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SIZE-DEPENDENT DELETERIOUS EFFECTS ON SPERM MOTILITY BY MICROPLASTICS OF DIFFERENT SIZES

Lei L.^{1,2,13,*}, Lin Z.^{1,2}, Li Z.², Ji S.², Lo H.S.³, Billah B.⁴, Sharmin A.⁵, Han X.⁶, Lui W.-Y.⁷, Tse W.K.F.⁸, Fang J.K.-H.^{9,10}, Shang X.^{11,*}, Lai K.P.^{12*}

¹ *University of Chinese Academy of Sciences, Beijing, China*

² *The Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China*

³ *Department of Environmental Science, Stockholm University, Stockholm, Sweden*

⁴ *Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh*

⁵ *Department of Chemistry, Bangladesh University of Engineering and Technology, Dhaka, Bangladesh*

⁶ *Core Laboratory, Shanxi Provincial People's Hospital (Fifth Hospital) of Shanxi Medical University, Taiyuan, China*

⁷ *School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR, China*

⁸ *Laboratory of Developmental Disorders and Toxicology, Center for Promotion of International Education and Research, Faculty of Agriculture, Kyushu University, Fukuoka, Japan*

⁹ *Department of Food Science and Nutrition, The Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR, China*

¹⁰ *State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China*

¹¹ *Department of Urology, Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China.*

¹² *Key Laboratory of Environmental Pollution and Integrative Omics, Guilin Medical University, Education Department of Guangxi Zhuang Autonomous Region, China*

¹³ *Guangdong Provincial Key Laboratory of Brain Connectome and Behavior, CAS Key Laboratory of Brain Connectome and Manipulation; Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen, China*

Growing concerns regarding the reproductive toxicity associated with daily-life exposure to microplastics (MP) have become increasingly prevalent. Even the presence of MP in the South China Sea and Hong Kong SAR beach has been repeatedly reported. In reality, MP exposure encompasses a heterogeneous mixture of varying sizes rather than a uniform size. In this study, an oral exposure mouse model was utilized to assess the effects of MP of three size ranges: 1 – 5 μm , 20 – 27 μm , and 125 – 150 μm . Our result showed that MP of different sizes all impaired sperm motility, with an elevated curvilinear velocity, and a decreased linearity and straight-line velocity of sperm motility. Notably, alterations did not manifest in animal locomotion, body weight, or sperm count. Linear regression analysis substantiated a negative correlation between the size of MP and sperm curvilinear activity. Immunohistochemical analyses unveiled the intrusions of only 1 – 5 μm MP into Leydig cells and testicular macrophages. Further testicular transcriptomic analysis revealed disruptions within pathways related to spermatogenesis, oxidative stress, and inflammation. Particularly within the 1 – 5 μm MP group, a heightened perturbation in pathways linked to spermatogenesis and oxidative stress was observed. Our data support the size-dependent impairment of MP on sperm functionality, underscoring the pressing need for apprehensions about and interventions against the escalation of environmental microplastic contamination. This urgency is especially pertinent to small-sized MP.

EVALUATION OF THE MICROPLASTIC INGESTION IN LARVAL FISH IN THE COASTAL WATERS OF TAIWAN: FIELD AND LABORATORY SYUDIES

Leu M.-Y.^{1,2}, Lin S.-H.², Chen T.-Y.² and Ko F.-C.^{1,2}

¹Department of Biology, National Museum of Marine Biology & Aquarium, Checheng, Pingtung, Taiwan, ROC

²Graduate Institute of Marine Biology, National Dong Hwa University, Shoufeng, Hualien, Taiwan, ROC

Microplastics (MPs) have been documented in marine environments worldwide, where they pose a potential risk to biota. Coastal shelf seas are rich in productivity but also experience high levels of MP pollution. In their early life stages, planktonic fish larvae are vulnerable to pollution, environmental stress and predation. To date, the effects of MP ingestion in fish are still unclear. Marine fish eggs and larvae is a crucial part in the recruitment, but environmental interactions between MPs and lower trophic organisms are poorly understood. Here we assessed the occurrence of MP ingestion in wild fish larvae. Fish larvae were taken across five sites (approximately 1–3 km from shore) in the coastal waters of Hengchun Peninsular, Taiwan from August 2022 to April 2023. We identified 0.62% of fish larvae (n = 159) had ingested MPs, of which were mainly fibres in shape (100%), clear in colour (100%), and 0.2–5 mm (100 %) in size. Ingestion was only observed in one species: goatfish (*Upeneus* sp., n = 1). MPs analysed by Fourier Transform Infrared Spectrometer (FT-IR) were confirmed to be polymeric, identified as polyester. Furthermore, this study used polyethylene microplastic beads (PE 100–125 µm and 250–300 µm) to conduct exposure experiments on the eggs and larvae of the golden trevally (*Gnathanodon speciosus*) and the juvenile clown anemonefish (*Amphiprion ocellaris*). Fertilized eggs were exposed from 18 hours post-fertilization until hatching, and the larvae were exposed on the 10 day post hatching (10 dph) and 20 dph. Both the larvae and juveniles were exposed for 5 days. The concentrations of MPs were 0 p/m³ (control group), 10⁴ p/m³ (1× concentration group), and 10⁵ p/m³ (10× concentration group), with a temperature control of 26 ± 0.5 °C. The experimental results showed that the hatching rate of *G. speciosus* eggs was not affected by MPs. However, in the 10× concentration group, the mortality rate of larvae significantly increased at both 10 dph and 20 dph. There were no significant differences in the growth rate of larvae among all groups, and the ingestion rate ranged from 0–13%. The mortality rate of *A. ocellaris* juveniles in the 10× concentration group was significantly higher than in the other groups. The results indicate that an increase in MP concentration may lead to higher mortality in larvae and juveniles. More than 80% of the MPs were excreted within 2 h of ingestion. Further research is needed to determine the exact causative factors. This study provides baseline ecological data illustrating the probability of MP ingestion in marine larval fish in the Taiwan seawater, which could have an adverse effect on fish health as well as marine biota.

ISOLATION AND IDENTIFICATION OF PUTATIVE EXPANDED POLYSTYRENE-DEGRADING BACTERIA FROM THE GUT OF *LIGIA CINERASCENS* (ISOPODA: LIGIIDAE)

Lee Y.M.¹, Yoo J.W.¹, Lee Y.H.¹, Park C.R.¹, Park J.S.¹, Lee H.S.¹, Cho S.H.¹ and Jung J-H^{2,3}

¹ Sangmyung University, Republic of Korea

² Korea Institute of Ocean Science and Technology, Republic of Korea

³ Korea University of Science and Technology, Republic of Korea

Expanded polystyrene (EPS) is one of the major plastics that is widely distributed in coastal areas around the world, including South Korea. Because plastics are difficult to degrade and persist in the environment for long periods, plastics pollution, including EPS, has become an important environmental concern. Previous studies have reported that some organisms, such as meal worms, and their gut microbes can biodegrade the plastics, but information on plastics degradation by gut microbiome of marine organisms are still lacking. In this study, we collected the sea slaters from highly plastic-polluted coastal region in Geoje, Korea. *Ligia cinerascens* was identified using 16S rRNA sequence, and exposed to EPS for 21 days under laboratory condition. Gut extracts were cultured for one month in a medium containing only EPS as the carbon source for the enrichment of plastic-degradable bacteria. Cultured bacteria were transferred to LB agar plate, and colonies were selected to identify using gram staining and bacterial 16S rDNA sequence. To characterize the plastic degradation of gut bacteria, their attachment on EPS film were examined using SEM. Morphological and molecular analysis of isolated microbes showed that they were gram-positive and closely matched with *Bacillus thuringiensis*., implying that this bacterium might have potential ability to degrade EPS. SEM showed that they can attached on EPS film, indicate that they can use EPS as a carbon source. Our findings suggest potential usage of *L. cinerascens* and their gut microbiome for plastics degradation.

SIZE-DEPENDENT TOXIC EFFECTS OF POLYPROPYLENE MICROPLASTICS ON REPRODUCTION, ANTIOXIDANT SYSTEM, AND METABOLOMIC PERTURBATION OF MONOGONONT ROTIFER *BRACHIONUS KOREANUS*.

Lee Y.-M.¹, Yoo J.-W.¹, Park J.-S.¹, Lee Y.-H.¹ and Jeong T.Y.²

¹ Sangmyung University, Republic of Korea

² Hankuk University of Foreign Studies, Republic of Korea

Microplastics (MPs; < 5 mm diameter) are ubiquitous pollutants in the ocean and have become a major threat to the marine environment due to their high bioaccumulation rate and toxicity on marine organisms. Small sized MPs, especially < 20- μ m, can be ingested by small marine organisms including zooplanktons, resulting in deleterious effects, and it is known that the size of MPs is the critical factor on their toxicity. Although polypropylene (PP) fragment is one of the major type of MPs in the ocean, the information of their toxicity on marine organisms are still limited. Thus, we investigated the toxic effects of two size of PP fragments (0.5- and 5- μ m) on reproduction, antioxidant system, and metabolomic modulation of monogonont rotifer *Brachionus koreanus*. The results of chronic toxicity test showed that 0.5- μ m PP significantly reduced the fecundity of F0 generation at 20 μ g/mL concentration, and the reproductive toxicity of 0.5- μ m PP was further enhanced in the following generations (F1 and F2). However, 5- μ m PP didn't induce significant effects on reproduction in all generation. After 24 h exposure of PPs, gene expression and enzyme activity of antioxidant-related enzymes were modulated depending on the size of PP. These results suggest that the 0.5- μ m PP more toxic than the 5- μ m PP, and size of MP is an important factor in their toxicity. After metabolomic analysis, 304 and 178 number of metabolites are significantly affected by 0.5- and 5- μ m PP exposure, respectively (total 360 metabolites). Enrichment analysis demonstrated that the carbohydrates, amino acids, lipids, vitamins, and drug metabolism pathway are significantly affected by PPs, and these pathways were more significantly affected by 0.5- μ m PP exposure. Our findings suggested that the PP fragments can induce reproductive disorder, oxidative stress, and metabolic disorder on *B. koreanus*, which can be enhanced as the size of the PP is smaller.

EFFECTS OF FOODS ON SIZE- AND SHAPE-DEPENDENT TOXICITY OF MICROPLASTICS ON TWO MARINE ZOOPLANKTONS: INGESTION OF PLASTICS AND ACUTE TOXICITY

Lee Y.-M., Park J.-S., Yoo J.-W., Lee Y.-H. and Park C.-R.

Sangmyung University, Republic of Korea

Microplastics (MPs) are one of the major pollutants in the ocean and can be accumulated in the digestive tract of marine organisms, resulting in adverse effects, such as interfere of feeding, and disorder of energy metabolism and reproduction. Although it is known that PP and PE fragments are the dominant type of MPs in the marine environments, information on their toxic effects on marine organisms is lack. MPs' ingestion by organisms can be affected by shape and size of MPs, and presence of foods and is important factor on toxicity of MPs. Thus, in this study, we investigated that the ingestion patterns and acute toxicity of MPs on two marine zooplanktons (rotifer *Brachionus koreanus* and brackish water flea *Diaphanosoma celebensis*) depending on the size and shape of MPs and presence or absence of foods. After MP exposure, the smaller the size of MPs, the higher the ingestion rate regardless the shape of MPs in both species. In addition, the presence of foods decrease ingestion of MPs (~ 1 mg/L), but didn't affect MPs ingestion at high concentrations of MPs (10 mg/L). Acute toxicity test showed that the fragment type of MPs was more toxic than the beads type, and the smaller their size, the stronger their toxicity. In addition, toxicity of MPs was alleviated by foods at the low concentrations of MPs. These results imply that the size and shape of MPs are the important factor in their toxicity, and presence of foods can interfere consumption of MPs competitively, reducing toxicity of MPs at low concentrations. Our findings suggest that the various factors, such as shape and size of MPs and presence of foods, should be considered for risk-assessment of MPs in marine environments.

METAGENOMIC ANALYSIS OF SEA SLATER *LIGIA* GUT MICROBIOME COLLECTED FROM THE COASTAL REGION IN SOUTH KOREA

Lee Y.M.³, Jung J-H^{1,2}, Choi K.M.¹, Mun S.H.¹, Yoo J.W.³

¹ Korea Institute of Ocean Science and Technology, Republic of Korea

² Korea University of Science and Technology, Republic of Korea

³ Sangmyung University, Republic of Korea

Plastic has been a great concern in the aquatic ecosystem due to increasing global usage and worldwide distribution by non-biodegradable properties. Expanded polystyrene (EPS) is one of the major plastics that is widely distributed in coastal areas of South Korea. Metagenomics analysis has been widely used to understand the dynamics, function, and interaction of microbial communities against environmental pollution in ecotoxicological studies. Ingested microplastic can modulate marine organisms' gut microbiome, leading to an increase in plastic-degrading bacteria. However, information on the modulation of gut microbial community by microplastic is still lacking in coastal invertebrates, Isopod. In the present study, *Ligia* collected from the clean site (Nae-do, ND) and polluted site (Maemul-do, MD) were identified using mitochondrial 16S rRNA sequence, and their accumulation of EPS was measured. In addition, the gut microbial community of *Ligia* was investigated using 16S amplicon sequencing and metagenomic analysis. Sequencing and phylogenetic analysis showed that *Ligia* collected from ND was closely related to *L. exotica*, whereas those from MD had a clade with *L. cinerascens*. EPS accumulation within the body of *Ligia* was higher in *L. cinerascens* (MD) than in *L. exotica* (ND), suggesting that *Ligia* can ingest microplastics. The total of operational taxonomic unit (OTU) of *Ligia* gut microbiome was higher in ND than in MD. Alpha diversity showed a higher richness of *Ligia* from MD, compared to those of ND. PCoA analysis revealed the distinct bacterial communities of *Ligia* gut from ND and MD. Abundant phylum commonly found at both sites was Proteobacteria (67.47%, ND; 57.30%, MD) and Bacteroidetes (13.63%, ND; 20.76%, MD) which are known to be dominant in plastic debris in marine environments and have a potential of hydrocarbon-degrading. Functional gene analysis showed that nineteen plastic degradation-related genes, such as PEG-hydrogenase, esterase, and carboxylesterase, were detected from the gut microbe of *Ligia*, indicating the role of *Ligia* gut microbiome in plastic degradation. This study provides a better understanding of the dynamic of the gut microbiome community of *Ligia* in different environmental conditions.

ENHANCING BEACH LITTER SURVEILLANCE IN HONG KONG WATERS THROUGH AERIAL DRONE MONITORING

Mou L.C., Chen J.J.*

Department of Applied Science, School of Science and Technology, Hong Kong Metropolitan University

The issue of environmental litter is a pervasive global challenge that impacts shorelines worldwide, giving rise to significant ecological concerns due to its potential impact on biodiversity and marine wildlife. This study specifically addresses this issue within the context of Hong Kong, where the western and southern waters are particularly vulnerable to the influx of marine debris, especially during the wet season, originating from the Pearl River Delta. Accurate quantification of beach litter is crucial for formulating effective marine conservation strategies.

To achieve this objective, we employed commercial aerial drones to conduct comprehensive beach litter surveys across 12 strategically chosen ecologically significant sites. These sites span from the northwestern New Territories to the southern reaches of Hong Kong Island and encompass areas classified as Coastal Protection Areas or Marine Parks/Reserves, highlighting their environmental importance and conservation value.

The study involved the assessment of site cleanliness using a systematic ranking process, providing a solid foundation for subsequent actions in marine conservation and litter mitigation. The methodology developed through this research offers a practical and reliable approach to beach litter monitoring, ready to be disseminated to interested public or organizational entities.

The insights gained from this study contribute to a deeper understanding of seasonal beach litter variations within the Hong Kong region, facilitating the development of waste management strategies, ecological conservation efforts, and the preservation of marine environments. By shedding light on the extent and nature of beach litter, this research supports informed decision-making and proactive measures to address this pressing environmental issue.

ESTABLISHING BASELINES AND ASSESSING RISK: DOCUMENTING THE COMPOSITION OF MICROPLASTICS IN INDIAN OCEAN PYGMY BLUE WHALE FAECAL SAMPLES

Liu M.¹, Yang K.¹, Brannan N.², Liu H.M.¹, Zhang K.¹, Porter L.^{2*}, Cai M.³, Yan M.^{1*}, Kudavidanage E.P.⁴, Leung K.M.Y.^{1,*}

¹ State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong, China

² Southeast Asia Marine Mammal Research, Hong Kong, China

³ State Key Laboratory of Marine Environmental Sciences, Xiamen University, Xiamen, China

⁴ Department of Natural Resources, Sabaragamuwa University, Sri Lanka

Microplastics (MPs), an emerging contaminant in the marine environment, have attracted widespread attention due to their ubiquitous occurrence and harmful effects, however, few data have been recorded on the quantity and composition of MPs ingested by baleen whales. In this study, we analysed microplastics from faecal samples obtained from pygmy blue whales (*Balaenoptera musculus brevicauda*) and seawater samples collected from the northern Indian Ocean. A total of 45 MP were detected in the faecal matter, with an average abundance of 0.82 items/g dry weight. Six polymer types were identified, using Fourier-transform infrared spectroscopy (FTIR), nearly half of which were polyethylene terephthalate (PET). The variety of shapes, colours, and polymer types of MP in blue whale faecal samples indicates the wide range of MP that are available within the marine environment that are being ingested. For pygmy blue whales, MP ingestion exposure is estimated according to typical filter-feeding rates estimated for this sub-species. This study provides essential baseline information on the potential volume of MP loads in pygmy blue whales and considers what adverse impacts of long-term exposure to microplastics.

COMPARISONS BETWEEN THE INGESTION AND REJECTION OF MICROPLASTIC BEADS BY BURROWING CLAMS, *MERETRIX MERETRIX* AND *PAPHIA UNDULATA*

Xu X.Y.^{1,2}, Wong L.Y.¹, Cheung S.G.^{1,2}

¹ Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

² The State Key Laboratory of Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

Microplastics (MPs) contamination has become one of the major environmental concerns, and monitoring MP pollution in marine ecosystems is essential for proper risk assessments. Bivalves, such as mussels and clams, are proposed as bioindicators for MP contamination in the marine environment. To test their potential as bioindicators for MPs, two clam species, namely *Meretrix meretrix* and *Paphia undulata*, were offered two sizes (small: 45-53 μm , and large: 106-125 μm) of fluorescent red polyethylene microbeads, and the ingestion (number of MPs in body tissue and faeces) and rejection (number of MPs in pseudofaeces) of MPs investigated. Overall, MP beads ingested were 36% more than those rejected (Two-way mixed model ANOVA, $p = 0.004$). There was also a significant interaction between the size and fate of MPs ($p = 0.003$). For both species, significantly more small beads were ingested than rejected ($p < 0.001$), but there was no difference for the large beads. The uptake of MPs by *P. undulata* was significantly more than that by *M. meretrix* ($p = 0.001$), which can be attributed to a higher filtration rate of *P. undulata*. As these two burrowing species tend to ingest MPs but not reject them, they have potential to be good bioindicators for MP pollution, which can be further confirmed by studies on the retention time of MPs in these clams.

EFFECTS OF SURGICAL MASKS WITH DIFFERENT WEATHERING CONDITIONS ON BEHAVIOR AND GROWTH OF WHITELEG SHRIMP (*LITOPENAEUS VANNAMEI*)

Chen T.H.^{1,2}, Lo Y.H.¹ and Chen M.C.²

¹ Graduate Institute of Marine Biology, National Dong Hwa University, Taiwan

² National Museum of Marine Biology and Aquarium, Taiwan

The COVID-19 pandemic has led to a surge in the global demand for surgical masks. This increase, coupled with the fact that these masks can release a significant amount of microplastics, has raised concerns about a new source of plastic pollution in the ocean. Discarded masks often end up in coastal areas, undergoing weathering and microbial attachment, eventually settling on the seabed and potentially impacting benthic marine life. This study focuses on whiteleg shrimp (*Litopenaeus vannamei*), a benthic species native to coastal areas and known to ingest microfibers. We exposed shrimp to new surgical masks, sun-exposed surgical masks, and seawater-soaked surgical masks, studying shrimp-mask interactions and the effects of chronic exposure on shrimp behavior and growth. Our findings indicate that seawater-soaked masks and sun-exposed masks significantly affected shrimp behavior. However, we observed no significant differences in microplastic bioaccumulation or shrimp growth after a 28-day chronic exposure. These results highlight the sensitivity of shrimp behavior to microplastic exposure.

DAILY TIDAL INFLUENCE ON MICROPLASTIC DISTRIBUTION IN TYPICAL TIDAL RIVER

Liu H.M., Cheung S.G.

City University of Hong Kong, China

Due to the influence of tidal movement and river flow, the distribution of MPs in tidal rivers undergoes large spatial and temporal variations. Shing Mun River (SM) is one of the largest tidal rivers located in the eastern part of Hong Kong. Four channelized tributaries feed the main river which flows into Tolo Harbour. It is estimated that around 17 million pieces of plastic debris is discharged from SM into Tolo Harbour every year. With an average flow rate of 0.012 m³/s to 0.192 m³/s, the flow direction and velocity of SM were controlled by the tidal movement in the Tolo Harbour, causing MPs to either 'stay' or 'leave' the river.

The influence of tidal movement on the distribution and composition of MPs was studied at different phases of tidal cycles at the upper (SM2) and lower courses (SM6) of SM. No significant differences were observed. Generally, more MPs were observed in the lower courses during flood tide, and more MPs was observed in the upper course during ebb tide. MPs flowed direction was same as tidal movement and no spatial differences were observed indicating that MPs 'stay' in the tidal river.

PFAS ABSORPTION ON THE MICROPLASTIC PARTICLES IN THE TOLO HARBOR REGION

Liu H.M., Ruan Y., Cheung S.G.

City University of Hong Kong, China

MPs were found heavily polluted to the Hong Kong aquatic environment. Rivers were one of the major carriers to transport MPs to the ocean. Shing Mun and Lam Tsuen River was located in the eastern part of Hong Kong and ether Tolo Harbor finally. They were found with high magnitude of MPs pollution. In the recent decade, MPs were also reported to carry different organic or inorganic pollutants, such as heavy metals, bisphenol a, PAHs, and PCBs, etc.

Per- and polyfluoroalkyl substances (PFASs) was one of the emerging pollutants. PFASs was found to be very persistent and bioaccumulated in the biota. They are widely used in water-resistant products and cookware in our daily life. Due to the toxicity, PFOS were banned to use in 2008.

Tolo Harbor was one of the water quality controlled region in Hong Kong monitored monthly by the Environmental Protection Department. In 2015, an intensive survey has been conducted to investigate the PFASs concentration Hong Kong surface seawater. PFOA (~26%), PFOS (~20%), and PFBA (~21%), were detected predominantly. Tolo Region is the shelter far away from the open sea. Seawater and pollutants were hardly pumped out. Higher magnitude of PFASs concentration was expected. MPs flushed from two major rivers might sink in the region, and finally flushed to the costal shore. Thus, we hypothesis the absorption of PFASs on the MPs found in the region.

In this study, we sampled MPs and sediment in the sandy beaches at Tolo Region from Wu Kai Sha (WKS), Sam Mun Tsai (SMT), Lai Chi Chong (LCC), and Tap Mun (TM). Extraction on PFASs was performed for the environment sample. Different polymer pellets, including PE, PP, and PS were also immersed in the field and laboratory to study the absorption effect.

The \sum PFASs mean concentration found in sediment samples were 3.674 mg/g (SMT), 2.786 mg/g (TM), 1.248 mg/g (WKS), and 0.202 mg/g (LCC), respectively. For the plastic samples, the \sum PFASs mean concentration were 51.680 mg/g (WKS), 44.228 mg/g (SMT), 20.728 mg/g (LCC), and 1.449 mg/g (TM), respectively.

Field samples showed that PE absorbed more \sum PFASs than PS and PP in most of the locations, while PS showed a higher absorption in the laboratory experiment.

THE INFLUENCE OF RAINFALL EVENTS ON MICROPLASTIC POLLUTION IN TIDAL AND NON-TIDAL RIVERS IN HONG KONG

Liu H.M., Cheung S.G.

City University of Hong Kong, China

MPs are pervasive in aquatic environments, including rivers, estuaries, lakes and open seas. Hong Kong is a hotspot for microplastics (MPs) pollution. However, there are very few studies on microplastic pollution in Hong Kong rivers, not to mention that many of them are located in urban areas and influenced by tidal movement. The microplastics distribution and abundance in two tidal (Shing Mun River (SM), Tuen Mun River (TM)) and two non-tidal rivers (Lam Tsuen River (LT), Silver River (SR)) were investigated in dry and wet seasons.

In the dry season, TM had significantly higher suspected MPs abundance than SM, LT, and SR. In the wet season, the two tidal rivers, SM (44.78 ± 43.36 items/L), and TM (29.23 ± 18.56 items/L) had significantly higher MPs abundance than the non-tidal river SR (12.46 ± 8.70 items/L) (Tukey test, $p < 0.05$). A higher MPs abundance was recorded downstream of all the rivers. Rainfall transported MPs from both the land and storm drains to the rivers, causing a higher pollution loading than in the dry season. The built-area ratio was positively correlated with the MPs abundance in both the dry ($R^2 = 0.6600$, p -value < 0.05) and wet seasons ($R^2 = 0.2699$, p -value < 0.05), but its effect decreased during the wet season because of the influence of the rainfall.

INSIGHTS INTO TISSUE-SPECIFIC BIOACCUMULATION OF NANOPLASTICS IN MARINE MEDAKA AS REVEALED BY A STABLE CARBON ISOTOPIC APPROACH

Yeo I.-C.¹, Shim K.-Y.¹, Kim K.¹, Lee J.-S.², Shin K.-H.³, Jeong C.-B.^{1*}

¹ Department of Marine Science, Incheon National University, Incheon 22012, South Korea

² Department of Biological Sciences, Sungkyunkwan University, Suwon 16419, South Korea

³ Department of Marine Sciences and Convergent Technology, Hanyang University ERICA Campus, Ansan 15588, South Korea

Despite the high bioavailability and potentially extensive presence of nanoplastics in aquatic environments, the biological fate of nanoplastics is largely unknown because of analytical limitations in detection and quantification. Fluorescently labeled nanoplastics are widely used to detect bioaccumulation, but this method is prone to false-positive results due to the leaching of fluorescent dyes. Here we propose a novel stable carbon isotopic approach to detect and quantify nano- and microplastics in a complex organic matrix. Because carbon is the major component of plastics (>87% in polystyrene), it is possible to investigate tissue-specific bioaccumulation of nano- and microplastics in the medaka *Oryzias melastigma* by quantifying the contribution of plastic particles as an end-member in the composition of stable carbon isotopes in different tissues. In addition to the digestive organs (e.g., the gut and intestines) that are constantly exposed to the water column via ingestion, nanoplastics were shown to selectively bioaccumulate in the gills and ovary, implying a unique mode of action of bioaccumulation based on the physicochemical properties of the nanoparticles. These findings should improve our understanding of the tissue-specific bioaccumulation of nano- and microplastics in aquatic organisms.

BIOACCUMULATION AND TOXICITY OF NANOPLASTICS TO FISH MEDAKA UNDER THE CONTEXT OF GLOBAL WARMING

Gao S., Zheng S., and Cai W.

State Key Laboratory of Marine Pollution and Department of Infectious Disease of Public Health, City University of Hong Kong, Hong Kong SAR, China

Nanoplastics (NPs) pollution in the marine environment under global climate change has become a growing concern and significantly impacted marine ecosystems. However, study on NPs bioaccumulation and toxicity to marine fish under elevated temperatures is not well understood. In this study, we investigated the primary route of absorption for polystyrene NP (PS-NP) in marine fish medaka, and evaluated its bioaccumulation and cytotoxicity under elevated temperature using histological, transcriptomic and biochemical approaches in vivo and in vitro. Our result indicated that the digestive tract was the dominant PS-NPs bioaccumulated organ, which exhibited an initial increase followed by a decreasing trend with time. Meanwhile, the internalized patterns and susceptibility to viral pathogens were evaluated in vitro using tilapia brain cell line with a flow cytometer and confocal imaging. Our findings provided insight into the cellular transportation mechanism of NPs and the potential toxicity of NPs on marine fish under the challenges of elevated temperature.

EFFECT OF MARINE DEBRIS ON THE INTERTIDAL BENTHIC ASSEMBLAGES ON THE SOUTHERN COAST OF KOREA

Kim T.L.¹, Kang T.W.², Kim H.¹, Kim H.G.^{1*}

¹ *Department of Marine Environmental Sciences, Chungnam National University, Daejeon, Republic of Korea*

² *Korea National Park Research Institute*

We aimed to investigate the effect of marine debris on intertidal macro-meiofauna assemblages at the garbage-dense area of Tongyeong, Gyeongsangnam-do, South Korea. Abiotic and biotic samples (macro and meiofauna) were collected before and after the removal of marine debris in various types of intertidal areas, including rocky, sandy, and mud flats, on a monthly basis from May to September. Additionally, a control area with relatively low marine debris impact was selected to compare the effects of marine debris on benthic fauna assemblages. As a result, the density and taxa richness of meiobenthos were significantly different between before and after removal of marine debris at all type of habitats. Nematoda were the most dominant taxa at all type of habitats. After the removal of marine debris, the density and taxon richness of meiofaunal assemblages increased to levels similar to those of the control group in both mudflat and rocky shore whereas a decrease in both density and taxa richness was observed at the sandy shore. The sediment from marine debris area exhibited higher concentrations of heavy metals and PAHs compared to the control group. Furthermore, a greater number of instances exceeding the threshold effect limit for heavy metals were observed in the marine debris area. Over time, a gradual decrease in the concentrations of heavy metals and PAHs was noted. The findings of this study can provide essential data for future methodologies and ecological assessments related to the marine debris.

ABUNDANCE AND DISTRIBUTION OF MICROPLASTICS IN THE COASTAL ENVIRONMENTS OF MACAO: ANALYSIS OF MANGROVE AND NON-MANGROVE AREAS

Saidu M. B. and Tagulao K. A.

Institute of Science and Environment University of St. Joseph, Macao SAR

Microplastics are small plastic particles less than 5 mm in size and are a growing environmental concern. This study investigated the prevalence and spatial distribution of microplastics in the unique ecosystems of mangrove and non-mangrove areas in Macao. The presence of microplastics in these habitats can be attributed to various human activities, including the disposal of household waste, daily product usage, and fishing practices.

The study highlights the role of mangrove vegetation in plastic entrapment, which helps reduce the abundance of microplastics in the environment. To investigate this, we conducted comprehensive sediment and water sampling in both mangrove and non-mangrove coastal sites in Macao. Our sample analysis involved sedimentation, digestion, and filtration procedures for effective microplastic extraction. Our findings revealed that mangrove areas exhibited a significantly higher abundance of microplastics in both sediments and water, with a prevalence of 59.67% and 56.09%, respectively, compared to non-mangrove areas. Notably, within the mangrove environment, samples from a site closest to a village displayed a remarkable 14.37% and 71.20% increase in microplastic content in the sediments and water, respectively, compared to the combined quantities from other sites. Moreover, our study identified a gradient increase in microplastic concentrations along the coastal shorelines.

These results underscore the urgent need to address microplastic pollution as a substantial issue in mangrove ecosystems, calling for immediate attention and conservation efforts.

INGESTION OF MICROPLASTICS IN BARNACLE AMPHIBALANUS AMPHITRITE VARIED WITH CONCENTRATION, SIZE AND FORM OF MICROPLASTICS BUT NOT THE PRESENCE OF BIOFILM

Wang Y.¹, Cheung S.-G.^{1,2*}

¹Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

²The State Key Laboratory of Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

More and more studies have demonstrated the impact of MP ingestion on marine organisms. Microbes rapidly colonize MPs when they are released to the ocean. The biofilm developed on MPs changes the MPs' properties and confuses consumers, such as filter-feeders, with some of which preferentially selecting MPs with biofilm for ingestion. Filter feeders such as barnacles and other bivalves have been suggested as potential bioindicators of MP pollution. Unlike bivalves, barnacles are considered as non-selective feeders without particle sorting capability. The striped barnacle *Amphibalanus amphitrite* was offered with two sizes (27-32 μm and 90-106 μm), forms (microspheres and microfibers) and types (polyethylene, polystyrene and polyester) of MPs at three concentrations (100 P/ml, 1000 P/ml and 10000 P/ml). These MPs were incubated in the sea for 0, 3 or 14 days for biofilm development. The presence of the biofilm did not affect the ingestion of MPs but the ingestion rate varied with the interaction between the concentration and form of MPs. The results indicate that the shape and concentration of MPs determine the number of MPs ingested but no preferential ingestion of MPs with biofilm was observed. The results are discussed with respect to the potential of barnacles being bioindicators of MPs.

MICROPLASTICS FROM FACE MASK IMPAIRS SPERM MOTILITY

Lin Z.^{1,2}, Li Z.², Ji S.², Lo H.S.³, Billah B.⁴, Sharmin A.⁵, Lui W.-Y.⁶, Tse W.K.F.⁷, Fang J.K.-H.^{8,9}, Lai K.P.^{10,*}, Li L.^{1,2,11,*}

¹ *University of Chinese Academy of Sciences, Beijing, China*

² *The Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China*

³ *Department of Environmental Science, Stockholm University, Stockholm, Sweden*

⁴ *Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh*

⁵ *Department of Chemistry, Bangladesh University of Engineering and Technology, Dhaka, Bangladesh*

⁶ *School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR, China*

⁷ *Laboratory of Developmental Disorders and Toxicology, Center for Promotion of International Education and Research, Faculty of Agriculture, Kyushu University, Fukuoka, Japan*

⁸ *Department of Food Science and Nutrition, The Hong Kong Polytechnic University, Hung Hom, Hong Kong SAR, China*

⁹ *State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China*

¹⁰ *Key Laboratory of Environmental Pollution and Integrative Omics, Guilin Medical University, Education Department of Guangxi Zhuang Autonomous Region, China*

¹¹ *Guangdong Provincial Key Laboratory of Brain Connectome and Behavior, CAS Key Laboratory of Brain Connectome and Manipulation; Shenzhen-Hong Kong Institute of Brain Science-Shenzhen Fundamental Research Institutions, Shenzhen, China*

The COVID-19 pandemic has resulted in unprecedented plastic pollution from single-used personal protective equipment (PPE), especially face masks. The increased use of single-use plastics PPE has resulted in a rise in plastic waste in coastal and marine environments. Unfortunately, improper disposal has led to the presence of PPE in beaches and oceans worldwide, posing significant environmental risks and potential threats to marine organisms, terrestrial organisms and even mammals like human. The secondary pollutants – the microfibers of microplastics from face masks (mask MP) heighten the rising concern about their detrimental effects on human health. The degradation of face masks results in the generation of microfibers spanning a spectrum of sizes. Within the scope of this investigation, the constituents comprising mask MP were subjected to characterization utilizing FTIR spectroscopy, wet-sieving size analysis, and scanning electron microscopy. Using a mouse model, oral exposure to mask MP at two doses, 0.1 and 1 mg MP/day per mouse, caused no change in animal locomotion, body weight, or sperm counts, but significant damage in sperm motility with increased curvilinear velocity (VCL). The high-dose mask MP exposure caused a significant decrease in the linearity (LIN) of sperm motility. Further testicular transcriptomic analysis showed perturbed molecular pathways related to spermatogenesis, oxidative stress, inflammation, metabolism and energy production after mask MP exposure. Collectively, our findings substantiate that these secondary pollutants—microplastics emanating from face masks—yield adverse effects on mammalian reproductive capacity. Better management of single-use plastic waste is required, and innovative technologies and more environmentally friendly materials should be developed.

ECOTOXICOLOGICAL ASSESSMENT OF BIODEGRADABLE PLASTICS: EFFECTS ON SKELETONEMA COSTATUM AND VIBRIO FISCHERI

Eunji Jung¹, Jeong-In Park^{1,2}, Won Bae Joh^{1,2}, Jun Kim¹, Mira Park² and Youn-Jung Kim^{1,2,*}

¹Department of Marine Science, Incheon National University, Incheon, 22012, Republic of Korea

²Research Institute of Basic Sciences, Incheon National University, Incheon, 22012, Republic of Korea

The deployment of plastic fishing gear in oceanic environments leads to the production of macro and microplastics, which in turn result in significant detrimental effects on marine ecosystems. The phenomenon of ghost fishing caused by various plastic gear has stimulated the development of biodegradable plastic gear. In this study, the ecotoxicological impacts of biodegradable plastics were evaluated using microalgae *Skeletonema costatum* and luminescent bacteria *Vibrio fischeri*. The toxicity of biodegradable plastics was evaluated by exposing the biodegradable plastic particles ($\leq 500 \mu\text{m}$) and seawater with decomposed biodegradable plastic to *S. costatum* and *V. fischeri*. The exposure durations for *S. costatum* and *V. fischeri* were 72 hours and 30 minutes, respectively. The study revealed that the type and shape of biodegradable plastics can have different effects on the two test species. However, considering the correlation between smaller microplastic size and toxicity to freshwater algae as outlined in recent research papers [1], additional experiments factoring in various plastic sizes are essential. And it is crucial to consider the presence of antioxidants and plasticizers added during plastic processing. Therefore, additional research is required to explore the potential hazards that various plastic sizes, antioxidants, and additives such as plasticizers may pose to marine organism.

NORFLUOXETINE, A NEW EDC (ENDOCRINE-DISRUPTING CHEMICAL) IN THE MARINE ENVIRONMENT, DISRUPTS THE REPRODUCTIVE PHYSIOLOGY OF BALTIC MUSSELS

Hallmann A.¹, Goździk P.¹, Leszczyńska D.¹, Roszkowska A.², Smolarz K.³, Sokołowski A.³, Caban M.⁴

¹Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdansk, Poland

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Gdansk, Gdansk, Poland

³Department of Functioning of Marine Ecosystems, Faculty of Oceanography and Geography, University of Gdansk, Gdynia, Poland

⁴Department of Environmental Analysis, Faculty of Chemistry, University of Gdansk, Gdansk, Poland

Antidepressants are introduced into the environment due to anthropogenic activities, and are driven by factors such as population growth, increased drug consumption, and the limited efficacy of their removal in purification processes. One notable antidepressant, fluoxetine, is a potent psychotropic drug that functions as a selective serotonin reuptake inhibitor (SSRI). In vertebrates, the demethylation of fluoxetine leads to formation of norfluoxetine (NFLU), an active metabolite with more potent inhibitory effects on selective serotonin reuptake. SSRIs persist in the environment due to continuous discharge from wastewater treatment plants, showing minimal biological degradation during sewage treatment. Antidepressants have been detected in various water sources, including drinking water, municipal reservoirs, and both fresh and marine waters. These compounds have the potential to accumulate in sediments, and SSRIs and their metabolites are considered emerging organic contaminants that impact non-target species in the marine environment. Marine organisms like mussels, which are commercially harvested as a food source, are consistently exposed to pharmaceuticals in their habitats.

The main aim of this study was to assess the effect of NFLU on the reproductive physiology of marine bivalves. Under laboratory-controlled conditions a model species *Mytilus trossulus* from the southern Baltic Sea was exposed to 500 ng/L NFLU over six days.

Results from the study revealed significant physiological responses in mussels exposed to NFLU. Serotonin levels decreased in the haemolymph and male gonads while increased in female gonads. Similar patterns were observed for the level of estrogen with a decrease in the concentration of 17 β -estradiol in males and an increase in females. However, there was no statistically significant increase in testosterone levels in either sex during 6-day exposure to NFLU. Interestingly, no significant differences in aromatase and monoamine oxidase activity were found in NFLU-exposed females and males.

These findings underscore the potential risks associated with NFLU exposure for consumers of mussels and shed light on the complex physiological effects of NFLU on bivalves, especially on the neurotransmitters' and hormones' systems. Additionally, these results suggest that norfluoxetine can be categorized among endocrine-disrupting compounds (EDCs).

CONTAMINATION OF LEGACY AND ALTERNATIVE PLASTICIZERS IN SEDIMENTS FROM ARTIFICIAL LAKES AND COASTAL WATERS NEAR HIGH-TECH INDUSTRIAL COMPLEXES: OCCURRENCE, DILUTION EFFECT, AND ECOLOGICAL RISK

Moon H.-B., Oh J.-S., Kim Y., Mok S., and Lim J.-E.

Department of Marine Science and Convergence Engineering, College of Science and Convergence Technology, Hanyang University, Republic of Korea

Phthalates have been used as conventional plasticizers in diverse industrial and consumer products since the 1950s. High-tech industries to produce automobile, semi-conductors, and pharmaceuticals need plastic components in their devices and products. Considering lipophilicity of plasticizers, sediment is acted as reservoirs and secondary sources in aquatic environment after migrating from sources. Artificial lakes (Lakes Asan, Sapgyo, Hwaseong, and Namyang) and Asan Bay, which connected by sea dike, are surrounded by about 4000 industries including traditional and high-tech industries. Despite increasing demand of alternative plasticizers (APs), few studies have been conducted on the occurrence and distribution of APs in the boundary between freshwater and saltwater environments. In this study, 14 legacy phthalates (LPs) and seven APs were measured in 48 sediments in an artificial four lakes and a bay, located on the western coast of Korea. LPs and APs were detectable in all freshwater and coastal sediment, indicating ubiquitous contamination in the aquatic environment. Sedimentary concentrations of Σ LP and Σ AP in the lakes were 1~3 orders of magnitude higher than those found in the bay ($p < 0.01$). This suggests that proximity to industrial complexes is crucial for the plasticizer contamination in the aquatic environment. Sediments from Lake Asan showed the higher concentrations of plasticizers. The lake is located near the largest industrial complexes and WWTP, confirming industrial activities as potential sources of plasticizers. Plasticizer concentrations in sediment were dependent on the increased organic carbon (OC) contents and finer grain size ($p < 0.01$). DEHP, DiNP, DiDP, and DEHTP were dominant in all sediments and their contributions accounted for > 90% of total plasticizers. Concentration ratio of Σ LP and Σ AP was not significantly different for sediments between lakes and bay. However, the compositional profiles of plasticizers varied between lakes and bay. We calculated the inventories of plasticizers in sediment to understand mobility of these contaminants from freshwater to saltwater environment due to environmental processes and dilution effect. Based on the calculated inventories of plasticizers, LPs (7.7) exhibited significantly higher dilution ratio than those found for APs (2.2). Dilution ratio of plasticizers in sediment ranged from 0.68 for DINCH to 14 for DEP, dependant on molecular weight and hydrophobicity (expressed as log K_{ow}). Our findings suggest that alternative plasticizers have limited mobility from terrestrial sources and preferentially are degraded under environmental processes. Sedimentary DEHP concentrations in lake sediments exhibited potentials for ecotoxicological risks to benthic organisms through comparison of several sediment quality guidelines.

IN VITRO IMPACTS OF BISPHENOL A ON IMMUNE FUNCTIONS OF PRIMARY CULTURED HEMOCYTE OF PACIFIC ABALONE (*HALIOTIS DISCUS HANNAI*)

Kim J.-H., Choi K.-S., Hong H.-K.

Department of Marine Life Science (BK21 FOUR) and Marine Science Institute, Jeju National University, Jeju 63142, Republic of Korea

Bisphenol A (BPA), 2,2-bis (4-Hydroxyphenyl) Propane, is one of the ubiquitous pollutants detected in coastal environments, posing great potential threats to marine species, especially mollusks. In this study, we investigated the toxic effects of BPA on Pacific abalone (*Haliotis discus hannai*) using *in vitro* assays with primary cultured hemocytes. Hemocytes were exposed to a wide range of BPA concentrations (up to 100 μ M) to assess cytotoxicity. Subsequently, hemocytes were exposed to sublethal concentrations (LC20=2.3 μ M and LC50=5.8 μ M) of BPA for 48 hours, and we evaluated cellular immune responses, including phagocytosis, oxidative capacities, and nitric oxide (NO) production of hemocytes using flow cytometry. Flow cytometry revealed that the hemocyte parameters did not differ between LC20 and the control. However, hemocytes exposed to LC50 of BPA exhibited significantly ($P<0.05$) reduced phagocytosis and oxidative capacities, along with significantly ($P<0.05$) increased NO production compared to control hemocytes. These findings suggest that BPA exposure may negatively impact the immune system of Pacific abalones, making them more susceptible to infections and other stressors in their natural environment. This study also suggests that *in vitro* assays with primary cultured abalone hemocytes could be used as a tool to quantify the cytotoxicity of chemical pollutants.

PRELIMINARY RESULTS FOR THE TOXICITY OF ARTIFICIAL TURF INFILL MATERIALS TO MARINE SPECIES

Wang Y.¹, Xu X.Y.^{1,2}, Liu Q.-H.^{1,3}, Xu S.-P.^{1,2}, Yan M.^{1,2}, Leung K.M.Y.^{1,2}

¹ *The State Key Laboratory of Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*

² *Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*

³ *School of Chemistry and Materials Science, University of Science and Technology of China, Hefei, Anhui, China*

The occurrence of artificial turf infill materials made from recycled tire crumbs (RTC) in the coastal marine environment have attracted increasing environmental concerns recently. For instance, a large amount of tire crumbs was reported on a beach at Lantau Island, Hong Kong. Although toxicity data of leaching solutions from RTC and /or RTC particles to many freshwater species has been documented in scientific literature, their toxicity to marine species remains largely unknown. This study, therefore, aims to investigate the acute toxicity of artificial turf infill materials made from the RTC to selected marine species, namely copepod, microalgal species, and marine medaka fish. Infill materials from three constructors were milled into small particles to prepare for the leaching solution. The marine species were exposed to not only the leaching solution but also a solution containing RTC particles, in order to assess the potential toxicity effect from particles as well. Preliminary results indicated that the RTC of two constructors did not cause severe mortality (<20 %) to the copepod *Tigriopus japonicus* at a concentration of 20 g particles/L. However, the leaching solution of RTC from the third constructor caused mortality in the adult copepod (LC₅₀ = 866.3 mg particles/L). The addition of RTC particles to the leaching solutions further enhance the toxicity to the adult copepod (LC₅₀ = 189.7 mg particles/L). However, further studies on other marine species are needed to comprehensively verify the toxicity effect of the particles.

RETROSPECTIVE IDENTIFICATION OF HYDROPHOBIC ORGANIC COMPOUNDS IN SUSPENDED PARTICLES OF FLOWBACK AND PRODUCED WATERS USING GAS CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY

Lin H. and He Y.

City University of Hong Kong, China

The chemical profiles in the suspended particles of hydraulic fracturing flowback and produced water (HF-FPW) are poorly understood but have important implications for their environmental fate and ecotoxicity. Here, nontarget screening using gas chromatography-tandem high-resolution mass was combined with toxicity predictor to identify and prioritize the hydrophobic organic compounds present in the suspended particulate phase of HF-FPW collected from China and Canada. Based on the nontarget screening workflow and filtering criteria, we putatively identified 420 compounds associated with different confidence levels. Based on the ClassyFire chemical taxonomy system, these compounds were primarily categorized into benzenoids, hydrocarbons, lipids and lipid-like molecules, organic oxygen, and organic nitrogen compounds. We further obtained the functional use information from several public sources, and the putatively identified compounds can be assigned into several functional categories, including fragrance, antioxidant, surfactant, plasticizer or processing aids, and catalyst. A prioritization ranking scheme was used to evaluate the ecological risks of the identified compounds, and 5 of them were identified as priority compounds based on their ecotoxicity risks. We also found that the chemical composition varied greatly across different wells, which can be ascribed to the geological strata and chemicals employed during the HF process. This study advanced our understanding on the environmental fate and ecotoxicity risks of organic contaminants present in the FPW samples and further facilitate the development or implementation of effective treatment strategies for the HF associated wastewater.

LIQUID CRYSTAL MONOMERS IN VENTILATION AND AIR CONDITIONING DUST: INDOOR CHARACTERISTICS, SOURCES ANALYSIS AND TOXICITY ASSESSMENT

Jin Q., Fan Y., Lu Y., Zhan Y., Sun J., Tao D., He Y.*

School of Energy and Environment and State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong, China

Indoor dust contaminated with liquid crystal monomers (LCMs) released from various commercial liquid crystal display (LCD) screens may pose environmental health risks to humans. This study aimed to investigate the occurrence of 64 LCMs in ventilation and air conditioning filters (VACF) dust, characterize their composition profiles, potential sources, and associations with indoor characteristics, and assess their *in vitro* toxicity using the human lung bronchial epithelial cells (BEAS-2B). A total of 31 LCMs with concentrations (Σ LCMs) ranging from 43.7 ng/g to 448 ng/g were detected in the collected VACF dust. Additional analysis revealed the potential interactions between indoor environmental conditions and human exposure risks associated with the detected LCMs in VACF dust. The service area and working time of the ventilation and air conditioning system, and the number of indoor LCD screens were positively correlated with the fluorinated Σ LCMs in VACF dust ($r=0.355\sim 0.511$, $p < 0.05$), while the associations with the non-fluorinated Σ LCMs were not found ($p > 0.05$), suggesting different environmental behavior and fates of fluorinated and non-fluorinated LCMs in the indoor environment. Four main indoor sources of LCMs (i.e., computer (37.1%), television (28.3%), Brand A smartphone (21.2%) and Brand S smartphone (13.4%)) were identified by positive matrix factorization-multiple linear regression (PMF-MLR). Exposure to 14 relatively frequently detected LCMs, individually and in the mixture, induced significant oxidative stress in BEAS-2B cells, where LCMs with higher BCF values and lower molar volume values wield greater influence over their toxic effects on organisms. Among them, non-fluorinated LCMs, specifically 3cH2B and MeP3bcH, caused dominant decreased cell viability. This study provides new insights into the indoor LCMs pollution and the associated potential health risks due to the daily use of electronic devices.

DISCHARGE OF ANTIBIOTIC RESISTANCE GENES (ARGS) FROM WASTEWATER TREATMENT PLANTS

Mao X., Zhang T.*

The University of Hong Kong, China

Antibiotic resistance genes (ARGs) discharged from wastewater treatment plants (WWTPs) have a significant effect on the downstream receiving environment. In this study, the antibiotic resistome of effluent from 14 WWTPs in Hong Kong was investigated using metagenomics. Results revealed that multidrug, β -lactam, bacitracin, tetracycline, and aminoglycoside were the predominant ARG types in the HK effluents. There was high spatial variation in the total abundance of ARGs, ranging from 0.24 to 2.74 copies per cell. Chemically enhanced primary treatment (CEPT) process WWTPs discharged a greater diversity and prevalence of ARGs than secondary treatment WWTPs. The resistome structure in Hong Kong effluents was similar to that of Singapore, Switzerland, and the United States. The results of this study highlight the need for monitoring ARGs in WWTP effluents, as well as the potential risks associated with reusing water.

PROMISING POTENTIAL OF THE SCYPHOZOAN JELLYFISH AURELIA AURITA AS A NEW ECOTOXICOLOGICAL MODEL

Wang Y.¹, Xu X.-Y.^{1,2}, Liu Q.-H.^{1,3}, Xu S.-P.^{1,2}, Yan M.^{1,2*}, Leung K.M.Y.^{1,2*}

¹ *The State Key Laboratory of Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*

² *Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*

³ *School of Chemistry and Materials Science, University of Science and Technology of China*

The purpose of this work was to conduct a preliminary investigation into the feasibility of using the common moon jellyfish ephyrae, *Aurelia aurita* (Linnaeus, 1758), as a new model organism in marine ecotoxicology. A series of laboratory experiments were conducted to establish a standardized testing protocol and assess the impact of various emerging chemicals from marine environments on both the individual level (mortality rate) and the molecular level (transcriptomic analysis). The organisms were exposed to two commonly found hazardous chemicals, N¹-(4-Methylpentan-2-yl)-N⁴-phenylbenzene-1,4-diamine (6PPD) and several PFASs, including hexafluoropropylene oxide-dimer acid (HFPO-DA), bis(trifluoromethylsulfonyl)imide (NTF2), and p-perfluorous nonenoxybenzene sulfonate (OBS), to observe their acute reactions under static exposure conditions. Among these pollutants, 6PPD showed potent toxicity with an LC₅₀ of 1.446 mg/L within 24 h, while OBS also demonstrated significant lethal effects with an LC₅₀ of 4.009 mg/L within 48 h. Additionally, we tentatively investigated the bioconcentration of toxic substances in jellyfish through a food chain. The findings of this study suggest that the *A. aurita* ephyrae are promising model animals for ecotoxicological research.

EFFECTS OF PHARMACEUTICAL ANTIBIOTIC MACROLIDE CLARITHROMYCIN ON COSMOPOLITAN BENTHIC DINOFLAGELLATE AMPHIDIINIUM CARTERAE (GENOTYPE 2)

Lam V.T.T.¹, Chen W.¹, Wu R.¹, Wang Q.¹, Mou L.C.¹, Leung K.M.Y.^{1,2}, Yan M.^{1,2*}

¹ State Key Laboratory of Marine Pollution, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, China

² Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, China

Amphidinium carterae (Genotype 2) is a benthic dinoflagellate found on various substrates in the marine environment. It is known to be toxic to some marine organisms and produces bioactive compounds such as Amphidinolides, which have properties like antitumor, antibacterial, antifungal, and anti-diabetes. These compounds have potential importance in the pharmaceutical industry. However, the widespread use of the macrolide antibiotic Clarithromycin has led to its accumulation as waste in the marine environment, causing harm to microalgae.

There is a lack of knowledge regarding the effects of Clarithromycin on *A. carterae*, particularly its survival and production of bioactive compounds, including algal toxins. This study aims to investigate these effects by exposing *A. carterae* to various environmental concentrations of Clarithromycin over three generations. The study focuses on a strain of *A. carterae* named SKLMP_Ve021 (Genotype 2) isolated from Hong Kong waters.

The experiment involved exposing the algae to six concentrations of Clarithromycin, ranging from 0.01 to 1000 ng/L, along with control groups. Morphological and physiological changes, antioxidant responses, enzyme activities, and changes in Clarithromycin concentrations in the algal culture medium were examined. The production of bioactive compounds, including biotoxins, was determined using UPLC-Q-TOF-MS/MS.

Results showed that algal cells exposed to the two highest concentrations of Clarithromycin (100 and 1000 ng/L) only survived for two generations, with a cell density of only 5-8% compared to the control group. As the exposure concentration increased, a decrease in maximum photosynthetic efficiency and variation in enzyme activities were observed. Further results regarding Clarithromycin concentrations in different parts of the algal cells and the production of bioactive compounds and biotoxins will be obtained soon.

This study provides valuable insights into the effects of Clarithromycin on *A. carterae*, emphasizing the threats of emerging pharmaceutical contaminants to benthic microalgae.

COMBINATORIAL IMMUNE AND STRESS RESPONSE, CYTOSKELETON AND SIGNAL TRANSDUCTION EFFECTS OF GRAPHENE AND TRIPHENYL PHOSPHATE (TPP) IN MUSSELS

Li F., Meng X.J., Wang X.Q.

Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences

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

THE DYNAMIC VARIATION OF MICROBIAL COMMUNITY COMPOSITION AND HG-RELATED GENE ABUNDANCE IN THE COMPLEX MANGROVE ECOSYSTEM

Liu G., Wang S., Tsui M., Lee P.

*School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T, Hong Kong SAR, China
State Key Laboratory of Marine Pollution, The City University of Hong Kong, Shatin, Hong Kong SAR, China*

Mangrove ecosystems are highly productive and situated at the transition between land and sea. They receive both natural and anthropogenic mercury (Hg) inputs, and some of the Hg can be converted to neurotoxic methylmercury (MeHg). Meanwhile, various abiotic factors such as water temperatures, dissolved oxygen, and organic carbon content in mangrove sediments can mediate the production of MeHg by facilitating the activities of microbial Hg methylation. As a result, mangroves are considered as biogeochemical hotspots for MeHg production by some researchers. Recent research has shown that microorganisms are crucial for Hg methylation and MeHg demethylation in mangrove sediments. Indeed, MeHg production is driven by the microbes containing the *hgcAB* gene pair, such as sulfate-reducing bacteria, iron-reducing bacteria, and methanogens. Microorganisms possessing Hg resistance are regulated by the *mer* operon, which primarily functions to degrade MeHg by facilitating Hg transport across the cell membrane and reducing Hg into its volatile form Hg⁰. Microorganisms face the challenge of adapting to the complexity of natural ecosystems, for example, the microbes develop and coordinate different growth forms biofilm in diverse aquatic ecosystems. Biofilm is a microbial population that is embedded in extracellular matrices made up of extracellular polymeric substances. Bacterial biofilm formation is often regulated by quorum sensing which is a population of density-based cell-cell communication process via signaling molecules and it also improves the Hg resistance and MeHg degradation ability. It is worth noting that microplastic pollutants pose a threat to mangrove ecosystems by altering the biogeochemical cycling of mercury in mangrove environments, adding to the complexity of these ecosystems. In this presentation, preliminary findings probing into the Hg-related genes in mangrove ecosystems in Hong Kong will be presented.

BIOACCUMULATION OF SILOXANE AND THE EFFECTS ON AMINO ACID METABOLISM OF PLANKTON, ROTIFER, AND FISH: AN INVESTIGATION INTO FEEDING RELATIONSHIP

Won E.J.¹, Cho H.E¹, Kim D.K., Yun H.Y.¹, Jeong H.S.², Byun E.J. ², Lee J.S. ², Moon H.B.¹, Shin K.H. ¹

¹Hanyang University, Republic of Korea

²Sungkyunkwan University, Republic of Korea

Methyl siloxanes are emerging persistent toxic compounds, and the ecological environment risks posed by them have been a cause of concern worldwide. In this study, we determined whether methyl siloxane, especially D4, transferred from water accumulates in three different aquatic organisms in terms of the feeding relationship (phytoplankton: *Tetraselmis suecica*, rotifer: *Brachionus plicatilis*, and fish: *Orizias melastigma*) and whether siloxane affects amino acid metabolism. A significant bioaccumulation potential was observed in phytoplankton. In addition, there was a difference in the nutrient concentration of the medium to which the phytoplankton were exposed. The concentration of NH₄ was lower than that of the control group, indicating that NH₄ was reused from phytoplankton exposed to siloxane. This finding may indicate changes in nitrogen metabolism in phytoplankton and is expected to be interpreted as the stable isotope ratio of amino acids in the near future. Fish exposed to siloxane had lower nitrogen isotope ratios of amino acids (e.g., valine leucine, isoleucine, proline, aspartate, methionine, and glutamic acid) than the control group, indicating changes in amino acid metabolism under stress conditions. Moreover, for zooplankton and fish, changes in bioaccumulated siloxane concentrations were assumed to affect the accumulation of siloxane through feeding. Rotifer also showed high bioaccumulation, but the concentration of D4 when exposure together with a lower trophic organism (phytoplankton) had lesser bioaccumulation than the group of only water-born; however, fish showed opposite trends.

EFFECTS OF BISPHENOL A ON REPRODUCTION, BEHAVIOR, AND ACYLTRANSFERASE GENE EXPRESSION IN THE ROTIFER *BRACHIONUS PLICATILIS*

Yoon D.S., Kim J.S., Hong M.S., Lee M.C., and Lee J.S.

Sungkyunkwan University, Suwon, South Korea

In this study, we examined the effects of bisphenol A (BPA) concentrations on the rotifer *Brachionus plicatilis*, focusing on aspects such as growth, reproduction, behavior, oxidative stress, and the expression of mono- and diacylglycerol acyltransferase (*MGAT* and *DGAT*) genes. Our findings reveal that higher concentrations of BPA resulted in a delayed peak in the daily number of offspring, indicating a disruption of the normal reproduction rate. At certain BPA concentrations, the cumulative number of progeny was found to decrease, whereas the average lifespan of *B. plicatilis* was relatively stable across exposures. At higher BPA concentrations, alterations in behavioral parameters, such as turn frequency, were observed, but there were no significant changes in the size. In addition, BPA exposure induced oxidative stress in *B. plicatilis*, leading to an increase in ROS production. Concurrently, it also triggered an increase in the activities of SOD (superoxide dismutase) and CAT (catalase). Genomic analysis identified three *MGATs* and two *DGATs* within the three *Brachionus* species (*B. plicatilis*, *B. rotundiformis*, and *B. koreanus*). The phylogenetic analysis revealed the links between these acyltransferases and their homologous genes in other species, emphasizing unique gene architectures and evolutionary pathways. In addition, BPA exposure was found to modulate the expression levels of *MGATs* and *DGATs* in *B. plicatilis*. These findings contribute to our knowledge of the effects of BPA on *B. plicatilis* and the function of acyltransferases in the physiology and adaptation of *Brachionus* species.

BIOGEOCHEMICAL AND OPTICAL PROPERTIES OF COASTAL WATERS OF THE NORTHERN MALACCA STRAITS

Tan. E.¹, Mohd Zanuri, N. B.¹ and Martin, P.²

¹ *Universiti Sains Malaysia, Malaysia*

² *Nanyang Technological University, Singapore*

Coastal waters are optically and biogeochemically complex, with a variety of terrestrial and anthropogenic inputs. As one of the busiest shipping routes, the Malacca Strait is subjected to intense human activities, and also receives huge terrestrial inputs from rivers. This results in strong spatial gradients in optical water quality along the coast, which may impact primary productivity and the distribution of benthic communities. However, our knowledge of the optical properties in this region is very limited. Our objective is to determine how the optical properties in the northern Malacca Strait are controlled by biogeochemical variability. Water samples were collected to analyse biogeochemical variables (dissolved organic carbon (DOC), coloured dissolved organic matter (CDOM), chlorophyll-a (Chl-a), total suspended sediment (TSS) and nutrients), and inherent optical properties (IOPs) were measured. Reflectance measurements using sensors were also collected and used to correlate with the biogeochemical variables to establish the underlying relationship. This study hypothesizes that water samples collected from different compartments (e.g., estuaries, coastal waters) demonstrate distinct biogeochemical and optical properties. This study is important to comprehend the influence of terrestrial input into nearby water bodies that may influence its optical properties and thus primary productivity.

PRELIMINARY STUDY ON URBAN COASTAL AREAS: A FINAL RESERVOIR FOR ANTIBIOTIC RESISTANCE GENES

Lai V. H. Y.

Hong Kong Metropolitan University, China

Antibiotic resistance genes (ARGs) have long served as adaptive defensive mechanisms among bacteria, enabling their survival and propagation in challenging environments. However, the threats arising from ARGs have skyrocketed over the past decades. The consequences of multiple factors, including reckless antibiotic applications, improper waste handling, and inefficient wastewater treatment, have culminated in the emergence of untreatable and lethal extensively drug-resistant (XDR) or pan-resistant bacteria.

While current methods employed in wastewater treatment can effectively eliminate most microorganisms, their efficacy against ARGs remains uncertain. To identify the relation between wastewater effluent and marine ecosystems, we embarked on a study to monitor the abundance and prevalence of common ARGs in Hong Kong's urban coastal areas. Our finding reveals Sul1 is the most abundant resistance gene, with an average relative abundance of 4.45E-02. Furthermore, temperature and salinity emerge as key factors to the seasonal variation in total ARG abundance. The correlation matrix shows that Sul1 has the strongest correlation with temperature with a coefficient of -0.74. Suggesting the temperature plays a major role in promoting horizontal gene transfer events on Sul1. By comparing our findings with other studies via meta-analysis, Sul1 is typically the most abundant gene with its strong correlation with the mobile genetic elements (MGE) while other resistance genes are less likely correlated to such MGE.

This preliminary study provides valuable insights for developing control measures to counteract the dispersal of ARGs in Hong Kong. To address the root cause of antibiotic resistance, it is imperative to explore antibiotic alternatives, such as phage therapy, which mitigate the selective pressures exerted by ARGs and ultimately alleviate the emergence of drug-resistant bacterial strains

REMOVAL EFFICIENCY OF ANTI-TUBERCULOSIS DRUGS IN PRIMARY WASTEWATER TREATMENT PLANT

Chen C.H. and Chen W.Y.

Department of Ecology and Environmental Resources, National University of Tainan, Taiwan

Tuberculosis is the 13th leading cause of death in the world. Anti-tuberculosis drugs, Isoniazid (INH), Rifampicin (RMP), and Ethambutol (EMB) can effectively treat tuberculosis. However, unmetabolized drugs will be excreted by the human body. If anti-tuberculosis drugs cannot be effectively processed in wastewater treatment plants (WWTPs), these drugs will enter the water environment. In order to know whether the removal efficiency of anti-tuberculosis drugs in WWTPs is related to water quality conditions, influent and effluent water were sampled at the Central District Sewage Treatment Plant, which is the largest primary sewage treatment plant in Kaohsiung, Taiwan. We measured INH, RMP, and EMB residues and water parameters (temperature, pH, dissolved oxygen, suspended solids, and biochemical oxygen demand). The research results show that the highest average concentration of INH during influent was 1772.22 ± 3828.49 ng/L, followed by EMB 74.09 ± 42.73 ng/L, and finally RMP 17.11 ± 6.08 ng/L; the removal efficiency of INH the highest is $60.86 \pm 27.59\%$, followed by EMB $19.48 \pm 28.33\%$ and RMP $13.89 \pm 18.25\%$. Results of correlation analysis show that the INH and EMB concentrations of influent are negatively correlated with the water temperature, and the removal efficiency of the two drugs is positively correlated with the water temperature; therefore, higher temperatures can enable better removal of anti-tuberculosis drugs. The concentrations of INH and RMP are positively correlated with suspended solids, and the removal efficiency of INH and RMP is also positively correlated with the removal efficiency of suspended solids. It indicates that INH and RMP may be adsorbed on suspended solids and decrease water residues. Further, the removal efficiency of anti-tuberculosis drugs is related to the temperature and suspended solids in water quality conditions. In conclusion, WWTPs can improve the removal of anti-tuberculosis drugs by regulating temperature or enhancing the treatment efficiency of suspended solids.

OXIDATIVE CONVERSION AS TOOL TO REVEAL UNKNOWN EXTRACTABLE ORGANOFUORINE IN COMPLEX MATRICES

Yuen C.N.T.¹, Kärroman A.²

¹ State Key Laboratory of Marine Pollution (SKLMP), City University of Hong Kong, China

² Man-Technology-Environment (MTM) Research Centre, Örebro University, Sweden

The current analysis of PFAS using target analysis has encountered several challenges, one of them being the lack of commercial standards. To gain a more comprehensive understanding of PFAS contamination in samples, complementary methods such as oxidative conversion, also known as total oxidizable precursor assay (TOPA) and extractable organofluorine (EOF) analysis are employed. In TOPA, perfluoroalkyl acid (PFAA) precursors undergo rigorous oxidation conditions by hydroxyl radical at alkaline condition and high temperature. This converts them into quantifiable perfluoroalkyl carboxylic acids (PFCAs) of varying chain lengths. (Houtz and Sedlak, 2012) Conversely, EOF analysis quantifies the fluorine content in sample extracts through combustion. (Yeung *et al.*, 2008) However there is limited literature on oxidative conversion applied to sediment and biota samples, as well as the complementary analysis of TOPA with EOF.

Research findings have indicated that wastewater treatment plants (WWTP) can be significant point source of PFAS and PFAS precursors contamination in marine environment upon discharge into surface water. (Elmoznino, Vlahos and Whitney, 2018) In this study, sediments and biota samples (*monoporeia*, *macoma* and *edulis*) were collected from background Baltic Sea region to the outlet and upstream of the WWTP. These samples were analysed comprehensively using target analysis, oxidative conversion and extractable organofluorine (EOF) analysis. The study aims to determine the current contamination status of PFAS and their precursors in the collected samples and to assess the contribution of both known PFAS and unknown PFAA precursors to the extractable organofluorine mass balance.

PFAS were detected in all collected samples, albeit constituting a small fraction of the extractable organofluorine. Oxidative conversion revealed significantly higher concentrations of short-chain and ultra-short-chain PFCAs in all samples, indicating the presence of short-chain and telomerization-manufactured precursors. Furthermore, the results identified another potential source of PFAS pollution in a sample that exhibited much higher concentrations of PFCAs in sediment after oxidation. Post-oxidation results increased known EOF by 25%. Although most EOF components remained unidentified, the comprehensive analysis demonstrated its strength in providing new information that target analysis alone could not offer. Oxidative conversion also proved particularly useful when EOF alone couldn't provide meaningful insights due to contamination or high detection limits.

FATES OF ANTI-TUBERCULOSIS DRUGS IN MUNICIPAL WASTEWATER AND RECEIVING WATER: OCCURRENCE AND REMOVAL

Huang X.R. and Chen W.Y.

Department of Ecology and Environmental Resources, National University of Tainan, Taiwan

According to statistics from the World Health Organization, tuberculosis is one of the largest infectious killers in the world and ranks as the thirteenth leading cause of death globally. Isoniazid (INH), Rifampicin (RMP), and Ethambutol (EMB) are first-line drugs used for the treatment of tuberculosis in various countries, and those are not easily degradable in wastewater treatment processes, which may pose risks to the ecological environment. This study measured the monthly residue levels of anti-tuberculosis drugs in influent and effluent of the Fengshan Water Resource Center and receiving water Fengshan River in Kaohsiung, Taiwan. We aimed to assess the removal efficiency of anti-tuberculosis drugs by secondary wastewater treatment plants and environmental fate in water environments. Results show that the monthly average concentration of INH in the influent was 1669 ± 3062.4 ng/L, representing the highest average concentration among the three drugs in the influent. The lowest detected average concentration was RMP, with a monthly average concentration of 19 ± 7.3 ng/L. The highest removal efficiency at the wastewater treatment plant is RMP ($88 \pm 21\%$), followed by INH ($76 \pm 38\%$), and lastly, EMB ($45 \pm 27\%$). In riverine environment, the highest monthly average concentration was detected for INH 95 ± 148.9 ng/L, while the lowest was observed for RMP 1 ± 1.3 ng/L. In terms of seasonal variations, we found that the concentrations of anti-tuberculosis drugs were below the average in autumn and increased above the average in winter; this suggests that these compounds' biodegradability (microbial activity suppression) may be reduced during colder seasons, leading to increased concentrations in the wastewater treatment plant samples. The removal efficiency of anti-tuberculosis drugs exhibited a significant positive correlation with influent temperature, with higher temperatures favoring drug removal. RMP and EMB exhibit similar chemical properties; their removal efficiencies are strongly correlated. Additionally, RMP and EMB show significant negative correlations with effluent pH, influent and effluent dissolved oxygen, and influent suspended solids, suggesting the adsorption characteristics of these drugs onto solid matter in wastewater. RMP and EMB also show a pronounced negative correlation with effluent pH in riverine environments. Overall, the removal efficiency of EMB is below 50%, signifying ineffective treatment, and it subsequently enters the surface water bodies in the environment. It may potentially re-enter human activities downstream through various processes. In summary, given the similar properties of RMP and EMB, and EMB's relatively lower removal efficiency, subsequent wastewater treatment facilities should be particularly attentive to regulating effluent pH, influent and effluent dissolved oxygen, as well as controlling the removal of suspended solids to achieve an optimal drug removal efficiency.

OCCURRENCE OF PHARMACEUTICAL CONTAMINATION IN GLOBAL ESTUARIES

Adedipe D.T., Chen C., Leung K.M.Y.

State Key Laboratory of Marine Pollution, City University of Hong Kong

The contribution of contaminants in estuaries is gradually becoming a bane to the aquatic system and environment at large. These contaminants include pharmaceuticals which are major emerging contaminants of global priority. Using meta-data analysis this review seeks to comprehensively understand the occurrence, risk, and influencing factors of pharmaceuticals contamination in global estuaries.

Over the last two decades, in order to understand the current status of these chemicals and also identify the locus of pollution, a global review has been carried out. In total, this review retrieved 2652 concentration data for 198 medicines from 87 estuaries in 25 different countries across six continents. Their respective ecological risks were also assessed.

Africa recorded the highest cumulative weighted average concentration level (WACL) values (145461.86 ng/L). The cumulative WACL values were followed by North America (24316.39 ng/L), South America (20784.13 ng/L), Europe (5754.67 ng/L), Asia (3121.69 ng/L), and Oceania (2844.04 ng/L). Carbamazepine, diclofenac, and paracetamol were detected in all continents. A total of 32 pharmaceutical categories were identified, and analgesics, antibiotics, and stimulants had the most reports in global estuaries. High acute and chronic toxicity concerns were observed in 4 and 12 pharmaceuticals, respectively. In addition, pharmaceutical pollution in estuaries was found to be positively correlated with regional unemployment and poverty ratio, but negatively correlated with life expectancy and GDP per capita.

From the review, the occurrence in estuaries is still vaguely understood in these continents, especially in Africa, Oceania, and South America. This study will subsequently contribute to the existing knowledge and in addition, provide researchers insights to further studies.

Keywords: *pharmaceuticals; global estuaries; ecological risk assessment; socioeconomic factors*

TARGET AND SUSPECT SCREENING AND TEMPORAL TRENDS OF LIQUID CRYSTAL MONOMERS IN MARINE MAMMALS FROM THE SOUTH CHINA SEA

Tao D., Leung K.M.Y., Lam P.K.S., He Y.

City University of Hong Kong

Liquid crystal monomers (LCMs) have been widely used in manufacturing display panels for over 30 years, and found to be widespread in marine sediments in the Greater Bay Area of China. Since these compounds are potentially persistent, bioaccumulative, and toxic, they may pose adverse impacts on wildlife, including marine mammals. Herein, target and suspect screening of LCMs is conducted using tissues from stranded individuals of the Indo-Pacific humpback dolphin (*Sousa chinensis*) and the finless porpoise (*Neophocaena phocaenoides*), two resident marine cetacean species inhabiting the South China Sea. The samples were collected from 2007 to 2020. In total, 41 LCMs were detected in muscle, brain, blubber, liver, melon, and kidney samples from these two species, with the highest levels found in blubber and muscle. The dolphins exhibited significantly higher total LCMs concentrations compared to the porpoises. No significant temporal trend was observed in blubber concentrations of either total LCMs or the five prominent LCMs in the two species. Additionally, two novel LCMs were identified by suspect screening using gas chromatography–Orbitrap. Overall, this study established a robust method for target and suspect screening of LCMs in biosamples, and provided the first evidence of LCMs contamination in various tissues, including brain, within the two marine mammal species, underscoring the need of subsequent ecotoxicological inquiries into LCMs, particularly on their neurotoxic effects.

IMPACT OF METABOLIC DISRUPTOR PERFLUOROCTANE SULFONATE ON THE APOPTOSIS AND SECRETION OF RAT PANCREATIC ISLET CELLS

Zhang X., Fu Y., Zheng P., Li M., Li R.

Guilin Medical University

Perfluorooctane sulfonate (PFOS) is a ubiquitous chemical compound widely utilized across the globe, representing one of the prevalent forms of perfluorinated compounds (PFCs). The correlation between exposure to PFOS and the onset of diabetes, which disrupts the insulin-secreting β cells within pancreatic islets, remains shrouded in uncertainty. In this study, we endeavored to elucidate the impact of perfluorooctane sulfonic acid (PFOS) on the apoptosis and secretion of pancreatic islet cells in rats. To achieve this, we subjected cultured INS-1 cells to varying concentrations of PFOS for durations of 24, 48, and 96 hours. Following treatment with 100 and 200 $\mu\text{mol/L}$ PFOS, a substantial increase in both mRNA and protein expression of Bax was observed, while the gene and protein expression of Bcl-2 exhibited a rapid decline across all treatment groups. Furthermore, PFOS exerted a concentration-dependent effect on both insulin expression and secretion. Notably, the expression of Glut2 and AMPK was significantly inhibited, whereas the levels of inflammatory mediators ERK1/2, MyD88, and TRIF experienced a marked increase. These findings shed light on the propensity of PFOS to promote apoptosis in INS1 cells, consequently impairing insulin expression, as well as hampering Glut2 and AMPK functionality. In addition, PFOS potentially instigates the activation of MyD88 and TRIF TLR-triggered signaling pathways, thereby fostering the production of inflammatory cytokines through ERK1/2 activation.

EFFECTS OF ANTI-TUBERCULOSIS DRUG RIFAMPIN ON REPRODUCTIVE TOXICITY OF *DAPHNIA MAGNA*

Huang T.H., Lee Y.Y., Chen W.Y.

Department of Ecology and Environmental Resources, National University of Tainan, Taiwan

The first-line anti-tuberculosis drugs, especially rifampin, is hard to dissolve and degrade. Based on the recent study, there is limited evidence regarding the effect of rifampin on aquatic organisms. Therefore, the study aims to assess the impact of rifampin on the reproduction of *Daphnia magna*. The study conducted a 21-day *Daphnia* reproduction toxicity bioassay with exposure to 0.78, 1.6, 3.125, 6.25, and 12.5 mg/L of rifampin. We observed average parental growth, parental survival, first-time birth, daily production, and offspring survival. Results show the parental growth of 0.78 mg/L and 1.6 mg/L significantly decreased compared to control ($p = 0.006$). The cumulative production and average production per time decreased by more than 20%, while exposure concentrations were more than 3.125 mg/L. A noticeable offspring survival decrease starts from 3.125 mg/L ($p < 0.001$). However, there is no significant difference in parental survival compared to control. On the basis of the results, we consider the most sensitive concentration to aquatic organisms is 3.125 mg/L. In conclusion, we can briefly infer that rifampin residues higher than 3.125 mg/L in the aquatic environment would adversely impact the reproduction of aquatic organisms.

REPRODUCTIVE EFFECTS OF CHRONIC EXPOSURE TO ANTI-TUBERCULOSIS ETHAMBUTOL ON *DAPHNIA MAGNA*

Lin Z.H., Wang Y.J., Lee C.W. and Chen W.Y.

Department of Ecology and Environmental Resources, National University of Tainan, Tainan, Taiwan

Ethambutol (EMB) is one of the anti-tuberculosis medications, and patients require long-term treatment with EMB. The EMB enters watershed environments after being absorbed and metabolized within the human body, potentially affecting aquatic organisms. This study aims to investigate the reproduction related to the safety threshold of the anti-tuberculosis medication, EMB, within aquatic ecosystems. A 21-day chronic toxicity test was conducted using the test organism, *Daphnia magna*, during which the following parameters were observed: parental survival, body length, reproductive time, offspring quantity, and offspring survival. Results showed that EMB had a significant impact on parental body length ($p < 0.05$), and the time to first brood was extended in the high-concentration exposures ($p < 0.05$). The high-concentration groups (25 mg/L, 50 mg/L) showed a significant decrease ($p < 0.01$) in reproductive frequency, offspring quantity per brood, total offspring production, and surviving offspring compared to the low-concentration group (6.25 mg/L, 12.5 mg/L). However, EMB did not significantly affect the offspring survival rate. This study highlights total offspring production as the most sensitive indicator for assessing the reproduction toxicity of EMB to *D. magna*, with a safety threshold identified as EMB concentrations below 12.5 mg/L. In conclusion, high concentrations of EMB negatively impact the reproduction of *D. magna*, particularly with respect to highlights total offspring. *D. magna* plays a crucial role as a primary consumer in the ecosystem, and elevated EMB concentrations in the environment could lead to ecological imbalance. Therefore, the toxicity assessment of EMB in this study can serve as a basis for future environmental risk assessments of anti-tuberculosis medications.

Keywords : Anti-tuberculosis medications; Ethambutol; *Daphnia magna*; Reproduction; Ecotoxicity

OCCURRENCE, BEHAVIOR AND FATE OF LIQUID CRYSTAL MONOMERS IN MUNICIPAL WASTEWATER

Zhan Y., Jin Q., Lin H., Tao D., Law L.Y., Sun J., & He Y.

City University of Hong Kong, China

Liquid crystal monomers (LCMs), the essential substances used in the display screen of electronic devices, have been proposed as a class of emerging chemicals of concern. Despite their detection in various environmental matrices, little is known about the presence of LCMs in municipal sewage systems. This study aimed to investigate the occurrence, distribution, and fate of 64 LCMs released into the aqueous environment from a municipal wastewater treatment plant (WWTP) in Hong Kong, China. In total 14 LCMs were detected in WWTP samples. Specifically, the Σ_{14} LCMs concentrations in crude influent, final effluent, and final sludge were found to be 16.8 ± 0.3 ng/L, 2.71 ± 0.05 ng/L, and 19.2 ± 1.0 ng/g dry weight, respectively. Among them, 10 fluorinated LCMs (F-LCMs) were determined to be present at concentrations of 8.90 ± 0.10 ng/L, 1.69 ± 0.05 ng/L, and 9.94 ± 1.00 ng/g dry weight, respectively. The predominant non-fluorinated LCMs (NF-LCMs) detected in all samples were 3OCB and EPhEMOB, while 2OdF3B was the dominant F-LCM. The overall removal rate of total LCMs was 83.8 ± 0.3 %, with 25.4 ± 4.8 % being removed by biodegradation and UV treatment. Compared to NF-LCMs, F-LCMs were more resistant to biodegradation. Despite the significant removal of LCMs through WWTP, the remaining LCMs in the final effluent could result in an annual emission of 3.04 kg of total LCMs from the population of Hong Kong. This study provides the first evidence of LCMs contamination in municipal wastewater, possibly arising from routine electronic devices usage. Further investigation is needed to elucidate the potential impact of LCMs emission via WWTP effluent on the aquatic receiving ecosystem.

A ROBUST APPROACH TO QUANTIFY 65 PHARMACEUTICALS IN A LARGE-SCALE MONITORING CAMPAIGN

Luo Q., Chen C., Lai R.W.S., Xu S., Adedipe D., Zhou G., Leung K.M.Y.

Stake Laboratory of Marine Pollution, City University of Hong Kong, China

We established and optimized a robust analytical method for water samples with a small volume was established for simultaneously detecting and quantifying 65 pharmaceuticals using the solid phase extraction (SPE) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). A sample volume of 20 mL was found to be optimal for achieving satisfactory recoveries. Potential environmental factors such as water pH, salinity, matrix, storage time and temperature were investigated to clarify their effects on the accuracies of this method. The results showed that the target compound recoveries under acidic condition (pH 3 - 5) were satisfactory. Although salinity would possibly have significant impacts on the detection of target compounds, the magnitude of such an effect was very limited and regarded as acceptable. The matrix of river water or seawater did not significantly interfere with the detection of most of the target compounds. The recoveries for most of the pharmaceuticals were still within the acceptable ranges after stored for seven days at room temperature, and relatively low temperatures were beneficial for mitigating the possible degradation. This method was further applied and validated by analysing water samples collected from Kai Tak River and its estuary in Hong Kong, and the range of cumulative pharmaceutical concentrations was found to be 4,812 – 34,077 ng/L. This cost-effective and user-friendly method established a solid foundation for the large-scale monitoring campaign, such as the Global Estuaries Monitoring (GEM) Programme which has been lunched to monitor pharmaceuticals in over 150 estuaries around the globe.

LONGITUDINAL ARGS PROFILES IN WASTEWATER TREATMENT PLANT INFLUENT REVEALED BY METAGENOMIC ANALYSIS

Li S., Yin X., Chen X., Yan W., Chen Y., Liu L., Wang Y., Jiang X., Ju F., Ye L., Wang C, and Zhang T.*

Environmental Microbiome Engineering and Biotechnology Lab, Center for Environmental Engineering Research, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China.

The imprudent antibiotics use accelerates antimicrobial resistance (AMR) caused by antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs), thereby endangering human and animal health. Sewage influents of wastewater treatment plants (WWTPs), containing thousands of people's gut microbiomes, are considered antibiotic-resistant hotspots. Although spatial studies have examined WWTP influents from diverse regions and countries to assess their resistomes, longitudinal studies that may measure temporal fluctuations are lacking.

This work examined temporal ARG profiles of Shatin WWTP influent in 13 years using metagenomics analysis of 97 monthly samples. The results show that the total ARG abundance significantly changed every three to four years, Cluster 1 (2010.10–2013.12), Cluster 2 (2014.01–2017.12), and Cluster 3 (2018.01–2022.03), with fluctuation in the range of 0.50~2.08 copies/cell, and eleven ARG types increased in abundance and proportion over the years, including beta-lactam, multidrug, and quinolone. Furthermore, PCoA analysis revealed a distinct resistome composition in Shatin sewage influent compared to various sample types, including human and animal specimens and other sewage samples. The time series has three temporal clusters, each spanning about four years. Through network examination, strong co-occurring connections were discovered among 71 specific Class one integrons and 198 subtypes of ARGs originating from 13 types, demonstrating their potential for horizontal gene transfer. Co-occurrence patterns were mostly found among related ARG types (multidrug, beta-lactam, and quinolone) or between two types, like beta-lactam and multidrug. Furthermore, the results also showed that 775 species from seven phyla could potentially serve as hosts for 196 subtypes of ARGs of 15 types with significant correlation levels at $p < 0.01$ and $r > 0.9$.

This longitudinal study used an 'omics-based' approach to assess high-risk ARGs' medical threats. The findings revealed that clusters 1 and 2 exhibited a seasonal increased risk for AMR. However, after implementing policies aimed at controlling AMR in Hong Kong, cluster 3's risk returned to pre-policy levels, signifying the efficacy of such interventions in reducing AMR. The findings in this longitudinal influent analysis provided insights into the risk assessment of sewage resistome regarding AMR threats to human health.

IDENTIFICATION OF TRANSFORMATION PRODUCTS OF ORGANIC UV FILTERS BY PHOTOOXIDATION AND THEIR DIFFERENTIAL ESTROGENICITY ASSESSMENT

Lu, Y., Chow, M.K., Sun, J., Tao, D., Jin, Q., Ren, Y., Wang, W.X. and He, Y.

School of Energy and Environment and State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong SAR, China

Organic ultraviolet filters (OUVFs) are extensively released into aquatic environments, where they undergo complex phototransformation. However, there is little knowledge regarding their transformation products (TPs) and associated endocrine disruption potentials. In the present study, we characterized the chemical and toxicological profiles of TPs for two common OUVFs, oxybenzone (BP3) and ethylhexyl methoxycinnamate (EHMC), by photooxidation under environmentally relevant conditions. It is hypothesized that TPs of the tested OUVFs will show varied estrogenicity at different reaction times. High-resolution liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) identified 17 TPs of 7 m/z for BP-3 and 13 TPs of 8 m/z for EHMC at confidence levels ≤ 2 . Five novel TPs of 2 m/z were reported for the first time with structure-diagnostic MS/MS spectra. Estrogenicity assessment using the MCF-7-*luc* cell line showed discrepant estrogenic activities exhibited by OUVF-TPs over time. Specifically, BP3-TPs exhibited significantly greater estrogenicity than the parent at several reaction times, whereas EHMC-TPs displayed fluctuating estrogenicity with a declining trend. Correlation analysis coupled with molecular docking simulations further suggested several TPs of BP3 as potential endocrine disruptive compounds. These findings underscore the necessity of considering mixtures during chemical testing and risk assessment and highlight the potentially greater risks associated with post-transformation cocktails.

CONCISE REVIEW ON DEGRADATION OF ORGANIC UV FILTERS IN THE WATER ENVIRONMENT

Sun J.J., Rene E.R., Tao D.Y., Lu Y.C., Jin Q.Q., Lam J.C.H., Leung K.M.Y., He Y.

City University of Hong Kong, China

Organic ultraviolet filters (OUVFs) have been used globally for over the past 20 years. Given the fact that OUVFs can be easily released from sunscreens applied on human skins, they have been frequently detected in aquatic environments and organisms. Some byproducts of OUVFs might be more recalcitrant and toxic compared to their parent compounds. To further assess the toxicity and potential risk of OUVFs' byproducts, it is necessary to determine the fate of OUVFs and identify their transformation products. This review summarizes and analyzes pertinent literature and reports in the field of OUVFs research. These published research works majorly focus on the degradation mechanisms of OUVFs in aquatic environments, their intermediates/byproducts, and chlorination reaction. Photodegradation (direct photolysis, self-sensitive photolysis and indirect photolysis) and biodegradation are the main transformation pathways of OUVFs through natural degradation. To remove residual OUVFs' pollutants from aqueous environments, novel physicochemical and biological approaches have been developed in recent years. Advanced oxidation, ultrasound and bio-based technologies have proven to eliminate OUVFs from wastewaters. In addition, the disinfection mechanism and the byproducts (DBPs) of various OUVFs in swimming pools are discussed in this review. Besides, further research in the field of OUVFs research are also mentioned.

TARGET AND NON-TARGET ANALYSIS OF ORGANIC POLLUTANTS IN SEWAGE EFFLUENT AND RECEIVING SEAWATER IN THE ARCTIC REGION OF KONGSFJORDEN

Kang D., Jeon J.

Changwon National University, Korea

The effluent discharged from Ny-Ålesund, where the Arctic research station is located, is polluted by various organic pollutants used by residents, and is flowing into Kongsfjord, an Arctic area. In this study, target and non-target screening based on LC-HRMS was applied to search for organic pollutants present in effluent, shorelines, and the fjord. Samples were collected once a day for five consecutive days starting May 26, 2023. In the case of effluent inflow and outflow, passive sampling was also performed. There were a total of 522 substances subject to target analysis, of which 97 were detected. Among them, 77 substances were quantitatively analysed above the LOQ. Quantitative results showed high concentrations of caffeine, PEGs, triethanolamine, and acetaminophen in the effluent. In the beaches and fjords around the discharge point, relatively high concentrations of organic pollutants were found in the discharged water. As a result of non-target analysis, the presence of at least 20 additional substances was recognized. Some of these substances showed higher concentrations on the beach or fjord than in the discharged effluent. This indicates that there are sources of pollution other than effluent from Ny-Ålesund in the Arctic marine environment.

STUDYING THE GUT MICROBIOME, RESISTOME, AND HUMAN PATHOGENIC BACTERIA IN MARINE FISH OF HONG KONG MARINE PARK

Ye L., Cai W., Yan M., Zhong L., Gao S., Lam V.T. T., Chen X., Heng H., Guo Z., Chan E.W.C., Li F., Leung K.M.Y., Chen S.

City University of Hong Kong, China

The Hong Kong Polytechnic University, China

The gut microbiome of marine fish plays a pivotal role in their health and overall ecosystem dynamics. In this study, we conducted a comprehensive analysis of the gut microbiomes of four different families of marine fish species in the Hong Kong Marine Park using metagenomic techniques. Our research focused on the prevalence of human pathogenic bacteria (HPB), the diversity of resistance genes (resistome), and the assembly of high-quality metagenome-assembled genomes (MAGs). Our findings revealed that among the HPB identified, *Photobacterium damselae* and *Selar Crumenophthalmus* displayed the highest abundance, primarily due to the presence of *Photobacterium damselae*. Interestingly, the HPB abundance in the other four fish species was generally below 10%. Notably, *Sardinella fimbriata* exhibited the highest diversity in HPB, with as many as 29 different species identified. In the context of the resistome, *Sardinella fimbriata* stood out, harboring 25 different types of antibiotic resistance genes (ARGs), including the crucial beta-lactam resistance genes. Other fish species also contained a significant number of ARGs, with *Alepes kleinii* having the fewest at 13 types. Importantly, we observed the widespread presence of beta resistance gene types across all fish species, indicating their potential significance in shaping the gut microbiome. Furthermore, we successfully assembled 252 high-quality metagenome-assembled genomes (MAGs) from the four different fish families. Notably, two of these MAGs carried ARGs associated with *Mycobacterium tuberculosis*. These MAGs will serve as valuable references for future research on fish gut microbiomes. In conclusion, our study provides valuable insights into the composition and diversity of the gut microbiome in marine fish from the Hong Kong Marine Park. The prevalence of HPB and the extensive variety of resistance genes in these fish species highlight the need for further research to understand the role of fish gut microbiomes in maintaining ecosystem health and to potentially mitigate the spread of antibiotic resistance. These findings will serve as a foundation for future investigations in this field.

ORGANOPHOSPHATE ESTERS IN SEAWATER AND SEDIMENTS FROM THE YANGTZE RIVER ESTUARY (YRE) AND EAST CHINA SEA (ECS)

Wei L. and Huang Q.H.

Tongji University

The widespread occurrence of organophosphate esters (OPEs) in estuary and ocean environment has been reported globally due to the gradual restrictions in use and phase-out of brominated flame retardants (BFRs). It is of great importance to monitor the occurrence of new emerging OPEs and assess the potential risk for aquatic organism, also identify the pollution source.

The Yangtze River Estuary (YRE) along with East China Sea (ECS) is typical estuarine and coastal sea environment, and the physicochemical properties of seawater varied widely associated with the mixing currents between freshwater input and marine currents. We conducted a field cruise along YRE and ECS during November 24th-29th, 2021, and collected 37 seawater samples (including 20 surface seawater samples and 17 bottom seawater samples), and 13 sediments samples. To be summarized, 12 out of 13 targeted OPEs (including TCEP, TCIPP, TDCIPP, TIBP, TNBP, TEHP, TBOEP, TPHP, EHDPHP, MDPP, CDP, BPDPP, and TPTP) were detected in seawater samples except MDPP, and the total concentrations of OPEs in surface and bottom seawater were in the range of 4.81-144 ng/L (mean: 57.1 ng/L), and 5.34-59.0 ng/L (mean: 25.7 ng/L), respectively. TCIPP and TCEP showed higher proportions in seawater. For the surface sediments samples, the total concentrations of OPEs varied between 9.49 and 83.0 ng/g dry weight (dw), with a mean value of 24.0 ng/g dw. TCIPP and TIBP were major contaminants.

The spatial distribution of OPEs was enormously impacted by the unique geochemical properties along YRE and ECS, which could be divided into three groups, as freshwater zone, mixing zone, and marine zone. Generally, the highest levels of OPEs in seawater were presented in mixing zone (site C4-C10), while marine zone showed the lowest concentration levels. We further explored the impacts of physicochemical properties of seawater, and the surface layer was more impacted by geochemical properties than the bottom layer, especially for salinity and density. The highest concentration in sediments was presented in the sand piled area of the YRE, which was enormously impact by the river sand input.

The complex and unique geochemical properties enormously impacted the occurrence and fate of OPEs in estuary and coastal areas, and we will further explore the potential mechanism.

ARSENIC SPECIES AND THEIR HEALTH RISKS IN EDIBLE SEAWEEDS COLLECTED ALONG THE CHINESE COASTLINE

Ran Bi^{ab*}, Zhangxun Huang^{ab}, Stanislav Musil^c, Ásta H. Pétursdóttir^d, Puhui Zhao^a, Yongfeng Jia^e, Wenhua Liu^{ab}

^a Guangdong Provincial Key Laboratory of Marine Disaster Prediction and Prevention, Shantou University, Shantou, 515063, China

^b Institute of Marine Sciences, Shantou University, Shantou 515063, China

^c Institute of Analytical Chemistry of the Czech Academy of Sciences, Veveří 97, 60200 Brno, Czech Republic

^d Matis, Vinlandsleid 12, 113 Reykjavik, Iceland

^e Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

Edible seaweeds with a relatively high total arsenic concentration have been a global concern. As the largest seaweed producer, China contributes about 60 % of the global seaweed production. The present study investigated 20 seaweed species collected from representative seaweed farming sites in the six provinces along the Chinese coastline, of which *Saccharina japonica*, *Undaria pinnatifida*, *Neopyropia spp.*, *Gracilaria spp.*, *Sargassum fusiforme* were listed as the most consumed seaweeds in Food and Agriculture Organization of the United Nations (FAO). The inorganic arsenic (iAs) concentration in most of the seaweeds was below maximum limits (0.3 mg iAs/kg) as seaweed additives for infant food in the National Food Safety Standard of Pollutants in China (GB2762-2017, 2017), except for the species *Sargassum*, in which the iAs concentration significantly exceeded the limit and ranged from 15.1 to 83.7 mg/kg. Arsenic speciation in 4 cultivated seaweeds grown in both temperate and subtropical zones is reported for the first time. No significant differences in total As and iAs concentration were identified, except slightly higher total As concentration were found in *Saccharina japonica* growing in the temperate zone. The estimated daily intake (EDI) of toxic iAs via seaweed consumption was generally below the EFSA CONTAM Panel benchmark dose lower confidence limit (0.3 µg/kg bw/day) except for all *Sargassum* species where the EDI was significantly higher than 0.3 µg/kg bw/day. Moreover, the first-ever reported data on As speciation indicated very high iAs concentrations in *Sargassum hemiphyllum* and *Sargassum henslowianum*. To minimize the food chain iAs exposure, reducing both human intake of *Sargassum spp.* and the used of *Sargassum spp.* for animal feed is highly recommended.

BIOACCUMULATION AND TROPHIC TRANSFER OF ANTHROPOGENIC PERSISTENT ORGANIC CHEMICALS IN MARINE COASTAL PLANKTON

Ko F.-C.^{1,2}, Chu C.-H.², Cheng J.-O.¹, Chang C.W.¹

¹ National Museum of Marine Biology and Aquarium, Taiwan

² Institute of Marine Biology, National Dong-Hwa University, Taiwan

As low trophic level organisms that sustain the food web, plankton, particularly phytoplankton, serve pivotal roles as both primary producers and carriers of persistent organic pollutants (POPs) from the atmosphere and seawater into the biota. This study aimed to comprehensively analyze the accumulation and distribution patterns of polycyclic aromatic hydrocarbons (PAHs) in phytoplankton and zooplankton across varying exposure durations within a simulated planktonic food chain. The study utilized a plankton food chain consisting of *Tetraselmis chuii* (phytoplankton), *Brachionus rotundiformis* (rotifers), and *Apocyclops sp.* (copepods) cultured within a gas purging system with a continuous supply of PAHs. The investigation revealed three distinct temporal phases of PAH accumulation: 0.2-1 hours, 1-24 hours, and 24-168 hours. Notably, PAH concentrations exhibited substantial fluctuations during the initial 0.2-1 and 1-24 hour intervals but approached a steady state after 24 hours of exposure. An intriguing observation was the differential accumulation of low molecular weight (LMW) PAHs, which were found in higher quantities in copepods compared to rotifers and phytoplankton. Conversely, high molecular weight (HMW) PAHs were significantly more abundant in phytoplankton, indicating potential selectivity in PAH uptake among planktonic organisms. Principal component analysis (PCA) further elucidated a clear demarcation between phytoplankton and zooplankton, suggesting distinct PAH accumulation patterns influenced by trophic levels. Notably, linear relationships were established between the bioconcentration factor (BCF) and PAH hydrophobicity (K_{ow}) for all PAHs within the planktonic food chain. The varying slopes in the linear regression between \log BCF and \log K_{ow} for phytoplankton, rotifers, and copepods underscored the existence of diverse pathways for PAH accumulation among planktonic organisms. Moreover, the assessment of biomagnification factors (BMF) revealed a potentially greater risk of biomagnification for LMW PAHs compared to their HMW counterparts within the planktonic community. This study offers valuable insights into the complex dynamics of PAH accumulation in planktonic food webs, with potential implications for understanding the broader ecological impacts of these persistent pollutants.

EFFECTS OF TERRESTRIAL ORGANIC MATTER ON METAL CONTAMINATION AND ECOLOGICAL RISKS IN PORT SEDIMENTS

Chen C.F., Lim Y. C., Wang M.H., Albarico F.P.J.B., Hsu C.W., Chen C.W., and Dong C.D.

National Kaohsiung University of Science and Technology, Kaohsiung City, Taiwan

Port sediments are an important sink for organic matter and metal deposition. There is usually a significant correlation between sediment organic matter (SOM) and metal concentrations in sediments, an important controlling factor in the distribution of metals in the aquatic environment. However, there are few reports on how SOM sources influence metal distribution in sediments. This study investigated the metal concentrations and the stable isotope compositions ($\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$, respectively) of SOM in the surface sediments (Kaohsiung Port, Taiwan). This study combined C and N stable isotope composition, correlations, and multiple linear regression analysis to explore the influence of SOM sources on metal pollution and toxicity risks in sediments. Results showed a similar spatial distribution pattern between SOM and metal contents in the sediments. Total organic carbon (TOC) and metals in estuarine sediments were high, gradually decreasing toward the port outlet. Estuarine sediments are hot spots for metals, with high pollution and potential toxicity risk levels, while channel and port outlets have low levels. The $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ and carbon/nitrogen ratio (TOC/TN) indicate that the SOM is mainly of terrestrial origin ($66.4\pm 13.0\%$). On average, $73.9\pm 12.9\%$ of the SOM in estuarine sediments came from terrestrial sources, while those in the lower level of the channel and port outlet is $58.9\pm 8.0\%$. This study proposes a prediction model for sediment metal pollution load index and toxicity risk index, which includes three variables: terrestrial organic carbon (TOC_{terr}), $\delta^{13}\text{C}_{\text{org}}$, and TOC/TN. These three variables reflected that the degree of metal pollution and toxicity risk in sediments were mainly affected by the terrestrial organic matter contents (i.e., source or composition of SOM). These results provide a clearer understanding of the relationship between SOM and metal distribution in port sediments.

APPLICATION OF PHYSIOLOGICALLY BASED TOXICOKINETIC (PBTK) MODELING FOR ASSESSING EXPOSURE AND TISSUE DISTRIBUTION OF BDE-47 IN NEOPHOCAENA PHOCAENOIDES

Tee K.A.¹, Xie M.¹, Jin L.¹, Kot B.C.W.¹, Lam P.K.S.², Ruan Y.¹

¹ City University of Hong Kong, China

² Hong Kong Metropolitan University, China

Due to the high level of urbanization and rapid growth of manufacturing activities, the Pearl River Delta receives an elevated burden of brominated flame retardant (BFR) pollution, which may pose significant risks to human health and the environment in this region. The persistent nature of these pollutants necessitates continued biomonitoring in order to comprehensively assess risks to the aquatic environment. In this regard, resident cetacean species such as the Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) may serve as a biomonitor for the spatiotemporal dynamics of BFRs in the Pearl River Delta because of their susceptibility to bioaccumulation. Moreover, their status as protected species under local, regional, and international laws makes BFR exposure biomonitoring pivotal for conservation efforts. However, due to prohibitions on sample collection from living individuals, the development of non-intrusive methods for exposure assessment is required. In this study, physiologically based toxicokinetic (PBTK) modelling was used as a non-intrusive tool for assessing the exposure of *Neophocaena phocaenoides* to 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), the major component in the commercial formula of penta-BDE technical mixture which has been banned in China since 2014. Models were compared to concentrations detected in various tissue samples collected from stranded individuals (2013–2022) for further validation. Adipose tissue was found to be the major compartment involved in the bioaccumulation of BDE-47 in the body, with predicted concentrations similar to samples taken from 2013–2020. Furthermore, sensitivity analysis indicates that parameters relating to adipose tissue were most likely to affect model outcomes due to its status as the main reservoir of BDE-47 in the body. Results show that PBTK modeling can be used to assess the exposure and tissue distribution of BDE-47 for *Neophocaena phocaenoides* in Hong Kong.

ASSESSMENT OF BIOACCUMULATION AND ECOLOGICAL RISK OF POTENTIALLY TOXIC METALS IN BENTHIC ECOSYSTEM ALONG COASTAL ZONE OF INDUSTRIALIZED CITY, SOUTHWESTERN TAIWAN

Lim, Y.C.¹, Chen, C.F.¹, Albarico, F.P.B.^{1,2,3}, Cayabo, G.D.B.^{1,2,4}, Wang, M.H.¹, Chen, C.W.¹, and Dong, C.D.^{1,2}

¹ Department of Marine Environmental Engineering, National Kaohsiung University of Science and Technology, Kaohsiung City, Taiwan

² Institute of Aquatic Science and Technology, National Kaohsiung University of Science and Technology, Kaohsiung City, Taiwan

³ College of Fisheries and Allied Sciences, Northern Negros State College of Science and Technology, Sagay City, Philippines

⁴ College of Fisheries and Aquatic Sciences, Western Philippines University, Puerto Princesa City, Palawan, Philippines

Due to rapid urban and industrial development, coastal waters are vulnerable to anthropogenic pollution, such as toxic metals and persistent organic pollutants. The impacts of anthropogenic pollution on the coastal environment and ecosystem have raised significant public concern. In this study, four species of benthic organisms were collected along the coastal zone of Kaohsiung City, the largest heavy industrial city in Taiwan, during March, October 2022, and April 2023, including mauxia shrimp (*Acetes intermedius*), penaeid shrimp (*Parapenaeopsis cornuta*), left-handed hermit crab (*Diogenes spinifrons*), and leucosiid crab (*Philyra platychira*). Potentially toxic metals (Cu, Zn, Pb, Cd, Cr, As) were determined in the tissues (whole tissue) of 85 organisms. Sediment and seawater samples were also collected to evaluate bioconcentration factors (BCF) and bioaccumulation factors (BSAF). Moreover, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the organisms were determined to understand the trophic level of the crustacean species. Results showed that the Cu, Zn, Pb, Cd, Cr, and As contents of crustaceans were 0.54–23.3, 2.88–46.8, 0.15–2.33, 0.05–1.59, 0.001–0.055, and 0.11–1.84 $\mu\text{g/g}$, respectively, indicating obvious inter-species and spatial variations. Among the species studied, the left-handed hermit crab (*D. spinifrons*) represented relatively high contents of Cu and Zn, with averages of 9.40 ± 4.16 and 25.0 ± 6.44 $\mu\text{g/g}$ w.w., respectively. In contrast, leucosiid crab (*P. platychira*) showed relatively high contents of Cd (0.035 ± 0.008 $\mu\text{g/g}$ w.w.). Regarding spatial distribution, the crustaceans collected from the adjacent Kaohsiung Harbor showed relatively high metal contents with a metal pollution index (MPI) ranging between 0.28–1.31, compared to those from the area around the river mouth (MPI: 0.22–0.89) and fishing port (MPI: 0.23–0.98). It indicated that shipping activities in Kaohsiung Harbor and the pollution from surrounding industrial areas significantly impact the metal contents in local crustaceans. Correspondingly, high sediment metal concentrations were observed around Kaohsiung Harbor, averaging 101, 297, 132, 31.7, 0.22, and 3.67 mg/kg for Cu, Zn, Pb, Cd, Cr, and As, respectively. Since mauxia shrimps (*A. intermedius*) and penaeid shrimps (*P. cornuta*) are edible, according to the health risk assessment, the metal contents of the shrimps collected in this study area were in the low-risk range. This study is the first report on a comprehensive pollution assessment of Kaohsiung's coastal benthic ecosystem, which can provide valuable information for coastal management.

QUANTIFYING NATURAL AND ANTHROPOGENIC FLUXES OF TRACE METALS FROM A SMALL MOUNTAINOUS RIVER AND ESTUARY (KAOPING) IN SOUTHWESTERN TAIWAN

Hung, J.-J.¹, Yang, C.-Y.¹, Lai, I.-J.¹, Li, Y.-H.²

¹ *Department of Oceanography, National Sun Yat-sen University, Kaohsiung, Taiwan*

² *Department of Oceanography, University of Hawaii Manoa, Honolulu, HI 96822, USA*

The Kaoping River is a tropical, high-standing island-type river having one of the global highest denudation rates in the river basin. The river also flows through urban and industrial regions in the downstream region affected by anthropogenic pollution. As most riverine metals are transported primarily by particulate matters, it is important to know the transport and flux conditions of metals under conditions of high denudation and human pollution. The experiments carried out by two periods with contrasting patterns of rainfall showing that the transport and annual fluxes of trace metals (Mn, Cd, Cu, Cr, Pb, Zn, and Hg) were apparently modulated by the local climatic pattern and anthropogenic pollution. The enrichment factors of metals also show a greater pollution status in the dry season than in the wet season, and in the downstream zone than in the upstream zone. The relationship between metal concentration and inverted flow rate (Q^{-1}) allows us to distinguish the natural from anthropogenic contributions. Although the gross fluxes of dissolved and particulate metals were mainly controlled by river discharge and sediment load, the net fluxes of metals through the estuary were modified due to non-conservative behavior of most dissolved metals.

SUBLETHAL IMPACTS OF HEBEI SPIRIT OIL SPILL ON THE REPRODUCTIVE PHYSIOLOGY OF PACIFIC OYSTER *CRASSOSTREA GIGAS* AT TAEAN ON THE WEST COAST OF KOREA

Shin J.-S.¹, Lee H.-J.^{1,3}, Keshavmurthy S.^{1,4}, Yim U.-H.², Hong H.-K.¹, Kang C.-K.⁵, and Choi K.-S.¹

¹ Department of Marine Life Science (BK 21 FOUR) and Marine Science Institute, Jeju National University, Republic of Korea

² Oil and POPs Research Group, South Sea Research Institute, Korea Institute of Ocean Science and Technology (KIOST), Republic of Korea

³ Southeast Sea Fisheries Research Institute, National Institute of Fisheries Science (NIFS), Republic of Korea

⁴ Biodiversity Research Center, Academia Sinica (BRCAS), Taiwan

⁵ School of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), Korea

Following the *Hebei Spirit* oil spill (HSOS) in December 2007, benthic organisms in the intertidal and shallow subtidal of the Taean coast on the west coast of Korea became devastated by the spilled crude oil. To understand the stresses caused by accident, we determined the growth and reproduction of the Pacific oyster *Crassostrea gigas* cultured on an oil-contaminated tidal flat over three years shortly after the accident. A month after the oil spill, the alkyl PAHs in the oyster body elevated to an extremely high level (13,500 ng/g dry wt), although the PAHs dropped to 5,335 ng/g dry wt in December 2008 and returned to the levels before the accident (547-858 ng/g dry wt) by 2010. In 2008, the oil-damaged oysters exhibited a declined condition and gonad somatic indices, exhibiting a sign of physiological stress. However, by 2009, a year after the HSOS accident, oysters in the study site exhibited a sign of recovery from the oil stress in terms of improvement in the condition index, storage reserves, and egg mass, suggesting a certain level of recovery from the oil spill-driven stresses. Gonad maturation, reproductive effort, and the carbohydrate content in the tissues in 2010 were comparable to the levels in 2009 and the levels observed from oysters in regular oyster farming areas. Our study suggested that the Pacific oysters recovered from the HSOS stresses within a year, although long-term monitoring of oyster health must be carried out to ascertain the recovery.

BIOACCUMULATION OF TRACE METAL ELEMENTS IN THE CRITICALLY ENDANGERED MAUGEAN SKATE (*ZEARAJA MAUGEANA*)

Mondon, J.A¹, Robertson Z.¹, Corbett, P.A¹, Bell J.² and Callahan, D.¹

¹Deakin University, Australia

²University of Tasmania, Australia

There is a paucity of research relating to the Maugean skate despite its critically endangered status. This is the first investigation to identify assimilation and compartmentalisation of non-essential metals bioaccumulation by this species.

The skate now only survives in Macquarie Harbour, on the southwest coast of Tasmania, Australia. It displays high site fidelity and preference for foraging small prey in shallow, soft sediment benthic habitats within the limited 70 km² habitat range of the harbour. Past mineral extraction waste from copper and gold mining have contributed significantly to legacy contamination of Macquarie Harbour sediments, predominantly from amalgam and smelting extraction processes, and acid drainage from exposed mineral-rich bedrock entering the harbour via the Gordon-Franklin River and King River catchments. Mean concentrations of Hg (3.38 +/- 0.25 mg/kg) and As (29.27 +/- 3.48 mg/kg) in muscle were respectively 4 to 5 times higher than in liver, whilst Cu (26.34 +/- 14.76 mg/kg) was eighteen times higher in liver compared to muscle. Cd (0.99 +/- 0.12) and Pb (0.42 +/- 0.05) were present at lower concentrations in liver indicating a broader suite of bioavailable non-essential metals in Macquarie Harbour. There was no significant correlation between total metals burden in muscle and length of animal, and no significant difference in metals burden between sexes. Mean Hg concentration in the Maugean skate was twice that recorded in White spotted skate from the heavily metals-contaminated Derwent River in southern Tasmania, however no ecotoxicological biomarkers of effect from non-essential metals exposure in the Maugean skate have been investigated. The evidence of non-essential metals assimilation provided here is critical to understanding the legacy and on-going metal stressors to the species and provides baseline contaminant bioaccumulation concentrations for future comparison.

INSUFFICIENT EVIDENCE TO LINK HUMAN EXPOSURE TO HEAVY METALS WITH BIOMARKERS OF GLIOMA IN COASTAL POPULATIONS

Xie M.Y.^{1,2}, Ruan Y.F.², Wang Q.², Leung K.M.Y.², Liu L.Y.¹, and Zeng E.Y.¹

¹Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China

²State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Kowloon, Hong Kong 999077, China

With the rapid development of the coastal economy, the impact of environmental factors on human health has attracted a growing concern. Intense anthropogenic activities in coastal areas have a significant impact on the accumulation of heavy metals in humans. Coastal residents well represent populations in economically developed areas and tend to have greater exposure to heavy metals. Glioma patients in coastal areas of Guangdong as the research subjects in the present study, we determined the content and distribution characteristics of heavy metals in brain tissue, and examined the associations between heavy metals and brain tumor. Thirteen heavy metals were measured in 137 glioma and 35 non-glioma samples collected from 161 alive patients in coastal populations. All target heavy metals were detected, they could cross the blood-brain barrier. Concentrations of Mn, Cu, and Zn were higher in glioma than in non-glioma samples, while those of Ni and Se were higher in non-glioma samples, probably suggesting that these five heavy metals are more prone to be altered by changing pathological conditions. In addition, Cu/Zn, Cr/Mn, Cr/Se, Ni/Se, Pb/Mn, and Pb/Se were statistically different between glioma and non-glioma samples by a difference test and a multiple logistic regression model. These concentration ratios may serve as chemical markers to assist pathological analysis for differentiating between tumor and healthy tissue. However, no direct link between heavy metal concentrations or concentration ratios and biomarkers of glioma (*i.e.*, tumor grade, P53, and Ki-67) was observed. No sufficient evidence was obtained to implicate the role of heavy metals in inducing glioma, largely caused by the limited number of samples. Different concentrations and concentration ratios of heavy metals may be the consequence rather than the cause of pathological changes in brain tumors.

THE POTENTIAL INFLUENCE OF SUBMARINE HYDROTHERMAL DISCHARGE ON BIOACCUMULATION OF TRACE METALS IN ZOOPLANKTONS

Peng S.H.¹, Hung J.J.², Hwang J.S.³, Chen Arthur C.T.² and Wei T.P.⁴

¹ *National Museum of Marine Biology and Aquarium, Pingtung, Taiwan*

² *Department of Oceanography, National Sun Yat-Sen University, Kaohsiung, Taiwan*

³ *Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan*

⁴ *Research Center for Water Science and Technology, Tainan, Taiwan*

This study evaluates the potential bioaccumulation of trace metals in zooplankton in the submarine hydrothermal field off Kueishan islet (KI), a volcanic islet located on the western margin of Kuroshio and 10 km away from the northeastern coast of Taiwan. A cluster of small shallow hydrothermal vents closing to the eastern KI emit hydrothermal fluid characterized by high temperature, low pH and high trace metals. The distribution and accumulation of trace metals in seawater and zooplanktons were investigated during the period of 2009–2011. The results show that most metal concentrations were about one to three orders higher in vent fluid than in the Kuroshio water. Metal discharges from hydrothermal vents were estimated to be 18.87, 2.73 and 0.87 ton/year for Al, Fe and Mn, respectively. The dissolved trace metal concentrations decreased rapidly after mixing the fluid with ambient seawater, resulted likely from the dilution and increase of pH. The concentrations of Cd · Co · Fe · Ni · Mn and Zn in zooplanktons display relatively high values in the northern side of KI, suggesting that the discharged metals may be transported by Kuroshio current toward the northeastern region off KI where the zooplanktons may uptake trace metals through seawater and/or food web.

DISTRIBUTION OF TIRE AND ROAD WEAR PARTICLES (TRWP)-RELATED POTENTIALLY TOXIC ELEMENTS (PTES) WITH PARTICLE SIZE IN THE PORT OF BUSAN: A POTENTIAL CONTRIBUTOR OF PORT SEDIMENT POLLUTION

Choi J.Y.^{1,2}, Park K.H.¹, Lee S.E.¹, Won E.J.^{3,4}, Kim K.T.¹, Hyun S.M.¹, Ra K.T.¹ and Hong G.H.⁵

¹*Korea Institute of Ocean Science and Technology, Republic of Korea*

²*National Korea Maritime & Ocean University, Republic of Korea*

³*Hanyang University, Department of Marine Science and Convergent Technology, Republic of Korea*

⁴*Institute of Marine and Atmospheric Sciences, Republic of Korea*

⁵*East China Normal University, Republic of Korea*

The Port of Busan is one of the busiest seaports in the world and in a metropolitan city that supports a vigorous economic activity of about three million residents. It has been reported that marine sediments around the Port of Busan, as well as major ports around the world, are contaminated with various pollutants, including potentially toxic elements (PTEs). Transporting inbound and outbound goods from and to the port via vehicles is known to be a significant source of pollution along with the incoming and outgoing ships at the onshore and offshore areas, respectively.

This study aimed to identify sources of PTEs of road-deposited sediment (RDS) in the port. RDS mainly results from the deposition of tire and road wear particles (TRWP), which are mechanical abrasion of brakes and tire erosion of road surfaces by passing traffic. These vehicle-generated particulate pollutants are deposited mainly on the road surface and interact with other solid materials. In recent years, the pollutants with TRWP have been an emerging environmental concern because of their toxicity and contamination of coastal sediment.

Notable findings of this study are 1) The traffic volume and vehicle type influenced the grain size of RDS, while it was negatively correlated with the accumulation of PTEs; 2) Geoaccumulation index (I_{geo}) of Zn, Cd, Cu, Pb, Sb, and Cr showed that the RDS were severely contaminated with PTEs-related TRWP; 3) RDS, which is <250 μm , was affected by the same anthropogenic activity traffic source, statistically ($p < 0.05$); 4) PCA results showed that Heavy-duty vehicle (HDV) activity in the port area may have affected the most abundant PTEs, such as Zn, in RDS in this area.

The fine particles of RDS on the road surface are potential coastal sediment because they can be readily washed off into the nearby water sphere by stormwater and settled down. Therefore, the results of this study suggested that it is necessary to mitigate TRWP, mainly induced by HDV, to control the sediment PTE quality in the port.

ROAD DUST AND TRAFFIC-DERIVED NON-EXHAUST PARTICLES: A PRELIMINARY STUDY FOR THE CHEMICAL CHARACTERIZATION AND MARINE ENVIRONMENTAL EFFECTS

Choi J.Y.^{1,2}, Won E.J.^{3,4}, Jung J.M.^{1,5}, Lee Y.J.^{1,2}, Kim M.S.⁶, Lee H.W.¹, Hong G.H.⁷ and Kim K.T.¹

¹Korea Institute of Ocean Science and Technology, Republic of Korea

²National Korea Maritime & Ocean University, Republic of Korea

³Department of Marine Science and Convergent Technology, Hanyang University, Republic of Korea

⁴Institute of Marine and Atmospheric Sciences, Republic of Korea

⁵Division of Earth Environmental System Science, Pukyong National University, Republic of Korea

⁶National Institute of Environmental Research, Republic of Korea

⁷East China Normal University, Republic of Korea

Road dust (RD) and traffic-related sources have recently been recognized as significant causes of environmental pollution in urban and coastal environments. As exhaust emission regulations for air quality have been strengthened in developed countries, non-exhaust emission (NEE) from transportation has been recognized as an important source of environmental pollution. In addition, the increase in the intensity and frequency of heavy and extreme rainfall due to climate change can significantly increase the marine inflow of RD and tire and road wear particles (TRWP) from urban impervious surfaces such as roads of cities and coastal infrastructure. Due to these effects, research on the environmental fate and risk of road dust and road traffic-related sources is expected to become more important shortly. Previous studies conducted in several coastal areas, including the Busan coast, showed that coastal sediment was contaminated with RD and TRWP. For environmental forensics and fate studies of RD and TRWP, Methods are needed to precisely identify and characterize individual road dust and TRWP in complex environmental samples.

In this study, we collected RD and NEE derived such as TRWP (road-deposited sediment (RDS) and tire-derived wear particles (TWP)) and brake pad-derived wear particles (BWP) in Busan and attempted its chemical characterization with stable isotopes and potentially toxic elements (PTEs). This preliminary study shows that BWP and TWP contain high concentrations of PTEs, including Zn, Mn, Cu, Sb, and Cr. Accordingly, investigations on the impact of their input on the marine environment have been conducted. Although the health risks due to exposure through the food chain in the marine environment are currently unclear, the heavy metal profiles and stable isotope signatures analyzed in this study can be used as important chemical tracers to track their fate in the marine environment.

SPATIAL DISTRIBUTION AND SOURCE IDENTIFICATION OF PERSISTENT TOXIC SUBSTANCES IN SEAWATER AND SEDIMENT OF GYEONGGI BAY, SOUTH KOREA

Kim T., Song H., Yoon S.J., Lee J., Kim B., Khim J.S.*

School of Earth and Environmental Sciences & Research Institute of Oceanography, Seoul National University

Persistent toxic substances (PTSs) derived from a range of anthropogenic activities transport to the marine environment through river runoff and wastewater from numerous sources. However, accurately identifying the sources of various pollutants and quantitatively assessing their ecological impact remains limited. The present study aimed to understand and evaluate the fate of pollutants in major pollution sources, including industrial complexes and wastewater treatment plants along the coastal areas of Gyeonggi Bay. Multi-media (sediments and seawater) collected from total 101 sites were analyzed for 16 polycyclic aromatic hydrocarbons (PAHs), including 6 alkylphenols (APs), and 10 styrene oligomers (SOs). The concentrations of PTSs (PAHs, APs, SOs) generally showed a decreasing (mean: 81%) trend from pollution sources to the open sea. The concentrations of PTSs in sediments and water layers were decreased (~98.6%) significantly greater in tidal flats and salt marshes (source > tidal flats and salt marshes). In the case of PAHs, polymer forms were more abundant in tidal flats and salt marshes, while APs and SOs consisted of a higher proportion of low-molecular-weight and metabolites. In some areas with ports, industrial complexes, or wastewater treatment plants (such as Incheon and Ansan), substantial pollution loads were observed, indicating that the spatial distribution characteristics are related to their origin and physico-chemical properties. The decrease in PTSs concentrations in tidal flats and salt marshes sediments suggested the potential for the purification of pollutants. This study suggested the origins of PTSs and a qualitative and quantitative assessment of the purification capacity of tidal flat in Gyeonggi Bay.

CADMIUM CONCENTRATION AND C, N STABLE ISOTOPE ANALYSIS OF FINLESS PORPOISES (*NEOPHOCAENA* SPP.) FROM MATSU

Fang, T.¹, Yao, C.-J.², Chou, L.-S.³, Chen, M.-H.^{1,4,5*}

¹ Institute of Marine Ecology and Conservation, National Sun Yat-sen University, Kaohsiung, Taiwan

² Department of Biology, National Museum of Natural Science, Taichung, Taiwan

³ Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan

⁴ Department of Oceanography, National Sun Yat-sen University, Kaohsiung, Taiwan

⁵ Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan

Twenty-two Matsu finless porpoises (MFP), *Neophocaena* spp., collected in 2004-2015 were analyzed for tissue cadmium (Cd) levels and stable nitrogen and carbon isotope ratios. No correlation was found between $\delta^{13}\text{C}$ ratios and body length, indicating no off-shore migration or benthic/pelagic diet shift at different life stages. Lower mean tissue Cd levels were found in the MFP (kidney: 6.700 ± 13.252 , liver: 1.150 ± 1.887 , lung: 0.061 ± 0.050 , muscle: 0.031 ± 0.036 mg/kg dw) compared to finless porpoises from other regions of the Chinese coast. As a Cd pollution bioindicator, linear regression lines were established between tissue Cd levels and body length. Observations of the $\delta^{15}\text{N}$ ratio and Cd levels corresponding to body length revealed maternal transfer, growth dilution and bioaccumulation. A body length of 110 cm was identified as the transition point dividing “calves and juveniles” from “subadults and adults” regarding MFP trophic level transition and tissue Cd bioaccumulation.

Keywords: non-essential element, growth dilution, maternal transfer, regression line, trophic level

CONCENTRATIONS OF FIVE HEAVY METALS AND C, N STABLE ISOTOPES IN THE TISSUES OF FOUR CETACEAN AROUND TAIWAN

Tien Y.-R.¹, Chen M.-H.^{1,2}, Chou L.-S.³, Yang W.-C.³, Yao C.-J.⁴

¹ National Sun Yat-sen University, Taiwan, ROC

² Kaohsiung Medical University, Taiwan, ROC

³ National Taiwan University, Taiwan, ROC

⁴ National Chung Hsing University, Taiwan, ROC

The concentrations of Fe, Zn, Cu, Mg, and Cd in the muscles, livers, kidneys, and lungs of four species of cetaceans, including *Kogia breviceps*, *Steno bredanensis*, *Tursiops aduncus*, and *Tursiops truncatus*, from the water around Taiwan in 2003 to 2020. Based on the muscle $\delta^{13}\text{C}$, it was found that *K. breviceps* and *S. bredanensis* primarily inhabit the continental shelf and deep-sea regions, while *T. aduncus* and *T. truncatus* inhabit coastal and shallow seas. Additionally, according to $\delta^{15}\text{N}$, *K. breviceps* predominantly feeds on deep-sea cephalopods, whereas the other three species mainly feed on fish. There were accumulation differences among the species. Fe concentrations in the muscle and kidney tissues of *K. breviceps* were higher than the other three species, likely due to their diving capabilities and physiological differences, resulting in varied requirements and utilization of heavy metals. The study revealed that Fe, Zn, Cu, and Mn elements were primarily accumulated in the liver tissues of the four cetacean species, while Cd was found the kidney tissues. Furthermore, in *K. breviceps*, Fe concentrations in the liver and kidney, and Cd concentration in the muscle, were positively linked to body length. On the other hand, Zn concentration in the muscle, Mn concentration in the kidney of *K. breviceps*, and Mn concentration in the muscle of *T. truncatus* and *S. bredanensis* showed negative correlations with body length. The accumulation patterns of these five heavy metals varied among the four cetacean species, likely due to their physiological requirements and different age-related elemental demands. In comparison to other marine areas in recent years, the concentrations of heavy metals in the coastal waters of Taiwan did not show a higher pollution trend.

Key words: *Kogia breviceps*, *Steno bredanensis*, Species differences, Essential element, Iron, Cadmium

ACCUMULATION LEVELS AND PROFILES OF PERSISTENT ORGANIC POLLUTANTS (POPS) IN BLUBBER AND FUR OF SPOTTED SEAL (*PHOCA LARGHA*) FROM PETER THE GREAT BAY, SEA OF JAPAN/EAST SEA

Mok S.¹, Ammatil A.R.¹, Trukhin A.M.² and Moon H.-B.¹

¹ Hanyang University, Republic of Korea

² V.I. Il'ichev Pacific Oceanological Institute, Russia

Accumulation of persistent organic pollutants (POPs) in marine mammals is of great concern and is associated with declining populations. Marine mammals (e.g., cetaceans and pinnipeds) have been utilized as sentinel species to assess POPs accumulation in marine ecosystem due to their feeding habits, long life-span, high fat content, and slow metabolic capacity. The spotted seal (*Phoca largha* Pallas, 1811) is distributed in northern Pacific Ocean and is considered as endangered species. Thus, it is essential to investigate the current accumulation of multiple POPs in these species to assess ecotoxicological risks in context of marine conservation issues. The concentrations of POPs and related chemicals were measured in blubber (n=19) and fur (n=41; only for PFASs) of spotted seal (*P. largha*) from Peter the Great Bay, Sea of Japan/ East Sea in 2015–2017. Polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and chlorobenzenes (CLBz) were determined by gas chromatography coupled with a mass spectrometer (GC-MS). Polybrominated diphenyl ethers (PBDEs) and alternative flame retardants were determined by GC-tandem mass spectrometer (GC-MS/MS). Per- and polyfluoroalkyl substances (PFASs) were determined by liquid chromatography coupled with tandem mass spectrometer (LC-MS/MS). Among the targeted POPs in blubber, PCBs were detected at the highest concentration (range: 299–7350 ng/g lipid), followed by OCPs (290–1340 ng/g lipid) and PBDEs (0.73–27.1 ng/g lipid). Although low concentration levels of PFASs in blubber, the excessive concentration was observed for 6:2 fluorotelomer sulfonate (6:2 FTS) used as an alternative substance for regulated PFASs. The accumulation patterns of PFASs in fur were similar to those of blubber. These results indicated that the habitat of spotted seals is severely contaminated to 6:2 FTS by the consumption and emission from the anthropogenic activity. Opposite to other POPs, the concentrations of PBDEs and alternative flame retardants in blubber were increased with age, which could be due to the capacity for the independent feeding activity. Considering that most samples were obtained from pups, placental and lactational transfer could be major mechanism governing their accumulation levels of POPs and related chemicals. Approximately 84% of spotted seals exceeded previously proposed threshold levels for PCBs, implying that PCBs continue to pose a potential health risk to wildlife. Continuous monitoring of POPs in spotted seals and their surrounding environment is necessary to manage and reduce risk to their population.

THE ACCUMULATION OF MERCURY IN DIFFERENT SIZES OF PHYTOPLANKTON IN THE EUTROPHIC ESTUARY, NORTHERN TAIWAN.

Chang F.W., Fang T.H.

National Taiwan Ocean University, Keelung, Taiwan.

Total mercury and methylmercury in estuarine water and in different meshes (70, 120, 200 and 420) of phytoplankton were analysed to study the accumulation of Hg by phytoplankton and Hg contamination status in the eutrophic estuary, northern Taiwan. The samples were collected at May and July, 2023. The concentrations of dissolved total Hg (DTHg) and dissolved methylmercury (DMeHg) in estuarine water ranged within 7.6-62.2 ng/L and 0.2-9.5 ng/L, respectively, and DMeHg contributed 1-49 % of DTHg pool. The sum of total Hg and MeHg concentrations of phytoplankton ranged within 0.6-54.2 mg/kg and 0.02-5.6 mg/kg, respectively. The Hg concentrations in all samples collected at May were significantly higher than those of July. The result of Hg concentration of phytoplankton in different meshes varied significantly but the result for MeHg concentration was consistent during the two surveys. The estuarine distribution of Hg concentrations in phytoplankton exhibited scatter, but higher concentration generally appeared in the upper estuary. The Hg concentration of phytoplankton in each mesh varied significantly and seemed to be independent with the mesh during two surveys. However, total MeHg concentrations in phytoplankton were generally dominated by phytoplankton in mesh 70 and the contribution of mesh 420 was the least among the different meshes. It is also found that Hg and MeHg concentrations of phytoplankton in mesh 200 correlated well with the both total concentrations in phytoplankton during two surveys. The MeHg bioconcentration factor of phytoplankton in different meshes varied widely and the log value ranged within 2.5-5.7. The highest value is found in mesh 70, suggesting that the larger the sample, the higher the bioconcentration factor.

PHOSPHORUS FRACTIONATIONS AND THEIR TRANSFORMATIONS IN SEDIMENT CORES OF THE EUTROPHIC ESTUARY, NORTHERN TAIWAN

Chen, X.C. & Fang, T.H.

National Taiwan Ocean University, Keelung, Taiwan

The present study employed the Ruttensburg sequential extraction method (SEDEX) to study the P fraction variation in three sediment cores (SC) collected from the eutrophic estuary, northern Taiwan. The SEDEX method chemically divides the sedimentary P into five fractions: (F1) loosely sorbed P, hereafter refers to as PSORB; (F2) ferric iron-bound P (PCDB); (F3) authigenic carbonate fluorapatite + biogenic apatite + CaCO₃-bound P (PCFA); (F4) detrital apatite P (PDET); and (F5) organic P (PORG). The sedimentary total P (STP) concentrations in SC at the upper estuary station ranged within 1748-2391 mg/kg and the concentration significantly decreased to 571-906 mg/kg at the lower estuary station, suggesting that the upper estuary was highly polluted with P. The percentage of each P fraction concentration to the total P pool in the three sediment cores followed the sequence: PCDB (45-75%) > PDET (9-32%) > PCFA (6-15%) > PORG (4-14%) > PSORB (1-4%). The variation of each P fraction with depth in each sediment core was not so significant. However, the percentage of PCDB, accounting for 61-75% of STP, in sediment core of the upper estuary, significantly decreased to 45-57 % of STP in sediment core of the lower estuary, which accompanied with the increase of PDET fraction in sediment core of the lower estuary. This result may suggest the anthropogenic P, probably from the domestic discharge effluent, in the estuarine water was adsorbed by particles and settled down to the sediment of the upper estuary.

(Keywords: phosphorus; sequential extraction method; sediment core)

CHANGES IN THE CONCENTRATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN FECAL PELLETS OF *MARPHYSA* SP. E AND REDUCED MUD IN THE YORO TIDAL FLAT, JAPAN

Yuichiro O., Satoshi A., Mayu O., Kenji O. and Atsuko N.

Toho University, Japan

Introduction

In tidal flat environments, benthic organisms play an important role in the material cycle through their burrowing and feeding activities. In our previous studies, we found that *Marphysa* sp. E *sensu* Abe *et al.* (2019) (Annelida, Eunicida), a benthic organism inhabiting the Yoro tidal flat (Ichihara, Chiba, Japan), selectively ingests reduced muds containing high concentrations of polycyclic aromatic hydrocarbons (PAHs) and subsequently excretes them as fecal pellets (Osaka *et al.*, 2023). After excretion, PAH concentrations in the fecal pellets rapidly decreased to half within 2 h (Onozato *et al.*, 2010, 2012). In this study, to investigate whether the rapid decrease in PAH concentrations observed in the fecal pellets of *Marphysa* sp. E also occurs in the reduced muds, we measured and compared the temporal changes in PAH concentrations in those samples. Based on these results, we discussed the contribution of *Marphysa* sp. E to the environmental remediation of tidal flats.

Materials and methods

Sample collections were performed in the Yoro tidal flat (Ichihara, Chiba, Japan) in April to October in 2021 to 2023. Fecal pellets immediately after excretion and reduced muds obtained by digging up the bottom sediment were collected. Each sample was divided into two, one of which was quickly frozen with dry ice, and the other was left in the tidal flat for 2 h and then frozen. After pretreatment of these samples, PAH concentrations were determined by gas chromatograph-mass spectrometer (QP-2010, SHIMADZU).

Results and discussion

Total PAH concentrations for the fecal pellets of the *Marphysa* sp. E and reduced mud were 596 to 2041 and 216 to 558 $\mu\text{g kg-dry}^{-1}$, respectively. The concentration in fecal pellets left for 2 h were 35 to 55 % lower than those in the fresh ones. However, the reduced mud showed no significant changes in either PAH concentrations and composition. In general, it has been reported that the half-life of PAHs in the sediment is between several weeks to months (Lu *et al.*, 2012), and that high molecular weight PAHs were hardly degraded in reduced deep layer sediments (Karickhoff *et al.*, 1979). The mechanism of the reduction of PAH concentrations over a short period of time remains unclear although it is possible that PAHs degradation was carried out by microbes and/or enzymes in the fecal pellets (Mrozik *et al.*, 2003; Jørgensen *et al.*, 2008). These results suggest that the passage of reduced mud through the body of *Marphysa* sp. E is required to the rapid reduction of PAH concentrations, and their feeding and excretion behaviors are making an important contribute to the purification in the tidal flat environment.

OCCURRENCE AND ECOLOGICAL RISK OF ORGANOTINS IN THE MARINE ENVIRONMENT OF HONG KONG

Jiang Y., Liu M.*, and Leung K.M.Y.*

State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong 999077, China

Organotin compounds (OTs), such as tributyltin (TBT) and triphenyltin (TPT), are highly toxic biocides and can cause morphological abnormalities in marine organisms at low concentrations (ng/L). Since the 1960s, they have been extensively used as active ingredients in antifouling paints on ship hulls, and biocides in agricultural and textile industries. Therefore, their contaminations in coastal marine environments are common and widespread. The Marine Environment Protection Committee of the United Nations' International Maritime Organization adopted a global prohibition on the use of OT-based biocides on sea-going vessels in 2008. Hong Kong is a densely populated coastal city and serves as the 7th busiest container port in the world. Historically, its marine environment received a large amount of OTs originating from shipping activities, surface runoff, partially-treated wastewater, and discharge from the Pearl River. In Hong Kong, a legislation on banning the use of OTs on ships came into force on January 1, 2017. This study, hence, aims to test if such a policy intervention is effective to reduce the OT contamination and their impact on the benthic community. We collected samples of seawater, sediment and the rock shell *Reishia clavigera* in the marine environment of Hong Kong during 2022–2023, and quantified the concentrations of six OT compounds in these samples. The results clearly indicated that there was a decreasing trend of OT contamination when compared with the data obtained in 2014–2015, indicating the positive effect of the local legislation. TPT was found to be the most predominant OT compound across the rock shell tissue, seawater, and sediment samples, which was consistent with the previous trend. The highest tissue concentration of total OTs (936.3 ng/g d.w.) was detected in *R. clavigera* from Waterfall Bay, while those collected from Po Toi island exhibited the lowest tissue concentration (1.6 ng/g d.w.). However, concentrations of OTs remained high in seawater and sediment in areas with busy marine traffic such as Sai Kung and Aberdeen.

SAEMANGEUM LAKE SEDIMENT ECOTOXICITY ASSESSMENT USING BENTHIC AMPHIPODS, POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) AND HEAVY METAL ANALYSIS, AND TOTAL ORGANIC CARBON ANALYSIS

Lee H.J.¹, Jeon G.E.¹, Kwon B.-O.¹, Baek J.M.¹, Hyeon S.W.¹, Cho J.H.¹, Yoon S.J.², Khim J.S.²

¹ Kunsan National University, Kunsan, Republic of Korea

² Seoul National University, Seoul, Republic of Korea

This study confirmed the presence or absence of toxicity through an ecotoxicity assessment of sediments from Saemangeum Lake, located in the West Sea of South Korea, from 2020 to 2023, and analyzed PAHs (Polycyclic Aromatic Hydrocarbons), heavy metals, and TOC (Total Organic Carbon) to identify the cause. From 2020 to 2023, sediment samples were collected from seven stations in Saemangeum Lake using Van Veen Grab Sampler on a Vessel, and sediments from two stations at the Mangyeong River Estuary and Sinsi Island, a control site, were collected using a Spatula. *Monocorophium uenoi* was selected as the ecotoxicity test species according to the marine environmental engineering test standards, and a sensitivity test was conducted to confirm that it was a suitable species for this experiment. As a result, it was confirmed that it was a suitable species with an average of 1.61 mg Cd L⁻¹. As a result of the ecotoxicity assessment, the relative average survival rate in 2020 was 16.5%, the relative average survival rate in 2021 was 10.8%, the average was 25.5% in 2022, and the average was 13.6% in 2023. As a result of the PAHs analysis, the average was 57 ng g⁻¹ in 2021, 61.7 ng g⁻¹ in 2022, and 39 ng g⁻¹ in 2023. As a result of Heavy Metal analysis, the concentrations of analytes (As, Cd, Cr, Hg, Ni, Pb, Cu, Zn, Li) did not exceed the cautionary standards at all peaks. As a result of TOC analysis, the average was 0.71% in 2023. Additionally, TOC showed a significant correlation with the relative mean survival rate. (p<0.001) Through this study, the presence or absence of toxicity of Saemangeum Lake sediments was confirmed from 2020 to 2023, and among a total of 9 peaks, St.2 in 2020, St.5 in 2022, and JG peak were confirmed to be 'toxic'. Through additional research, we plan to determine whether there are other toxic substances in Saemangeum Lake sediments.

TOXICITY OF POLYETHYLENE TEREPHTHALATE MICROFRAGMENTS ON THE MONOGONONT ROTIFER *BRACHIONUS KOREANUS*: INGESTION, ACUTE AND CHRONIC TOXICITY, AND TRANSCRIPTOMIC AND METABOLOMIC MODULATION

Lee Y.-M.¹, Yoo J.-W.¹, Park J.-S.¹, Lee Y.H.¹, Choi T.J.¹, Jeong T.Y.² and Kim C.B.¹

¹ Sangmyung University, Republic of Korea

² Hankuk University of Foreign Studies, Republic of Korea

Due to increasing production and usage of plastics, plastic pollution has been considered one of the most important environmental problems. In the ocean, plastic debris can be degraded into smaller particles, such as microplastics (MPs; < 5 mm), which can be consumed by marine organisms, including small zooplanktons, inducing deleterious effects such as metabolic disorder, neurotoxicity, and reproductive disorder. Although polyethylene terephthalate (PET) particles are dominant type of MPs in the ocean, its toxic effects on marine organisms are poorly understood. Thus, we investigated the toxic effects of PET particles (mean diameter: 15- μ m) on monogonont rotifer *Brachionus koreanus*. Although ingestion test showed that PETs were easily ingested by *B. koreanus*, there was no significant toxicity of PETs on survival of *B. koreanus* up to 400 μ g/mL. Chronic toxicity test also indicated that PETs up to 200 μ g/mL did not affect development, reproduction, and population growth. These results suggest less toxicity of PETs on rotifer at individual level. However, transcriptomic and metabolomic analysis showed that 489 number of genes and 109 number of metabolites were significantly affected after exposure to 200 μ g/mL of PETs. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis confirmed that metabolic pathway, choline metabolism in cancer, and glutamatergic synapse related genes, and starch and sucrose metabolism, galactose metabolism, pantothenate and CoA biosynthesis, glycerophospholipid metabolism, and various amino acid biosynthesis related metabolites were significantly affected by PETs exposure. Our findings suggest that the toxicity of PETs on *B. koreanus* was not severe at the individual level, but there was potential toxicity of PETs on metabolic disorder and neurotoxicity.

A CHROMOSOME-LEVEL GENOME ASSEMBLY OF THE MARINE MEDAKA *ORYZIAS MELASTIGMA*: GENOME-WIDE IDENTIFICATION OF 778 G PROTEIN-COUPLED RECEPTOR (GPCR) GENES FOR A FULLY ANNOTATED FISH GPCR REPERTORIE FOR ECOPHYSIOLOGY

Kim D.-H.¹, Kim M.-S.¹, Wu R.S.S.² and Lee J.-S.¹

¹Department of Biological Sciences, College of Science, Sungkyunkwan University, Suwon 16419, South Korea

²The Education University of Hong Kong, China

The marine medaka *Oryzias melastigma* is considered to be a useful fish model for marine and estuarine ecotoxicology studies and has good potential for field-based population genomics because of its geographical distribution in Asian estuarine and coastal areas. In this study, we generated a chromosome-level assembly of the *O. melastigma* genome using Hi-C sequencing. The total length of the assembled genome was 843.30 Mb, with 794.00 Mb (94.15%) anchored into 24 chromosomes. The chromosome-level genome contained 25,215 protein-coding genes, of which 20,570 (%) genes were functionally annotated. Moreover, a total of 778 GPCRs were fully identified and annotated based on the chromosomal-level genome of *O. melastigma*. Comparison of the GPCRs of *O. melastigma* to those in both human and other fish species reveals a high level of orthology of class A receptors; however, significant differentiation of chemosensory receptors (ORs, GRs) was found in fish species. Our results provide a better understanding of the advanced repertoires of GPCR and expand our knowledge of fish eco-physiological mechanisms in response to various environmental stimuli.

CRISPR/CAS9-MEDIATED GENE TARGETING OF TWO DESATURASE GENES IN THE WATER FLEA *DAPHNIA MAGNA*: IMPLICATION TO FATTY ACID AND EXPRESSION PROFILES

Byeon E.¹, Yoon D.-S.¹, Jeong H.¹, Park H.G.², Shin K.-H.³, Lee M.-C.⁴, and Lee J.-S.¹

¹ Sungkyunkwan University, Suwon, South Korea

² Gangneung-Wonju National University, Gangneung, South Korea

³ Hanyang University, Ansan, South Korea

⁴ Gachon University, Seongnam, South Korea

Fatty acid desaturases are a class of enzymes that facilitate the introduction of a double bond (unsaturation) into a fatty acid chain. This particular group of enzymes is nearly ubiquitous in most living organisms, where they modulate the fluidity of lipids inside cellular membranes. In addition, these enzymes play a crucial role in manufacturing polyunsaturated fatty acids (PUFAs), which serve as precursors for several biologically active compounds, such as hormones and growth regulators. Here, we first identified lipid metabolism-related genes (elongase and desaturase) in the freshwater flea *Daphnia magna*, where 15 elongase and 6 desaturase genes were found. Among them, to investigate the possible role of desaturase in fatty acid synthesis and the biological function of organisms, fatty acid composition, *in vivo* parameters, and enzyme activation were confirmed using the *desaturase 9 (D9)* and *5/6 (D5/6)* genes knock-out *D. magna* generated using the CRISPR/Cas9 system. As a result, a daphnia-specific fatty acid profile was observed in the fatty acid composition analysis of wild-type *D. magna*. When exposed to thermal stress to confirm the desaturase function, the total fatty acid (TFA) synthesized in the mutation compared to the wild type was similar, while the ratio of each fatty acid composition was shown to be different. In addition, *in vivo* investigations found high mortality and decreased fecundity due to high-temperature stress in the *D5/6* knock-out line. Taken together, we have identified the lipid metabolism-related genes in *D. magna* and confirmed that the function of the enzyme is daphnia-specific. Furthermore, a potential possibility has been observed that the *D5/6* gene is primarily involved in fatty acid synthesis and physiological function in *D. magna*.

Keywords: Fatty acid, desaturase, CRISPR/Cas9, *Daphnia magna*

INSIGHTS INTO THE EFFECTS OF ANTI-TUBERCULOSIS COMPOUNDS AT GENE EXPRESSION LEVEL IN DAPHNIA MAGNA

Chen W.Y.¹, Su Y.C.², Huang S.T.^{1,3}, Huang H.M.¹

¹ Department of Ecology and Environmental Resources, National University of Tainan, Taiwan

² Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Taiwan

³ Civil and Environmental Engineering, University of Massachusetts-Amherst, USA

Isoniazid (INH), rifampicin (RMP), and ethambutol (EMB) are the first-line drugs for tuberculosis treatment and have been detected in aquatic environments. There is a potential exposure risk to aquatic organisms. Knowledge of the molecular mechanism of anti-TB drugs provides insight into ecotoxicity and benefits the environmental risk assessment of epigenetic-modulating compounds. We investigated the molecular effects of anti-TB drugs, INH, RMP, and EMB on *Daphnia magna* using RNA sequencing. The constitutive expression of differentially expressed molecular pathways among these anti-TB drugs is detected. We compared transcript expression of exposure treatment with control (total number of transcript expression 12,759) and 439 genes with significantly differentially expressed genes (DEGs) shared by the three anti-TB drugs. Gene ontology enrichment analysis divided DEGs into three categories (molecular function, cellular components, and biological process); anti-TB drugs shared the toxic mechanism on the metabolic process (biosynthesis process and organic substance metabolic process) and establishment of protein localization. Further, these three anti-TB drugs have distinct toxicological mechanisms of action; EMB primarily targets neurotoxicity-related processes, including regulation of neuronal synaptic plasticity, neurotransmitter receptor transporter, and central nervous system development. INH influences the reproduction processes related to oocytes. This study used gene expression signatures to improve our understanding of the mechanism of toxicity of anti-TB drugs on aquatic organisms.

UNVEILING THE GILL SYMBIOTIC COMMUNITY IN *THYASIRA TOKUNAGAI* KURODA & HABE AND INSIGHTS INTO SULFIDE DETOXIFICATION IN THE EAST SEA-BYEONG WASTE DUMPING SITE, KOREA

Shin J.-S.¹, Kwon K.K.², Kim C.-J.³, Choi K.-S.¹

¹ Department of Marine Life Science (BK 21 FOUR) and Marine Science Institute, Jeju National University, Republic of Korea

² Marine Biotechnology Research Center, Korea Institute of Ocean Science and Technology, Republic of Korea

³ Marine Environment Research Department, Korea Institute of Ocean Science and Technology, Republic of Korea

The continuous influx of organic matter into marine benthic environments leads to the accumulation of sulfides in sediments and water columns. High concentrations of sulfides act as toxins to benthic organisms, inhibiting oxygen-transporting enzymes such as chrome c oxidase. However, the marine bivalve family Thyasiridae has successfully adapted to toxic environments because the sulfur-oxidizing symbiotic bacteria inhabiting their gills neutralize sulfide into comparatively less toxic sulfate. In this study, we collected the opportunistic Thyasirid species, *Thyasira tokunagai*, from the East Sea-Byeong waste dumping area in South Korea and analyzed the gill symbiotic microbial community involved in sulfide metabolism. To identify symbiotic bacteria, 16S rRNA metagenome sequencing was performed on gill tissues of *T. tokunagai*, and the operational taxonomic unit (OTU) was clustered with a similarity threshold of 97% or higher. The analysis of the gill symbiotic bacterial diversity in *T. tokunagai* at the class level revealed that there were 20 OTUs with a relative abundance of more than 1%, with Epsilonproteobacteria being the most dominant at an average of 55.2%. At the genus level, there were 44 OTUs with a relative abundance of more than 1%. The *Sulfurovum*-like OTU and *Sulfurimonas*-like OTU, belonging to the Epsilonproteobacteria, were the most dominant types in the gills of *T. tokunagai*. Phylogenetic analysis of these OTUs revealed that they were clustered within the group of sulfur-oxidizing bacteria. In addition, metagenome sequencing revealed bacteria that use various materials for metabolisms, such as *Spirochaeta* spp. and *Methylophaga* spp. (chemo-organotrophic) and the genus *Methanomicrobium* spp. (Methanotrophic) and are thought to have additional symbiotic mechanisms as well as sulfur-oxidation. Although there is limited information on the analyzed symbiotic bacteria, further analysis, such as whole genome sequencing, is required. The dominance of sulfide oxidizing symbionts in *T. tokunagai* gills strongly suggested a successful adaptation mechanism in a reducing environment.

TEBUCONAZOLE INDUCES REPRODUCTIVE TOXICITY VIA FOXL2-MEDIATED PHOSPHORYLATION SIGNALING IN ZF4 CELLS OF ZEBRAFISH

Wang A.X., Ma Y.F. and Gui W.J.

Zhejiang University, China

Tebuconazole is a widely used fungicide. Our previous study found that tebuconazole exposure could inhibit the expression of aromatase Cyp19A in zebrafish, negatively regulate FoxL2, and induce the male bias sex differentiation of zebrafish larvae. To evaluate the mechanism of anti-estrogen endocrine disruption induced by tebuconazole, particularly the effect of tebuconazole on FoxL2 protein phosphorylation. A strategy of heterologous expression zebrafish protein (zFoxL2) in *Escherichia coli* (*E. coli*) BL21(DE3) host cells was demonstrated and FoxL2 rabbit anti-fish polyclonal antibody was prepared. Further, the method of in vitro experiment of zebrafish fibroblast cell line (ZF4 cells) was adopted. qRT-PCR and Western blotting analysis indicated that there was no significant change in FoxL2 protein expression in the tebuconazole exposed group for 8h, which was consistent with the results of *foxl2* gene in the 100 μ M tebuconazole exposure groups for 2, 4, 8 and 24 h. Interestingly, exposed to tebuconazole for 8h significantly decreased the pFoxL2(ser238) levels by 42.4% ($p=0.001$) that suggested that inhibiting effect of tebuconazole on FoxL2 protein phosphorylation. Transcriptome results showed that a total of 312 (103 up-regulated and 209 down-regulated) differentially expressed genes were observed, among which 15 genes were related to phosphorylation. A total of 65 subunit phosphorylation sites of kinases was predicted on the zFoxL2. Co-immunoprecipitation assay found that the binding ability of zFoxL2 and pFoxL2(ser238) to other proteins were decreased by 17.02% ($p=0.029$) and 31.39% ($p=0.027$) after exposure to 100 μ M of tebuconazole for 8h, respectively. The pull-down results showed that the amount of binding protein in the tebuconazole exposed group was reduced by 17.96% ($p=0.021$), but not the expression of FoxL2 protein. The present study elucidated the inhibition effect of tebuconazole on FoxL2 protein phosphorylation in zebrafish and provided relevant new basis for comprehensive evaluation of environmental health risks of triazole fungicides.

WATERBORNE TEBUCONAZOLE EXPOSURE INDUCES MALE-BIASED SEX DIFFERENTIATION IN ZEBRAFISH (DANIO RERIO) LARVAE VIA AROMATASE INHIBITION

Yang S.Y., Qiao K., Gui W.J.

Zhejiang University, China

Tebuconazole is a widely used fungicide for various crops that targets sterol 14-*a*-demethylase (CYP51) in fungi. However, attention has shifted to aromatase (CYP19) due to limited research indicating its reproductive impact on aquatic organisms. Herein, zebrafish were exposed to 0.5 mg/L tebuconazole at different developmental stages. The proportion of males increased significantly after long-term exposure during the sex differentiation phase (0–60, 5–60, and 19–60 days postfertilization (dpf)). Testosterone levels increased and 17 β -estradiol and *cyp19a1a* expression levels decreased during the 5–60 dpf exposure, while the sex ratio was equally distributed on coexposure with 50 ng/L 17 β -estradiol. Chemically activated luciferase gene expression bioassays determined that the male-biased sex differentiation was not caused by tebuconazole directly binding to sex hormone receptors. Protein expression and phosphorylation levels were specifically altered in the vascular endothelial growth factor signaling pathway despite the possibility of tebuconazole directly interacting with kinases. Aromatase was selected for a potential target analysis. Molecular docking and aromatase activity assays demonstrated the interactions between tebuconazole and aromatase, highlighting that tebuconazole poses a threat to fish populations by inducing a gender imbalance.

IMMUNOTOXICITY OF BPA AND ITS REPLACEMENT CHEMICALS

Li M., Li R., Li R.*, Lai K.*

Key Laboratory of Environmental Pollution and Integrative Omics, Guilin Medical University, Guangxi, China

Bisphenol A (BPA), a well-documented endocrine disrupting chemical, is widely used in daily plastic products. Beside the endocrine disrupting effect, BPA was also reported to exhibit immunotoxicity. Because of its adverse effects on the human health, many countries have banned the use of BPA. In the recent years, many BPA replacement chemicals such as bisphenol B (BPB), bisphenol E (BPE), bisphenol S (BPS), and bisphenol fluorene (BHPF) were produced to replace BPA. Because these replacement chemicals have similar chemical structure with BPA, they may also harm the human health. However, their immunotoxicity and the molecular mechanisms underlying the toxicity is still largely unknown. In the present study, we exposed the primary human lymphocytes to BPA and its replacement chemicals. Our results showed that the exposure of BPA and its replacement chemicals altered the cytokine production, such as IL-1b, IL-5, IL-6, IL-8, IFN-a2B, and TNF-a, in the lymphocytes. And, BPA and BHPF exhibited a larger effect. By using the comparative transcriptomic analysis, we further investigated the altered biological processes and signaling pathways caused by BHPF exposure. Our data highlighted the alteration of immune response, T cell's functions, and cytokine-cytokine receptor interaction in the human lymphocytes through the deregulation of gene cluster including *il21*, *il3*, *il23r*, *il27*, *ifnl2*, *ifnl3*, *ccl19*, *fut7*, *cd160*, *eda*, *ccr8*, and *nodal*. Our result, for the first time, showed the immunotoxicity of BHPF, and unfold the mechanisms underlying the immunotoxicity.

Biological responses to chemical mixtures and/or multiple stressors: P80

PENAEID SHRIMP COUNTERACT HIGH AMMONIA STRESS BY GENERATING AND USING FUNCTIONAL PEPTIDES FROM HEMOCYANIN, SUCH AS HMCs27

Zhao M.M., Zheng Z.H., Wang C.Q., Yao D.F., Lin Z.Y., Zhao Y.Z., Chen X.L., Li S.K., Aweya J.J., and Zhang Y.L.

Shantou University, China

Agricultural and anthropogenic activities release high ammonia levels into aquatic ecosystems, severely affecting aquatic organisms. Penaeid shrimp can survive high ammonia stress conditions, but the underlying molecular mechanisms are unknown. Here, total hemocyanin and oxyhemocyanin levels decreased in *Penaeus vannamei* plasma under high ammonia stress. When shrimp were subjected to high ammonia stress for 12 h, 24 hemocyanin (HMC) derived peptides were identified in shrimp plasma, among which one peptide, designated as HMCs27, was chosen for further analysis. Shrimp survival was significantly enhanced after treatment with the recombinant protein of HMCs27 (rHMCs27), followed by high ammonia stress. Transcriptome analysis of shrimp hepatopancreas after treatment with or without rHMCs27 followed by high ammonia stress revealed 973 significantly dysregulated genes, notable among which were genes involved in oxidation and metabolism, such as cytochrome C, catalase (CAT), isocitrate dehydrogenase, superoxide dismutase (SOD), trypsin, chymotrypsin, glutathione peroxidase, glutathione s-transferase (GST), and alanine aminotransferase (ALT). In addition, levels of key biochemical indicators, such as SOD, CAT, and total antioxidant capacity (T-AOC), were significantly enhanced, whereas hepatopancreas malondialdehyde levels and plasma pH, NH₃, GST, and ALT levels were significantly decreased after rHMCs27 treatment followed by high ammonia stress. Moreover, high ammonia stress induced hepatopancreas tissue injury and apoptosis, but rHMCs27 treatment ameliorated these effects. Collectively, the current study revealed that in response to high ammonia stress, shrimp generate functional peptides, such as peptide HMCs27 from hemocyanin, which helps to attenuate the ammonia toxicity by enhancing the antioxidant system and the tricarboxylic acid cycle to decrease plasma NH₃ levels and pH.

Biological responses to chemical mixtures and/or multiple stressors: P81

AMMONIA STRESS AFFECTS THE STRUCTURE AND FUNCTION OF HEMOCYANIN IN *PENAEUS VANNAMEI*

Zhao M.M., Aweya J.J., Feng Q., Zheng Z.H., Yao D.F., Zhao Y.Z., Chen X.L., and Zhang Y.L.
Shantou University, China

Anthropogenic factors and climate change have serious effects on the aquatic ecosystem and aquaculture. Among water pollutants, ammonia has the greatest impact on aquaculture organisms such as penaeid shrimp because it makes them more susceptible to infections. In this study, we explored the effects of ammonia stress (0, 50, 100, and 150 mg/L) on the molecular structure and functions of the multifunctional respiratory protein hemocyanin (HMC) in *Penaeus vannamei*. While the mRNA expression of *Penaeus vannamei* hemocyanin (PvHMC) was up-regulated after ammonia stress, both plasma hemocyanin protein and oxyhemocyanin (OxyHMC) levels decreased. Moreover, ammonia stress changed the molecular structure of hemocyanin, modulated the expression of protein phosphatase 2 A (PP2A) and casein kinase 2 α (CK2 α) to regulate the phosphorylation modification of hemocyanin, and enhanced its degradation into fragments by trypsin. Under moderate ammonia stress conditions, hemocyanin also undergoes glycosylation to improve its *in vitro* antibacterial activity and binding with Gram-negative (*Vibrio parahaemolyticus*) and Gram-positive (*Staphylococcus aureus*) bacteria, albeit differently. The current findings indicate that *P. vannamei* hemocyanin undergoes adaptive molecular modifications under ammonia stress enabling the shrimp to survive and counteract the consequences of the stress.

Biological responses to chemical mixtures and/or multiple stressors: P82

SINGLE AND COMBINED EFFECTS OF MERCURY AND POLYSTYRENE BEADS ON MULTIXENOBIOTIC RESISTANCE (MXR) IN THE BRACKISH WATER FLEA *DIAPHANOSOMA CELEBENSIS*

Lee Y.-M. and Yoo J.-W.

Sangmyung University, Republic of Korea

Due to their wide distribution in the ocean and toxicity on marine organisms, nano- and microplastics (NMPs) and mercury (Hg) are considered major contaminants in the marine ecosystem. Although NMPs are toxic to marine organisms by itself, they can act as a vector of ambient pollutants, including Hg, due to high adsorption capacity of their surface. Previous studies reported that toxicity of Hg can be modulated by NMPs co-exposure, but the results of their toxicological interaction were controversial. Because smaller the size of the NMPs, the larger the surface/volume ratio, the size of NMPs is an important factor in interaction with ambient pollutant. Thus, for better understanding the toxicological interaction between NMPs and Hg, study on the size-dependent effects of NMPs on interaction with Hg should be conducted. In this study, we investigated the Hg adsorption onto three different sized polystyrene beads (PS; 0.05-, 0.5-, and 6- μm), and Hg bioaccumulation and multixenobiotic resistance (MXR) response conferred by ATP-binding cassette (ABC) transporters in the brackish water flea *Diaphanosoma celebensis* after single and combined exposure to Hg (0.2 to 0.8 $\mu\text{g/L}$) and PS beads (1 mg/L) for 48 h. After 24 h incubation, Hg were adsorbed onto 0.05-, 0.5-, and 6- μm PS at 17.80, 3.32, and 2.61 $\mu\text{g/g}^{\text{PS}}$, respectively. After 48 h exposure to Hg, Hg bioaccumulation in *D. celebensis* was 6.04 $\mu\text{g/g}^{\text{biota}}$, which was increased by 0.05- μm PS co-exposure (21.03 $\mu\text{g/g}^{\text{biota}}$) and decreased by 0.5- and 6- μm PS (5.39 and 3.76 $\mu\text{g/g}^{\text{biota}}$, respectively). In addition, activity of ABCB and ABCC was increased by Hg exposure but inhibited by 0.05- and 0.5- μm PS co-exposure. Similarly transcriptional modulation of ABCBs and ABCCs (except *ABCB1-1* and *ABCC1-1*) was also significantly decreased upon Hg+NP exposure compared to sole-Hg exposure. These results suggest that the PS beads, especially 0.05- μm PS, can disrupt MXR, resulting in increase of Hg toxicity on *D. celebensis*. Our findings will be helpful for a better understanding of toxicological interaction between Hg and NMPs on marine zooplanktons.

Biological responses to chemical mixtures and/or multiple stressors: P83

METHODOLOGICAL ADVANCES AND FUTURE DIRECTIONS OF MICROALGAL BIOASSAYS FOR ENVIRONMENTAL RISK ASSESSMENTS

Lee J., Hong S., Khim J.S.

Kongju National University, Republic of Korea

Microalgal bioassays are widely applied to screen the toxicity of various chemicals due to multiple advantages, including high sensitivity, short test duration, and cost-effectiveness. Yet, information on how microalgal bioassays are applied for environmental risk assessments remain limited, despite the wide variety of methods used. Here, we summarize and explore published articles on microalgal bioassay methods for environmental risk assessments, including the types of samples, sample preparation methods, and endpoints, highlighting key scientific advancements. Eighty-nine published articles since 2002 were collected and reviewed, and keyword analysis was performed using bibliographic analysis. Traditionally, most studies implementing microalgal bioassays focused on water samples (44%) with passive samplers (38%). Studies using the direct exposure method (41%) of injecting microalgae into sampled water mainly evaluated toxic effects by growth inhibition (63%). With the development of new instruments, various automatic sampling techniques, targeted and non-targeted analyses, and in situ bioanalytical methods with multiple endpoints have emerged. Future research is needed to predict and identify causative toxicants affecting microalgae, and to quantify the cause-effect relationships between contaminants and adverse effects. This study provides the first comprehensive overview of recent advances in microalgal bioassays performed with environment samples, identifying future research directions based on our current understanding and limitations.

Biological responses to chemical mixtures and/or multiple stressors: P84

THERMAL EFFLUENT REDUCTION EFFECTS ON THE PLANKTON COMPOSITION AND LEVELS IN A TROPICAL NUCLEAR POWER PLANT

Chen T.-C.¹, Wong S.-L.², Chen A.C.T.¹ and Chen M.-H.^{1,3,*}

¹ National Sun Yat-sen University, Taiwan, ROC

² National Pingtung University of Science and Technology, Taiwan, ROC

³ Kaohsiung Medical University, Taiwan, ROC

Using 26 years of plankton monitoring data, we investigated the influence of the reduction in thermal effluent from a tropical nuclear power plant, the third nuclear power plant (NPP3) in Nanwan Bay, southern Taiwan, with an annual water temperature range of 22~28°C, on the variation of plankton composition and their levels. Our findings revealed that there is a natural seasonal variation in the plankton composition, based on 96 genera of phytoplankton and 34 categories of zooplankton, in any year. The seasonal phytoplankton density was 971±1125, 4,603±15,969, 2,067±4,341, and 331±414 cells/l, and zooplankton abundance was 417±225, 336±193, 300±158, and 328±199 ind./m³, for spring, summer, autumn and winter, respectively. During the maintenance shutdowns in winter, the thermal effluent reduced to half, and the third dominant taxon of zooplankton, appendicularia, has found to replace with chaetognaths. There were no significant differences in plankton abundance between normal operation and maintenance periods in the spring and autumn. Overall, the influence of power plant operation and maintenance on plankton composition and abundance in Nanwan Bay appears to be limited, due to the good water circulation in the Bay. This study offers valuable insights into the impacts of reducing the thermal effluent of a nuclear power plant on marine ecosystems, and the ecological consequences.

Keywords: zooplankton; phytoplankton; assemblages; thermal effluent; Nanwan Bay

Biological responses to chemical mixtures and/or multiple stressors: P85

METAGENOMICS SURVEILLANCE REVEALS DIFFERENT STRUCTURE AND FUNCTION OF PROKARYOTIC MICROBIAL COMMUNITY ASSOCIATED WITH MANGROVE PNEUMATOPHORES

Lee F.W.F.^{*,1,2,3}, Bohra V.^{1,2}, Lai K.K.Y.^{1,2}, Lam K.L.^{1,2}, Tam N.F.Y.^{1,2,3}, Xu S.J.L.^{1,2}, Zhou H.C.^{2,4}, Li F.L.^{1,2}

¹ School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

² Greater Bay Area Mangrove Wetland Research & Development Centre, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

³ State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong SAR, China

⁴ College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, 518071, China

Mangrove wetlands located in intertidal zone along tropical and subtropical coasts are subject to frequent tidal flooding, and mangrove plants are submerged at high tides. Aerial roots grown against gravity of mangrove plants distributed at the foreshore help to breathe air thus adapt to oxygen-deprived mud. A common type of these roots in mangrove wetlands is pneumatophores and their surfaces can host diverse microbes. Pneumatophores and their surrounding matrices are characterized by periodic tidal cycles that induce gradual variation in environmental conditions across small spatial scales, leading to the formation of characteristic microbial zones where microbial community structure and function vary. However, the roles of these microbial communities in various biogeochemical processes, and their variations and relationships with pneumatophores remain unexplored.

In this study, we aimed to investigate the structure and function of the prokaryotic microbial community associated with pneumatophores and compare with their surrounding matrices in the Ting Kok mangroves of Hong Kong. We analyzed two types of pneumatophore samples: microbes directly on the pneumatophore surface (pneumatophore epiphytes, PE) and those on the sediment adhered to the pneumatophore surface (pneumatophore sediment, PS). The surrounding matrices included mangrove sediment (MS), mudflat sediment (MF), and tidal seawater (SW). Results revealed that bacteria made up over 95% of the total microbial DNA in all samples, with Proteobacteria being the most abundant phylum, followed by Bacteroidetes. The bacterial community profile associated with pneumatophores (both PE and PS) was similar to that of SW but significantly differed from that of MF and MS. The archaeal community remained uniform across different matrices and was dominated by Euryarchaeota. Pneumatophore-associated microbes (PS and PE) had a higher relative abundance of genes for both assimilatory and dissimilatory nitrate reduction pathways involving nitrate reductase. However, genes for nitrogen fixation and denitrification pathways were more abundant in MF and MS, which also had more genes for methanogenesis, particularly heterodisulfide reductase. Genes encoding enzymes for sulfur metabolism showed different patterns of variation in abundance among different matrices, with higher abundance in SW, MF, and MS. These findings demonstrated the uniqueness of microbial community associated with mangrove pneumatophores. To our knowledge, this is the first attempt to reveal the prokaryotic functions on pneumatophores and their surrounding matrices in mangrove ecosystems using metagenomic sequencing.

Biological responses to chemical mixtures and/or multiple stressors: P86

HABITAT VARIATION OF PROKARYOTIC COMMUNITIES AND THEIR CONNECTION WITH ENVIRONMENTAL FACTORS IN MAI PO RAMSAR SEDIMENTS

Lam K.L.¹, Tam N.F.Y.^{1,2}, Xu S.J.L.¹, Mo W.Y.¹, Chan P.L.¹, Lee F.W.F.^{1,2}

¹ School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

² State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong SAR, China

The Mai Po Nature Reserve, part of the Ramsar site in Hong Kong, consisting of mudflat, mangrove, and inter-tidal shrimp ponds (*gei wai*) habitats is crucial for the protection of biodiversity, particularly waterbirds. However, the Reserve is also polluted by anthropogenic activities, particularly antibiotic and heavy metals. Previous research focused on the degree of pollutants in water and sediments, and the effects to animals and plants. However, the effects of pollutants on microbial composition, diversity, and functional profiles among different habitats within the Reserve are seldom reported, despite the ecological significance of sediment microbes. This study examined the community structure and diversity of sediment microbiome in three different habitats: mudflat, mangrove and *gei wai* within the Reserve using high-throughput sequencing of 16S rRNA amplicon. Results revealed that *Proteobacteria* was the most abundant bacterial phylum in all three habitats, ranging from 44% to 61%. *Acidobacteria*, *Gemmatimonadetes*, and *Thaumarchaeota* were significantly enriched in mangrove sediments, while *Nitrospirae* was predominantly found in *gei wai*. Notably, pathogenic *Vibrio* species were detected in all sediment samples, posing a potential threat to the health of waterbird and other organisms. Mangrove sediments exhibited higher microbial diversity compared to mudflat, suggesting habitat type could influence microbial diversity. Microbial functions were predicted through bioinformatic analysis with Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). Carbohydrate and amino acid metabolism were the prevalent metabolic pathways, with nitrogen metabolism pathways significantly higher in mangrove sediments than that of the other two habitats. Approximately 800 intrinsic antibiotic resistance genes (iARGs) were identified in sediment samples using functional gene annotation and then leveraging the Comprehensive Antibiotic Resistance Database (CARD). Antibiotic inactivation was the most prevalent resistance mechanism, with beta-lactam resistance being the most prevalence resistance family. Significant variations in the relative abundance of specific iARGs were also observed across different habitats. Furthermore, variation partitioning analysis was determined that the combined effect of antibiotics, heavy metals and sediment characteristics explained greater variation of the composition of microbiome and iARGs compared to their individual effects, indicating a synergistic relationship.

This study offers insights into the relationships among sediment characteristics; anthropogenic pollution (heavy metals and antibiotics); the composition, diversity and predicted functions of sediment microbiome; and the abundance of antibiotic resistance genes. The findings not only enhance our understanding on the ecological importance of sediment microbiomes but also consequences of anthropogenic pollution in Ramsar, that are essential scientific information guiding the conservation strategy and management of coastal wetlands.

Biological responses to chemical mixtures and/or multiple stressors: P87

COMPARE EXOTIC AND NATIVE MANGROVE PLANT SPECIES ON COMPOSITION AND FUNCTION OF SOIL MICROBIAL COMMUNITY IN FUTIAN, SHENZHEN, CHINA

Tian T. T.¹, Yang Q.^{2*}, Tam N. F. Y.^{3,4,5*}, Li F.L.^{3,5}, Wong Y.S.³, Hang L.N.⁶, Luan T.G.⁶, Lan C.Y.⁶

¹ National-Regional Joint Engineering Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-environmental and Soil Sciences, Guangdong Academy of Sciences, Guangzhou, China

² Guangdong Neilingding Futian National Nature Reserve, Shenzhen, China

³ Greater Bay Area Mangrove Wetland Research & Development Centre, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

⁴ School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

⁵ City University of Hong Kong Shenzhen Research Institute, Shenzhen, P. R. China, Shenzhen

⁶ State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-Sen University, Guangzhou, China

Mangrove restoration by the exotic mangrove genus *Sonneratia* has been practiced as a strategy to reconstruct mangrove wetlands along South China coasts, particularly in Guangdong Province. However, studies on how *Sonneratia* affects the soil quality in mangrove wetlands are still limited. In the present study, we compared the variations in soil physicochemical properties, microbial biomass, microbial community composition and function between exotic mangrove plant species (*Sonneratia apetala* and *Sonneratia caseolaris*) and native species (*Aegiceras corniculatum* and *Kandelia obovate*) at stand ages of 10-years in Futian National Nature Reserve, Shenzhen Bay, China. Surface soils (0-10 cm) were collected from the mangrove-dominant area planted with four mangrove species. Compared with the native mangrove species, *S. caseolaris* and *S. apetala* had lower soil microbial biomass, but had greater microbial respiration (SMA), metabolic quotient (qCO₂) and enzymatic activities, including cellulase, β-glucosidase and protease. Microbial communities based on phospholipid fatty acid analyses (PFLAs) revealed that the ratios of Gram-positive to Gram-negative bacteria of both *Sonneratia* species were significantly lower than the two native mangrove species, however, the ratios of monounsaturated bacteria to saturated bacteria of *S. caseolaris* were significantly higher than the other mangrove species. Redundancy analyses (RDA) revealed that pH, carbon and nitrogen were significant environmental factors (P < 0.05) affecting microbial community compositions and driving the soil enzymatic activities. These results indicated that the changes in microbial community compositions were accompanied by changes in the activity of some enzymes involved in carbon and nitrogen cycling, which may be of great significance in understanding the soil microbial responses to the exotic mangrove species introduction.

Biological responses to chemical mixtures and/or multiple stressors: P88

COMPARISON OF FREE-LIVING MARINE NEMATODE COMMUNITY IN INTERSPERSED HABITATS OF MANGROVES AND *SPOROBOLUS ALTERNIFLORUS* IN ZHANJIANG · CHINA

Zhu H.L.^{1,3}, Gan K.Y.^{1,4}, Chen Z.T.⁴, Li F.L.^{1,2}, Lee F.W.F.^{1,2}, Xu S.J.L.^{1,2}, Zhou H.C.^{1,4*}, Tam N.F.Y.^{1, 2, 3*}

¹ Greater Bay Area Coastal Mangrove Wetland Research & Development Centre, Guangdong Neilingding Futian National Nature Reserve, Shenzhen, China

² School of Science and Technology, Hong Kong Metropolitan University, Hong Kong, China

³ City University of Hong Kong Shenzhen Research Institute, Shenzhen, State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong, China

⁴ College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China

Since the spread of a grass species, *Sporobolus alterniflorus* to the Leizhou Peninsula, China in 2006, there has been an overall incremental trend in its growth and spread into intertidal mangrove habitats. As an exotic invasive species, *S. alterniflorus* could compete with native mangrove species, replace native mangrove plants and become the dominant species, affecting the animal community in mangrove wetlands. However, the effects of *S. alterniflorus* on marine nematode community and how they differ from that of mangrove plants have seldom been studied, even though nematode community is important in ecological functions of intertidal habitats. The present study aims to compare the free-living nematode community in interspersed habitats of mangrove plant species dominated by *Avicennia marina* and *Sonneratia apetala*, and a grass species, *Sporobolus alterniflorus* in Zhanjiang, China. Bulk sediments were collected from the areas of *A. marina*, *S. alterniflorus*, *S. apetala* and mudflat in Leizhou Peninsula, Zhanjiang, in autumn 2021. The rhizosphere of *A. marina* and *S. alterniflorus* were also collected. The results showed that the invasion of *Sporobolus alterniflorus* resulted in a slight decrease in meiofauna abundance compared to the mudflat, but there was an increase in the number of genera of marine nematodes, as well as a change in the dominant genera and a decrease the index of trophic diversity (ITD). The difference in marine nematode communities collected from bulk sediment and rhizosphere in *S. alterniflorus* habitats was smaller compared to that in mangrove habitats. The nematode community structure was also more comparable between the same type of sediments in *S. alterniflorus* and native mangrove habitats. These findings indicate the impacts of the exotic invasive *Sporobolus alterniflorus* spreading into intertidal mangroves cannot be ignored and in-depth researches related to its effects on benthic macro- and micro-animal community should be conducted.

Biological responses to chemical mixtures and/or multiple stressors: P89

PHYSIOLOGICAL EFFECTS AND MOLECULAR RESPONSE IN THE MARINE ROTIFER *BRACHIONUS PLICATILIS* AFTER COMBINED EXPOSURE TO NANOPLASTICS AND COPPER

Byeon E.¹, Wang C.¹, Jeong H.¹, Lee J.-S.¹, Maszczyk P.², Sayed A.E.-D.H.³, Hwang U.-K.⁴, Kim H.S.¹ and Lee J.S.¹

¹ Sungkyunkwan University, Suwon, South Korea

² University of Warsaw, Warsaw, Poland

³ Assiut University, Assiut, Egypt

⁴ National Institute of Fisheries Science, Gunsan, South Korea

Because nanoplastics (NPs) can transport pollutants, the absorption of surrounding pollutants into NPs and their effects are important environmental issues. This study shows a combined effect of high concentrations of NPs and copper (Cu) in the marine rotifer *Brachionus plicatilis*. Co-exposure decreased the growth rate, reproduction, and lifespan. The highest level of NP ingestion was detected in the co-treated group, but the Cu concentration was higher in the Cu single-exposure group. ERK activation played a key role in the downstream cell signaling pathway activated by the interaction of NPs and Cu. The increased sensitivity of *B. plicatilis* to Cu could be due to the impairment of MXR function caused by a high concentration of NPs, which supports our *in vivo* experiment results. Our results show that exposure to NPs could induce the dysfunction of several critical molecular responses, weakening resistance to Cu and thereby increasing its physiological toxicity in *B. plicatilis*.

Keywords: Nanoplastics, Copper, Detoxification, Combined effect, Marine rotifer, *Brachionus plicatilis*

Biological responses to chemical mixtures and/or multiple stressors: P90

EFFECTS OF NANO- AND MICROPLASTICS WITH THE COMBINED EXPOSURE OF ZINC OXIDE NANOPARTICLES ON TOXICITY, OXIDATIVE STRESS, AND ENERGY METABOLISM IN THE MARINE ROTIFER *BRACHIONUS KOREANUS*

Byeon E.¹, Sanpradit P.², Jeong H.¹, Lee J.-S.¹, Kim H.S.¹, Peerakietkhajorn S.², Lee J.-S.¹

¹ Sungkyunkwan University, Suwon, South Korea

² Prince of Songkla University, Hat Yai, Thailand

Microplastics interact with other pollutants in aquatic environments, especially nanoparticles that can attach to surfaces and potentially threaten biological systems. In this study, the toxic effects of individual and combined exposure to zinc oxide (ZnO) nanoparticles and two sizes (nano and micro) of polystyrene microplastics (NPs and MPs) were assessed in the marine rotifer *Brachionus koreanus*. The results of *in vivo* experiments showed an overall adverse effect on population growth and reproduction in the NPs+ZnO group. Co-exposure to plastics and ZnO tended to increase NP accumulation in the body while decreasing MP accumulation. Compared to the control group, all treatment groups showed increased ROS levels and antioxidant enzyme activities, especially when NPs were co-treated with ZnO, resulting in increased activity of oxidative stress and antioxidant-related genes. In addition, upregulation of the MAPK signaling pathway was observed following NPs and ZnO exposure. Thus, overall, compared to individual exposure, the combined exposure of nano-size plastics (NPs) and ZnO showed a change in antioxidant enzyme parameters and a decrease in metabolic capacity with reduced energy. The results of this study show that exposure to ZnO and the effect of combining NPs and MPs differ by particle size and provide a deep understanding of both environmental pollutants.

Keywords: Micropalstics, nanoplastics, zinc oxide, combined effect, marine rotifer, *Brachionus koreanus*

Biological responses to chemical mixtures and/or multiple stressors: P91

SUBTROPICAL CORAL EXPANSION WITHIN HIGH-LATITUDE MARGINAL CORAL COMMUNITIES IN JEJU

Kim T.¹, Pons L.M. T.¹, Conti-Jerpe I.², Jöst A.B.¹, Choi S.K.¹, Park S.R.³, Kang D.-H.¹, Baker D.M.⁴

¹ Tropical & Subtropical Research Center, Korea Institute of Ocean Science & Technology, Republic of Korea

² Department of Integrative Biology and Museum of Vertebrate Zoology, University of California Berkeley, USA

³ Department of Marine Life Sciences, Jeju National University, Republic of Korea

⁴ School of Biological Sciences, The University of Hong Kong, Hong Kong

While most concerns about corals focus on their decline in low-latitude areas, the expansion of corals to temperate regions is currently gaining more attention. Jeju, South Korea, is at the northern limit of coral environmental tolerance, yet coral colonies have been extending in some areas for years. Surprisingly, they have colonized a wide range of habitats, from the warm and relatively oligotrophic tropics to the more eutrophic coastal area of Jeju Is. This indicates that corals are capable of switching their trophic strategies from autotrophy to primarily heterotrophy. Rapid environmental changes occurring in Jeju waters offer an unprecedented opportunity to better understand the trophic plasticity of corals, which is allowing them to survive and expand in very different environments. Here, we applied carbon and nitrogen stable isotope values, and related modelling (SIBER: Stable Isotope Bayesian Ellipses in R) to evaluate trophic strategies and plasticity across sites and seasons of four common coral species around Jeju Island. Our results showed that *Alveopora japonica* is relatively autotrophic, while *Montipora millepora* and *Psammocora* spp. are mixotrophic, and *Oulastrea crispata* is relatively heterotrophic. In addition, comparing the trophic strategies between newly-expanded corals in unfavourable, as well as pristine sites highlights their nutritional adaptation to prevailing environmental conditions. It may also shed light on the species-specific limits of trophic plasticity. Our assessment of the trophic niches of corals in Jeju may aid and improve predictions on future expansion of these species in temperate areas in general.

Biological responses to chemical mixtures and/or multiple stressors: P92

INFLUENCE OF TEMPERATURE ON REPRODUCTION, AND GROWTH OF THE BROWN SHRIMP, *CRANGON HAKODATEI* (CARIDEA, CRANGONIDAE) IN THE EAST SEA OF KOREA

Lee I.O.¹, Kwon B.-O.², Oh C.-W.³, Khim J.S¹

¹ Seoul National University, Seoul, Republic of Korea

² Kunsan National University, Kunsan, Republic of Korea

³ Pukyong National University, Republic of Korea

It is well known that the changing environmental temperature has a profound effect on the reproduction and growth of marine organisms. However, few studies have investigated the biological characteristics of *Crangon hakodatei* and the effect of temperature on them. Here, we aimed to investigate 1) the reproduction and growth of *C. hakodatei*, and 2) the effect of temperature on it, based on over 1,500 samples collected from August 2010 to July 2011 in the East Sea of Korea. The samples contained more females than males, and the number of shrimps had a positive correlation with the temperature. The observed morphometric measurements clearly showed that females reached both larger size and higher growth rate than males, indicating sex-specific growth. The Von Bertalanffy growth function parameters were $CL_{\infty} = 18.2$ mm, $K = 0.5 \text{ year}^{-1}$, $C = 0.7$, and $WP = 0.1$ for females, and $CL_{\infty} = 15.7$ mm, $K = 0.6 \text{ year}^{-1}$, $C = 0.6$, and $WP = 0.3$ for males. The examined growth performance index was 2.22 for females and 2.17 for males. The maximum gonadosomatic index (GSI) coinciding with the breeding period was examined in March (12.2 ± 0.26) and a minimum of it in July (0.19 ± 0.15). There was a difference between the mean GSI of females with non-eyed and those with eyed eggs, indicating that *C. hakodatei* is a consecutive breeder. In particular, the monthly sampled *C. hakodatei* were divided into three groups according to seasonal water temperature, size and the number of the mature females, and most mature females were mainly observed in the low-temperature period. Overall, our novel findings on the vital influence of temperature on shrimp reproduction, suggest that *C. hakodatei* can be used as a potential sentinel species for biomonitoring water temperature changes in South Korea.

Biological responses to chemical mixtures and/or multiple stressors: P93

SYMBIOTIC BACTERIAL COMMUNITIES AND CARBON METABOLIC PROFILES OF CORAL ACROPORA AT DIFFERENT HEALTH STATUS RESPONSE TO HEATWAVE

Zhou J.¹, Qin Y.¹, Cai Z.¹, Wu J.² and Chan L.L.²

¹ Shenzhen International Graduate School, Tsinghua University, China.

² City University of Hong Kong, China

Thermal-induced coral bleaching is a topic that has received much attention, but the dynamics of coral-associated bacterial communities in symbiont have remains under explored. Meanwhile, studies of coral bleaching have largely ignored the intermediate status of corals, focusing only on the differences between the healthy and bleached corals. Here, we investigated three healthy status coral *Acropora pruinosa* (non-bleached, semi-bleached, and fully-bleached) under heatwave stress, involving related symbiotic bacterial profiles, including composition, diversity, network relationship, and the C-functional features. After 16S rRNA gene sequencing, high-throughput quantitative PCR (qPCR)-based chip, and related biochemical analysis, the results shown that the zooxanthellae number gradually decreased with the increase of bleaching. The tissue section of coral slices shows that bleaching exacerbates tissue lesions and symbiont cells decreasing. The α -diversity of microbial communities was increasing with the seriousness of bleaching. Some specific bacteria taxa, including Nitrososphaeria, Bacteroidia and Deltaproteobacteria were enriched in semi-bleached host. Network analysis revealed significantly different degrees of modularity among the non-, semi- and fully-bleached individuals at the bacterial class level, and more complex microbial interactions appeared in semi-bleached group. Additionally, functional analysis illustrated that the carbon metabolic abilities (fixing, degradation) were significant lower in fully-bleached individuals (only half of the healthy coral). Structure equation modeling revealed that the bacterial community diversity and C-metabolic function were directly related with the healthy status. These findings suggested that coral-associated bacterial responses to bleaching occurs under heatwave stress in a healthy-dependent manner. It can help us better understand the response mechanism of coral symbionts under bleaching pressure and develop novel strategies for restoring coral from microbial prespective.

Biological responses to chemical mixtures and/or multiple stressors: P94

EFFECT OF TRACE METAL ENRICHMENT AND THERMAL STRESS ON THE PHYSIOLOGY OF THE SCLERACTINIAN CORAL

Chen C.C., Meng P.-J. and Tu T.H.

National Applied Research Laboratories, Taiwan Ocean Research Institute, Taiwan

National Dong Hwa University, Taiwan

National Taiwan Normal University, Taiwan

Over the past few decades, there has been a notable surge in coral bleaching events, primarily attributed to shifts in climate and heightened marine pollution. While corals are commonly regarded as sensitive to metals, certain trace metals are essential in minute quantities for various physiological functions, such as photosynthesis and antioxidant defenses. This research delves into the implications of thermal stress and metal exposure, specifically iron (Fe) and manganese (Mn), on parameters like growth rates, photosynthetic efficiency, zooxanthellae density, coral bleaching levels, and chlorophyll a concentration in the scleractinian corals *Turbinaria irregularis* and *Montipora mollis*. The aim is to unravel the intertwined impacts of temperature, manganese, and iron on the physiological characteristics of these corals. The findings highlight that temperature exerts the primary influence on coral growth rates, with iron demonstrating the capacity to enhance short-term growth rates. Additionally, both iron and manganese contribute to heightened rates of photosynthesis and increased chlorophyll concentrations, while also retarding the onset of heat stress-induced coral bleaching. The insights gleaned from this study provide a deeper understanding of how corals respond to metal enrichment in seawater. Furthermore, they offer valuable insights into the variations in susceptibility to environmental stress observed among different coral species.

Biological responses to chemical mixtures and/or multiple stressors: P95

THE SUBLETHAL EFFECTS OF TRACE METALS COMBINED WITH VARIOUS SEDIMENT PARTICLE SIZE GROUPS ON THE SALT-TOLERANT CHIRONOMID LARVAE OF *KIEFFERULUS LONGILOBUS*

Hsieh C. Y.^{1,2,3}, Haung G.C.¹, Chang Y.T.¹ and Wu M.C.^{1,2}

¹ Department of Environmental Science and Engineering, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan, R.O.C.

² Center for Water Resources Education and Research, National Pingtung University of Science and Technology, Pingtung 912, Taiwan, R.O.C.

³ Department of Biomedical Science and Environmental Biology, College of Life Science, Kaohsiung Medical University, Kaohsiung 80708, Taiwan, R.O.C.

The salt-tolerant native Taiwanese benthic invertebrate *Kiefferulus longilobus* served as the test species for sediment toxicity in this investigation. Sediment samples collected from Hehua Bridge (HH), Qianfeng Road (QF), Qianzhou Bridge (QZ), sewage treatment plant discharge (STP), about 1 km downstream from the STP (STP2), Weiren Bridge (WR), and Jiugang Bridge (JG), which is within the tidal reach of the Agongdian River Basin in Southern Taiwan. By filtering sediments of various particle sizes (<63 μ m, 63-149 μ m, >149 μ m) and using resin to chelate heavy metals in the sediments, we are investigating the effects of two factors (sediment particle size and metal concentration) on the survival rate and sublethal effects (head capsule width, body length, and mentum abnormalities) of *Kiefferulus longilobus*.

According to our study's findings on sediment toxicity, of the seven sample locations, site HH had the highest average survival rate of chironomids, while site STP2 had the lowest. The lack of a clear pattern in survival rates across collection sites suggests that sediment particle size had little bearing on *K. longilobus* survival. When *K. longilobus* was exposed to sediment treated with resin chelation, the survival rate of larger particles (>149 μ m) at sites HH, STP, and STP2 considerably increased ($p < 0.05$). Furthermore, the survival rates of none of the samples differed noticeably from those of the control group, proving that trace metal concentrations affected *K. longilobus* survival.

The average head capsule width and body lengths of the test organisms exposed to WR and STP sediments without the addition of resin were smaller than at other sites. Given that growth was greatly reduced, it seemed likely that the sediments at these two sites contained trace metals that inhibited *K. longilobus* growth. The average head capsule width and body length of chironomids exposed to resin-chelated metals with various sediment particle sizes did not significantly change, proving that the growth of *K. longilobus* was unaffected by diverse sediment particle sizes. Prior to the sediment being treated with resin, chironomids exposed to site QF exhibited the highest rates of mentum deformation (10.53%) and wear (31.40%).

Different particle sizes showed a decrease in the deformation rate and wear rate of the mentum after sediments were treated with resin, but the biological mentum deformation rate and wear rate at each site were not significantly correlated, showing that trace metals possibly caused deformation and wear of the lower mentum of *K. longilobus*, but this was not the only factor. To completely comprehend the effect factor of deformity and wear in salt-tolerant chironomids, toxicity testing on laboratory samples spiked with various contaminants is advised.

Biological responses to chemical mixtures and/or multiple stressors: P96

INFLUENCE OF SALINITY ON THE BIOCHEMICAL AND PHYSIOLOGICAL RESPONSE IN *TRACHINOTUS BLOCHII* EXPOSED TO SELENIUM

Gopi N^{1,2}, Vaseeharan B², Darbha G.K1

¹ Environmental Nanoscience Laboratory, Department of Earth Sciences, Indian Institute of Science Education and Research Kolkata, Mohanpur-741246, West Bengal, India.

² Biomaterials and Biotechnology in Animal Health Lab, Department of Animal Health and Management, Alagappa University, Science Block, 6th floor, Burma colony, Karaikudi-630004, Tamil Nadu, India.

Salinity shifts in estuarine and coastal areas are becoming a major topic of concern and are one of the main factors affecting metal bioavailability and stress factors on marine organisms. Selenium (Se) is an essential trace metal element, but many aspects of its toxicity remain unclear, particularly in the marine environment. Physiological and biochemical-related biomarkers are of great interest due to their responses to environmental stressors, which provide valuable data for the biological monitoring of marine pollution.

Hence, we studied the effects of different salinity levels (5, 15, and 35 PSU) on the response of the silver pompano *Trachinotus blochii* when exposed to different concentrations of selenium (0, 5-100 µg/L) for 96 hours. Survival rates and accumulation of Se in different tissue parts were recorded. Biochemical and physiological analyses were performed to assess the antioxidant enzymes Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), biotransformation enzyme Glutathione-S-transferase (GST), scavengers of Metallothionein (MTs) and Reduced glutathione (GSH), oxidative stress effect of malondialdehyde (MDA) and protein carbonyl (PC), Na⁺/K⁺-ATPase (NKA) activity in the gill and liver tissues, and neurotoxicity (AChE) responses in the brain tissue.

Selenium-exposed fishes showed an alteration in antioxidant enzymes, NKA activity, induced oxidative stress effect, and neurotoxicity. These results clearly showed that higher salinity levels have mitigated the harmful effects of selenium. Salinity acted as a strong protective factor against increased oxidative damage (protein carbonyls and lipid peroxidation), and neurotoxicity (AChE) in low salinity (5 PSU). We applied an overall index, an integrated biomarker response (IBR), which increased under high concentrations of selenium condition but recovered to normal levels under high salinity treatment. Our results confirmed that exposure to sublethal concentrations of waterborne selenium alters biochemical and physiological responses in fish, highlighting the important protective role of higher salinities in ameliorating selenium toxicity associated with enhancing biochemical and physiological response in this model estuarine teleost.

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

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Biological responses to chemical mixtures and/or multiple stressors: P96S

SYNERGISTIC EFFECTS OF TEMPERATURE, SALINITY, AND NANOPLASTIC BEADS ON THE LIFE HISTORY TRAITS OF THE MARINE ROTIFER *BRACHIONUS PLICATILIS*

Yoon D.S., Song S.E., Lee M.C., and Lee J.S.

Sungkyunkwan University, Suwon, South Korea

In this study, we investigate effects of the increased temperature, salinity, and nanoplastic (NP) contamination on the life history characteristics of the marine rotifer *Brachionus plicatilis*. The findings indicate that an elevated temperature led to a decrease in the longevity of *B. plicatilis*, whereas the total number of offspring remained unaffected by the increased reproductive activity. However, when *B. plicatilis* was exposed to NP under conditions of elevated temperature and salinity, its lifespan and reproductive rate decreased. Subsequently, under the same conditions, we measured their levels of reactive oxygen species (ROS), superoxide dismutase (SOD), and catalase (CAT). According to the results, exposure to NP in conjunction with high temperatures and high salinity resulted in increased levels of ROS, SOD, and CAT. Additionally, an increase in temperature, salinity, and nanoplastic pollution significantly altered the fatty acid profile. At last, we conducted multigenerational experiments to validate the recovery of life history traits. The pre-reproductive day showed recovery within a single generation when exposed to high temperatures alone. However, when exposed to high temperatures, high salinity, and NP, it took three generations for recovery. Yet all other biomarkers demonstrated recovery after just one generation. Our findings did not entirely parallel the observed effects of elevated salinity, temperature, or nanoplastic contamination on *B. plicatilis* from previous studies. However, these findings improve our knowledge of how these three abiotic factors combine to create a new and important stress.

Biological responses to chemical mixtures and/or multiple stressors: P97

ANTIFOULING EFFICACY OF CHLORINE DIOXIDE ON THE PHYSIOLOGICAL AND CELLULAR RESPONSE IN ADULT GREEN MUSSEL *PERNA VIRIDIS*: A MULTIMARKER STUDY

Bandita Badakumar^{1, 2}, Dinakaraswami Inbakandan^{2*}, Srinivas Venkatnarayanan¹, T.V. Krishna Mohan^{1, 3}, Y. Venkata Nancharai^{1, 3}, Nitish Kumar Pandey¹, P. Veeramani¹ and P. Sriyutha Murthy^{1, 3*}

¹Water & Steam Chemistry Division, Bhabha Atomic Research Centre, Kalpakkam - 603 102, India

²Centre for Ocean Research, Sathyabama Institute of Science and Technology, Chennai - 600 119, India

³Homi Bhabha National Institute, Anushaktinagar, Mumbai - 400 094, India

Continuous low-dose chlorination (CLDC) is adopted in tropical coastal electric power station for biofouling control. The CLDC (0.2 ± 0.1 ppm) otherwise called “exomotive chlorination” has been effective in preventing mussel larvae from settling in the cooling water system. However, heavy infestation by the green mussel *Perna viridis* has been observed in the cooling water systems (CWS) of Madras Atomic Power Station (MAPS), located at Kalpakkam, East coast of India, which is not effectively controlled by in-plant administered chlorine concentrations (0.2 continuous & 0.5 shock dose mg/L, TRO). Various size ranges (10 – 110 mm) have been recorded in spite of the biocidal regime in vogue at different sections of the CWS indicating tolerance to the biocidal regime, periodical settlement and growth inside the CWS. The ‘exomotive’ chlorine in action, only helps in driving out the veliger larvae from the cooling circuit and is found to be insufficient to kill the settled adult mussels. In-lieu the present study was carried out to investigate the efficacy of chlorine dioxide as an alternate biocide to combat adult green mussels. The efficacy of continuous chlorine dioxide (ClO₂) dosing (0.1 and 0.2 mg/L, TRO) on adult green mussels was studied by; determining the time taken for mortality and its effect on different organelles and cellular processes viz: physiological, genetic, and neuronal using stress biomarkers. Tested concentrations revealed 100% mortality after 6 days (0.1 mg/L) and 3 days (0.2 mg/L). ClO₂ exposure to green mussels showed complete valve closure even at the lowest tested concentration and showed increased ammonia production (no Pseudofeces) indicating oxidative metabolism. This in turn showed reduced condition index (54 – 85 %) in green mussels exposed to ClO₂ compared to controls. Genotoxicity assessment showed increase in % tail DNA fraction with increase in ClO₂ concentration and incubation time. Generation of reactive oxygen species (ROS) was observed in all the tested tissues, with highest activity recorded in the digestive gland and mantle cavity. A marginal increase in Superoxide dismutase (SOD) and Catalase (CAT) activity was observed on all days with increase in ClO₂ concentration in all tissue samples tested which resulted in weakening of the physiological and metabolic functions. The smallest antioxidant molecule i.e. reduced glutathione (GSH) also showed prominent activity in the digestive gland (247 – 785 nmol/mg protein) of green mussels exposed to ClO₂. An important observation in the present study was that ClO₂ was found to act on the nerve synapse and inhibit acetylcholinesterase (AChE) activity. The AChE activity reduced in treatments viz: 75% (0.1 mg/L) and 82% (0.2 mg/L) compared to control. In alignment with the Best Available Technology (BAT) standards for cooling water systems, it is crucial to collect data regarding the biocidal impact of ClO₂ on mature green mussels. Consequently, this current dataset will prove valuable not only to industries seeking to formulate efficient antifouling approaches but also to regulatory bodies as they establish discharge guidelines for effluents containing ClO₂.

Biological responses to chemical mixtures and/or multiple stressors: P98

NATURAL ORGANIC MATTER ENHANCED NATURAL TRANSFORMATION OF EXTRACELLULAR ANTIBIOTIC RESISTANCE GENES IN SUNLIT WATER

Liu Q.-H., Yuan L., Li Z.-H. and Sheng G.-P.

University of Science and Technology of China, China

The global spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in aquatic systems has emerged as a critical public health concern. Existing water treatment technologies are often unable to effectively remove extracellular ARGs (eARGs), which can subsequently be acquired by competent bacteria in downstream water environments, thereby perpetuating antibiotic resistance. Natural organic matter (NOM), a common component of aquatic systems, has been underexplored with regard to its role in ARG spread. Upon solar irradiation, NOM generates various reactive species that may act as environmental stressors for the transformation of eARGs in competent bacteria occurring in natural aquatic environments. In this study, we have employed the pBR322 plasmid as a model and demonstrated that solar radiation-reactive NOM could facilitate the transfer of eARGs via natural transformation in *Acinetobacter baylyi* ADP1, which is a well-established model organism for studying competence and transformation. The natural transformation frequency of the pBR322 plasmid increased by 7.6 and 23.2 folds upon exposure to NOM under 0.5 h simulated solar irradiation, in comparison to exposure to solar radiation or NOM alone, respectively. The underlying mechanism was associated with the generation of reactive oxygen species (ROS), especially hydroxyl radicals ($\bullet\text{OH}$), induced by NOM exposure to simulated solar irradiation. These ROS promoted bacterial oxidative stress responses, increased cell membrane permeability, and upregulated the expression of related genes. Our findings reveal that exposure to simulated solar irradiation in the presence of NOM can accelerate the horizontal gene transfer of ARGs via the natural transformation pathway, exacerbating the risk of ARB proliferation in aquatic environments.

Biological responses to chemical mixtures and/or multiple stressors: P99

EXPLORING FACTORS SHAPING THE MICROALGAL COMMUNITY STRUCTURE ON PNEUMATOPHORES

Lai K.K.Y.^{1,2}, Li F.L.^{1,2}, Tam N.F.Y.^{1,2,3}, Xu S.J.L.^{1,2}, Zhou H.C.^{2,4}, Lee F.W.F.^{1,2,3}

¹ School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

² Greater Bay Area Mangrove Wetland Research & Development Centre, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

³ State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong SAR, China

⁴ College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, 518071, China

Pneumatophores are upright pencil-like or cone-like roots possessed by some mangrove plant genera such as *Avicennia* and *Sonneratia*. These aboveground structures facilitate the aeration necessary for root respiration and are submerged at high tide but exposed at low tide. Due to deposition from tidal waters, mature pneumatophores are often coated with a layer of sediment together with planktons such as microalgae. Thus the microhabitats on pneumatophores might depend on mangrove species, submersion duration and properties of sediment coats. However, very little is known about the difference in microalgal taxa on pneumatophores between microhabitats. In this study, we examined the compositions of epiphytic microalgae on pneumatophores of three mangrove species found in Futian Mangrove Nature Reserve, Shenzhen, China (a designated Ramsar site), namely *Avicennia marina*, *Sonneratia apetala* and *S. caseolaris*. Pneumatophores were randomly collected from each species and divided evenly into two regions (upper and lower). Sediment coats of both regions were removed from pneumatophores and bare pneumatophores were further washed to detach epiphytic microalgae. The compositions of microalgae from sediment coats and bare pneumatophores were identified and cell densities of identified genera were counted. Results revealed that *Oscillatoria* was the most abundant genus in all except for two samples, probably due to its filamentous structure for easier attachment and the preference of relatively low salinity (<10 ppt) as measured in situ. For both sediment coats and bare pneumatophores, algal species richness was always higher in the lower region of pneumatophores regardless of mangrove species, but the sediment coats harboured on average four times higher algal cell density. Among the three mangrove species, algal cell density and diversity were overall higher in *S. caseolaris* than the other two mangrove species. Pearson correlations showed that the algal diversity in sediment coats and bare pneumatophores was positively correlated with their respective water contents. These findings implied that drought stress could be a key factor shaping the algal community on pneumatophores and the dominance of *Oscillatoria* in most samples might be due to its drought tolerance related-traits. To obtain a more comprehensive picture, multi-omics approaches could be used as the next step to depict the stress tolerance mechanisms of microalgae and any biological interaction between mangrove plants and microalgae.

Biological responses to chemical mixtures and/or multiple stressors: P100

MULTIGENERATIONAL EFFECTS OF ELEVATED TEMPERATURE ON HOST-MICROBIOTA INTERACTIONS IN THE MARINE WATER FLEA *DIAPHANOSOMA CELEBENSIS* EXPOSED TO MICRO- AND NANOPLASTICS

Kim M.-S., Lee Y.H., Lee Y., Jeong H., Wang H., Wang D., and Lee J.-S.

Department of Biological Sciences, College of Science, Sungkyukwan University, Suwon, 16419, South Korea

Rising ocean temperatures are driving unprecedented changes in global marine ecosystems. Meanwhile, there is growing concern about microplastic and nanoplastic (MNP) contamination, which can endanger marine organisms. Increasing ocean warming (OW) and plastic pollution inevitably cause marine organisms to interact with MNPs, but relevant studies remain sparse. Here, we investigated the interplay between ocean warming and MNP in the marine water flea *Diaphanosoma celebensis*. We found that combined exposure to MNPs and OW induced reproductive failure in the F2 generation. In particular, the combined effects of OW and MNPs on the F2 generation were associated with key genes related to reproduction and stress response. Moreover, populations of predatory bacteria were significantly larger under OW and MNP conditions during F2 generations, suggesting a potential link between altered microbiota and host fitness. These results were supported by a host transcriptome and microbiota interaction analysis. This research sheds light on the complex interplay between environmental stressors, their multigenerational effects on marine organisms, and the function of the microbiome.

Biological responses to chemical mixtures and/or multiple stressors: P101

IDENTIFICATION OF SNPS ASSOCIATED WITH SALMON LICE (*LEPEOPHTHEIRUS SALMONIS*) RESISTANCE IN ATLANTIC SALMON (*SALMO SALAR*) USING GENOME-WIDE ASSOCIATION ANALYSIS (GWAS)

Zhang Y.¹, Garber A.F.², Li R.¹, Fast M.D.³, and Ca W.¹

¹ Department of Infectious Diseases and Public Health and State Key Lab of Marine Pollution, City University of Hong Kong, Hong Kong SAR, China

² Aquatic Biosciences, Huntsman Marine Science Centre, St. Andrews, New Brunswick

³ Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada

The Atlantic salmon (*Salmo salar*) holds significant economic value in the aquaculture industry and it is an important species in the marine ecosystem. However, its sustainable development is hampered by the recurrent infestation of salmon lice (*Lepeophtheirus salmonis*) infestation. This underlines the need for selective breeding program that targets enhanced lice resistance. Genome-wide association analysis (GWAS) has emerged as a powerful tool for the identification of genetic markers suitable for selective breeding purpose. In collaboration with scientists from the industry and academia in Canada, we performed a family-based GWAS on Atlantic salmon to identify single nucleotide polymorphisms (SNPs) associated with salmon lice resistance. Our experimental population included a total of 960 Atlantic salmon from 20 families, which were measured for lice resistance in the lice challenge experiment. RNA sequencing was used as a cost-effective method to identify SNPs in five selected families, i.e., the two most susceptible families and the three most resistant families. After quality control, 80,140 high-quality SNPs were retained for further analysis. GWAS for lice resistance using a generalized linear model identified 12 SNPs based on the suggestive threshold, predominately located on chromosomes 8, 14, and 22. Accordingly, seven lice resistance candidate genes were further identified, which were associated with cell-tight adhesion dynamics, cell migration, stress, and inflammatory modulation. Overall, this study offers novel insights into the molecular mechanisms underlying salmon lice resistance and contributes to the knowledge of SNP marker-assisted selection in the selective breeding practice of North American Atlantic salmon.

Biological responses to chemical mixtures and/or multiple stressors: P102

THE EFFECTS OF TITANIUM DIOXIDE NANOPARTICLES (NANO-TiO₂) UV FILTER ON FERTILIZATION RATE AND LARVAL SURVIVAL OF SCLERACTINIAN CORAL *ACROPORA TUMIDA*

Leong J.C.H.^{*}, Cheung B.C.T.^{*}, Chan E.Y.Y., Cheng T.K.T., Wong E.L.C., Chan M.H.C., Lau A. S.U., Tse I.W.Y., Lee C.G.W., Chui A.P.Y.

Simon F.S. Li Marine Science Laboratory, School of Life Sciences, Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China

In recent years, there has been an increased research focus on investigating the potential adverse effects of Ultraviolet (UV) filter pollution on the marine environment and ecosystems. Currently, common wastewater treatment is not effective in removing organic UV filters like oxybenzone, octinoxate, and octocrylene, causing them to accumulate in sewage and eventually get discharged into the sea. These pollutants sometimes lead to coral bleaching and mortality. Recently, inorganic UV filters like Titanium dioxide nanoparticles (nano-TiO₂) are used as a solution to the pollution problem, despite the uncertain effects on marine organisms, among which are the vulnerable scleractinian corals. Although studies have been done on the effects of inorganic nanoparticles in adult-stage scleractinian corals, those on sensitive early stages of the corals are underexplored. This study presents the effects of nano-TiO₂ UV filter on fertilization success, embryonic development, and larval survival of the scleractinian coral *Acropora tumida*, a dominant branch form corals in Hong Kong. *A. tumida* gametes were exposed to different concentrations of nano-TiO₂ suspension (10 mg/L, 5 mg/L, 1 mg/L, 0.1 mg/L, and 0.01 mg/L) and then fixed with 2.5% formalin at the time T=4 hours to examine fertilization success. Preliminary results showed that there was no significant difference ($p = 0.859$) in the fertilization success between treatments and control (0.1 micrometers filtered seawater) groups. There was also no significant difference in the proportion of abnormal development under different concentrations of nano-TiO₂. For larval survival, *A. tumida* larvae (3 day post-fertilization), were collected from mass culture and exposed to the same scale of concentrations of nano-TiO₂ suspension, and their survival rate was recorded every 24 hours over three days. The result showed that there were interactive effects of TiO₂ concentration and duration of exposure to larval survival, and with higher survivorship in control compared with TiO₂ concentration 1 mg/L to 10 mg/L. This study reports preliminary findings on the effects of nano-TiO₂ on multiple early life stages of *A. tumida*, no significant effect was observed in fertilization success and embryonic development, however, prolonged exposure to high concentration of nano-TiO₂ might have potential negative effects on coral larval survival. Further studies on larval settlement, as well as long-term coral juvenile performance, are essential to ensure that the inorganic UV filter does not undermine reef health.

Biological responses to chemical mixtures and/or multiple stressors: P103

SYNERGISTIC EFFECT OF SALINITY AND SUSPENDED SEDIMENT ON FERTILISATION SUCCESS AND EMBRYONIC DEVELOPMENT OF *ACROPORA TUMIDA* AND *PLATYGYRA CARNOSA* IN A MARGINAL CORAL ENVIRONMENT, HONG KONG

Chang T.K.T., Chan J.T.C., Cheung B.C.T., Fong T.H.W., Wong E.L.C., Lee C.G.W. and Chui A.P.Y.

Simon F.S. Li Marine Science Laboratory, School of Life Sciences, Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China

Coral spawning event in Hong Kong coincides with the SE monsoon season. Heavy rains brought by monsoons or tropical cyclones may lead to a decline in salinity and increased terrestrial sediment runoff. As a result, coral spawning may occur in an environment of reduced salinity and increased level of suspended sediment. In this study, we manipulated the two stressors to investigate the interactive effect of salinity and suspended sediment on fertilisation success and embryonic development of two dominant broadcasting scleractinian corals in Hong Kong, *Acropora tumida* and *Platygyra carnosa*. Low salinity level treatment of 26 psu with high concentration of suspended sediment (100 NTU of turbidity) had significantly reduced fertilisation success of *A. tumida*, while the effect on *P. carnosa* was insignificant. In terms of embryonic development, the proportion of abnormal development increased with increased suspended sediment concentration under low salinity treatment (26 psu) for both species, while a negative correlation between sediment concentration and normality of embryonic development was observed in *A. tumida* under both normal (32 psu) and reduced (26 psu) salinity. These observations suggested a potential synergistic effect of low salinity and high suspended sediment concentration on the reproductive success of broadcasting scleractinian corals. As occurrence of extreme weather and intense rainfall is projected to become more frequent under climate change, reproductive success of hard corals is likely to be compromised under the projected scenario in the near future.

Biological responses to chemical mixtures and/or multiple stressors: P104

CORAL CHIMERISM: STRATEGY TO BOOST FIELD SURVIVORSHIP IN EARLY OUTPLANTS OF SEXUALLY-REARED *ACROPORA TUMIDA* IN HONG KONG

Wong E.L.C., Lai I.Y.Y., Kong C.,K.W., Chui A.P.Y.

Simon F.S. Li Marine Science Laboratory, School of Life Sciences, Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China

Coral chimerism may occur between genetically distinct larvae that settle side by side and fuse during the early stage of their lives. This has been reported as a common feature in the wild and is hypothesised to enhance early recruit survivorship due to the multiplication of tissue area which possibly reduces size-specific mortality. In addition, the more diverse genetic combination allows the expression of alternative phenotypes, which could be an evolutionary rescue mechanism to advance the adaptive potential to contrasting disturbances. Therefore, incorporating coral fusion into sexual coral propagation, it might offer a novel approach for enhancing survivorship of small sexually-reared coral recruits. This technique could have a much higher implication for marginal coral environment which demonstrates a low post-settlement survivorship and slow growth rates of coral recruits. In this study, we outplanted 1.5-month fused and unfused recruits of *Acropora tumida* corals at Knob Reef (KR) and Tung Ping Chau Marine Park (TPCMP) in northeastern Hong Kong, and investigate the survivorship and growth rate. Results revealed that fused recruits had higher survivorship and growth rate at both sites. In a more protected environment as represented by TPCMP, single polyp recruits had 11.9% survivorship through the first two months of deployment in the field, while up to 60.5% of the fused recruits survived. These findings suggest that coral chimeras could enhance early post-settlement survivorship in the field and improve restoration efficiency by reducing the duration of laboratory culture of the juveniles, and thus the related costs and risks, before their outplantation.

Biological responses to chemical mixtures and/or multiple stressors: P105

ENVIRONMENTAL TOLERANCE OF GORGONIAN CORAL *GUAIAGORGIA* SP.

Tong Y.Y.A., Lau S.U.A., Wong L.C.E., Cheng K.T.T., Cheung C.T..B., Chan H.C.M., Lee G.W.C., Chui P.Y.A.

Simon F.S. Li Marine Science Laboratory, School of Life Science, The Chinese University of Hong Kong, Hong Kong

Since the last decade, there has been an increased awareness of anthropogenic pressures on octocoral (including gorgonian) populations in Hong Kong. In 2016, the construction of the Hong Kong International Airport (HKIA) Third Runway affected around 3300 gorgonian *Guaiagorgia* sp. colonies due to reclamation. *Guaiagorgia* sp. is the only gorgonian species found in western waters and not anywhere else within Hong Kong. Gorgonian assemblages have a fundamental ecological role as a habitat that provides a complex three-dimensional structure to support high biodiversity. Hence, it is urgent to preserve their populations. Hong Kong western water is brackish with large fluctuation in salinity due to the influence of Pearl River outflow. It also experiences large fluctuation in water temperature throughout the year. To fill our knowledge gap on our understanding on the environmental tolerances of *Guaiagorgia* sp. in Hong Kong, laboratory experiments were conducted to identify the salinity and thermotolerance thresholds of this species.

Salinity threshold experiment showed that long- term exposure to 8 psu appears to be lethal for *Guaiagorgia* sp., whereas 20-32 psu is the optimal growth range demonstrated by significantly increased buoyant weight. The wide best-performing salinity range matches the fluctuating salinity in its natural habitat. It also suggested that *Guaiagorgia* sp. distribution is not limited to low-salinity seawater. This led to a new hypothesis that the *Guaiagorgia* sp. distribution in Hong Kong is limited by other factors. Insufficient food availability in eastern and northern waters, and geographical isolation due to ocean current might be some of the factors that contributed to its very localized distribution in western Hong Kong.

Temperature threshold experiments showed that *Guaiagorgia* sp. performed best in lower temperature range of 12-24°C, but can also tolerate higher temperatures below 33°C for up to 28 days of exposure. All branches in lower-temperature threshold experiment showed positive weight growth, while in the upper-temperature threshold experiment, the branches experienced negative growth (i.e. die-back) at 27°C or above. Long exposure to 33 °C appears to be lethal for *Guaiagorgia* sp.. In general, exposure to elevated temperature resulted in an increase in tissue necrosis and a decrease in both polyp activity and skeletal growth rates. This indicate that *Guaiagorgia* sp. is likely to grow better in winter and become stressed in summer.

All these information will form the baseline for further evaluation of the adaptation of this species under the global climate change scenarios. These information are also essential in the design of an adaptive strategic plan for the conservation and protection of this octocoral species.

Biological responses to chemical mixtures and/or multiple stressors: P106

TRANSCRIPTOME ANALYSIS AND IDENTIFICATION OF POTENTIAL BIOMARKERS IN HUMAN CELL LINES EXPOSED TO JELLYFISH SHK-LIKE PEPTIDE

Park J.-I^{1,2}, Jung E.¹, Joh W.B.^{1,2}, Kim J.¹, Park M.², Yum S., ³Youn-Jung Kim^{1, 2*}

¹Department of Marine Science, Incheon National University, Incheon, 22012, Republic of Korea

²Research Institute of Basic Sciences, Incheon National University, Incheon, 22012, Republic of Korea

³Ecological Risk Research Division, Korea Institute of Ocean Science and Technology (KIOST), Geoje 53201, Republic of Korea

To mitigate jellyfish blooms and develop first-aid treatments for jellyfish stings and medical treatments, research to utilize jellyfish as a resource and identify the components of venom has been underway for decades. Recently, the combination of transcriptome and proteomic techniques has proven to be the most effective strategy for identifying marine venom and the complexity of its venom composition, as well as predicting its function through bioinformatics pipelines. Dr. Yum from KIOST has identified ShK-like peptides from jellyfish, g5156.t1-3 and CBRV1-10493, that exhibit Kv1.3-blocking activity, utilizing the jellyfish's genomic data. We utilized QuantSeq 3' mRNA-Seq to discern the differentially expressed genes (DEGs) in A549 (human lung adenocarcinoma cell), BEAS-2B (human bronchial epithelial cell) and U373MG (human glioblastoma cell) cells exposed to each peptide at a concentration of 20% cytotoxicity. In the annotation cluster analysis of the DEGs, a process related to mitosis was confirmed in the A549 and BEAS-2B exposed to g5156.t1-3. DEGs of A549 cell exposed to CBRV1-10493 were mainly enriched in biological processes related to lung development, positive regulation of cellular extravasation, and TRIF-dependent pathway. For U373MG cell group, upregulated six genes and downregulated eight genes were commonly expressed among the two treatments, and several of these genes are also considered to be cancer diagnosis biomarkers. We selected five genes (ASPM, CENPF, CENPE, KIF20B, KIF15, and KIF5C) that were identified in BEAS-2B cell, eight genes (MMP14, ICAM1, CD40, TICAM2, ADAM19, EGR1, PPARG, PROX1) that were identified in A549 cell, and six genes (SLC16A1-AS1, RCBTB2, SCNN1G, GRIK2, ADAMTS6, and FEZF1) that were identified in U373MG cell for quantitative real-time qPCR (RT-PCR) analysis. From the identified DEGs expression results, we believe that it is necessary to confirm the potential value of the peptide as a treatment biomarker.

*Corresponding author: duckyj@inu.ac.kr

Biological responses to chemical mixtures and/or multiple stressors: P106S

MARINE HEATWAVES AND LIGHT LIMITATION INDEPENDENTLY ALTER THE GROWTH AND PRODUCTIVITY OF THE TROPICAL SEAGRASS *HALOPHILA OVALIS*

Bass A.V.,¹ Falkenberg L.J.,^{1,2} Luo H.W.,¹ Thibodeau B.¹

¹ *The Chinese University of Hong Kong, Hong Kong SAR*

² *University of South Australia, South Australia*

Seagrasses are important marine foundation species, which support high coastal biodiversity and provide many socioeconomic benefits. However, seagrasses are threatened by anthropogenic change, both climate and non-climate related, particularly marine heatwaves (MHWs) which are having negative impacts on seagrasses and seagrass ecosystems globally. With the combined impacts of other anthropogenic stressors, such as light limitation from eutrophication or increased sediment load, the growth, productivity, and elemental cycling of the seagrass may be altered. This change in seagrass function can be due to an underlying shift in the seagrass microbiome.

In this study, we exposed individuals of the tropical seagrass *Halophila ovalis* to a 10-day MHW under three different light intensities to examine the impacts of these two stressors on the growth, productivity, elemental cycling, and leaf microbiome of the seagrass. We found that both MHW and decreased light had independent negative impacts on the growth of the seagrass, particularly the rhizome elongation rate, and the leaf growth rate, as well as increased the rate of leaf loss. Similarly, chlorophyll concentration was altered by both stressors, with the typical responses to reduced light (i.e., increasing chlorophyll concentration) being inhibited by the MHW. Nitrogen assimilation rate also decreased under both MHW temperature and reduced light availability. We found no interactive effects of MHW and light limitation on *Halophila ovalis*.

From the results obtained so far, we show that MHWs can drive decreased productivity of seagrass, particularly when combined with low light availability. Furthermore, MHWs can reduce the ability of *H. ovalis* to adapt to lower light levels. Poor water clarity and habitat health can therefore increase the susceptibility of seagrasses to extreme climatic events.

SEASONAL VARIATION IN FISH ASSEMBLAGES AND HABITAT UTILIZATION IN CHIKU LAGOON, TAIWAN

Chen M.-H.^{1,2,3}, Lai C.-C.¹, and Hanafi N.^{1,4}

¹ Department of Oceanography (Marine Biology Group), National Sun Yat-sen University, Taiwan

² Institute of Marine Ecology and Conservation, National Sun Yat-sen University, Taiwan

³ Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Taiwan

⁴ Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia

From September 2021 to December 2022, fyke nets were used each month to examine the fish biodiversity, seasonal variation, and habitat utilization of dominant species in Chiku Lagoon, Tainan, Taiwan. The study recorded a total of 13,301 individuals and 525 kg of fish, representing 149 species from 102 genera and 55 families. Canonical Analysis of Principal Coordinates (CAP) identified four distinct seasonal clusters in the fish assemblages of Chiku Lagoon. The dominant species varied by season: *Nuchequula mannusella*, *Nematalosa come*, and *Sillago sihama* were dominant in the spring; *Eubleekeria splendens*, *Leiognathus equulus*, and *Gerres macracanthus* in the summer; *L. equulus*, *Pomadasys argenteus*, and *P. kaakan* in the autumn; and *E. splendens*, *Planiliza macrolepis*, and *Taenioides cirratus* in the winter. Chiku Lagoon serves as a spawning ground for *E. splendens*, *N. come*, *N. mannusella*, *G. erythrourus* in spring, and for *S. sihama* from summer to winter. Additionally, it acts as a year-round nursery ground for *L. equulus*, *P. argenteus*, *P. kaakan*, and *G. macracanthus*, and a feeding habitat for *P. macrolepis* in summer, and for *T. cirratus* and *Plotosus lineatus* in the autumn and winter. Compared to an earlier study in 1995-1998, the fish community markedly changed with only 65 consistent species and 84 new marine species, and with the absence of 43 estuarine species. The dominant species have changed over 25 years, and the fish community has changed from being dominated by large-bodied Mugilidae species, such as *P. macrolepis*, *Moolgarda cunnesius*, and *Pelates quadrilineatus* to small-bodied Leiognathid species, i.e. *E. splendens* and *L. equulus*. While Chiku Lagoon remains a vital breeding and nursery ground for numerous tropical and subtropical fish species, the fish community has shifted towards small pelagic species, primarily from the Leiognathidae family, reflecting ecological changes due to extreme weather driven by climate change and the shrinkage of Chiku Lagoon.

Key words: Assemblage change, Spawning ground, Nursery ground, Extreme weather, Habitat shrinkage

SEASONAL VARIATION OF BENTHIC FISH ASSEMBLAGES IN CHANGHUA'S OFFSHORE WIND FARM AREA, CENTRAL WESTERN TAIWAN

Chen M.-H.^{1,2,3*}, Lai C.-C.¹, Hung T.-S.⁴, and Chang, C.-W.⁴

¹ Department of Oceanography (Marine Biology Group), National Sun Yat-sen University, Taiwan

² Institute of Marine Ecology and Conservation, National Sun Yat-sen University, Taiwan

³ Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Taiwan

⁴ Marine Ecology and Conservation Research Center, National Academy of Marine Research, Taiwan

Biological surveys were conducted using a local commercial beam trawler at depths of 20m at the inshore site and 40m at the offshore site during 2017-2018 and 2020-2021. Across 10 separate voyages, each with two net trawls, we collected a total of 8,026 fish, classified into 51 families, 84 genera, and 114 species. Although the species count remained consistent at 77 for both inshore and offshore areas, the composition of dominant fish species differed significantly. The top three dominant species at the inshore site were *Equulites absconditus*, *E. rivulatus*, and *Secutor ruconius*, whereas at the offshore site they were *Pennahia macrocephalus*, *Upeneus japonicus*, and *Plotosus lineatus*. Notably, fish abundance was higher at the inshore site, whereas biomass was greater at the offshore site. The fish assemblages exhibited both seasonal and locational variation, dividing into winter, spring (offshore), and summer-autumn groups, thereby highlighting the ecosystem's dynamic response to environmental conditions. Key species in these groups were *P. pawak*, *S. ruconius*, *Benthoosema pterotum* for the winter group, *Arius arius*, *Terapon theraps*, *P. pawak* for the spring-offshore group, and *E. absconditus*, *E. rivulatus*, *U. japonicus* for the summer-autumn group. The area was identified as a potential spawning and nursery ground for several key species. Specifically, *E. absconditus*, *E. rivulatus*, and *P. pawak* primarily utilize the area for spawning and nursery grounds during the spring and summer seasons, while *U. japonicus* does so during the summer and autumn seasons. The identified key species act as potential biological indicators for the marine ecosystem in the area. Their seasonal presence and spawning behaviour offer insights into the overall health and stability of the marine ecosystem. These baseline data serve as crucial references for future assessments of the ecological effects related to thousands of wind farms planned to be constructed in this area after 2030. Moreover, such data are particularly valuable for ongoing studies and conservation efforts in the context of climate change and sustainable marine development.

Keywords: Baseline, Spawning ground, Nursery ground, *Upeneus japonicas*, *Pennahia macrocephalus*

CEPHALOPOD ASSEMBLAGES IN RELATION TO THE ENVIRONMENTAL FACTORS IN THE COASTAL WATERS OFF WESTERN TAIWAN

Wu Y.-L.¹, Ho C.-W.², Hsu Y.-W.³, Chen H.-S.⁴, Chen K.-S.⁵, Chen C.-Y.⁶, and Chen M.-H.^{1,7,8*}

¹ Department of Oceanography, National Sun Yat-sen University, Taiwan

² Department of Life Sciences, National Chung Hsing University, Taiwan

³ Interpretation and Education Section, Taijiang National Park, Taiwan

⁴ Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan

⁵ Marine Ecology and Conservation Research Center, National Academy of Marine Research, Taiwan

⁶ Department of Marine Environmental Engineering, National Kaohsiung University of Science and Technology, Taiwan

⁷ Institute of Marine Ecology and Conservation, National Sun Yat-sen University, Taiwan

⁸ Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Taiwan

R/V *New Ocean Researcher III* and R/V *Ocean Researcher III* with the beam trawl on-board were used to investigate cephalopods and to measure the hydrological data along the coastal waters off western Taiwan from June 2007 to November 2021. In total, 1,545 individuals from 34 taxa, 15 genera, and 6 families of cephalopods were identified, accounting for 33% of records in Taiwanese water. The top six dominant species were *Acanthosepion pharaonis*, *A. lycidas*, *A. esculentum*, *Euprymna berryi*, *Inioteuthis japonica*, and *Loliolus beka*, accounting for 73.4% of the total catch. The assemblages showed significant geographic differences, separating into the "north group" and the "south group" in the Chiku–Jiading area along the coastal waters less than 100 m in depth, as well as the "deep-water group" at water depths greater than 100 m. The assemblages did not change over the past 15 years, but the abundance of *Sepia vietnamica* decreased significantly in the later period (2016–2021) compared to the earlier period (2007–2010), and there was a trend that *Amphioctopus marginatus* was distributed in deeper water, which may be related to global warming and reductions in fishery resources. This is the first recording of cephalopod assemblages along the coastal waters off western Taiwan, which can lay the foundation to elucidate cephalopods' reaction to rising sea temperatures in tropical and subtropical waters in the future.

Keywords: Environmental adaptation, Water depth, Distribution, *Acanthosepion pharaonis*, *Acanthosepion lycidas*

FEASIBILITY STUDY OF DETECTING LOCAL SEAHORSE AND PIPEFISH SPECIES IN THE COASTAL AREA OF HONG KONG BY ENVIRONMENTAL DNA (EDNA)

Chan M.H.C.^{*1}, Yeung L.T.C.^{*1}, Chan J.T.C.¹, Tse I.W.T.¹, Tsang K.K.K.¹, Lau A.S.U.¹, Leung J.C.H.¹, Cheng T.K.T.¹, Fong T.H.W.¹, Cheung B.C.T.¹, Lee C.G.W.¹, Wong E.L.C.¹, Cheang F.C.C.², Leung K.M.Y.³, Chui A.P.Y.¹

¹ Simon F.S. Li Marine Science Laboratory, School of Life Sciences, Chinese University of Hong Kong, Hong Kong, Hong Kong SAR, China

^{2b} Department of Science and Environmental Studies, The Education University of Hong Kong, Taiipo, N.T., Hong Kong, China

³ Department of Chemistry, City University of Hong Kong, China

The global populations of seahorse and pipefish species are in rapid decline due to habitat loss, overexploitation for trade, and marine pollution. To protect and conserve them, a comprehensive and systemic survey should be conducted in Hong Kong as the results can provide the government with invaluable information for the development of an adequate conservation management plan. The underwater visual census (UVC) is a conventional way to survey these species, but the method may not be ideal since the divers may overlook the species for their small size and cryptic lifestyle, especially with the poor visibility in the waters of Hong Kong. Environmental DNA (eDNA), which refers to the genetic materials present in environmental samples that allow the detection of the target species without direct observation, has been an emerging tool in marine biodiversity monitoring and conservation worldwide. This research aims to investigate the feasibility of detecting local seahorse and pipefish species in the coastal area of Hong Kong by two eDNA methods – a newly developed qPCR specific for the yellow seahorse (*Hippocampus kuda*), and metabarcoding. Using a Smith-Root ANDe™ backpack, a system for in-situ water filtration and collection of eDNA, a standardised sampling strategy and an eDNA extraction method were optimised and determined. The detection of the target species can be achieved by collecting 5L of seawater at each of the 9 sampling points along transects and precipitating the eDNA with multiple membranes. Thereafter, eDNA from seawater was extracted from 12 sites covering the eastern, western and southern regions of Hong Kong during both dry and wet seasons, and UVC was conducted simultaneously in four of the sites for results comparison. The preliminary results showed that the qPCR method could give positive results either in the presence or absence of yellow seahorses during the UVC. After the identification of seahorse and pipefish species in the samples by metabarcoding, the two eDNA methods will be compared, analysed, and further discussed in the presentation. It is believed that the developed eDNA methods can complement the traditional survey method and may facilitate the environmental impact assessment in Hong Kong and nearby regions.

DIVERSITY OF PENNATULOIDEA AROUND LANTAU ISLAND, HONG KONG

Heung B.Y.W.¹, Li Y.X.¹, Loke H.X.¹, Kei K.², Lai V.C.S.³, Chan L.L.⁴, Qiu J.W.¹

¹ Department of Biology, Hong Kong Baptist University, China

² Reef Check Foundation (Hong Kong)

³ Ecosystems Ltd

⁴ State Key Lab of Marine Pollution, City University of Hong Kong, China

Sea pens in the superfamily Pennatuloidea (Anthozoa: Octocorallia) are common inhabitants of marine environments from shallow water to deep sea. However, little is known about the diversity of Pennatuloidea. In Hong Kong, only 5 species belonging to 5 genera and 4 families of sea pens have been recorded. This study aims to expand our knowledge of the diversity of Pennatuloidea in Hong Kong waters based on samples collected from the waters around Lantau Island by implementing an integrated molecular and morphological approach. For each collected specimen, we extracted their DNA and amplified their mitochondrial MutS and ND2 sequences by PCR or obtained these sequences by genome skimming. Phylogenetic analyses of our samples revealed eight species of sea pens, including two species belonging to *Veretillum* and *Virgularia* – two newly recorded genera of sea pens from Hong Kong. We are conducting morphological analyses to obtain information on their sclerites and zooids. Overall, our preliminary results indicate that the diversity of sea pens in Hong Kong is much higher than previously thought and warrants further studies with higher sampling efforts.

AN INTEGRATED MORPHOLOGICAL AND MOLECULAR STUDY CLARIFIES THE IDENTITIES OF TWO SPECIES OF PORCELAIN CRABS IN THE GENUS PORCELLANELLA (ANOMURA: PORCELLANIDAE)

*Loke H.X.*¹, *Heung Y.W.*¹, *Li Y.X.*¹, *Hosie A.*², *Chan T.Y.*³, *Wang Z.*⁴, *Mendoza J.C.E.*⁵, and *Qiu J.W.*¹

¹ Department of Biology, Hong Kong Baptist University, China.

² Department of Aquatic Zoology, Western Australian Museum, Australia.

³ Institute of Marine Biology, National Taiwan Ocean University, Taiwan.

⁴ State Key Laboratory of Marine Environmental Science, College of Ocean and Earth Sciences, Xiamen University, China.

⁵ Lee Kong Chian Natural History Museum, National University of Singapore, Singapore.

During biodiversity surveys in the urbanized waters of Hong Kong, we collected the porcelain crab specimens of the genus *Porcellanella*. They are often commensals of sea pens and are widely distributed across the Indo-Pacific. Two members of this genus, *P. triloba* White, 1851 (originally described in Australia) and *P. picta* Stimpson, 1858 (originally described in Hong Kong) have a confusing taxonomic history due to high morphological similarities. *Porcellanella picta* was synonymized with *P. triloba* by Sankarankutty (1961). Although this decision was rejected by Johnson (1964), in the WoRMs website *P. picta* is recognized as a synonymy of *P. triloba*. Our study aimed to clarify this taxonomic issue using an integrated molecular phylogenetic and morphological approach. We examined 24 specimens of *Porcellanella* collected from Taiwan, Xiamen, Hong Kong, Thailand, Singapore, and Australia. We found two main morphotypes, with distinct morphological features that can be used to separate the Australian species *P. triloba* and South China Sea (SCS) species *P. picta*. We extracted their mitochondrial 16S rRNA gene and COI gene sequences data and performed Maximum Likelihood and Bayesian Inference phylogenetic analyses, which revealed the Australian in two to three distinct clades, and the SCS specimens in another distinct clade. For the most dominant Australian clade and the SCS clade, sequence comparison revealed a mean percentage genetic divergence of 15.2% for COI and 4.3% for 16S. BLASTn analysis showed the intra-clade genetic similarity ranges between 99.28 – 99.46 % for COI and 98.32 – 100 % for 16S in *P. triloba*, whereas 98.01 – 100% for COI and 99.04 – 99.52 % for 16S in *P. picta*. Overall, our study indicates that most of the Australian specimens belong to *P. triloba* whereas all the SCS specimens belong to *P. picta*. Two Australian specimens exhibited substantial divergences in 16S and COI sequences, which may warrant more detailed morphological and molecular analyses to determine if they belong to undescribed species.

THE INFLUENCE OF OCEAN WARMING ON THE BIOFILTRATION FUNCTION OF THE REEF-BUILDING MUSSELS AND OYSTERS (BIVALVIA, MYTILIDAE AND OSTREIDAE): A META-ANALYSIS

Lo C.C.^{1,2}, Minuti J.J.², Astudillo J.C.^{1,2} and Leung K.M.Y.¹

¹ *City University of Hong Kong, China*

² *Hong Kong Metropolitan University, China*

Biofiltration by reef-building bivalves, such as mussels and oysters, plays a crucial role in enhancing water quality and controlling harmful algal blooms, providing an essential ecosystem service. This physiological trait is known to be temperature dependent, implying that long-term impacts may arise due to climate change-induced ocean warming. In this study, a meta-analysis was conducted to investigate the latitudinal effect on the thermal regime of biofiltration in bivalves, utilising data from previous studies. Furthermore, projections were made to assess the future biofiltration capacity of these two groups of reef-building bivalves under different warming scenarios.

Data from a total of 22 studies were adopted for the meta-analysis with 8 studies focusing on oysters and 14 studies on mussels. The findings revealed a significant negative correlation between the absolute latitude and the optimal temperature for bivalves' biofiltration. However, the latitudinal effect was more pronounced on oysters than on mussels. In contrast, the critical thermal maximum for bivalves' biofiltration remained relatively consistent between 30 to 50 °C, regardless of latitude.

Ocean warming's impact on bivalves' biofiltration efficiency was also projected, considering different warming scenarios. Results indicated that, under most conditions of RCP 2.6 or RCP 8.5 by 2100, the annual mean biofiltration capability of temperate and tropical mussel populations, as well as temperate oyster populations, would remain unchanged or experience a slight enhancement. However, the biofiltration efficiency of tropical oyster populations would be significantly reduced in 2100 under the RCP 8.5 scenario. Furthermore, during the warmest three consecutive months in 2100 under RCP 8.5, both tropical mussel and oyster populations would exhibit a notable depression in biofiltration capacity.

This study demonstrates that biofiltration, a vital ecosystem function facilitated by reef-building bivalves, exhibits a geographically specific thermal regime. The findings suggest that the response of bivalve populations to ocean warming will vary depending on their geographical location and the warming scenarios. Consequently, some populations may experience benefits, while others could face potential risks.

AUTOMATIC DETECTING, IDENTIFYING AND MEASURING OSTRACODS WITH DEEP LEARNING

Hu J., Hong Y., Chen Y., Moriaki Y.

The University of Hong Kong

Microfossils of ostracods provide abundant information to understand past climate and environmental changes. The species distribution and size data for ostracods can be useful protocols for accessing the information. But identifying and measuring enormous amounts of ostracod specimens are time-consuming tasks for researchers. Recent advancement of digital microscopy and deep-learning technology provided the ground to automate micropaleontological identification processes. Here we introduced a workflow to integrate deep learning technology as an automation tool for ostracod genus and species identification detection. In the workflow, we created a dataset with 69630 images of Hong Kong marine ostracods containing 79 genera and 139 species then developed a framework to detect and identify ostracods' genera and species with dataset. We also measured each ostracod's height, width and area by applying segmentation on top of the detection result. We reached 95.01% accuracy in genus identification and 91.63% in species identification. In the object detection task, the trained YOLO v8 model reached 76.53% and 89.81% average precision in species detection and genus detection task. The promising result reveals the potential to apply deep learning to power the future automatic identification system for ostracods. We will detail the workflow to encourage researchers with similar needs to adapt deep learning in their future research.

RESTORING DEGRADED ARTIFICIAL SHORELINES IN A POLLUTED ENVIRONMENT WITH ECO-ENGINEERING: A CASE STUDY FROM HONG KONG

Bradford T.E.¹, Astudillo J.C.², Lai C.¹, Leung R.W.S. ³, Lo C.C.², Minuti J.J.¹, Leung K.M.Y.¹

¹ *State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*

² *School of Science and Technology, Metropolitan University of Hong Kong, Hong Kong, China*

Urbanisation and coastal armouring are particularly evident in cities and ports, with large proportions of coastlines transformed to artificial vertical and sloping seawalls. Hard eco-engineering structures, which provide microhabitats (e.g. crevices, water retention), can mitigate heat and desiccation stress to marine organisms, enhancing biodiversity on seawalls. A polluted marina in Hong Kong with both sloping and vertical seawalls was chosen as a case study for hard eco-engineering in a degraded ecosystem with continuous influence of contaminated surface runoff and the maritime industry. Eco-engineering fixtures that provide physical refuge and water retention or high complexity via an oyster shell reef were placed on the sloping seawall with respective control boulders, and two designs of complex eco-panels were placed on the vertical seawall, with a control panel and scraped seawall control (n=4). Following 18 months of deployment, the species diversity found on most of the sloping seawall fixture types and both panel types were significantly higher than the respective controls. The significantly different community composition of the fixtures also shows that eco-engineering fixtures can improve the biological community and ecosystem functioning on the seawalls in a polluted marina, pushing the limit of implementation of eco-engineering in marinas and busy ports of urbanized coastal cities.

ASSESSING BIODIVERSITY POST-ARTIFICIAL BEACH CONSTRUCTION AT LUNG MEI, TOLO HARBOUR

Leung R.W.S.^{1,2}, LO C.C.^{1,3}, BRADFORD T.E.¹, WONG C.K.M.¹, ASTUDILLO J.C.³, LEUNG K. M.Y.¹

¹*State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China*

²*The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China*

³*School of Applied Science & Technology, Hong Kong Metropolitan University, Homantin, Hong Kong*

Tolo Harbour is recognized for its diverse coastal habitats and rich marine biodiversity. However, there have been concerns about habitat degradation and biodiversity loss associated with coastal development, land reclamation, and the construction of a man-made beach. The study aims to investigate the marine biodiversity between the Lung Mei area and the natural reference site after the construction of the Lung Mei artificial beach. Two study sites located on the east and west sides of the Lung Mei artificial beach and one reference site (Ting Kok East) were selected and surveyed from February 2021 to January 2022 to understand the spatial and temporal differences in biodiversity. Seasonal quantitative surveys, including transect and quadrat surveys, beach seining, and purse seining, were conducted twice in the dry and wet seasons. Monthly qualitative monitoring (i.e., rapid assessment) was also conducted throughout the one-year study period to complement the species recorded at each site.

A total of 327 marine and coastal species were recorded in the study. Ting Kok East (TKE), the reference site, had the highest biodiversity of marine organisms among all three sites, with a total of 243 species. Of the two study sites, Lung Mei West (LMW) demonstrated a higher marine species richness than Lung Mei East (LME), with 239 and 208 species respectively, as well as higher abundance and biomass of epifauna and infauna. The most diverse taxonomic groups recorded in this project were bivalves, gastropods, crustaceans, and fishes, which contributed 74.3% of the total recorded species in this study. In all three study sites, species richness, abundance, and biomass were higher in summer than in winter.

The study findings suggested there might be a varying degree of impact towards LME and LMW. This one-year systematic biodiversity assessment allowed a better understanding of coastal biodiversity in a post-construction scenario in the Lung Mei area. However, the study findings only represent the biodiversity soon after the construction of the artificial beach, and longer-term monitoring warrants verifying any possible recovery or positive change in biodiversity over time.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P118

PHYTOPLANKTON BLOOMS ALONG THE CHINESE COAST IN THE EAST CHINA SEA

Chen C.-C., Shiah F.-K., Gong G.-C.

National Taiwan Normal University, Taiwan

Academia Sinica, NanKang, Taiwan

National Taiwan Ocean University, Taiwan

The extent and duration of phytoplankton blooms, particularly harmful algal blooms, have witnessed a substantial increase in coastal ecosystems worldwide, primarily attributed to escalating eutrophication. In the East China Sea, similar trends of heightened phytoplankton blooms are evident, occurring more frequently, especially in the vicinity of the Changjiang River estuary and along the Zhejiang coast. This study investigates a severe phytoplankton bloom with a chlorophyll *a* concentration exceeding 20.0 $\mu\text{g L}^{-1}$ that was observed off the Zhejiang coast during the summer of 2014. Additionally, substantial phytoplankton biomass was documented along the Fujian coast. These blooms exhibited diverse biological responses linked to their occurrence. The causative factors for these blooms appear to be associated with various nutrient sources along the Chinese coast. These findings underscore the need for tailored regulatory strategies to mitigate the frequency and magnitude of phytoplankton blooms along the Chinese coast.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P119

ALLELOPATHIC EFFECTS OF THE LEAF EXTRACTS FROM TWO MANGROVE SPECIES ON HARMFUL DINOFLAGELLATES

Xu S.J.-L.^{1,4}, Wong B.Y.-K.¹, Chen Y.-H.², Zhou H.-C^{3,4}, Li F.-L.^{1,4}, Tam N.F.-Y.^{1,4,5}, Lee F.W.-F.^{1,4,5}

¹ School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

² Laboratory of Marine Biodiversity, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen, China

³ College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China

⁴ Greater Bay Area Mangrove Wetland Research & Development Centre, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

⁵ State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong SAR, China

Harmful algal blooms (HABs) occur frequently and globally, causing ecological threat and economic loss. Physical and chemical methods have been developed to control HABs, however, these approaches have certain limitations. It is essential to explore ecologically safe and commercially viable HAB mitigation strategies. Mangrove is a unique inter-tidal wetland along tropical and subtropical coastlines with high primary productivity and litter production. It is interesting that no HAB events have been reported despite its surroundings are usually nutrient rich, which it may be related to allelopathy of mangrove leaf and leaf litter. In this study, we investigated the allelopathic effect of senescent leaves of two dominant mangrove plants in China, *Kandelia obovata* and *Aegiceras corniculatum* on inhibiting the growth of two harmful dinoflagellates (*Karenia mikimotoi* and *Alexandrium tamarense*). Results revealed that the leaf extract of both plants affected algal growth, with *K. obovata* exerted stronger inhibitory effect than *A. corniculatum*. Not only these two algal species, the leaf extract of *K. obovata* also posed a broad-spectrum inhibitory effect on the other three harmful algal strains, but its allelopathic effect was algal species-specific, and leaf dose-dependent. The inhibitory effect of low dose repeated exposure of *K. obovata* leaf extract was more effective, stable and long-lasting in inhibiting harmful dinoflagellates than high dose single exposure. This study demonstrated that leaves of mangrove plants, especially *K. obovata* could repeatedly be applied at a low dose to inhibit algal growth, and may have the potential to be developed as an alternate strategy for HAB control in future.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P120

PHYTOPLANKTON COMMUNITY IN AN ESTUARY DURING *KARENIA MIKIMOTOI* BLOOM AT LOW AND HIGH TIDES – A CASE STUDY IN AN MAN-MADE CHANNEL IN SHENZHEN, CHINA

Li F.L.^{1,2,3}, Zhu H.L.^{1,3}, Wong Y.S.^{1,2}, Tam N.F.Y.^{1,2,3,4}, Lee F.W.F.^{1,2}, Yang Q.⁵, Zhou H.C.^{1,6*}, Xu S.J.L.^{1,2*}

¹Greater Bay Area Mangrove Wetland Research & Development Centre, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

²School of Science and Technology, Hong Kong Metropolitan University, Ho Man Tin, Kowloon, Hong Kong SAR, China

³City University of Hong Kong Shenzhen Research Institute, Shenzhen, P. R. China, Shenzhen

⁴State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

⁵Guangdong Neilingding Futian National Nature Reserve, Shenzhen, China

⁶College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China

The estuary is an important link between the sea/bay and land rivers subject to tidal flooding, and its ecological location and function are extremely important. However, this is also a place commonly affected by red tide blooms. The impact of red tides on this habitat, particularly phytoplankton, the primary producer, has not been sufficiently examined. Phytoplankton community in the estuary of a man-made channel in Guiwan River, Shenzhen was studied during *Karenia mikimotoi* bloom at low and high tides to reveal the response of microalgae to red tide in this artificial riverine estuary. Results showed that a total of 51 microalgae species belonging to 6 phyla and 36 genera were found in the late stages of *K. mikimotoi* bloom. Most species were Bacillariophyta (diatom, 54.9%) but the dominant species was *K. mikimotoi* (Pyrrophyta) with the relative abundance of 85-99.4% at high tide and 67.8-93.1% at low tide. *K. mikimotoi* blooming greatly reduced the population density and dominance of other microalgae, and also significantly reduced the biodiversity index (H') and evenness index (J') of the microalgal community, thus weakened the ecological function of the phytoplankton community in the estuary of the man-made Guiwan River. Under the *K. mikimotoi* blooming, most water quality showed extremely eutrophic level and was at the very heavy pollution state according to China National Standard. No significant relationship was found between the relative abundance of *K. mikimotoi* and the water quality parameters of Guiwan estuary, suggesting the *K. mikimotoi* blooming might be mainly brought by tidal action. The water environment of Guiwan estuary was susceptible to the impact of *K. mikimotoi* blooming, and there were signs of *K. mikimotoi* retained in the river for some time even though the salinities were low after the ebb tide, which might cause the risk of continuous outbreak/hazard of red tide bloom in estuaries. More attention to the response of estuarine phytoplankton community and the impact of post-red tide blooms is needed.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P121

TEMPORAL AND SPATIAL CHANGES OF DINOFLAGELLATES AND THEIR SPOROCCYSTS IN THE NORTH YELLOW SEA IN SUMMER 2019

Sun X.*, Zhou Z., Chang Q.

Shandong University, Weihai, China

In the summer of 2019, an investigation was conducted in Yantai coastal water, and the community characteristics and spatial-temporal distribution of dinoflagellates in the water column and cysts in the surface sediments were studied. In total, 31 taxa of dinoflagellate belonging to 24 genera were identified in water column. The dominant species were *Protoperidinium* spp., *Prorocentrum minimum*, and other 9 taxa. A total of 28 dinoflagellate cysts, belonging to 13 genera, were found in the surface sediments, including 6 dominant species, such as *Protoperidinium* spp. and *Alexandrium* spp. In the water column, the abundance of *Gonyaulax* spp., *Alexandrium* spp., *Protoperidinium* spp. and *Scrippsiella trochoidea* increased from July to August, while that of cysts of these four algae decreased in the sediments. Then, the cysts abundance of the above four dinoflagellates increased from August to September. The changes in the number of cysts of the main dominant groups (*Gonyaulax* spp., *Alexandrium* spp., *Protoperidinium* spp. and *Scrippsiella trochoidea*) in the sediment had a significant negative correlation with the changes in the number of the corresponding dinoflagellates in the water column. We speculated that cysts in sediments germinate into the water column in July, which can provide vegetative cells for the high abundance of dinoflagellates in the water column in August. The increase of dinoflagellate cyst abundance in sediment in September was related to the supplement of dinoflagellates in water in August. Therefore, the process of excystment and encystment of dinoflagellates is closely related to the change of algae cell abundance in water, which deserves further attention in order to prevent negative impact on local fishery resources.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P122

OCCURRENCE AND SEASONAL DISTRIBUTION OF THE NEUROTOXIN B-N-METHYLAMINO-L-ALANINE IN MUSSELS IN THE SOUTH SEA COAST OF KOREA

Kim S.-Y.¹, Kim M.¹, Rydberg S.², Baek S.H.³, Hong S.^{1*}

¹ Chungnam National University, Republic of Korea

² Stockholm University, Sweden

³ Korea Institute of Ocean Science and Technology, Republic of Korea

The marine biotoxin β-N-methylamino-L-alanine (BMAA), a neurotoxin, has been hypothesized as a causative factor to amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) and Alzheimer's disease. Cyanobacteria and diatoms, ubiquitously present in various aquatic ecosystems, are reported as the main algae producing BMAA. The present study investigated the occurrence and seasonal distribution pattern of BMAA in mussels collected from the South Sea Coast of Korea. Mussels were collected monthly at 11 sites along the South Sea Coast (n=118) from January to December 2021. BMAA was extracted through the subsequent steps: protein precipitation, protein hydrolysis, and clean-up process. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was used for BMAA quantification. In addition, a correlation analysis was conducted between the concentrations of BMAA in mussels and environmental factors (i.e. salinity, water temperature, the concentration of dissolved oxygen, Chlorophyll-a, and nutrients) to find the key factors related to the distribution of BMAA. As the results, the highest concentration was detected in December, at 1239 ng g⁻¹ dry weight (dw), followed by March (850 ng g⁻¹ dw). The lowest concentration of BMAA in mussels was found in October, with a concentration of 36 ng g⁻¹ dw. The concentration of BMAA in mussels was positively correlated with dissolved oxygen concentration in water and salinity. Water temperature and Chlorophyll-a concentrations were negatively correlated with the BMAA concentration. The present study has shown the occurrence of BMAA and its seasonal distribution pattern in mussels in Korean coastal waters. Further studies are needed to identify the causative microalgae of BMAA in the South Sea Coast of Korea and to evaluate the potential risk of BMAA to human health associated with seafood consumption.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P123

TOXICITY EFFECTS OF HYDROPHILIC ALGAL LYSATES FROM *COOLIA TROPICALIS* ON MARINE MEDAKA LARVAE (*ORYZIAS MELASTIGMA*)

Yan M. ^{1,2,3,*}, Gu J. ^{1,2}, Leung P.T.Y. ^{1,2,4,*}, Tian L. ^{1,5}, Lam V.T.T. ¹, Cheng S.H. ^{1,5}, Leung K.M.Y. ^{1,2,3,4}, Lam P.K.S. ^{1,4,5}

¹ State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong, China

² Department of Chemistry, City University of Hong Kong, Hong Kong, China

³ Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, China

⁴ Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai, China e Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China

⁵ Office of the President, Hong Kong Metropolitan University, Hong Kong SAR, China

Coolia tropicalis is a species of benthic and epiphytic toxic algae, which can produce phycotoxins that intoxicate marine fauna. In this study, the potential toxic effects of *C. tropicalis* on fish were investigated using larval marine medaka (*Oryzias melastigma*) as a model to evaluate fish behavior, physiological performance, and stress-induced molecular responses to exposure to two sublethal concentrations (LC₁₀ and LC₂₀) of hydrophilic algal lysates. Exposure to *C. tropicalis* lysates inhibited swimming activity, activated spontaneous undirected locomotion, altered nerve length ration, and induced early development abnormalities, such as shorter eye diameter, body as well as axon length. Consistent with these abnormalities, changes in the expression of genes associated with apoptosis (*CASPASE-3* and *BCL-2*), the inflammatory response (*IL-1 β* and *COX-2*), oxidative stress (*SOD*), and energy metabolism (*ACHE* and *VHA*), were also observed. This study advances our understanding of the mechanisms of *C. tropicalis* toxicity in marine fish in the early life stages and contributes to future ecological risk assessments of toxic benthic dinoflagellates.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P124

EPIPHYTIC COMMON CORE BACTERIA IN THE MICROBIOMES OF CO-LOCATED GREEN (ULVA), BROWN (SACCHARINA) AND RED (GRATELOUPIA, GELIDIUM) MACROALGAE

Lu D.-C.

Marine College, Shandong University

Macroalgae-colonizing bacteria are of great interest, both from ecological and biotechnological points of view. Currently, it is not well understood, which factors determine the initial colonization and maintenance of macroalgal epiphytic microbial communities once they are established, as there is general lack of understanding of the diversity and ecophysiological roles of common macroalgal epiphytic bacteria.

In my report '*Epiphytic common core bacteria in the microbiomes of co-located green (Ulva), brown (Saccharina) and red (Grateloupia, Gelidium) macroalgae*', we conducted a broad study on the epiphytic communities of four distinct macroalgal species from all three major lineages. These were sampled during four seasons in 2018/19 at a reef in Weihai, China, together with corresponding seawater and sediment controls. While previous studies largely focused on microbial community composition analysis using 16S rRNA amplicon or limited metagenome sequencing of single macroalgal species, we combined 16S rRNA amplicon sequencing (92 libraries), metagenomics (23 metagenomes, >1,600 metagenome-assembled genomes), massive cultivation (>5,500 strains), and sequencing of selected taxa (>960 draft genomes) for a large number of samples across four algal species.

We identified 14 core genera (persistently present on all macroalgal species) and 14 dominant genera (persistently present of three of the four macroalgal species), of which we obtained 230 strains and 153 species-level genomes and metagenome-assembled genomes. Based on functional annotations, we then focused on two key metabolic traits of algal epiphytic bacteria that were particularly prevalent in core/dominant bacteria, namely the potential to degrade algal polysaccharides and to synthesize bioactive secondary metabolites. We demonstrate a considerable functional redundancy among epiphytic community members in terms of polysaccharide degradation potential (>4,000 polysaccharide utilization loci), which likely represents an adaptive mechanism facilitating colonization of different algal hosts. We also show that, epiphytic marine bacteria, in particular *Flavobacteriia*, might have been underestimated as a source for bioactive substrates (>8,000 biosynthetic gene clusters), and we highlight core genera that likely control and maintain algal colonization.

This report is a collaborative effort of the Marine College of the Shandong University in China and the Max Planck Institute for Marine Microbiology in Germany and combines expertise in algal-associated bacteria, bacterial cultivation, taxonomy and physiology, genomics, metagenomics, and bioinformatic analysis. With this report we also communicate a large number of bacterial strains, many of them novel, as well as draft genomes and MAGs, many of as yet uncultured but relevant phycosphere species, for future studies, including candidates for biotechnological prospecting.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P125

MODULATION OF ICHTHYOTOXICITY OF *KARENIA MIKIMOTOI* BY ITS CO-CULTURING ASSOCIATED BACTERIA

Lam W., Lee T.C.H., Tam N.F.Y., Xu, S.J.L., Chung, W.L., and Lee, F.W.F

Hong Kong Metropolitan University, China

Harmful Algal Blooms (HABs) caused by *Karenia mikimotoi* frequently result in significant mortality of fish and shellfish. Several studies have shown that the toxicity of HAB species can be influenced by the bacteria associated with the algae. However, research on the association between *K. mikimotoi* and its associated bacteria is limited, resulting in a poor understanding of its toxicity to marine life. This study aimed to investigate how the ichthyotoxicity of *K. mikimotoi*, a Hong Kong strain isolated from HAB water (denoted as KMHK), is affected by its associated bacteria. We compared the difference in ichthyotoxicity between xenic (with associated bacteria) and axenic KMHK (without any associated bacteria) cultures based on an *in vitro* fish gill cell line bioassay, as well as isolated the KMHK associated bacteria from the xenic culture. The results revealed that the axenic culture exhibited a 2-fold increase in ichthyotoxicity compared to the xenic culture. When the associated bacteria were reintroduced to the axenic culture to form the "re-xenic" culture, the ichthyotoxicity of KMHK did not return to the same level as that in the xenic KMHK and the "re-xenic" culture was even more toxic than the axenic one. We randomly selected 30 algal-associated bacterial isolates which could be divided into three groups according to the colony colour, that is, red (R), yellow (Y) and white (W), and co-cultivated each isolate with axenic KMHK. The modulatory effects of bacterial isolates on algal ichthyotoxicity varied and could be categorized into three clusters based on 16S rDNA sequencing. The first cluster consisted of isolates belonging to *Alteromonas* genus, the second one was *Marinobacter* and *Ponticoccus* formed the third cluster. Results showed that three pairs of bacterial isolates belonging to the same genus had opposite effects on algal ichthyotoxicity, that is, R2 vs Y8 belonging to *Alteromonas*, R1 vs W7 in *Marionobacter*, and R10 vs W1 in *Ponticoccus*. The differences in ichthyotoxicity between each pair of bacterial isolates in the same genus were affected by growth phases of bacteria and algae, with the highest difference found when log phase bacterial isolate was co-cultured with log phase KHHK.

The gill cell viability of the bacterial isolate in the three pairs, R2, R1 and R10 added to axenic KMHK increased by 64.9%, 69.9%, and 63.0% compared to the other bacterial isolate in the same pair, Y8, W7 and W1, respectively. On the other hand, the contact mode between bacterial and algal cells, whether direct or indirect, did not significantly affect the ichthyotoxicity differences between the pair of R2 vs Y8, R1 vs W7, and R10 vs W1, with difference varied from 6.5 to 27.9%. The effect of associated bacterial isolates on algal toxicity depended on bacterial cell concentration. When R2, R1, R10, and Y8 cells were diluted 100-fold of its initial concentration, the gill cell viability significantly reduced with reduction of 15.6%, 28.7%, 12.6% and 9%, respectively. On the contrary, the cell concentration of W7 and W1 did not significantly alter algal ichthyotoxicity. Reducing the nitrate or phosphate concentration also resulted in significant reduction in gill cell viability in both xenic and axenic KMHK, as well as axenic KMHK co-cultured with R2 bacteria, but such reduction was not observed in the axenic KMHK with Y8. The present study demonstrates that the bacteria associated with harmful algal cells play a significant role in modulating the ichthyotoxicity of *K. mikimotoi*, affecting the damages of HABs. Further investigations and field studies are necessary to understand algal-bacterial interaction during HABs. This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (UGC/FDS16/M01/22)

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P126

PROTEOMIC INSIGHTS OF INTERACTION BETWEEN ICHTHYOTOXIC DINOFLAGELLATE *KARENIA MIKIMOTOI* AND ALGICIDAL BACTERIA *MARIBACTER DOKDONENSIS*

Lee T.C.H.¹, Lam W.¹, Tam N.F.Y.^{1,2}, Xu S.J.L.¹ and Lee F.W.F.^{1,2}

¹ School of Science and Technology, Hong Kong Metropolitan University, Hong Kong

² State Key Laboratory of Marine Pollution, City University of Hong Kong, Hong Kong

Harmful Algal Blooms (HABs) are a global environmental problem caused by the excessive growth of algae, leading to significant economic losses and health impacts. Algicidal bacteria have been proposed as a potential solution to control HABs, but the understanding of the interactions within this process is limited. We have isolated an ichthyotoxic *Karenia mikimotoi* (KMHK) and algicidal bacterium *Maribacter dokdonensis* (P4) from a *Karenia mikimotoi* bloom in Hong Kong 2016, and revealed that P4 had an indirect algicidal effect, excreting substances into the extracellular matrix to kill axenic (bacteria-free) KMHK. The team has also successfully obtained an axenic culture of KMHK, without any algal-associated bacteria. The present study aimed to further investigate the algicidal interaction between KMHK and P4 based on their proteomic responses in several exposure experiments using a cells co-culturing model system, which could separate bacterial and algal cells with a 0.1 µm membrane. The proteome responses of both KMHK and P4 were determined after 8 and 24 hours of exposure. The proteome changes in KMHK after 24 hours of exposure to P4 culture (bacterial cells and medium) and supernatant (medium), as well as that of P4 cells, in the presence or absence of axenic KMHK, were also explored.

The results showed that the proteins of KMHK were downregulated starting at 8 hours and remained so at 24 hours. These downregulated proteins were involved in energy metabolism (including photosynthesis, oxidative phosphorylation and carbon fixation), carbohydrate metabolism, cysteine and methionine metabolism, and cytoskeleton. Differentially expressed proteins of KMHK were related to the response to reactive oxygen species (ROS) and necroptosis, suggesting that KMHK cells were likely suffering from ROS and possibly dying via necroptosis. The proteome responses of KMHK exposed to P4 culture were almost identical to the supernatant exposure, indicating no additional stimulation from the bacterial cells. In terms of the proteome responses of P4 cells, results after 8 and 24 hours of co-cultured with axenic KMHK culture primarily showed the effects of the bacteria at stationary phase such as cell division arrest and adaptation to exposure conditions, including enhanced ferric ion uptake, utilization, and storage. Only bacterial proteins associated with oxidative stress and heat responses were downregulated in after exposure for 8 and 24 hours, and under the presence of KMHK compared with the absence of KMHK, suggesting that KMHK did not induce any stress in P4. This study provides insights into the algicidal interaction between bacteria and algae, without the additional interference from algal-associated bacteria, as the research was conducted in an axenic microalgal culture.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P127

A STUDY ON THE DETECTION OF SAXITOXIN(STX) IN REAL SEAFOOD USING NEURO-2A ASSAY

Kim J.¹, Hong S.Y.¹, Kim Y.³, Park J.^{1,2}, Joh W.B.², Kwon D.H.¹, Kim S.², Park M.², Han Y.S.³, Kim Y.-J.^{1,2*}

¹ *Department of Marine Sciences, Incheon National University, Incheon, Korea*

² *Research Institute of Basic Sciences, Incheon National University, Incheon, Korea*

³ *Institute of Environmental Protection and Safety, NeoEnBizCo., Bucheon, 14523, Republic of Korea*

In recent times, there has been a notable increase in the incidence of marine organisms containing the toxin saxitoxin (STX) in natural environmental samples. Among the various analytical methods available, the Neuro-2a (N2a) cell analysis has emerged as a promising experimental approach that not only offers precision and reproducibility but also facilitates the quantification of toxins. This study is primarily focused on the establishment of an N2a analysis method for the quantification of STX.

The methodology involved setting the concentration of environmental samples and assessing the survival rate of cells subjected to Ouabain and Veratridine (O/V) treatment. Through repeated experiments, we developed a toxin quantification method based on EC50 values, which included a cell density of 70,000, an O/V concentration of 600/60, 24 hours of incubation, and a maximum concentration of 25 mg TE/ml. Subsequently, we explored the extraction of STX from actual marine products, and Solid Phase Extraction (SPE) techniques that could efficiently isolate STX. Applying these methods to the actual marine products samples, we were able to establish a process for detecting and quantifying STX in real seafood products. To implement this method in a real-world setting, it's essential to conduct studies on the correlation with detection techniques such as N2a-based CBA (Cell Based Assay), instrumental analysis, and MBA (Mouse Bioassay). Additionally, it's crucial to devise extraction techniques specifically designed for the isolation of STX from samples. This study suggests that if the N2a analysis method continues to evolve, it holds the potential to become a feasible technology for the detection of STX in domestic seafood. This study suggests that if the N2a analysis method continues to evolve, it holds the potential to become a feasible technology for the detection of STX in domestic

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Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P128

DETECTION OF TETRODOTOXIN (TTX) AND ITS ANALOGUES IN MUD SNAILS *NASSARIUS LIVESCENS* OCCURRING ON A SANDY BEACH IN JEJU ISLAND, KOREA USING A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS)

Kajino N.¹, Hong H.-K.¹, Park B.K.², Kim J.-H.¹, Kim Y.¹, Lee J.², Lee W.-O.¹ and Choi K.-S.^{1*}

¹Department of Marine Life Science (BK21 FOUR) and Marine Science Institute, Jeju National University, Republic of Korea

²Department of Food Science and Technology, Chung-Ang University, Republic of Korea

Recent studies have reported on the occurrence of tetrodotoxin (TTX), the sodium channel-blocking neurotoxin commonly found in pufferfishes in various marine gastropods in tropical and temperate shallow marine environments, alarming potential seafood poisoning as the sea snails are consumed. The intertidal beaches and rocky shores in Jeju Island include numerous subtropical and temperate gastropods including mud snails, they are commonly exploited as seafood. Accordingly, surveillance of TTX-bearing gastropods in intertidal Jeju Island is necessary to ensure seafood safety. In this study, we first screened TTX in fifteen gastropod species from the Jeju coast using TTX-specific competitive ELISA. The ELISA indicated that mud snail *Nassarius livescens* contained a certain level of TTX in their muscle and visceral mass, whereas 14 species of gastropods were free from TTX. Accordingly, TTX and its analogues in thirty individuals of *N. livescens* were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS applied in this study showed a quantification limit (MDL/MQL) of 18–19 µg/kg and 56–57 µg/kg respectively, indicating that the LC-MS/MS was sensitive enough to detect a small quantity of TTX in the tissues. The TTX levels in the muscle ranged from below the detection limit to 3,006.9 µg/kg and below the detection limit to 1,457.2 µg/kg in the viscera, respectively. The detection frequency of TTX in mud snails was 83.3% in muscle and 70.0% in visceral mass, indicating that most *N. livescens* contain TTX. The LC-MS/MS also revealed several TTX analogues, including *epi*TTX, anhydroTTX, deoxyTTX, dideoxyTTX, trideoxyTTX, and norTTX in the tissues. This research was supported by a grant (20163MFDS641) from Ministry of Food and Drug Safety in 2024.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P129

QUANTIFICATION OF TETRODOTOXIN (TTX) AND ITS ANALOGUE IN THE BLUE-LINED OCTOPUS *HAPALOCHLAENA FASCIATA* (HOYLE, 1886) FROM JEJU ISLAND, KOREA

Kajino N.¹, Hong H.-K.¹, Park B.K.², Shin J.-S.¹, Subramaniam T.¹, Lee J.², Lee W.-O.¹ and Choi K.-S.^{1*}

¹Department of Marine Life Science (BK21 FOUR) and Marine Science Institute, Jeju National University, Republic of Korea

²Department of Food Science and Technology, Chung-Ang University, Republic of Korea

The highly venomous blue-lined octopus, *Hapalochlaena fasciata* Hoyle 1886, which possesses tetrodotoxin (TTX), has traditionally been found in shallow tropical and subtropical waters ranging from southern Australia to Japan. Recently, its distribution has expanded to the southern coast of Korea, leading to growing concerns regarding toxin poisoning incidents due to the increased occurrence of this species in the region. Due to the limited data available on the toxicity of this species distributed in Korean waters, we conducted a study to quantify the levels and distribution of TTX and its analogues in the blue-lined octopus collected from Jeju Island, South Korea. The blue-lined octopuses collected in this study were identified as *H. fasciata* through the analysis of 16S ribosomal RNA (16S rRNA) and cytochrome oxidase subunit I (COI) genes. The levels of TTX and its analogues in the posterior salivary gland (PSG), muscle, and skin of *H. fasciata* were analyzed using LC-MS/MS and UHPLC-Orbitrap MS. The LC-MS/MS results indicated that the PSG had the highest TTX level ranging from 259.4 to 883.5 µg/g, followed by the skin (2.5–32.0 µg/g) and muscle (2.9–19.0 µg/g). Additionally, five TTX analogues, including *epi*TTX, anhydroTTX, deoxyTTX, dideoxyTTX, and norTTX, were identified in PSG of *H. fasciata*. In PSG, the concentrations of TTX analogues were as follows: deoxyTTXs had the highest levels, ranging from 78.2 to 664.4 µg/g, followed by dideoxyTTXs (57.2–257.97 µg/g), *epi*TTXs (58.5–100.37 µg/g), anhydroTTXs (10.8–50.7 µg/g), and norTTXs (N.D.–17.6 µg/g). The results of this study provide toxicity information on *H. fasciata* occurring in Korean waters for the prevention of poisoning incidents. This research was supported by a grant (20163MFDS641) from Ministry of Food and Drug Safety in 2024.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P130

OKADAIC ACID-INDUCED CELL CYCLE INHIBITION IN EA.HY926 HUMAN VASCULAR ENDOTHELIAL CELLS

Joh W.B.¹, Kim Y.¹, Kim J.H.³, Park J.¹, Jung E.¹, Kim J.¹, Park M.², Kim Y.J.^{1,2*}

¹ Department of Marine Sciences, Incheon National University, Incheon, Korea,

² Research Institute of Basic Sciences, Incheon National University, Incheon, 22014, Korea

³ Korea Polar Research Institute 26 Songdomirae-ro, Yeonsu-gu, Incheon, 21990 Republic of Korea

Okadaic acid, a shellfish toxin, is a diarrhea-causing toxin that causes various digestive diseases when exposed to the human body, and induces growth inhibition or death in enterocytes, nerve cells, hepatocytes, and blood cells. Differential expression genes (DEGs) were compared through transcriptome analysis in human vascular endothelial cells (EA.hy926 cell line) exposed to Okadaic acid (OA). investigation at the molecular and cellular levels was performed using qRT-PCR and FACS analysis, respectively. In this study, the cytotoxicity of EA.hy926 cell line exposed to OA was investigated. Differentially expressed genes (DEGs) were selected through transcriptome analysis, and the toxic mechanism when exposed to marine-derived toxins was identified by functionally analyzing the selected DEGs. When comparing unigene expression in EA.hy926 cells exposed to OA, the comparison between Control (Con) and OA revealed 2,778 DEGs. (P value < 0.05 and fold change ≥ 1.5). As a result of the annotation and clustering of DEGs, genes related to apoptosis and cell cycle were mainly discovered. To verify these analysis results, five representative apoptosis-related genes and five cell cycle genes were selected, and their gene expression was analyzed by qRT-PCR. It was investigated that these target genes were upregulated or downregulated, and the investigation of changes at the cellular level through flow cytometry investigated apoptosis of EA.hy926 cells exposed to OA. These results revealed the mechanisms of apoptosis and cell cycle toxicity in EA.hy926 cells exposed to OA. In addition, it is expected to be helpful in studying the mechanism of toxicity when OA, a shellfish toxin, is exposed to the human body as well as EA.hy926 cells.

*Corresponding author: duckyj@inu.ac.kr

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FEEDING BEHAVIORS RESPONSES OF TWO COPEPODS-*PARACALANUS PARVUS* AND *CALANUS SINICUS* TO THREE TYPICAL RED TIDE ALGAE WITH DIFFERENT CONCENTRATIONS

Ding Z., Sun X.

Shandong University, Weihai, China

The top-down control of copepods to phytoplankton has immense ecological significance. This study investigated the feeding selectivity of copepods via their feeding behaviors using high-speed camera. Two copepoda species, *Paracalanus parvus* as a small copepod and *Calanus sinicus* as a large copepod, were used as their dominance in the nearshore of Chinese seas. Their feeding behaviors separately fed on three algae—*Heterosigma akashiwo*, *Prorocentrum donghaiense*, and *Skeletonema costatum*—were studied at five different concentrations; the factors characterizing feeding behavior, including the beating frequency (BF), beating time (BT), and rejection behavior, were analyzed. The results showed that all the average BFs and BTs of *P. parvus* fed on three algae were significantly higher than those of *C. sinicus*, but there were no significant differences in rejection behavior between these two copepods among all the three algae during the short period of experimentation. These results indicate that, comparing with *C. sinicus*, *P. parvus* displays more active feeding behavior than *C. sinicus*. Moreover, the average BF of *P. parvus* fed on diatom (*S. costatum*) was significantly higher than that on *H. akashiwo* and *P. donghaiense*, and there were no significant differences in the average BTs of *P. parvus* fed on these three algae. These indicate that *P. parvus* could exhibit a stronger top-down control over diatom. However, this feeding control did not show in *C. sinicus*, as the average BF of *C. sinicus* fed on diatom was significantly higher meanwhile the average BT was relatively shorter than those of the other two algae. Further comprehensive analysis to copepods feeding behaviors on three algae at different concentrations revealed that the feeding control of *P. parvus* on the three algae did not decrease with the increase of the concentration (except the BF on *P. donghaiense*), while the feeding control of *C. sinicus* represented a significant decreasing trend with increasing concentration of three algae (except *P. donghaiense*). These results further verify that comparing with *C. sinicus*, *P. parvus* exhibits a stronger feeding control on red tide algae. This study clarifies the strong feeding-control ability of small copepods to red tide algae, and also provides evidence for accurate analysis of top-down control and feeding niches of copepods in different body sizes.

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DIFFERENTIAL SUSCEPTIBILITY TO HYPOXIA IN HYPOXIA-INDUCIBLE FACTOR 1-ALPHA (HIF-1A)-TARGETED FRESHWATER WATER FLEA *DAPHNIA MAGNA* MUTANTS

Lee Y.¹, Byeon E.¹, Kim D.-H.¹, Lee J.-S.², Kim H.S.², and Lee J.-S.¹

¹ Department of Biological Sciences, College of Science, Sungkyunkwan University, Suwon 16419, South Korea

² School of Pharmacy, Sungkyunkwan University, Suwon 16419, South Korea

The water flea *Daphnia magna* serves as a key model organism for investigating the response of aquatic organisms to environmental stressors, including hypoxia. Hypoxia-inducible factor 1-alpha (*HIF-1a*) is a central regulatory protein involved in the cellular response to hypoxic conditions. In this study, we used CRISPR/Cas9 gene editing to create a panel of *D. magna* mutants with targeted alterations in the *HIF-1a* gene. These mutants exhibited differential susceptibility to hypoxia. Our results revealed a complex interplay of *HIF-1a*-mediated responses in *D. magna*. The *HIF-1a* mutants showed decreased survival and reproduction due to the down-regulated expression of *HIF-1a*-mediated pathways in low-oxygen conditions. These findings suggest that the *HIF-1a* pathway is a critical component of the hypoxia resistance in *D. magna*. This study provides novel insights into the molecular basis of hypoxia tolerance in *D. magna* and contributes to our broader understanding of how aquatic organisms may adapt to the changing oxygen levels caused by global environmental change.

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OXIDATIVE STRESS-MEDIATED DELETERIOUS EFFECTS OF HYPOXIA IN THE BRACKISH WATER FLEA *DIAPHANOSOMA CELEBENSIS*

Lee Y., Kim D.H., Byeon E., Lee J.-S., Kim H.S., and Lee J.-S.

Department of Biological Sciences, College of Science, Sungkyunkwan University, Suwon 16419, South Korea

Global oxygen content is decreasing due to climate change, including global warming and ocean acidification, which pose serious problems for the aquatic environment. The substantial decrease in oxygen level leads to hypoxia, which is defined as dissolved oxygen levels less than 2 mgO₂/L. Although it is widely known that hypoxia induces oxidative stress and reproduction decreases, there is a lack of studies of the toxic effects of hypoxia on brackish water cladocerans. In this study, we investigated the acute toxicity, *in vivo* effects, oxidative stress, and gene expression changes caused by hypoxia on the brackish water flea *Diaphanosoma celebensis*. The no-observed-effect concentration (NOEC) of 48 h of hypoxia exposure was found to be 2 mgO₂/L. Also, chronic exposure to NOEC caused a significant decline in lifespan and had no effect on total fecundity. The reactive oxygen species (ROS) induced by 48-hour hypoxia increased in a time-dependent manner, whereas the content of antioxidant enzymes (superoxide dismutase and catalase) decreased. The transcription and translation levels were modulated by hypoxia exposure, which is conducted biologically in triplicate. In particular, a significant increase in hemoglobin level was followed by up-regulation of *HIF-1a* gene expression and activation of the mitogen-activated protein kinase pathway. In summary, our findings provide a better understanding of the molecular mechanism of the adverse effects of hypoxia in brackish water zooplankton.

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INVESTIGATION OF DINOFLAGELLATE *GAMBIERDISCUS* AND CORAL REEF FISH OF THE REPUBLIC OF KIRIBATI

Zhu J.^{1,2}, Wu J.^{1,3}, Lee W.H.^{1,2}, Liu X.^{1,2} and Chan L.L.^{1,2,3*}

¹ State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

² Department of Biomedical Sciences, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

³ 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, Shenzhen 518057, China

Ciguatera poisoning (CP), a widespread foodborne illness, is caused by consuming fish contaminated with ciguatoxins (CTXs) produced by the dinoflagellate *Gambierdiscus*. The Republic of Kiribati, a Pacific Island nation with limited land resources and abundant marine resources, heavily relies on the fishing industry. However, contaminated fish-caused ciguatera outbreaks greatly affect its export trade. This study aims to investigate the ciguatoxicity, hemolytic activity, and toxin profile of 19 strains of *Gambierdiscus* and one strain of *Fukuyoa* using neuro-2a (N2a) by hemolytic assays and high-resolution mass spectrometry. Additionally, analysis of 17 species of snappers and groupers using N2a assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was conducted to determine their contamination with CTX. *Gambierdiscus* strains from Kiribati exhibited detectable ciguatoxicity and hemolytic activity. Putative 44-methylgambierone was identified as part of the contributors to the hemolytic activity, and other unknown hydrophilic toxins produced can be potentially linked to higher CP incidence in Kiribati. LC-MS/MS analysis on the fish samples revealed that the most toxic groupers had high level of CTXs (equivalents to 53 times higher than the safety threshold of P-CTX-1). The results of N2a assay suggested that the fish specimens also contained high levels of other CTX-like toxins or sodium channel activators. These findings provide valuable information on the occurrence and toxicity of *Gambierdiscus* in Kiribati and groupers and snappers can be served as bioindicator for risk assessment in ciguatera endemic areas, which contributes to overall environmental monitoring initiatives.

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SPATIOTEMPORAL DISTRIBUTION AND DRIVING MECHANISMS OF ALGAL TOXINS AND THEIR PRODUCERS IN HONG KONG COASTAL WATERS

Zhang L.¹, Xiong A.¹, Liu X.¹, Jin L.^{1,2,3}

¹ Department of Civil and Environmental Engineering, The Hong Kong Polytechnic University, China

² Department of Health Technology and Informatics, The Hong Kong Polytechnic University, China

³ State Key Laboratory of Marine Pollution, City University of Hong Kong, China

The occurrence and impact of harmful algal blooms (HABs) have been on the rise globally due to anthropogenic pollution and climate change in coastal areas. Lipophilic algal toxins (LATs) produced by toxigenic microalgae pose serious threats to marine life, shellfish, and human health. Hong Kong, renowned for its diverse marine ecosystems and thriving aquaculture industry, lacks comprehensive knowledge about the spatial and temporal distribution of algal toxins and their producers. In this study, we investigated nine selected LATs, phytoplankton communities, and potential toxin producers in relation to environmental parameters during wet and dry seasons in the coastal waters of Hong Kong. Among the nine targeted LATs, pectenotoxin-2 (PTX-2), okadaic acid (OA), dinophysistoxin-1 (DTX-1), and gymnodimine (GYM) were detected. Each LAT exhibited a distinct spatiotemporal pattern. PTX-2 concentrations were significantly higher in the dry season compared to the wet season throughout the entire study area. GYM concentrations were notably higher in the eastern waters compared to other regions in both seasons, with no significant seasonal difference in the same waters. OA concentrations in the eastern waters were significantly higher than in the southern and western waters, but only during the wet season. DTX-1 was detected exclusively during the dry season, with consistent concentrations across the study area, except for sporadic spikes in specific hotspots within Mirs Bay. The distribution of *Dinophysis* spp. and *Gymnodium* spp., known producers of PTX-2 and GYM, respectively, corresponded with the spatiotemporal pattern of their respective LATs. The relative abundance of *Dinophysis* spp. and *Gymnodium* spp. was positively associated with PTX-2 and GYM concentrations, respectively. In contrast, However, the spatiotemporal pattern of *Dinophysis* spp., which also includes DTX-1-producing species, was inconsistent with that of DTX-1 concentrations. *Prorocentrum* spp., which includes OA- and DTX-1- producing species, exhibited an inconsistent spatiotemporal pattern with OA and DTX-1 and displayed higher relative abundance in the eastern waters compared to the southern waters during the dry season. Further research is needed to identify LAT producers at the species level and understand their toxin-producing capacity over spatiotemporal scales. Among the various environmental factors screened, temperature and salinity showed strong correlations with the potential LAT-producing community, indicating their role in regulating the spatial and temporal distribution of toxins and toxin producers. Our findings provide valuable insights for predicting responses of toxigenic marine algae to future changes in the coastal environment.

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INVESTIGATION OF THE EFFECTS OF 44-METHYLGAMBIERONE ON MARINE MEDAKA (*ORYZIAS MELASTIGMA*)

Liu X.^{1,2}, Wu J.^{1,2}, Qin X.¹, Zhu J.^{1,2} and Chan L.L.^{1,2,3*}

¹ State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

² Department of Biomedical Sciences, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

³ Shenzhen Key Laboratory for the Sustainable Use of Marine Biodiversity, Research Centre for the Oceans and Human Health, City

Benthic dinoflagellate genus *Gambierdiscus*, widely distributed in tropical and subtropical regions of the oceans, can produce diverse array of ladder-shaped polyether toxins, including ciguatoxins (CTXs), maitotoxins (MTXs), gambierones, gambierol, gambieroxide, and gambieric acids. Some of these toxins are responsible for ciguatera food poisoning (CFP), which is the most common non-bacteria seafood-related illness worldwide. Among these toxins, 44-methylgambierone is the dominant toxin in *Gambierdiscus* toxin family. Like CTXs, 44-methylgambierone can activate voltage-gated sodium channels and disrupt ion conductance in excitable tissues of animals, and it has potential to bioaccumulate and undergo biotransformation through marine food webs. However, there are only few reports on the accumulation kinetics and toxicological properties of this toxin. In the present study, we use to marine medaka (*Oryzias melastigma*) as model to investigate the tissue-specific uptake of 44-methylgambierone, and its altered physiological functions and transcriptome. Our results showed that 44-methylgambierone could be distributed from yolk sac to all parts of medaka larval body after 18 hours of exposure. The levels of 44-methylgambierone accumulation in the head were significantly higher than those in the trunk, which is consistent with the observed impairment of fish behaviours. In addition, the genes associated with eyes, nervous systems, and heart were found to be altered in the transcriptome analysis. These findings provide valuable information on the potential bioaccumulation tendencies and toxicological properties of 44-methylgambierone, the main toxin of different *Gambierdiscus* species. Furthermore, these findings provide a better understanding on the impact of the entire *Gambierdiscus* community on marine animals.

Coastal eutrophication, hypoxia, harmful algae blooms and algal toxins: P137

ANTHROPOGENIC ALTERATION IN ARTIFICIAL SEAWATER LAKE WATER QUALITY AND PLANKTONIC ECOSYSTEM

Kim S.H.¹, Kim K.², Kim M.S.³, Cho H.E.¹, Shin K.H.¹, Won E.I.¹

¹Hanyang University, Republic of Korea

²Human and Ecocare Center, Konkuk University, Republic of Korea

³National Institute of Environmental Research, Republic of Korea

In recent decades, expansion of human activities (e.g., residential, industrial, agricultural, etc.) around watershed boundaries (e.g., estuary, river, and lake boundary) has enhanced the discharge rate of terrestrial anthropogenic nutrients to aquatic environments. These excess nutrient inputs may cause a deterioration in water quality and alter the planktonic ecosystem sustained by nutrient status. In this study, we analyzed the isotopic composition of nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$) and amino acid nitrogen ($\delta^{15}\text{N}_{\text{AAs}}$) to identify the anthropogenic N transportation from land surface to the planktonic ecosystem. Additionally, dominant zooplankton species were compared to identify the effect of species composition on the $\delta^{15}\text{N}_{\text{AAs}}$ composition of plankton samples. To compare the anthropogenic nutrient input from various land-use types, surface water samples from tributaries and lake were collected before and after the typhoon events (BT and AT, respectively). Zooplankton samples were also collected using zooplankton nets (net pore size, 60 μm). The dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) of lake water represented similar ranges in BT (2.7 \pm 0.8 mg/L for DOC and 0.8 \pm 0.7 mg/L for DTN) and AT (2.5 \pm 0.7 mg/L for DOC and 0.8 \pm 1.0 mg/L for DTN) samples. However, nutrients and DON showed different ranges according to typhoon events. DON (0.6 \pm 0.4 mg/L), $\text{NH}_4^+\text{-N}$ (0.1 \pm 0.2 mg/L), and $\text{SiO}_4^{2-}\text{-Si}$ (1.0 \pm 0.4 mg/L) demonstrated relatively higher ranges, and $\text{PO}_4^{3-}\text{-P}$ (0.2 \pm 0.3 mg/L) and $\text{NO}_3^-\text{-N}$ (0.5 \pm 0.8 mg/L) show higher ranges in AT. For the zooplankton, the total individual number for the lake sites showed higher ranges (20 \pm 18 Ind./L for BT and 7736 \pm 9598 Ind./L for AT) in the AT than in BT, with different dominant species, Nauplius of Copepoda for the BT and Protozoa (*Tintinnopsis sp.*) for the AT. These significant changes in dissolved components and zooplankton composition may indicate that typhoon events transport inland anthropogenic nutrients (N/P ratio; 9.4 \pm 8.4) to lake systems via tributary input. The nitrate dual isotopic composition ($\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$) in the BT significantly varied between industrial areas and other land-use groups. The nitrate dual isotopes in the lake sites (5.1 \pm 0.9 ‰ for $\delta^{15}\text{N}_{\text{NO}_3}$ and 12.4 \pm 1.6 ‰ for $\delta^{18}\text{O}_{\text{NO}_3}$) had values similar to those in urban sites (4.9 \pm 0.4 ‰ for $\delta^{15}\text{N}_{\text{NO}_3}$ and 13.0 \pm 1.0 ‰ for $\delta^{18}\text{O}_{\text{NO}_3}$) and wetlands (4.4 \pm 0.4 ‰ for $\delta^{15}\text{N}_{\text{NO}_3}$ and 12.1 \pm 0.8 ‰ for $\delta^{18}\text{O}_{\text{NO}_3}$), indicating that they were the dominant nitrate source in the BT. Meanwhile, the industrial area demonstrated significantly heavy and light $\delta^{15}\text{N}_{\text{NO}_3}$ (7.3 \pm 3.3 ‰) and $\delta^{18}\text{O}_{\text{NO}_3}$ (0.3 \pm 2.8 ‰) ranges, respectively, with high nitrate concentration (0.8 \pm 1.0 mg/L), indicating that nitrates in industrial areas may originate from different water sources used to manufacture machinery and petrochemicals. In the future, comparing $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ BT and AT may estimate the dominant specific anthropogenic source changes according to typhoon events. Furthermore, comparing $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{AAs}}$ plankton may provide an approximate amount of anthropogenic nitrogen transportation to planktonic ecosystems.

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POLYMORPHIC CHANGE IN MICROALGAE AND ITS ASSOCIATED ENVIRONMENTAL FACTORS IN KHAO CHAU OCEAN, HUIZHOU

Chen Y., Sun J., He S., Xie X., Zhou L., Gong H.

Huizhou University, China

As an important part of the wetlands in the Guangdong-Hong Kong-Macao Bay area, and planting a large number of mangroves is also one of the important aquaculture bases. However, there are fewer studies on the polymorphism of microalgae in the Khao Chau Ocean. This research adopts 23S rDNA high-throughput sequencing method to study the species composition, dominant taxa, abundance and diversity of microalgae communities from the survey data of microalgae communities and environmental factors in the offshore waters of the Khao Chau Ocean in April 2021, on the basis of which multivariate statistical analysis was applied to explore the effects of environmental factors on the characteristics of zooplankton communities. The results of the study showed that 806 species of microalgae were detected in the five areas, belonging to 2 kingdoms, 30 phyla, 56 orders and 118 orders, and the microalgae were mainly distributed in 8 phyla, including Cyanobacteria, Metazoan, Green Algae and Diatoms. Among the seven selected physicochemical factors, total phosphorus (TP) had a significant effect on microalgae, while ammonium salt (NH₄⁺) had no significant effect. The microenvironment was also correlated with microalgae polymorphism, and the microalgal plant diversity index (H') was highly significantly and positively correlated with chromium (Cr) and copper (Cu), while it was highly and negatively correlated with lead (Pb) and zinc (Zn). The results of this study will provide a reference for biodiversity analysis and fisheries development in the offshore area of Khao Chau ocean.

ADVANCED OXIDATION TECHNOLOGY FOR EFFICIENT TREATMENT OF EMERGING POLLUTANTS

Ming L.

City University of Hong Kong, China

In recent years, estrogen causes can affect the normal hormone metabolism of organisms, causing widespread concern. In water treatment, it is difficult to achieve good removal efficiency through traditional water treatment technology, and it flows into people's daily lives, damaging human health. Therefore, there is a need to develop efficient and feasible wastewater treatment methods for estrogen pollution in water bodies. Mn_3O_4 nanomaterials were successfully prepared by a hydrothermal method using green tea extract as a reducing agent, and the prepared Mn_3O_4 activated disulfide (PDS) was used to remove both estriol and 17 α -ethinylestradiol (E3/EE2). It was found that the activation of PDS by Mn_3O_4 was more effective than that of H_2O_2 , and the Mn_3O_4 /PDS system was 72.0% and 72.1% efficient for the simultaneous removal of E3/EE2, respectively. The pathways of hydroxyl achieved the reduction of estrogens radical ($\bullet OH$), sulfate radical ($SO_4^{\bullet -}$), superoxide anion ($O_2^{\bullet -}$), and singlet oxygen (1O_2) by Mn_3O_4 /PDS. Pathways to achieve the removal of estrogen, which is mainly dominated by single-linear oxygen (1O_2). Finally, the degradation pathways of E3 and EE2 were further analyzed by liquid chromatography-mass spectrometry (LC-MS), and the degradation products of E3/EE2 were small molecules containing hydroxyl and sulfate. In contrast, the degradation products' ecotoxicity was analyzed using the ESCOSAR model. The mechanism of green synthesis of Mn_3O_4 /PDS for the simultaneous removal of E3/EE2 by generating a large amount of 1O_2 was proposed, which provides a theoretical basis for further studies on the reduction of endocrine disruptors.

LITHIUM RECOVERY FROM SALINE WATER THROUGH H₃LiTi₅O₁₂-BASED CAPACITIVE DEIONIZATION TECHNOLOGY

Lin L.^{1,2}

¹ Tsinghua University, Shenzhen International Graduate School

² State Key Laboratory of Marine Pollution, The City University of Hong Kong

The rapid consumption of lithium ion (Li⁺) battery for electronic devices has significantly increased the demand for lithium resource in recent years. The saline water from sea and salt-lake is considered as the ideal source for Li⁺ extraction, but the co-existing cations, such as Na⁺ and K⁺ limit its recovery efficiency when the adsorption method is applied. In this study, the H₃LiTi₅O₁₂ nanoparticles (HTO) with a spinel structure were synthesized as Li⁺-selective adsorbent. The static adsorption experiments showed that the Li⁺ absorption capacity of HTO was significantly affected by the pH of bulk solution, and exhibited a high separation factor of Li⁺/Na⁺ and Li⁺/K⁺ at 37 and 16, respectively. When HTO was mixed with activated carbon (AC) and coated on the cathode plate of capacitive deionization (CDI) cell, the Li⁺ adsorption kinetics (rate constant) was significantly enhanced from 0.18 to 0.25 under 1.2 V, due to the accelerated rate of the film diffusion driven by the electric field. Density functional theory (DFT) calculation result showed that applying electricity can strengthen the hydrogen bond between Li and O atom on the HTO (1 1 1) facet. When the AC mixing ratio was increased from 10% to 50%, the Li⁺ desorption efficiency increased from 47% to 90%, but at the expense of sacrificing the adsorption capacity, which decreased from 21.6 mg/g to 9.8 mg/g. In terms of the overall recovery efficiency of Li from brine, the weight ratio of HTO:AC at 9:1 was suggested for the CDI method. Finally, the Li⁺ recovery from actual brine was successfully demonstrated, with 7.1 mg/g of adsorption capacity and well stability.

DEVELOPMENT OF A PLATFORM FOR RAPID IDENTIFICATION OF MICROALGAE AND MICROPLASTICS IN SEAWATER

Yan M.^{1,2*}, Liao R.³, Leung P.T.Y.^{1,2}, Liu M.Y.¹, Lam V.T.T.¹, Chen W.¹, Zhang K.⁴, Ruan R.F.^{1,2}, Leung K.M.Y.^{1,2}

¹ *State Key Laboratory of Marine Pollution and Department of Chemistry, City University of Hong Kong, Hong Kong SAR, China*

² *Research Centre for the Oceans and Human Health, City University of Hong Kong Shenzhen Research Institute, Shenzhen, China*

³ *Shenzhen Key Laboratory of Marine IntelliSensing and Computation, Institute for Ocean Engineering, Shenzhen International Graduate School, Tsinghua University, Shenzhen, China*

⁴ *National Observation and Research Station of Coastal Ecological Environments in Macao, Macao Environmental Research Institute, Macau University of Science and Technology, Taipa, Macao SAR, China*

The ocean is home to a significant number of suspended particles, which include microalgae and microplastic particles. These particles have become a growing concern worldwide due to their negative impact on the marine environment, fisheries, and human health. This negative impact is particularly evident in response to human activities and climate change.

The ongoing project aims to establish a highly efficient and effective rapid detection platform based on polarization measurement. This advanced platform will enable the timely identification and continuous monitoring of microalgae and microplastics in the ocean. By providing fast and reliable information on the composition, quantity, and distribution of these suspended particles, as well as generating early warning signals, the platform can significantly contribute to the protection of marine environments and the sustainable development of the fishing industry.

In order to achieve this goal, the project collected comprehensive data on various identified marine microalgae and microplastic particles. These data were associated with physical parameters obtained through polarization measurement and used to create a robust and extensive database. Through advanced big data analysis techniques and algorithm optimization, the platform will be able to rapidly and accurately classify target particles. One of the key advantages of this platform is its cost-effectiveness. Moreover, the platform has the potential to be expanded to detect additional target particles by enriching the information stored in the database, making it a versatile and adaptable tool for ongoing research and monitoring efforts.

OCEAN MAPPING TECHNOLOGY PROMOTING CARBON NEUTRALITY - A CASE STUDY IN TUNG PING CHAU, HONG KONG

Chung J.T.H.¹, Wu J.^{1,3}, Qin X.¹, Ng P.K.P.¹, Chan J.Y.T.¹, Foo Y.H.¹, and Chan L.L.^{1,2,3*}

¹ State Key Laboratory of Marine Pollution, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

² Department of Biomedical Sciences, City University of Hong Kong, Kowloon Tong, Hong Kong SAR, China

³ 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, Shenzhen 518057, China

According to a report in 2023, more than 90% of global greenhouse gas emissions were in the form of CO₂, which was attributed to the consumption of energy, and agriculture forestry and land use. More importantly, by 2050, it is estimated that the global population will be increased to 9.9 billion, which will induce more eco-unfriendly human activities. Therefore, it is necessary to achieve a balance of greenhouse gas emissions and absorption. Ocean is a major contributor to carbon neutrality; advanced technologies such as satellite observation and digital earth have been applied to provide baseline information for the estimation and prediction of oceanic carbon storage. However, these technologies are limited by disturbances of weather during monitoring and incomplete baseline information in the construction of digital Earth. By using a case study in Tung Ping Chau, we aim to develop a novel ocean mapping technology to provide comprehensive information for the calculation and prediction of carbon sink storage capability. Acoustic, optical, and aerial surveys are performed to depict a complete ocean benthic map including both coverage and distribution of macroalgae, seagrass, and coral reefs. Also, the areas of the hard coral community, octocoral community, and sargassum bed are determined. The constructed ocean benthic map of Tung Ping Chau provides updated information for carbon storage capacity estimation.

MEIO- AND MACROFAUNA AS INDICATORS IN THE ECOLOGICAL MONITORING PROGRAM, DO THEY BEHAVE THE SAME?

Zhou H., Abdul MD K., Montero Taboada R.

Ocean University of China, China

In the Jiaozhou Bay, one of the most important semi-enclosed waters along the southern Yellow Sea coast of China, anthropogenic activities like bivalve aquaculture and oil spills have severely impacted ecological environment of the bay in the past years. Meio- and macrofauna are the two dominant and diverse components of marine benthos, typically used as indicators in monitoring and assessing benthic ecosystem health. However, the response of the two components to the anthropogenic disturbance and environmental changes were not well understood. During an autumn cruise of 2018, meio- and macrofaunal samples were simultaneously collected at 11 stations from the north to south in the bay. We compared the behaviours of meio- and macrofaunal components for their community structure, diversity, function and trait based indices as indicators. The results suggesting that they do behave in some inconsistency, implying differential response of meio- and macrofauna to different environmental conditions and anthropogenic disturbances. As in the case of the Jiaozhou Bay, meiofauna indicated a generally moderate/poor environmental condition of the bay while macrofauna indicated a better condition as high/good. Meiofauna and macrofauna indicated the same results for the spatial variation of environmental conditions for most of the stations within the bay, except for the north-east station J1 where opposite assessment results were obtained. However, both meiofauna and macrofauna are good indicators for ecological status of the semi-enclosed bay; they consistently indicated the negative (J3, *Ruditapes philippinarum*) and positive (J4, *Crassostrea gigas*) effects of shellfish aquaculture on the ecosystem health. Our study suggested that the two components of meio- and macrofauna should be used interactively to produce more comprehensive and concrete assessment on the benthic ecosystem health during a routing monitoring program.

SOLID-PHASE MICROEXTRACTION AS A NOVEL TECHNIQUE TO EXTRACT CARBAMAZEPINE AND ITS METABOLITES FROM *CHLAMYS ISLANDICA* AFTER EXPERIMENTAL EXPOSURE

¹Leszczyńska D.², Roszkowska A¹, Lipiński M.¹, Hallmann A.^{1,3}, Mazurkiewicz M.⁵, Husøy I.S.⁴, Smolarz K.⁴, Sokołowski A.

¹ Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland

² Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland

³ Department of Marine Ecology, Institute of Oceanology Polish Academy of Sciences, Sopot, Poland

⁴ Department of Marine Ecosystems Functioning, Faculty of Oceanography and Geography, University of Gdańsk, Gdynia, Poland

⁵ Norwegian University of Science and Technology, Trondheim, Norway

Human and veterinary pharmaceuticals are present in the environment worldwide, mainly due to rising global consumption of pharmaceuticals, their improper disposal and poor efficacy of purification processes in wastewater treatment plants. These factors contribute to the continuous presence of pharmaceuticals and the products of their metabolism in the aquatic environments, including marine waters. Carbamazepine (CBZ) is an antiepileptic drug which is constantly released into the environment and has low removal rates during wastewater treatment processes and low biodegradability, thus it is persistent in many water basins. Due to its constant release to the marine environment, CBZ can be detected at levels up to µg/L and it is also present in tissues of bivalves (up to ng/g dry mass). Reported effects of CBZ exposure in marine bivalves have been related to cytotoxicity and genotoxicity, including oxidative stress.

Most extraction techniques for the isolation of CBZ from tissues and biofluids are based on traditional techniques such as solid phase extraction (SPE), pressurized liquid extraction (PLE) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). However, these extraction methods are time-consuming and require relatively large volumes of organic solvents. Solid phase-microextraction (SPME), a novel sample preparation technique, utilizes minimal volumes of organic solvents and thus is considered as a simple, rapid and green technology that can be applied to monitor the exposure of aquatic organisms to pharmaceuticals and other environmental pollutants. SPME also facilitates the quenching and storage of analytes. The aim of this study was to develop and apply the optimised SPME conditions to detect and quantify CBZ and its two active metabolites (10,11-epoxide carbamazepine (CBZE) and 10,11-dihydro-10-hydroxycarbamazepine (CBZD)) in tissues of marine bivalve, the Iceland scallop *Chlamys islandica*. The scallops were exposed to CBZ at two concentration levels: 50 ng/L and 1 µg/L in aquaria for 7 days. The experiments were performed in The Kings Bay Marine Laboratory (Ny-Ålesund, Svalbard, Norway). After exposure, the organisms were dissected into 4 tissues: gonads, muscle, gills and digestive system. SPME fibers coated with C18 extraction phase were inserted for 10 minutes into bivalves' tissues to extract CBZ and its metabolites. Fibers with retained (quenched) analytes were transported to Poland and desorbed for 10 minutes in the ACN:H₂O (80:20) mixture. The obtained extracts were subjected to LC-MS/MS analysis. Results have shown the presence of CBZ and its metabolites in *C. islandica* tissues at ng/g wet mass levels. The applied SPME-based technique can be successfully performed on-site as compounds are quenched on the coating which facilitates secure transportation of the samples without any loss of analytes. In addition, the biocompatibility of the extraction phase of SPME fibers provides the opportunity for *in vivo* sampling of the living organisms.

NATIONWIDE EVALUATION OF PURIFICATION CAPACITY FOR WATERBORN NITROGEN AND PHOSPHOROUS IN COASTAL SEDIMENTS OF SOUTH KOREA

Lee C., Kwon I., Kim B., Kim T., Lee J., Park S.Y. and Khim J.S.

School of Earth and Environmental Sciences, Seoul National University, Korea

Human activity is assumed to impair the regulating function required to maintain the marine ecosystem viz. coastal protection, prevention of erosion, water purification and carbon storage. Despite the significance of the marine ecosystem service, the complex interactions with human disturbance remain poorly understood. Here, using an artificial tide control system, we test how human activities impact the water purification value of Korean coastal areas.

Intertidal flats of South Korea were extensively surveyed in 2018–21. The sediment textural type (sand, mixed, and mud) classified from satellite images were significantly correlated to that identified from field data, warranting a nationwide estimate of total N and P purification values.

Our results suggest that water purification amount of nitrogen and phosphorus in the tidal flat was confirmed to be 1,557 Mg N yr⁻¹ and 4,633 Mg P yr⁻¹, respectively. The purification of total N and P varied significantly depending on the region, province, morphology, and population size of the adjacent tidal flats. Finally, we confirmed that population density had a particularly damaging effect on the regulating services in Korea. We find that food web and service robustness are highly correlated, but that robustness varies across services depending on their trophic level and redundancy. Overall, our results confirmed the valuable ecosystem service of tidal flat's cost-efficient N and P purification abilities, highlighting marine ecosystem service.

DO APPLICATION OF ETHICAL PRACTICES IN FISH SAMPLING AFFECTS PHYSIOLOGICAL CONDITIONS AND STABLE ISOTOPE RATIOS IN THE FISH SAMPLES?

Cho H.E., Yun H.Y., Won E.J., and Shin K.H.

Hanyang University, South Korea

Amino acid (AAs) composition and AA-specific isotope ratios in vertebrate sample including fish are frequently used for ecological, physiological and toxicological studies. Various sample types such as fish blood, liver and muscle or whole body are generally collected, but such collection process should be accompanied with specimens sacrifice under stressful conditions. Recently, investigators start to select sampling methods and experimental procedures based on bioethics for the humane care and use of animals for research purposes. Nonetheless, it is still unknown whether anesthesia, to minimize the lasting suffering moment in fish, can cause confounding effects on AAs. Using anesthetic drugs, respectively, 2-phenoxyehtanol 0.30 mL in water 1L and benzocaine 40mg dissolved in ethanol in water 1L, here we tested for a putative effect of these chemical methods as well as non-chemical methods (cooling to 10°C) on AA compositions in fish liver and blood, respectively, in fish a Mud Loach (*Misgurnus mizolepis*), and compared with no treatments (control). In 45 min application, the fish from all treatment aquaria lay down motionless contrast to the fish from control aquaria. Moreover, there were no significant effects on quantities of 15 common AAs, which were found in both liver and blood. These results suggest that anesthesia of fish is not confounded with the AAs metabolic processes in liver and blood tissues, and therefore the use of samples collected from anesthetized fish may be suggested in context of animal welfare in our lab and field work.