm motility as well as impair testicular development and sperm production in male fish, and retard gonadal development and oogenesis in female fish. Notwithstanding this, a genome-wide transcriptomic study to investigate the mechanism(s) underlying hypoxia-induced reproductive impairments in fish has not been reported. Here, we report the transcriptome sequencing (RNA-seq) and bioinformatics analysis of ovary and testis tissues of marine medaka (*Oryzias melastigma*) to determine the differential molecular responses of male and female gonads to hypoxic stress. Functional GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway and IPA (Ingenuity Pathway) analyses demonstrated differential gender-specific gene and signaling pathway responses in male and female fish to hypoxic stress. Specifically, our findings revealed the dysregulation of ovarian follicle development, gonad development and steroid metabolic processes through the modulation of activators including leukemia inhibitory factor (LIF), insulin-like growth factor 1 receptor (IGF1R) and follicle stimulating hormone (FSH) in ovary of hypoxic female medaka fish. In contrast, hypoxia caused the deregulation of the steroid hormone biosynthetic pathway and the stimulation of the apoptotic process through TP53 activation in the testis of hypoxic male medaka fish.

**TOXIC METALS/METALLOIDS POLLUTION IN THE YELLOW SEA AND BOHAI SEA, CHINA: DISTRIBUTION, CONTROLLING FACTORS AND POTENTIAL RISK****C L Xiao and Y B Li****The Ocean University, China**

The Bohai Sea and Yellow Sea are abutting to one of the highly urbanized and industrialized zones in China. The rapid economic development in this region has caused a variety of environmental problems in both seas, e.g., eutrophication, oil spilling, as well as toxic metals/metalloids pollution. Several potential sources of toxic metals exist in the Bohai Sea and Yellow Sea, including industrial waste, mariculture, mining, farming and atmospheric deposition. However, previous studies investigated only very limited portions of the two systems and part of the commonly concerning metals/metalloids. There is a lack of a comprehensive study on investigating the current pollution status of metals/metalloids in both ecosystems. In this study, two cruises were conducted in November 2013 and May 2014, respectively. Eight commonly concerning toxic metals (As, Cd, Cr, Cu, Ni, Pb, Zn and Hg) in Bohai Sea and Yellow Sea sediments were investigated. The eight toxic metals did not present a uniform spatial distribution pattern. Arsenic, Cd and Hg showed a decreasing trend from nearshore to offshore areas, while Cr, Cu, Ni, Pb and Zn displayed a similar spatial distribution pattern, with several “hot spot” widely located in the Bohai Sea and Yellow Sea in both seasons. Multiple regression analysis was performed to identify the factors controlling the distribution of toxic metals/metalloids in the two ecosystems. The results suggested that As, Cd and Hg may be mainly controlled by anthropogenic loading, while for Cr, Ni and Pb, environmental factors, especially the minerals, played a more important role. For Cu and Zn, the effects of enrichment factor and environmental factors were comparable, suggesting the combined effects of anthropogenic loading and sediment characteristics. In addition, three risk assessment methods, including potential ecological risk index (PERI), Sediment Quality Guidelines (SQGs) and the geoaccumulation index ( $I_{geo}$ ), were used to evaluate the ecological risk of toxic metals/metalloids in the Yellow Sea and Bohai Sea sediments. The results showed that Hg should be listed as the priority contaminant metals/metalloids in the Bohai Sea and Yellow Sea sediments, while As, Cr, Ni and Cu could also be an environmental problem and should draw more attention.

## **THE GREEN OYSTER EVENT OCCURRED IN AN INDUSTRIAL HARBOR IN THE CENTRAL TAIWAN**

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Green oyster was found in the Mailiao industrial harbor located in the Yulin County in the central Taiwan. The concentrations of trace metals (Cd, Cr, Cu, Ni, Pb and Zn) in seawater, including dissolved and particulate phases, in sediment and in oyster collected within the harbor were investigated. The concentrations of trace metals in particulate and sediment were chemically divided into leachable (1N HCl acid extracted) and detrital fractions (aqua regia + HF digested). The Cu concentrations in seawater, in particulate matter, in sediment and in oyster ranged within 0.55-1.47  $\mu\text{g l}^{-1}$ , 41.5-69.3  $\text{mg kg}^{-1}$ , 10.4-36.2  $\text{mg kg}^{-1}$ , and 274-1237  $\text{mg kg}^{-1}$  (dry W), respectively. The concentrations of the studied metals in the all samples generally exhibited increase with landward. The concentrations of particulate Cu and Pb were dominated by the leachable fraction, accounting for 49.9-64.8% and 55.9-80.9 of the total concentration pool, respectively. In contrast, particulate Cd and Cr were dominated by the detrital fraction. The concentration of leachable fraction of particulate Ni and Zn nearly equalized to the detrital fraction. The similar result was also found in the sediment samples. The analytical results suggest that the Mailiao industrial harbor were slightly contaminated by trace metals. The reasons for the green oyster occurred within the harbor are attributed to that the growth life of oyster generally exceeded two years and to that particulate Cu was dominated in the leachable fraction, which is bioavailable metal.

## COMPARATIVE QUANTITATIVE PROTEOMICS UNVEILS PUTATIVE MECHANISMS INVOLVED INTO PHENOTYPIC PLASTICITY OBTAINED BY *TIGRIOPUS JAPONICUS* UNDER MULTIGENERATIONAL EXPOSURE TO MERCURY CONTAMINATION

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Here, through a multigenerational life-cycle test, *Tigriopus japonicus* were exposed to different mercuric chloride treatments in seawater (nominal concentrations of 0, 0.5, 1, 10, and 50 µg/L) for five generations (F0–F4), and subsequently all the treatments were recovered in clean environments for one generation (F5). Six life history traits (survival, developmental time for nauplius phase, developmental time to maturation, fecundity, number of clutches, and number of nauplii/clutch) were examined for each generation. Mercury (Hg) accumulation was also analyzed for the adult copepods in the F1, F3, and F5. Specifically, we analyzed the proteome profiles in the F0, F2, and F5 copepods of the control and 50 µg/L Hg treatment, respectively, so as to explore the action mechanism for Hg toxicity/tolerance in the copepod. The results indicated that Hg accumulated in a dose-dependent manner for the F1, F3, and F5 generations. Moreover, higher Hg contents were observed in F3 than F1 at the same exposure levels. Among the six life history traits, only fecundity and number of nauplii/clutch showed a greater sensitivity to Hg toxicity, and the inhibitory effects worsened from F0 to F3, which was explained by a trend for higher metal accumulation with increasing generations. In the recovery generation (F5), none of the traits differed from the control, highlighting that Hg might not induce any epigenetic or parental effects in the following generations. Thus, we hypothesized that although cumulative effects might have been involved in Hg multigenerational toxicity, physiological acclimation, that is, phenotypic plasticity could explain Hg tolerance obtained by this copepod. Functional enrichment analysis showed that a quite different proteomic response was found in F5 when compared to the F0 and F2. Namely, the vast majority of enrichments were correlated with the down-regulated proteins in F0 and F2, however the enrichments for F5 were mostly attributable to the up-regulated proteins, suggesting that different underlying mechanisms are responsible for Hg toxicity and tolerance (i.e., phenotypic plasticity), respectively, during the long-term exposure. In detailed, Hg toxicity strongly prohibited many proteins in F0 and F2 which are related to several critical processes/pathways, e.g., ribosome, protein translation, gene expression, DNA replication, macromolecule metabolic process, cell cycle, cuticle organization, vitellogenesis, and so on. In the recovery generation (F5), many up-regulated proteins were enriched into the energy-production or pathogen-related defence pathways, such as carbohydrate metabolic process, amino sugar and nucleotide sugar metabolism, biosynthesis of antibiotics, and microbial metabolism in diverse environments, indicating a probably acclimatory strategy to fight against Hg toxicity. Taken together, proteomics **provides novel mechanistic** insights into phenotypic plasticity used by marine copepod *T. japonicus* when challenged with cumulative effects due to Hg multigenerational toxicity.

**Keywords:** mercury; phenotypic plasticity; proteomics; multigenerational toxicity

## STUDY ON DAMAGE AND TOXICITY MECHANISM OF CADMIUM IN THE DIGESTIVE SYSTEM OF THE FRESHWATER CRAB *SINOPOTAMON HENANENSE*

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Digestion is important physiological function of organisms. It controls the nutrient absorption and there after affects the growth of animals. Cadmium (Cd) is a heavy metal which is considered as one of the most toxic pollutants in aquatic ecosystems, which causes a series of toxic effects in aquatic organism. The present research investigated the structures of various organs of digestive system in the freshwater crab *Sinopotamon henanense* used a stereoscopic microscope and optical microscope, and studied systematically its morphological structure and histological characteristics of the digestive system at first. To explore the effects of Cd on the digestive system of aquatic animals, we applied acute toxicity experiment (Cd concentrations being 7.25, 14.50, 29.00 mg/L; exposure time being 4 days) and subchronic toxicity experiment (Cd concentrations being 0.725, 1.450, 2.900 mg/L; exposure time being 21 days), and examined the effects of Cd on the activities of 7 digestive enzymes in hepatopancreas, intestine and stomach in *S. henanense*. To further explore the mechanism of the changes in digestive enzyme activities, the levels of Cd accumulation in hepatopancreas, intestine and stomach after the two Cd exposure modes were assessed using the flame atomic absorption method. The changes in microscopic and submicroscopic structures of esophagus, midgut, hindgut and hepatopancreas in *S. henanense* in 29.00 and 2.900 mg/L Cd treatment groups were documented. Finally, colorimetric method was used to quantify the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) level of two main digestive organs (hepatopancreas and intestine) after the two different modes of Cd exposure. Moreover, the real-time fluorescent quantitative PCR technology was used to detect the mRNA of metallothionein (MT) gene expression in the same two tissues, to explore the mechanism of digestive system responses to Cd toxicity. The results showed: The results indicated that:

1. The digestive enzyme activities of *S. henanense* were reduced after Cd exposure, which seriously affected the digestive ability of crabs. The sensitivity of digestive enzyme activities to Cd stress can reflect the toxicity level on the digestive system of crustaceans, and thus can be used as biological indicators of water ecological environmental pollution caused by Cd;
2. Cd accumulation and damage degree of digestive system in *S. henanense* were higher in acute Cd treatment group than subchronic group. The pathological changes of hepatopancreas and intestine were most serious, and the large area epithelial necrosis was even observed, which showing that the two tissues are target organs of Cd toxicity. These injuries may influence the synthesis and secretion of digestive enzyme, leading to a loss of its normal digestive function;
3. The changes of SOD, CAT, GPx activities and MDA contents indicated that, in the two mode of Cd treatment, the antioxidant defense system of *S. henanense* was damaged. The degree of Cd toxicity to aquatic animals is related to oxidative stress and lipid peroxidation;
4. In the subchronic low Cd concentration, MT mRNA expression in the hepatopancreas and intestine were higher than acute groups, which indicate that acute Cd toxicity impairs the defense system. The ability to detoxify of MT may provide main self-protection mechanism for digestive system of *S. henanense* after chronic Cd stress.

## SEASONAL EFFECTS TO METALLOTHIONEIN RESPONSES TO METAL EXPOSURE IN A NATURALISED POPULATION OF *RUDITAPES PHILIPPINARUM* IN POOLE HARBOUR, UK

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Metallothionein (MT) is a protein that is induced by metal exposure in organisms, acting as a detoxification mechanism by binding to metal ions. Therefore, it can be used as a biomarker for metal pollution. The Manila clam (*Ruditapes philippinarum*) is an invasive species that has naturalised in Poole Harbour, UK. MT response to metal exposure has been evaluated in this species in the past showing increased concentrations when exposed to metals, but seasonal effects to this process have not been considered at the northernmost extent of its European distribution. This study assesses the biotic and abiotic effects on MT and metal concentrations in *R. philippinarum*, from January to October 2015 in areas of Poole Harbour with a pollution gradient. Concentrations of MT and metals were measured in digestive gland, gills, and whole tissue. Tissue weight, whole weight, and condition index, as well as variables such as pH, salinity, dissolved oxygen, were also monitored throughout the study period. During winter, linear regression analysis showed significant positive relationships between tissue metals and MT concentrations in all tissues ( $0.391 < R^2 < 0.787$ ,  $0.001 < P < 0.042$ ). However, during spring, when MT concentrations were highest, and summer, these relationships were mostly insignificant. MT concentrations in all tissues during spring had significant positive relationships with tissue weight ( $0.477 < R^2 < 0.577$ ,  $0.001 < P < 0.013$ ) and whole weight ( $0.491 < R^2 < 0.723$ ,  $0.001 < P < 0.011$ ). Significant positive relationships were also observed between MT in all tissues and condition index, during summer ( $0.349 < R^2 < 0.374$ ,  $0.035 < P < 0.043$ ). During spring and summer, biotic factors seem to have more influence over MT concentrations than metal exposure. This is probably due to an increase in MT production in spring caused by hormonal induction during gametogenesis, associated with increased tissue mass as the gonads expand. Furthermore, large MT concentration in the gonads may also add to elevated MT concentrations in spring. A depletion of energy resources, or physical stressors such as heat, may be attributed to the reduced MT production in clams of poor condition in summer. Abiotic factors, such as temperature, pH, and salinity did not show consistent effects on MT concentrations in each site. Hence it is likely that specific localised environmental conditions can govern MT production in *R. philippinarum*. It is also possible that localised populations may be adapted to chronic exposure from pollutants, or genetically distinct, and may exhibit a specialised defensive response. The evidence from this study provides an indication that MT may only be a useful biomarker of metal pollution during winter in *R. philippinarum* in the UK. Other processes probably override the role of MT as a detoxification mechanism for metal exposure during spring and summer. It is also important to consider localised effects when comparing sites. This information should be considered for the application of *R. philippinarum* in biomonitoring studies. The verification of biotic and abiotic controls over biomarkers in this species help to promote a universally available bioindicator of metal pollution.

## HIGH TOLERANCE OF VEGETATIVE BRANCH AND GERMLINGS OF THE BROWN ALGA *SARGASSUM HEMIPHYLLUM* TO LOW SALINITY EFFECTS

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Increasing intensity and frequency of storms has been predicted as one of the consequences of global climate change. In which case, changes in salinity are likely to become an important factor affecting the survival of coastal marine organisms. Macroalgae are important primary producers along the coast. In Hong Kong, the brown alga *Sargassum hemiphyllum* is an important canopy species. It occupies a critical niche in the low intertidal and shallow subtidal. It is therefore susceptible to low salinity exposure during low tide. We examined two growth stages of *S. hemiphyllum*, the vegetative and germling stages, for their vulnerability to low salinities. Secondary shoots with length of around 5 cm were collected from separate *Sargassum* individuals and kept in 250 ml conical flasks, each with 150 ml seawater culture medium. They were then subjected to salinity treatments at 0 psu, 10 psu, 20 psu, 30 psu and 33 psu (as ambient control). Likewise, germlings of *Sargassum* were induced to settle on Petri dishes and exposed to 0 psu, 5 psu, 10 psu, 15 psu, 20 psu, 25 psu and 33 psu (as ambient control). The ambient control was the salinity level of the seawater at the time when the samples were collected from the field. Both experiments lasted for 40 days and growth and mortality, as indicated by complete loss of pigments or decay of the branch or germlings, were used as the end points. All vegetative branches survived their exposure to different salinities, except at 0 psu. Vegetative branches attained the best growth under the ambient condition of 33 psu. On the other hand, all germlings cultured under different salinities demonstrated a type three survivorship curve, in which high mortality occurred early in the developing stage. All germlings died at day 6 under 0 psu, whereas most germlings could survive under 15 psu or higher salinities. The highest survivorship (80%) was surprisingly recorded at 15 psu as germlings exposed to higher salinities needed also to compete with the growth of epiphytic algae. Germlings under 25 psu obtained the highest increase in growth of 7 times of their original size at Day 0. Our results indicated that *S. hemiphyllum* is very tolerant to low salinities. They are likely to survive in the future even with lowering of salinity due to global climate change.

**EFFECTS OF DIFFERENT WATER pH ON MORTALITY AND ULTRASTRUCTURE OF GILLS AND SKINS IN JAVANESE MEDAKA, *ORYZIAS JAVANICUS***

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This study was conducted to determine the effects of different water pH on mortality and ultrastructure of gills and skins in Javanese medaka *Oryzias javanicus*. The fish were divided into five groups containing 12 fish in each group and in duplicates. The groups were treated with water pH 5, pH 6, pH 7, pH 8 and pH 9 for 14 consecutive days. The daily mortality of the fish was recorded, while the death fish were collected for histological examinations. The mortality were recorded highest in treatment group of pH 9 (100%), followed with pH 5 (25%) and pH 8 (4.17%), while no mortality recorded in groups of pH 6 and 7. The most frequent histological findings in gills were epithelium lifting and edema, which observed in all treatment groups except for groups treated with water pH 6 and 7. Epithelium hyperplasia, lamella fusion and aneurysm were mildly observed in gills of fish treated with pH 8, while only gill of fish treated with pH 9 showed severe desquamation and necrosis lesion. No histopathological changes of skin were observed in groups treated with pH 6 and 7. Fish from groups of pH 5 and 8 demonstrated mild lesions. The most severe skin lesions were epithelium sloughing and necrosis observed in group of pH 9. This study provides further information on the influences of water pH on Javanese medaka survival.

## **DIVERGENT RESPONSES OF TWO INTERTIDAL MEIOFAUNA COMMUNITIES TO CO<sub>2</sub>-INDUCED SEAWATER ACIDIFICATION**

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Ocean acidification poses an ongoing threat to marine organisms and ecosystems. A microcosm study on the potential effects of ocean acidification on two intertidal meiofauna communities was conducted. Sediment cores containing meiofauna were collected from the extreme low tidal zone of a sandy beach and a silty sandy beach in Qingdao (China). Microcosms were incubated for 0, 1, 2, 4, 8 weeks in natural seawater (as control) and acidified seawater (pH 7.3) which simulating the predicted ocean acidification level in 2300 in a carbon dioxide climate incubator (CO<sub>2</sub> concentration: 1900ppm). Meiofauna communities inhabited in the two types of sediment were distinctly different. The dominant meiofauna taxon was harpacticoid in sandy sediment while nematode dominated in silty sand sediment. In both sandy and silty sand sediment, nematode abundance increased in response to low pH in the first two weeks but was not significantly affected by acidification in the following weeks. In contrast, harpacticoid abundance at pH 8.1 showed a very significant increasing in the last week in both sandy and silty sand sediment. The nauplius abundance at pH 8.1 also showed a significant increasing in the last week in silty sand sediment but not in sandy sediment. The abundance of harpacticoid and nauplius were significantly suppressed by acidification in silty sand sediment. Exposure time and pH significantly changed the community structure and biodiversity of harpacticoid in sandy sediment. These differences were attributed to reduction in the numerically dominant species in the acidification treatments. The influence of acidification on community structure and biodiversity of harpacticoid in silty sand sediment was not significant. The community-based microcosm study indicated that ocean acidification could lead to changes in meiofauna communities in intertidal sediments under extreme acidification scenario, but the communities inhibited in different types of sediment might response divergently.

**LARVAL RESPONSES OF A PREY AND A PREDATOR TO OCEAN ACIDIFICATION****C Campanati<sup>1</sup>, S Dupont<sup>2</sup>, V Thiyagarajan<sup>1</sup> and G A Williams<sup>1</sup>****<sup>1</sup>The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China****<sup>2</sup>Department of Biological and Environmental Sciences, University of Gothenburg, Kristineberg, Fiskebäckskil, Sweden**

In future climate change scenarios, the survival and responses of key coastal foundation species are critical for the persistence of biodiversity in near-shore ecosystems. Particularly, under near-future acidified waters the most vulnerable early life stages could act as population “bottlenecks”. Moreover, organismal fitness in a high-CO<sub>2</sub> world is not absolute, as eventually the individuals’ success would also depend on the responses of co-interacting species. Differential dispersal capacities and tolerances to low pH between a prey and a predator at the early-life stages could alter their interspecific dynamics at the adult stage. Future acidification responses of the rock oyster *Saccostrea cucullata*, a dominant ecosystem engineer in the Indo-Pacific region, and its predator, the muricid gastropod *Reishia clavigera*, were evaluated in their planktonic early-life stages. We exposed newly hatched larvae of *S.cucullata* and *R. clavigera* to 3 different levels of pH ranging from  $\Delta\text{pH} = -0.3$  to  $-0.7$  pH units for 12 and 15 days of development respectively. Several endpoints, such as larval survival, growth, metabolic rate and shell dissolution were measured for the species throughout their developmental exposure to low pH. Under the same pH exposure, the larvae of the two species showed different sensitivities, with more obvious shell dissolution at the lowest pH conditions. Mortality was not affected by pH in *S.cucullata* larvae whereas *R.clavigera* larval survival decreased significantly at the most extreme pH/pCO<sub>2</sub> levels. In both species veligers growth rate was not altered under the range of pH tested. Unvaried shell growth in *R.clavigera* larvae exposed to undersaturated CaCO<sub>3</sub> conditions was probably supported by the higher metabolic rates observed. This, was further accompanied by the development of up to ~60 % shell and soft body abnormalities.

**CLEAVAGE OF AROMATIC RING ON THE METHYL-OPPOSITE END WAS THE PRIMARY PATHWAY FOR 1-MP DEGRADATION IN BACTERIUM *NOVOSPHINGOBIUM* SP. SA925**

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Alkylated polycyclic aromatic hydrocarbons (APAHs) comprise up to 98% of the total PAH fraction of crude oil. Among them, alkylated phenanthrenes account for about 13%. A pure bacterial strain of *Novosphingobium* sp. SA925 has been isolated from a refinery soil sample in Guangzhou, which is able to use 1-methylphenanthrene (1-MP) as a growth substrate. The removal rate of 1-MP by Strain SA925 was about 70% after five days with an initial 1-MP concentration 100 mg/L. In order to understand the degradation processes, we elucidated the metabolic pathways of 1-MP degradation. Identified by gas chromatography coupled to mass spectrometry and BSTFA for derivatization, metabolites of 1-MP belong to two distinct metabolic pathways, the hydroxylation of the methyl side chain and double hydroxylation of aromatic ring in opposite side of 1-MP, which were similar to the degradation pathways of 2-MP reported before. Due to the oxidation of methyl substituent after the first ring cleavage, a more complex metabolic network might exist in the present study. Identification of several key metabolites demonstrated that 1-MP degradation was mainly performed via an initial oxidation on the aromatic ring, and this was totally different with the metabolism in macroinvertebrates which primarily oxidise the methyl substituent forming polycyclic aromatic acids.

## SO<sub>2</sub> REMOVAL WITH A SIMULTANEOUSLY IMPROVED SOLAR-TO-H<sub>2</sub> ENERGY CONVERSION

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Sulfur dioxide (SO<sub>2</sub>) emitted from fossil fuels burning and chemical industries can cause lots of harmful issues in the environment. In a conventional method for SO<sub>2</sub> removal, aqueous sodium hydroxide (NaOH) solution is typically utilized to absorb SO<sub>2</sub> from flue gas. The resulted Na<sub>2</sub>SO<sub>3</sub> solution is then purged with air for an oxidation of Na<sub>2</sub>SO<sub>3</sub> before disposal. The energy of Na<sub>2</sub>SO<sub>3</sub> is wasted in this process. Herein, we propose that the solar-to-H<sub>2</sub> energy conversion efficiency can be greatly improved with a simultaneous removal of venenous SO<sub>2</sub> via a photoelectrochemical water splitting process, since Na<sub>2</sub>SO<sub>3</sub> possesses lower activation energy and faster kinetics as compared with the direct oxidation of water on the photoanode. A H<sub>2</sub> production rate of 41 μmol h<sup>-1</sup>cm<sup>-2</sup>, with a Faradaic efficiency of 98% is obtained in 0.075 M Na<sub>2</sub>SO<sub>3</sub>. This work provides a new method for the SO<sub>2</sub> removal with a simultaneous H<sub>2</sub> production.

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## **THE EFFECT OF TOTAL LOAD CONTROL SYSTEM ON WATER QUALITY IMPROVEMENT IN THE JIAOZHOU BAY, CHINA**

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Continuously increasing anthropogenic pollution to the coastal areas resulting in the deteriorating quality of seawater has been primarily stressor in Jiaozhou Bay (JZB), China. To overcome the limitations of the water quality improvement policy relying only on the concentration regulation, the total load control system (TLCS) has been carried out in China at the beginning of 2007 for chemical oxygen demand (COD) and 2011 for ammonia nitrogen, respectively. To establish the impact of the TLCS on the water quality, 11 water quality parameters were determined and analysed 13 monitoring stations of 156 water samples collected in the JZB between 2006-2007 (pre-TLCS) and 2012-2013 (5 year post-TLCS). The application of different multivariate statistical approaches for the interpretation of a large and complex data matrix obtained during pre-TLCS and 5 year post-TLCS periods in JZB is presented in this study. The significant decreasing trends of land-based load of COD and ammonia nitrogen from 2006 to 2013 indicated the TLCS played an important role in land-based pollutant management, but the response of seawater quality to load reduction was not clearly observed in JZB based on the cluster analysis and Mann-Whitney test. The monitoring stations were divided water quality control zone I and II (WQCZ I and II) during pre-TLCS and 5 year post-TLCS. The seawater quality in WQCZ II was clearly worse than WQCZ I, especially higher COD and dissolved inorganic nitrogen chemical contaminants concentrations which were observed in the northeastern estuaries in the WQCZ II. Based on the contribution in the factor analysis, four latent factors were identified as responsible for the data structure explaining 75% in pre-TLCS and 76% in 5 year post-TLCS of the total variance of the dataset, and the TLCS had influenced composition of the latent pollution sources. This study not only shows the necessity and usefulness of multivariate statistical assessment of large and complex databases to get better understanding about the effects on seawater quality during the TLCS implementation, but also indicates that the present mode of TLCS in Qingdao should be changed, in which the reduction of land-based pollution is depended on water quality in the JZB.

## BIOREMOVAL OF TRACE METALS FROM RHIZOSEDIMENT BY MANGROVE PLANTS IN INDIAN SUNDARBAN WETLAND

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The study investigates a detailed account of absorption, accumulation and partitioning of trace metals in individual plant tissues (leaf, bark and root/pneumatophore) of mangrove species (n=13) from Indian Sundarban Wetland to establish their phytoremediation capacity. The rhizosediments showed unique potential in manifold increase for all the metals (n=11; except cadmium), with the following descending order (expressed in mg kg<sup>-1</sup>): Fe (29746.59 ± 8717.26) > Mn (901.60 ± 285.90) > Zn (62.85 ± 22.2) > Cu (47.64 ± 14.78) > Ni (34.91 ± 12.1) > Pb (25.44 ± 19.62) > Cr (23.42 ± 13.56) > Co (9.51 ± 4.58) > As (3.56 ± 1.28) > Cd (0.32 ± 0.14) > Hg (0.20 ± 0.04). An overall similar trend of enrichment was also observed in plant compartments by adopting complex and coherent mechanisms. Root/pneumatophore as well as bark exhibited a higher capability to retain majority of the trace metals. Based on the sediment quality guidelines (SQGs), the prescribed effect range- low (ER-L) values were exceeded by Cu, Ni and Pb implying that adverse consequences to biota may occasionally occur, in contrast, Hg and Ni values exceeded the effect range- medium (ER-M) and hence adverse effects will be frequent for these toxic metals. Translocation factor (TF) and bio-concentration factor (BCF) values were too high (>1), suggesting that mangroves can be considered as hyperaccumulators. The study highlights the key role of two hyperaccumulators, namely *Sonneratia apetala* and *Avicennia officinalis* which could be efficiently used for future management strategies of Sundarban.

## ENVIRONMENTAL IMPACT ON DIVERSITY AND DISTRIBUTION OF TINTINNID (CILIATA: PROTOZOA) ALONG HOOGLHY ESTUARY, INDIA: A MULTIVARIATE APPROACH

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The spatiotemporal distribution, diversity and biomass of choreotrich ciliate tintinnid, the ubiquitous planktonic protist, was analysed from nine sampling sites ( $n = 252$ ) of diverse environmental stresses along the Hooghly Estuary, eastern coastal part of India during March 2012 to August 2014. Among 32 identified tintinnid species, the agglomerated genera *Tintinnopsis* (20 sp), dominated the community (~62%) followed by *Tintinnidium* (2 sp), *Leptotintinnus* (2 sp), *Codonellopsis*, *Stenosemella*, *Helicostomella*, *Favella*, *Eutintinnus*, *Metacylis*, *Dadayiella* and *Wangiella* (each comprising single species). A wide range of seasonal variations in tintinnid abundance was observed with maximum value (~1995 ind.  $l^{-1}$ ) during post-monsoon month in mouth of the estuary (Gangasagar) and minimum (~52 ind.  $l^{-1}$ ) during monsoon in brackish water site (Diamond Harbour). The biomass and daily production rate of tintinnid ranged from 0.004-2.764  $\mu\text{g C } l^{-1}$  and 0.04-3.54  $\mu\text{g C } l^{-1}\text{day}^{-1}$  respectively. An overall dominance and diversity of the small-sized tintinnids (lorica length  $<76\mu\text{m}$ ) belonging to the genera *Tintinnopsis* sp., *Tintinnidium* sp., *Codonellopsis* sp., *Wangiella* sp., *Eutintinnus* sp., *Metacylis* sp. and *Helicostomella* sp. was pronounced, accounting ~66% of the total tintinnid abundance. Results of ANOVA reveal a significant variation between species abundance and months ( $F= 2.21$ ;  $P\leq 0.041$ ). K-dominance curves were plotted against log rank  $k$ , showed species dominance over the investigated sites. The multidimensional scaling (MDS) represents a Kruskal's stress of 0.034, thus highlighting a significantly different spatial distribution. Principal Component Analysis (PCA) map showed clustering of core species with chl  $a$  and nitrate and could be considered as the crucial factors controlling the distribution and seasonal patterns of tintinnids. Biota-environment (BIOENV) analyses reveal that chl  $a$  and nitrate were the significant causative factors, suggesting that tintinnids may be used as a bioindicator for discriminating water quality status in this estuarine system. Both the human-induced and natural stresses on tintinnid community were also pronounced, disrupting the phytoplankton productivity as well as fishery potentialities. The study provided detailed information on the specialized group of microzooplankton which enhances our understanding regarding its crucial role in marine ecosystem and complex biotic interactions for maintaining the ecological and economic stability.

## A 3-YEAR MONITORING OF RECRUITMENT FAILURE OF SCLERACTINIAN CORALS IN HONG KONG, A SUBTROPICAL MARGINAL ENVIRONMENT FOR CORAL GROWTH

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Coral recruitment is one of the key processes involved in maintaining coral communities and facilitating reef recovery following disturbance. In this study, recruitment pattern of corals was monitored for three years in Hong Kong, a subtropical marginal environment for coral growth because of its large annual variation in sea water temperature from 14°C in winter to around 30°C in summer. Terracotta tiles were used as settlement substrata in two core coral areas in Tung Ping Chau Marine Park, A Ye Wan and A Ma Wan, where coral diversity is one of the richest in Hong Kong. The settlement and post-settlement survival of coral recruits were monitored at multiple spatial (site, depth) and temporal (season) scales using fluorescence census technique and microscopic examination starting in May 2009. The results revealed the extremely low natural recruitment success of corals, with a total of only 10 recruits recorded on 384 tile pairs, equivalent to 0.58 recruitm<sup>2</sup> throughout the study period. Recruits of *Platygyra acuta* pre-seeded on tiles that were subsequently grown *in situ* showed that these coral recruits experienced very high mortality following settlement, with average mortality of 80% within half a month and <0.1% survival through the first four months of deployment. The data obtained suggest that very low recruitment of corals in the monitored sites may be due to the lack of arrival of competent coral larvae, low larval settlement; or low post-settlement survival of new recruits due to high sedimentation, intense competition for space with other fouling organisms and predation effects. Presence of extensive fouling may be typical of a polluted environment characteristic of Hong Kong. Although this study was focused only on two sites within Hong Kong, the patterns observed could likely be true for other sites around the Pearl River Delta region. Further studies are in place to confirm this observation.

## **THE 2014 SUMMER CORAL BLEACHING IN SUBTROPICAL HONG KONG**

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Subtropical coral reefs are usually considered to be less affected by thermal stress than tropical coral reefs. Here we provide the first report of coral bleaching and its recovery in subtropical Hong Kong. This incident occurred in eastern Hong Kong waters during the hottest month of August in 2014. We conducted a video transect survey in September 2014 at eight sites that were affected by bleaching, tagged 48 coral colonies that suffered from bleaching, and observed their recovery six months after the survey. The bleaching was independent of coral growth forms, with *Platygyra* and *Porites* (massive), *Montipora* and *Pavona* (plate-like), and *Acropora* (branching) as the common victims. Observing the videos showed that the bleaching was in general mild, but there was substantial site difference, which varied from 0.4% colonies at Lo Fu Ngam to 13.4% at Sharp Island East. Sites with higher levels of bleaching (i.e. > 3% colonies) were confined to Port Shelter, a shallow protected bay. Follow-up observation showed that 76% of the tagged colonies had fully recovered, 12% partially recovered, and 12% mortality. These results indicate that the subtropical reefs of Hong Kong are not immune to bleaching, and there is a need to determine the thermal stress limits of coral species in Hong Kong.

## **GROWTH OF TWO MASSIVE CORALS ALONG ENVIRONMENTAL GRADIENTS IN SUBTROPICAL HONG KONG**

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Coral growth, a parameter sensitive to environmental conditions, has been widely used to indicate coral health. Here we reported a study of the growth of two dominant reef structure builders, *Porites lutea* and *Platygyra carnosus* in the subtropical waters of Hong Kong. We collected the coral colony samples from ten sites representing diverse environmental conditions, prepared coral slabs along their maximum growth axis, examined the slabs using X-ray, and determined their growth rates by quantification of annual growth bands. The average growth rate of *Porites lutea* was 5.2 mm/year with a range of 3.2-7.4 mm/year, while that of *Platygyra carnosus* was 5.7 mm/year with a range of 2.1-8.4 mm/year. These growth rates are low when compared with those of the corresponding species in tropical waters, perhaps due to the low temperatures in winter, which limit coral growth. The growth rate in both coral species had significant negative correlation with sedimentation rate. Overall, our study should that both regional and local stressors have posed limits to coral growth in Hong Kong.

**PREDATOR-PREY INTERACTION BETWEEN MURICID GASTROPODS AND MUSSELS UNDER OCEAN ACIDIFICATION**

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Predation of the muricid gastropod *Thais clavigera* on two sized groups (7-11 mm and 14-18 mm shell length) of the mussel *Brachidontes variabilis* was studied under three pCO<sub>2</sub> levels: 380, 950, 1250 μatm. At 950 μatm pCO<sub>2</sub> level, the prey handling time decreased significantly, with large-sized mussels being preferred by *T. clavigera*. The prey consumption rate, however, was independent of pCO<sub>2</sub> levels, although the prey searching time increased significantly at elevated pCO<sub>2</sub>. The present findings indicated that the predator-prey interaction between *T. clavigera* and *B. variabilis* was altered under ocean acidification that will result in a long-term impact on the population dynamics of the interacting species at the same habitat.

## **ANTHROPOGENIC OCEAN ENVIRONMENTAL CHANGES IN THE COASTAL URBAN ZONE OF SOUTH CHINA**

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Rapid urbanization and economic development in coastal areas exacerbates changes in local environments, including rising temperature and sea level, and declining in pH in near-shore waters of south China. Using Hong Kong-Shenzhen corridor in south China as an example, there was a steep rise in near-shore temperature and sea level in the past two decades. From 1990 to 2012, temperature increased  $\sim 0.060^{\circ}\text{C}/\text{yr}$ , sea level rose  $4.4\text{ mm}/\text{yr}$  and pH decreased from 8.2 – 7.8, much faster than offshore in the South China Sea and the global estimates. Many of the observed changes in the coastal zone were closely related to human activities including large increases in local population, gross domestic product and land fill. Empirical analyses suggest that large increase in population affected local temperature, and economic development had major impact on local pH. Results also show that pH and temperature were significantly correlated with local sea level rise but pH had more predictive power for local sea level rise, suggesting pH could be considered a predictor for changes in local sea level. Future developments in coastal areas should consider the increased risk posed by urbanization and related human activities.

**COMPLEX TOXIC IMPACTS OF HEAVY METALS AND PAHs TO MARINE MUSSELS****Y Zhang, S Chen and Y Di****Institute of Marine Biology, Ocean College, Zhejiang University, China**

Rapid economic development and marine exploration have placed the ocean under increased multiple stresses. Environmental pollutants, especially heavy metals and polycyclic aromatic hydrocarbons (PAHs) can cause detrimental impacts to marine ecosystems because they are stable and difficult to break down after introduced into water. A few studies were focused on the toxic effects of single kind of pollutants to marine environment and organisms, which cannot truly reflect the combined pollution in real world. The aim of this study is to investigate the complex toxic effects at different biological levels and tissues of marine mussels. *Mytilus spp.* were selected as model organisms to analyse the complex toxic impacts of heavy metals and benzo(a)pyrene. Farmed mussels from Zhoushan island, East China Sea, were selected in the experiments. Species were identified by specific designed molecular probes to avoid the viability caused by species difference. Both *in vivo* and *in vitro* exposure to different composition of heavy metals and B(a)P were constructed after 2 weeks accumulation in the lab. Triplicated mussels were collected and haemolymph, gill and digestive gland were dissected for the detection of series of biomarkers, including DNA damage detected by comet assay or micronucleus, stress related mRNA expression and histopathological damages. The results showed relative higher damage under complex exposure condition comparing to single chemical exposure, suggesting that marine organisms may employ different response system or biotransformation mechanisms to survive. Tissue specific-responses were also detected for all the biomarkers selected in this study, indicating that the function of tissues may play a key role during anti-stress procedure. In addition, both *in vivo* and *in vitro* exposure were applied to justify if the selected biomarkers can be developed as a quick tool during ecosystem health evaluation in the field. For most biomarkers, it is showed similar responses when exposed to same conditions, suggesting *in vitro* separated cells can be applied to mimic *in vivo* response and significantly shorten the test time in the field. The detailed correlation in cell response between *in vivo* and *in vitro* exposure will be focused in the future study. To our knowledge, this is the first research to include both *in vivo* and *in vitro* exposure together with the purpose of exploring the complex effects of heavy metals and B(a)P. The study will shed the light on developing a quicker environmental monitoring tools by using isolated marine organisms cells in the future.

## COMPARISON OF BDE-47 DEGRADATION PATHWAYS UNDER AEROBIC, ANAEROBIC AND ALTERNATING AEROBIC AND ANAEROBIC CONDITIONS IN MANGROVE SEDIMENT

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Polybrominated diphenyl ethers (PBDEs), a group of newly emerged toxic and persistent organic pollutants, are likely to accumulate in sediments when discharged and disposed to the environment. How to completely remove them from contaminated sediments without generating secondary contamination from the production of debromination products is always a challenge question for researchers. We compared the degradation pathways of BDE-47, one prevalent congener in environment, under aerobic, anaerobic and alternating aerobic and anaerobic conditions in mangrove sediment. The following results were obtained:

(i) Under aerobic condition, the concentration of BDE-47 did not show any significant changes in 5 months, and no debromination product was detected, which indicated that degradation of BDE-47 did not occur after 5 months of aerobic incubation.

(ii) Under anaerobic condition, significant reduction of BDE-47 was found during 5 months of incubation, while the debromination products, like BDE-28, -17 (Tri-BDE), BDE-15, -8, -7 (Di-BDE) increased significantly with time, which indicated that BDE-47 could be removed anaerobically but might produce secondary contamination due to debromination products.

(iii) Under alternating aerobic and anaerobic condition, Tri-BDE and Di-BDE were still the main debromination products, while the proportion of Di-BDE, especially BDE-15, varied among treatments with different durations and frequencies of aerobic and anaerobic periods. It was found that the proportion of BDE-15 was significantly less in treatments with longer duration of aerobic period, indicating that BDE-15 might go through aerobic degradation pathway during the aerobic period.

These findings suggested that BDE-47 under alternating aerobic and anaerobic conditions were removed through two mechanisms, reductive debromination of BDE-47 under anaerobic condition while the debrominated products, like BDE-15, were subsequently mineralized under aerobic condition. My future work will focus on the investigation of the metabolites and functional genes involved, aiming to confirm the aerobic mineralization process of the debromination products of BDE-47.

## NON-DESTRUCTIVE DETECTION FOR COMBINED STRESSES USING HIGH-RESOLUTION THERMAL INFRARED REMOTE SENSING AND “THREE-TEMPERATURE MODEL”: A CASE STUDY ON MANGROVE PLANT *KANDELIA OBOVATE*

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Pollution is one of the most important factor for mangroves degradation. The problem that the extra nutrient supply alleviates/deteriorates the effects of heavy metal stresses on physiological status of the mangrove plants should cause attention. The “thermal infrared remote sensing + three temperature model” technology has been successfully used in calculation of evapotranspiration under various underlying surface conditions, and limitedly used in non-destructive detection for environmental stress. A study quantifying the role of the interactive effects of heavy metal cadmium and nitrogen stress on photosynthetic parameters, including net photosynthetic rate ( $P_n$ ), stomatal conductance ( $G_s$ ) and transpiration rate ( $T_r$ ) in *Kandelia obovata* was conducted. And the feasibility of high-resolution thermal infrared remote sensing and the three-temperature model (3T model) for monitoring the effects of combined stresses on *K. obovata* was assessed. The experiment consists of four levels of  $\text{CdCl}_2$  stress (0, 1, 5 and 10  $\text{mg}\cdot\text{L}^{-1}$ ) in each of four  $\text{NH}_4\text{Cl}$  stress levels: 0, 10, 50 and 100  $\text{mg}\cdot\text{L}^{-1}$ , respectively.  $P_n$ ,  $G_s$  and  $T_r$  were tested by a portable photosynthesis system. The non-destructive testing indices, including leaf surface temperature ( $T_c$ ), plant transpiration transfer coefficient ( $h_{at}$ ) and estimated instant transpiration rate ( $T-3T$ ), were calculated by thermal images and the three-temperature model (3T model). The results showed that (1) contrary to our expectation, extra nitrogen addition led to a decrease in  $P_n$ ,  $G_s$  and  $T_r$  under Cd stresses ( $P < 0.05$ ). (2) With increasing nitrogen addition, there were significant decreases for  $T-3T$  and increases for  $h_{at}$  ( $P < 0.05$ ) under Cd stresses. The effects of Cd, N, and their interaction on photosynthetic parameters and non-destructive indices were significant ( $P < 0.05$ ). (3) The regression equation for  $T_r$  and  $T-3T$  showed a relatively high correlation coefficient of  $R^2 = 0.337$ , which can be viewed as a measure of the level of accuracy attained in the analysis, and then the model estimate is within acceptable. (4) The  $h_{at}$  was significantly negatively correlated with photosynthetic parameters and the  $T-3T$  was significantly positively correlated with photosynthetic parameters ( $P < 0.05$ ). (5) Daily changes of  $T_c$ ,  $h_{at}$ , and  $T-3T$  showed that the higher combined stress led to greater  $T_c$  and  $h_{at}$  and lower  $T-3T$ , especially for the increasing of nitrogen stresses. Thus, the extra nitrogen addition decreased heavy metal tolerance of *Kandelia obovata*, and the  $h_{at}$  and  $T-3T$  detecting by infrared thermography device reflected the stress conditions and could be employed to evaluate leaf transpiration status impaired by combined stresses.

**ANTIOXIDATIVE RESPONSE SYSTEMS OF KANDELIA OBOVATA, A TRUE MANGROVE PLANT SPECIES, TO POLYBROMINATED DIPHENYL ETHERS (BDE-99 AND BDE-209) DURING GERMINATION AND EARLY GROWTH****S Farzana, J Chan and N F Y Tam****Department of Biology and Chemistry and State Key Laboratory in Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong**

Polybrominated diphenyl ethers (PBDEs), commonly used flame retardants, are found in environmental samples worldwide. Due to urban and industrial discharges, coastal sediments become potential sinks for PBDEs but their effects on coastal ecosystems especially mangrove plants have rarely been reported. A 3-month sediment microcosm experiment made up by pots with mature propagules of *Kandelia obovata* was conducted to investigate the effects of PBDEs on antioxidative response systems of this dominant mangrove plant species. Sediment was contaminated with two PBDE congeners, that is, BDE-99 at 400  $\mu\text{g kg}^{-1}$  freeze dry sediment and BDE-209 at 50  $\text{mg kg}^{-1}$  freeze dry sediment. The pots were placed in a greenhouse and were subject to tidal flushing with one day high tide and one day low tide using artificial sea water at a salinity of 15 ‰. Results showed that all propagules successfully germinated in PBDE-contaminated sediment, but propagules in BDE-99 treated sediment had significantly lower rate of germination than the control and more time was need to have the formation of the 2<sup>nd</sup> and 3<sup>rd</sup> pair of leaves. The total dry biomass of the seedlings was also significantly reduced by BDE-99. The super oxide ( $\text{O}_2^-$ ) release and malondialdehyde (MDA) content in leaves and roots of BDE-99 treated seedlings were significantly higher than that in the control. BDE-99 also increased peroxidase (POD) activity and total polyphenol (TP) content in leaves and roots, but significantly decreased activities of leaf and root superoxide dismutase (SOD), while no effect on leaf and root catalase (CAT). Similar results were found in BDE-209 treatment except the malondialdehyde content in roots was less than the control. Different from BDE-99, BDE-209 increased superoxide content, activities of all three antioxidative enzymes, CAT, POD and SOD, in roots, and such enhancement was only found in leaf superoxide content and CAT. PBDEs were found in leaf, stem, propagule and root tissues, but higher uptake was in root tissues, indicating some plant uptake. These results indicated that *K. obovata* was tolerant to mangrove sediments contaminated with BDE-99 up to 400  $\mu\text{g kg}^{-1}$  and 50  $\text{mg kg}^{-1}$  of BDE-209, and its antioxidative enzymes and TP were important mechanisms to defend the plants against PBDEs stress. Because of its tolerance to PBDEs, *K. obovata* could be used for the bioremediation of PBDE-contaminated environments.

**Keywords:** Polybrominated diphenyl ethers, Germination, Growth, Antioxidative enzymes, MDA

## UTILITY OF DELAYED LUMINESCENCE AS AN INDICATOR FOR DETECTION OF INFLUENCE OF IRGAROL 1051 IN HARDCORAL

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We are investigating utilities of delayed luminescence (DL) from photosynthetic organisms as a new parameter to detect the growth inhibition on these organisms in rapid and simple measurement method. The DL is a special type of chlorophyll fluorescence; it detects only photosynthetic system (cells, chloroplasts or photosystems) in the dark condition after light irradiation. The DL signal relates with back reaction of photosynthetic electron transport reactions via quinone B (Q<sub>B</sub>) site in photosystem II that is target of triazine type herbicides (i.e. Irgarol 1051). The DL signal indicates activity of entire photosynthetic activity including linear and cyclic electron transport reactions. Since the DL correlate with amount of active photosystems, the DL is good to estimate growth potential of the photosynthetic organisms. Namely, DL can be one of the endpoint to evaluate chemical toxicity on alga and other photosynthetic organisms.

Recently herbicides including Irgarol 1051 from agriculture and antifouling paints are suspected to adverse effects on corals and its symbiotic dinoflagellates. Several µg/L of Irgarol 1051 were detected from ports areas of the world. In this study we report our first approach that applied the DL technique to detect influence of herbicide (Irgarol 1051) on hard coral *Acropora digitifera*.

The small branches of *A. digitifera* were exposed to 1 µg Irgarol 1051/L seawater. Then DL signal from each branch was measured before and, 1 to 24 hours exposure using the high sensitivity luminometer type-6110 (Hamamatsu Photonics K.K., Japan). The DL signal from *A. digitifera* decreased according with exposure time. The decreasing of DL in time-course experiment may indicate binding rate of Irgarol 1051 to Q<sub>B</sub> site in photosystem II of *A. digitifera*. The present result suggests the usefulness of measurement on DL to detect influence of herbicides in short time.

**EFFECT OF LOW CONCENTRATIONS OF IRGAROL 1051 ON COLOUR OF THE HARD-CORAL *ACROPORA TENUIS***

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Coral reefs worldwide are suffering from various anthropogenic threats, including high water temperature, ocean acidification, and anthropogenic chemicals, etc. Thus, establishing the physical health of coral reefs is an urgent need in coral ecosystem studies. The present study examined colour change in the branching, hard-coral *Acropora tenuis*, one of the representative species of hard-corals in the Indo-Pacific region, exposed to a low concentration of Irgarol 1051. Exposure levels were similar to those detected in several port areas, and a digital imaging system quantified the coral colour as RGB values (R, red; G, green; B, blue). Each R, G and B value ranged from 0 to 255, with black represented by R=G=B=0 and white as R=G=B=255. Concentrations of Irgarol 1051 at several  $\mu\text{g/L}$  have been recently recorded from port areas worldwide. Branches of *A. tenuis* were exposed to 0, 1, and 10  $\mu\text{g Irgarol 1051/L}$  for two weeks. The seawater temperature was set at 27.5 °C, and the PPF (photosynthetic photon flux density) was at 50  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . RGB values of coral were recorded daily using a digital single-lens reflex camera. RGB values increased, moving toward the ‘white’ end of the scale with the increase in Irgarol 1051 concentrations. The present study suggests that ambient levels of Irgarol 1051 recorded from port environments would lead to coral bleaching, if reported concentrations are found over the reef zone itself.

**THE RECENTLY INTRODUCED BIVALVE *XENOSTROBUS SECURIS* HAS HIGHER THERMAL AND SALINITY TOLERANCE THAN THE NATIVE *BRACHIDONTES VARIABILIS* AND ESTABLISHED *MYTILOPSIS SALLEI***

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The distribution of the recorded marine non-native invertebrates in Hong Kong is practically restricted to habitats with environmental stress, such as typhoon shelters which are often affected by salinity changes and pollution. Habitats with environmental stress may provide a niche opportunity for non-native species with high stress tolerance due to a lower tolerance of local species. The recently introduced bivalve *Xenostrobus securis* and the previously introduced *Mytilopsis sallei* (~30 years) have become dominant over native bivalve *Brachidontes variabilis* in estuarine fouling communities in Hong Kong. This study aimed to test whether the non-native species have higher thermal and salinity tolerance than the native species. Survival, attachment, clearance rate and byssal thread production of the non-native and native bivalves were examined through 96-h acute tests for representative combinations of temperature and salinity conditions in Hong Kong. The results indicate that *X. securis* responds normally in a wide temperature and salinity range, which could explain its fast population growth and spread in estuarine environments in Hong Kong. Though *M. sallei* also has a wide salinity tolerance, cold seawater conditions may reduce its physiological activity in winter, resulting in a disadvantage for them to compete with *X. securis*. The narrow temperature and salinity tolerance of *B. variabilis* may explain its low abundance in habitats dominated by the non-native bivalves. The high tolerance of *X. securis* suggests that it could become highly invasive in Hong Kong, impacting potentially estuarine communities and farming of oysters.