PROGRAMME

AoE Sympoisum Programme
6 January, 2011
T4 Meng Wah Complex, University Campus

9:30-9:40	Rudolf Wu	Welcoming Remarks and Overview
	Presentation by Task Team 1 (Chair: Paul Lam / Norman Woo)	
9:40-9:45	Paul Lam	Highlight on achievements of **Task Team 1**
9:45-10:00	M.S. Yang / C.C. Fong	Development of biosensors for direct detection of endocrine disrupting chemicals
10:00-10:15	Alice K.Y. Chan	Accumulation and maternal transfer of PBDE 47 in the marine medaka (*Oryzias melastigma*) following dietary exposure
10:15-10:30	Michael H.W. Lam	Can bromophenol metabolites in human urine be used as population exposure markers for PBDEs?
10:30-10:45	K.C. Liu	Luteinizing Hormone Receptor (*lhcgr*) as a Potent Estrogenic Responsive Gene in the Zebrafish Ovary for Studying Action Mechanisms of Bisphenol A
10:45-11:00	S.X. Chen	Proteomics Analysis for Estrogen-responsive Genes in Fish Ovarian Follicles
11:00-11:15		***Coffee Break***
11:15-11:30	Anna Tse	Deciphering the specific effects of EDC's along HPG Axis – an *in vitro* study
11:30-11:45	Andy C.K. Cheung	Analysis of estrogen receptors (ERs) in medaka and optimization of medaka cell lines for *in vitro* assays
11:45-12:00	G. Chaturvedi	Effect of Hypoxia–inducible Factors (HIFs) on microRNA expression and steroidogenesis in the H295R human adrenocortical cell line
12:00-12:15	L.H. Chu	Human leukocyte antigen (HLA) homologues: a key to understanding hypoxia-mediated modulation of reproductive functions in vertebrates
12:15-12:30	Andy K.H. Lo	Hypoxia Impairs Primordial Germ Cells (PGCs) Development in Zebrafish (*Danio rerio*) Embryos
12:30-12:45	Natalie Degger	Validation of Artificial Mussels for metal monitoring: Field studies along the coastlines of South Africa and China
12:45-14:15		***Lunch***
14:15-14:30	N. Yang	Detection of hepatitis A virus and norovirus in seawater
14:30-14:45	S.H. Cheng	Knowledge transfer of transgenic marine medaka technology
14:45-15:00	N.K.M. Cheung	Development of a high-throughput embryo-chip for quantitative molecular and histological profiling of chorionated medaka eggs and eleutheroembryos
15:00-15:15	Paul Lam	***Summary / Questions & Answers***

Presentation by Task Team 2 (Chair: P.Y. Qian / Doris Au)

15:15-15:20	P.Y. Qian	Highlight on achievements of **Task Team 2**
15:20-15:35	Bingzhang Chen	Does eutrophication reduce the effect of top-down control on phytoplankton in Hong Kong waters?
15:35-15:50	Jie Xue	Utilization of dissolved organic carbon by bacterial at two contrasting sites in Hong Kong waters
15:50-16:05	S.G. Cheung	Mortality and oxygen consumption of polychaete *Hydroides elegans* under hypoxic conditions
16:05-16:20		***Coffee Break***
16:20-16:30	Joanne Lee	Overview on larval biology research programe;
		Characterization of biofilm microbial community for larval settlement
16:30-16:40	Jill Chiu	Effects of hypoxia and PBDEs on larval settlement of marine benthic polychaetes
16:40-16:50	Amy Li	Effects of hypoxia and PBDEs on larval biology of marine invertebrates
16:50-17:00	Kondethimma H. Chandramouli	Two-dimensional proteome and phosphoproteome maps: a new insight into cytoskeleton dynamics and oxidative stress during larvae development in marine invertebrates
17:00-17:10	Tim Wong	Quantitative proteomics analysis of the metamorphosis of the marine bryozoan *Bugula neritina*
17:10-17:20	Magic Zhang	Multiplexed proteomic approach to investigate the effects of the anti-fouling agent butenolide on the development of the barnacle *Balanus amphitrite*
17:20-17:30	Kelvin Wong	Protein expression signatures in settling larvae of barnacles and oysters for hypoxia and ocean acidification stressors
17:30-17:40	Lydia Chen	Transcriptome profiling and gene characterization in the settlement of *Balanus amphitrite*
17:40-17:50	Jill Chiu	Summary of larval biology research
17:50-18:00	P.Y. Qian	***Summary / Questions & Answers***

AoE Sympoisum Programme
7 January, 2011
T4 Meng Wah Complex, University Campus

Presentation by Task Team 3 (Chair: X.Y. Li / Joseph Lee / W.X. Wang)

9:30-9:35	X.Y. Li	Highlight on achievements of **Task Team 3**
9:35-9:50	Kenneth Leung	Development of Chronic Life-Cycle Toxicity Tests for Emerging Chemical Contaminants Using the Marine Copepod *Tigriopus japonicus*
9:50-10:05	Gui Ming Zhai	Transport and Removal of Nanoparticles in Porous Media
10:05-10:20	Gilbert C.S. Lui	Determination of Hazardous Concentration for Species Densities from Field-Based Community Sensitivity Distributions
10:20-10:35	Jianping Gan	Numerical Investigation of Circulation and Nutrient Transport in the Mirs Bay
10:35-10:50	W K Li	Time-series Study on Marine Water Quality Monitoring Data in Hong Kong: Implications of the Effectiveness of Environmental Policy and Management
10:50-11:05		***Coffee Break***
11:05-11:20	Joseph Lee	Real-time Forecasting of Beach Water Quality by Data Assimilation Model Based on Bacterial Load
11:20-11:25	Joseph Lee	***Summary / Questions & Answers***

Presentation by Task Team 4 (Chair: M.H. Wong / Nora Tam)

11:25-11:30	M.H. Wong	Highlight on achievements of **Task Team 4**
11:30-11:45	N. Pi	The significance of Fe plaque formation on mangrove plants in the removal of wastewater-borne nutrients, heavy metals and persistent organic pollutants
11:45-12:00	P. Wang	Tolerance and toxicity of estradiol and ethinylestradiol in green microalgae
12:00-12:15	K.L. Chow	Combined TiO_2 photocatalysis and phytoremediation for efficient removal of flame retardants (PBDEs)
12:15-12:30	S. Ng	Photodegradation and phytoremediation of perfluorooctanoic acid Part I: Photodegradation
12:30-12:45	Y. Qiu	Photocatalytic activity for degradation of organic pollutants under visible light
12:45-13:00	N.F.Y. Tam	***Summary / Questions & Answers***
13:00-13:15		***Feedback from International Advisory Committee***

ABSTRACTS

OF

ORAL PRESENTATION

PRESENTATION

BY

TASK TEAM 1

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Development of biosensors for direct detection of endocrine disrupting chemicals

Chi-chun Fong, Lan Zou, Wing-Leung Wong, and Kwok-Yin Wong[1], Mengsu Yang[1]
[1]The Hong Kong Polytechnic University

The objective of this project is to develop a biosensor platform using piezoelectric high frequency resonators coupled with chemical/biochemical recognition elements for direct detection of endocrine disrupting chemicals. We have designed and fabricated the prototype biosensor system including the resonator-based sensor unit with sample introduction and detection modules and a table-top measuring instrument for signal collection. In this study, a feasible protocol using a bridging ligand for covalent linking with certain EDCs, for example bisphenol A (BPA) was developed, the mixture of synthesized mercapto-11-bisphenol A conjugate and 6-mercapto-1-hexanol were self-assembled on the sensor surface, and the optimal ratio of the mixed SAM layer for target binding was determined. Different concentrations of estrogen related receptor gamma (ER-γ), which has strong binding affinity with BPA and other EDCs were incubated with the sensing surface to obtain the binding responses of the biosensor. The detection of BPA was performed with the biosensor using binding inhibition detection assay. Samples containing different concentrations of BPA were incubated with fixed concentration of ER-γ, and the amount of un-reacted ER-γ was detected by the biosensor, which generated a calibration curves for BPA in solution. This study demonstrated the feasibility of using piezoelectric biosensor for the rapid and sensitive detection of EDCs in environmental samples.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Accumulation and maternal transfer of PBDE 47 in the marine medaka (*Oryzias melastigma*) following dietary exposure

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The accumulation and maternal transfer of 2,2',4,4'-tetrabromodiphenyl ether (PBDE 47) were investigated in the marine medaka (*Oryzias melastigma*) following dietary exposure, in which PBDE 47 was bioencapsulated into brine shrimp (*Artemia* sp.) and fed daily to male-female pairs of medaka. In the accumulation experiment, 2-month-old (pre-breeding) medaka were provided with dietary PBDE 47 at 1.3 ± 0.2 g/day for 21 days. Accumulation of PBDE 47 in the medaka was initially very rapid and reached a steady state within 10 days. Final concentrations were similar for males and females after 21 days (227 ± 33 and 249 ± 31 g g^{-1} wet weight, respectively), accounting for some 90% PBDE 47 provided in the diet. In the maternal transfer experiment, 3-month-old (breeding) medaka were provided with dietary PBDE 47 at 1.2 ± 0.2 g/day for 18 days, and reached body burden of 76 ± 3 (males) and 61 ± 6 (females) g g^{-1} wet weight. Female PBDE 47 concentrations were significantly lower than males by day 12 ($P < 0.05$), and egg PBDE 47 concentrations were up to 25 ng/egg by day 18. Our results showed that maternal transfer is an important offloading mechanism for female fish and highlights the potential effects of flame retardants on embryonic development and the F1 generation. Furthermore, lipid normalized egg:female PBDE ratios of 0.5 – 1.2 indicated that the maternal transfer of PBDE 47 is associated with lipid mobilization during egg production.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Can bromophenol metabolites in human urine be used as population exposure markers for PBDEs?

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Polybrominated diphenyl ethers (PBDEs) are commonly used brominated flame retardant additives in polymers and textiles and are present in many household appliances and furnishings. Because of their wide application, these compounds are entering our global ecosystem, and humans, at an alarmingly rapid rate, leading them to be formally recognized as persistent organic pollutants (POPs) by the Stockholm Convention. Monitoring human exposure to this class of pollutant is, therefore, very important to the assessment of their risk to society. Although numerous studies have been carried out to trace and assess population exposure to PBDEs in different parts of the world, it can be argued that the reliability of some of these results is limited, mainly due to the difficulty in the acquisition of adequate human tissue samples for measurement, and the bias inherent in limited sample sizes and sampling schemes. Blood and breast milk are the most frequently used human tissues for exposure quantification. Nevertheless, sampling of blood is an intrusive operation and large-scale sampling is usually difficult to achieve. Although breast milk sampling can be regarded as a non-intrusive process, samples are restricted to lactating women within a relatively narrow age distribution, and therefore these results may not reflect population-level exposure. Sampling human urine, on the other hand, is a truly non-intrusive process and it is much easier to obtain a large number of urine samples from voluntary donors for a large-scale population survey. Thus, it would be desirable if metabolites of PBDEs in human urines can be used as exposure markers. We have synthesized and purified glucuoride and sulfate conjugates of 2,4-dibromophenol and 2,4,6-tribromophenol to be LC-MS standards for their monitoring in human urine samples. Analytical protocol and preservation methodology have also been developed for the determination of these four bromophenol metabolites in human urine. Parallel blood plasma and urine samples from 100 voluntary donors (50 males, 50 females) from Hong Kong were analyzed for their levels of selected BDE congeners, OH-BDEs, MeO-BDEs, bromophenols and bromophenol glucuoride and sulfate metabolites. Data obtained were analyzed by multiple regression to reveal any correlation between the brominated compounds in human blood plasma and bromophenol metabolites in urine. In this presentation, we will report our preliminary results that suggest urinary bromophenol metabolites are potential biomarkers for the estimation of population exposure to PBDEs.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Luteinizing Hormone Receptor (*lhcgr*) as a Potent Estrogenic Responsive Gene in the Zebrafish Ovary for Studying Action Mechanisms of Bisphenol A

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Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are gonadotropins that control all major events in the gonads, including folliculogenesis and steroidogenesis in females. FSH and LH signal through their cognate receptors, FSH receptor (FSHR) and LH/choriogonadotropin receptor (LHCGR) respectively, across vertebrates. Using a zebrafish follicle cell culture system, we have demonstrated that estradiol (E2) is a potent regulator that differentially controls the expression of *fshr* and *lhcgr* during folliculogenesis. E2 stimulates the expression of both *fshr* and *lhcgr*; however, its effect on *lhcgr* mRNA level is much greater than that on *fshr*. Interestingly, we have obtained several lines of evidence suggesting that E2 may signal via both membrane-located and intracellular estrogen receptors (ERs). The expression of *lhcgr* in response to E2 treatment in time course experiments exhibits a unique biphasic response. Further pharmacological studies have shown that the short-term acute response (< 3 hr) and the long-term chronic response (24 hr) may involve different signal transduction pathways. The characteristic biphasic response of *lhcgr* to E2 therefore provides an excellent model system for analysing the action mechanisms of various estrogenic chemicals including bisphenol A (BPA).

BPA is one of the major chemicals produced worldwide as a component of polycarbonate plastics and plastic additives. Recent animal studies of the detrimental effects of BPA in the endocrine system have raised global concerns about the potential harmful impact of this chemical on humans. Although BPA has been a major focus in the field of endocrine disrupting chemicals, its action mechanisms remain controversial. In our studies, we demonstrated that BPA, like E2, also up-regulated *lhcgr* expression both time- and dose-dependently. Similar to E2, BPA displayed an acute effect on *lhcgr* expression, which reached peak level at 1.5 hr of treatment. The effect dropped to but sustained at a lower level afterwards until 12 hr. In contrast to E2, BPA showed little chronic effect at 24 hr of treatment. This has led us to hypothesize that while BPA and E2 exhibit a similar short-term effect on *lhcgr* expression, the difference in their long-term effect may imply different signaling mechanisms for these two molecules. The platform we established using the zebrafish follicle cell culture system and the marker gene *lhcgr* will be a useful tool for analyzing other endocrine disrupting chemicals with estrogenic activities.

Proteomics Analysis for Estrogen-responsive Genes in Fish Ovarian Follicles

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Endocrine-disrupting chemicals (EDCs) are substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action in animals and humans. Despite the growing public interest during past decades, our understanding of how these EDCs work at the cell and molecular levels remains limited.

To provide a comprehensive global picture about estrogen-responsive genes during folliculogenesis, proteomics approach (2-DE plus MALDI TOF/MS) was applied in this study using zebrafish ovarian follicles of previtellogenic (PV) stage when aromatase expression and estradiol (E_2) production increase significantly, marking the activation of follicles and the beginning of vitellogenic growth. In addition to E_2, bisphenol A (BPA) was selected as a model EDC molecule for evaluating its interference effects, because of (1) its high production volume, (2) widespread existence in the aquatic environment, (3) evidence of reproductive toxicity in laboratory animal studies, and (4) public concern for possible health effects from human exposures. In the present study, we focused our efforts on exploring and defining target proteins that are responsive to both E_2 and BPA in PV follicles.

Proteins from untreated and E_2 or BPA-treated PV follicles were analyzed by 2-DE. The proteins that displayed a clear increase in E_2 and BPA-treated PV follicles were identified by MALDI TOF/MS. Interestingly, among these proteins, creatine kinase brain B (Ckbb) has been reported as one of the most sensitive markers of estrogen exposure in the rat uterus, and constructs containing CKBB gene promoter inserts are also estrogen responsive in transient transfection experiments in multiple cell types. Besides, biological activities of some of these proteins have been shown to increase in rat uterus after administration of E_2, i.e. (1) glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and Enolase 3 (Eno3), and (2) peroxidase enzyme, peroxiredoxin 2 (Prdx2). Moreover, one of these proteins, procollagen-proline, 2-oxoglutarate 4-dioxygenase, beta polypeptide (P4hb) is the same enzyme as disulfide isomerase (PDI), a protein folding catalyst and a molecular chaperone. Earlier studies showed that PDI could bind E_2 *in vitro*, and in a recent study, it was identified by direct binding assay as a novel target of BPA, and the binding of BPA to PDI results in the disruption of its actions.

In summary, we have identified several proteins that show clear response to both E_2 and BPA in zebarfish PV follicles. Despite that the functions of these proteins during folliculogenesis still remain limited, BPA most likely interferes with E_2 actions, which could, in turn, adversely affect many cellular processes during folliculogenesis. Further research will characterize spatial and temporal expression of these proteins during folliculogenesis, and investigate the molecular mechanisms of the regulation of these estrogen-responsive target genes using primary cell cultures and pharmacological approaches. In addition, we will extend our study to marine medaka model so that our platform can be applicable to evaluate the endocrinological risks of EDCs in both freshwater and marine fish.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Deciphering the specific effects of EDCs along the HPG Axis – an *in vitro* study

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The vast majority of studies on endocrine disrupting chemicals are based on *in vivo* studies. The inherited complexity of feed back mechanisms and physiological regulation in *in vivo* system makes it impossible to decipher the target site and effect of endocrine disrupting chemicals (EDCs) on specific organs. As such, studies on *in vitro* system are required to provide information complementary to *in vivo* studies, and to provide important insights into the actions and mechanism of EDCs on specific organs. We have successfully developed primary cell cultures (pituitary, ovarian follicular and testicular cells) for marine medaka (*Oryzias melastigma*), and based on these *in vitro* systems, experiments were further carried out to elucidate the effect of different EDCs along individual compartments along the medaka HPG axis, particularly on steroidogenesis. The cell culture protocol was principally in compliance with zebrafish cell cultures, which had undergone numerous physiological studies and are able to provide repeatable and reliable result. To validate the system, the cells were challenged by hypoxia as well as several extensively studied EDCs including polybrominated diphenyl ethers (PBDE) and 4-nonylphenol (NP). The expression level of various steroidogenic genes were analyzed by quantitative real-time RT-PCR and the results were compared to those obtained from *in vivo* exposure of whole fish as well as from H295R, a human cell line. Although different systems did not exhibit identical expression patterns upon the same chemical exposure, the results were largely comparable. Our results suggest that medaka primary cultured cells can serve as a useful tool for screening EDCs and provide a platform for studying their specific actions on different compartments along the HPG axis.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Analysis of estrogen receptors (ers) in medaka and optimization of medaka cell lines for *in vitro* assays

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The objective of this study is to investigate tissue- and gender-specific expression of estrogen receptors (ERs) in medaka. Three classic estrogen receptor (omERα , omERβ and omERβ2) and five estrogen-related receptors (omERRα, omERβ1, omERβ2, omERRγ1 and omERRγ2) have been sequenced and identified in the marine medaka (*Oryzias melastigma*). Except for the omERRα, the full-length cDNA sequence of these ER receptors has not yet been completed. In the Japanese medaka (*Oryzias latipes*), the three classic estrogen receptor (olERα , olERβ and olERβ2) and the five estrogen-related receptors (olERRα, olERβ1, olERβ2, olERRγ1 and olERRγ2) have also been identified and the full-length cDNA sequences are all available. Comparison of nucleotides between the incomplete cDNA sequence of marine medaka and the cDNA sequence of Japanese medaka ERs revealed over 95% identity, ranging from 92%-99%, indicating the ERs and ERRs in Japanese medaka and marine medaka are highly similar at the genomic level.

Tissue expressions of ERs and ERRs transcripts in adult Japanese medaka were studied by Real-Time PCR. The endogenous levels of olERs transcripts among each other varied remarkably in the same tissue, some olERs (such as olER, olER β2) exhibited a distinct tissue-specific expression pattern. Gender specific expression was also found for the three classic ERs in specific organs e.g. liver and heart.

Estrogens/estrogen mimics bind to ER and mediate transcription of estrogen responsive gene (e.g. the telomerase TERT). Findings of this study provide strong scientific evidences to explain the observed tissue and gender specific effects of ERs and estrogen (and for the prediction of estrogen mimics) on transcription of TERT gene. The potential involvement of estrogen in transcriptional regulation of olTERT and omTERT is suggested by the existence of putative EREs in the promoter region of the olTERT gene and omTERT gene (total 8 and 7, respectively). The involvement of ERs and functional analysis of EREs in the TERT promoter are ongoing, using the Japanese medaka HdrR-e3 (embryo at the stage of 5 days after fertilization) and the DIT29 (derived from chemical-induced hepatoma) cell lines (kindly provided by Prof. Hiroshi Mitani). The growth rate for HdrR-e3 cells and DIT29 cells is only 4 – 5 days for a passage (at split ratio of 1:3 and 1:2, respectively), which is much faster than that of the commercial medaka fin fibroblast cell line (ca. 2 weeks). We have established standard operating procedures (SOPs) for maintenance and optimal growth for both HdrR-e3 and DIT29 cell lines. These two medaka cell lines are very useful *in vitro* tool for toxicity assessment and understanding the mode(s) of action of environmental contaminants in medaka.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Effect of Hypoxia–inducible Factors (HIFs) on microRNA expression and steroidogenesis in the H295R human adrenocortical cell line

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Living organisms possess a variety of adaptive and survival mechanisms to help them cope and survive under hypoxia. The Hypoxia-Inducible Factors (HIFs) are a family of transcription factors that mediate many of the molecular and cellular adaptative processes in response to hypoxia. Hypoxia is known to impair reproductive functions in vertebrates and population sustainability in natural environments. Hypoxia can affect steroidogenesis and may pose a threat to reproductive success of a population. MicroRNAs are post-transcriptional regulators involved in numerous cellular processes, including proliferation, differentiation, metabolism, and motility. So far, very little is known about the role(s) of HIFs and microRNAs in steroidogenesis. Using the H295R cell line, we test the hypothesis that HIFs and specific microRNA molecules are involved in regulating specific genes of the steroidogenic pathway in vertebrates. Human HIF-1α, -2α and -3α were overexpressed in H295R cells by transient and stable transfection using a lentiviral delivery system. HIF protein expression was monitored by Western blot analysis, and effects on steroidogenic enzyme gene expression patterns and hormone levels were examined by quantitative RT-PCR and ELISA, respectively. Previous studies have reported the expression patterns of specific microRNAs in various human cell lines in response to hypoxia. Here, microRNA profiling was also carried out in control and HIF-transfected H295R cells. Computational analysis revealed several distinct groups of microRNAs (upregulated and downregulated) in response to hypoxia or overexpression of a specific HIF-α protein. The results of these experiments will be described and discussed in this presentation.

Human leukocyte antigen (HLA) homologues: a key to understanding hypoxia-mediated modulation of reproductive functions in vertebrates.

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Genes in the major histocompatibility complex (MHC) such as the human leukocyte antigen B (HLA-B) and HLA-DR are implicated in the regulation of parental sex hormone levels and offspring sex ratios in humans. Differential regulation of HLA-G expression by hypoxia via the Hypoxia-inducible Factor-1 (HIF-1) transcription factor was recently demonstrated in several human cell lines. To further explore the relationship between hypoxia-mediated regulation of HLA and the biosynthesis of sex hormones, human H295R cells (which display active steroidogenesis) were exposed to hypoxia and expression levels of six HLA genes belonging to the class I subtypes (*HLA-A, HLA-B, HLA-C, HLA-E, HLA-F* and HLA-G) measured by quantitative RT-PCR. Hypoxia (1% O_2, 24 h) was found to induce expression of *HLA-A, HLA-B, HLA-C* and *HLA-E* but not *HLA-F*. A similar expression pattern was also observed for these four hypoxia-inducible HLA genes following exposure of H295R cells to cobalt chloride ($CoCl_2$, a HIF-1 inducer). The results suggest that hypoxic induction of these four HLA genes in H295R cells are likely mediated by the HIF-1 transcription factor. Overexpression and knockdown experiments of HIF-1α, HIF-2α and HIF-3α in H295R cells are now underway to confirm the specificity of the different HIF isoforms in HLA gene regulation. The effect of ectopic overexpression of each of the four HLA genes on testosterone and estrogen levels will also be examined in the H295R cells to establish the causal link between HLA expression and sex hormone production. Bioinformatics analysis revealed 8 class I HLA homologues in the zebrafish genome. Expression of these genes in response to hypoxia was measured by quantitative RT-PCR in zebrafish testis explants. Our findings indicated that zHLA-8 expression was significantly increased under hypoxia (1% O_2, 8 h) while no change (or suppression) in gene expression levels were detected for the remaining seven HLA homologues. Characterization of the functional role of HIF in zHLA-8 regulation is now underway in our lab using a "gain- and loss-of-function" approach in zebrafish embryos. Overall, our findings indicate that HLA gene expression is regulated by hypoxia in both humans and fish. Further efforts will be directed at investigating how changes in HLA expression may be linked to alteration in sex hormone levels and in turn changes in offspring sex ratios.

Hypoxia Impairs Primordial Germ Cells (PGCs) Development in Zebrafish (*Danio rerio*) Embryos

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Hypoxia is an endocrine disruptor and affects the sex ratio in adult fish. Little is known about the effect of hypoxia on germ line development during embryonic stage. Primordial Germ Cells (PGCs) are germ cell precursors. They become gametes during gametogenesis upon sexual maturation in animals. Therefore, alteration of PGC development could also impair reproduction. PGC development has been studied in many organisms. In zebrafish, *vasa* and *nanos1* are two well known PGC marker genes, and they are both essential for PGC development. By using whole mount *in situ* hybridization with *vasa* and *nos1* riboprobes to label PGCs, two approaches have been adopted to test the hypothesis that hypoxia causes a reduction of PGCs in terms of number and volume. Direct cell counting showed fewer numbers of PGCs in hypoxic stressed embryos (21.8 +/- 5.4) when compared with the control (30.6 +/- 5.2). Confocal microscopy also revealed a reduction in the volume of PGCs in embryos exposed to hypoxia (20874 +/- 4603μm^3), as compared with the control (31142 +/- 1831 μm^3). GFP nos1 3'UTR-injected embryos exposed to hypoxia showed mis-migrated PGCs in cranial regions, dorsal caudal regions and yolk cell extension. Real-time PCR showed that expression of *igfbp1* was up-regulated; suggesting that *igfbp1* induction under hypoxia may be the cause of the observed PGCs migration defect. With IGFBP1 morpholino, the PGC migration defect was rescued evidenced by no significant difference was found between IGFBP morphant exposed to hypoxia and WT under normoxia. For the first time, evidence has been provided that hypoxia induced IGFBP1, which suppresses IGF signalling and results in PGC migration failure, leading to fewer numbers and lower volumes of PGCs in the genital ridge.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Validation of Artificial Mussels for metal monitoring: Field studies along the coastlines of South Africa and China

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Validation of the AM under field conditions involved the transplantation of indigenous reference mussels (*Perna perna*) alongside the passive device. Field studies were carried out for six weeks at five localities along the South African coastline ranging from temperate to sub-tropical environment. Two-way analysis of variance, Pearson correlation and Discrimination Function Analysis were used to compare the concentrations of contaminants in AMs and mussels across sites and exposure periods. While uptake patterns between the AM and transplanted mussels were significantly comparable for majority of the analyzed metals, no positive correlations were observed at the reference site. The results indicated positive correlations for Cd, Cu, Pb and Zn in Cape Town; Cd and Pb in Port Elizabeth; Pb in Saldanha Bay; Cd, Cu, Pb and Zn in Richards Bay and no positive relationships from the reference site in Tsitsikamma National Park.

Similar field study was carried out at nine locations along the Chinese coastal line with different levels of metal pollution. Results showed very high concentrations of Cu, Cr and Hg at Qingdao, an industrial city with heavy traffic (194-202, 3610-5010 μg/g and 85-139 ng/g. respectively). The concentration of Pb in this location was nearly double of other locations. The second high concentrations by metal pollution were found in Shanghai (Cu, Zn, Cr and Pb: 21.2-36.7, 20.9-37.7, 4.44-13.1 and 3.85-8.61 μg/g respectively). High concentrations of Zn was identified at Dalian and Shantou, which can be attributable to industrial pollution at these two sites.

The overall field results in South Africa and China showed that the AM can provide a good estimate on metal levels in the marine environment, and the uniformity in biomonitoring which mussels cannot.

With the support from the International Atomic Energy Agency (IAEA), our "Global Artificial Mussel Watch" has now extended to eight African countries.

Detection of hepatitis A virus and norovirus in seawater

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Enteric viruses may be present naturally in aquatic environments or introduced through leaking sewage systems, urban runoff, agricultural runoff, and in the case of marine waters, sewage outfall and vessel wastewater discharge. Many types of pathogenic enteric viruses are excreted in human and animal wastes, which can be released into the environment through groundwater, estuarine water, seawater, rivers, and aerosols from sewage treatment plants. In addition to causing acute diseases, enteric viruses are of public health concern because of their low infectious dose, whereby it has been estimated that the risk of infection caused by consuming viruses in drinking water is 10- to 10,000-fold greater than that of pathogenic bacteria at similar exposures. Hepatitis A virus (HAV) and noroviruses (NoVs) are the leading cause of nonbacterial acute gastroenteritis throughout the world and together, they are estimated to be responsible for 90% of acute viral gastroenteritis cases worldwide. In this study, we used a negatively-charged membrane adsorption-elution method to facilitate optimal recovery of enteric viruses from marine waters. DNA sequencing and phylogenetic analysis of the HAV- and NoV-specific PCR products obtained from Hong Kong waters indicated that the major HAV type is of genotype IIB, while the major NoV types belong to genotypes GI/1, GI/2, GI/3, GII/4, GII/3. Detection and quantification of HAV were subsequently performed using a TaqMan-based real-time PCR technique. Here, we describe the optimization and evaluation of a HAV-specific TaqMan-based PCR assay for rapid and sensitive detection of HAV in Hong Kong marine waters. The TaqMan PCR method is capable of detecting HAV in seawater down to 1 PFU within 12 hours. We are presently experimenting with several novel molecular methods to facilitate more rapid concentration and detection of HAV and NoVs in seawater.

Knowledge transfer of transgenic marine medaka technology

Xueping Chen, Li Li, Richard Mam Kit Yu, Masato Kinoshita, Shuk Han Cheng

After the development a transgenic marine medaka that is highly sensitive to estrogen and estrogenic-like substances, we did lots of studies to explore its potential application. We found that this transgenic fish can be applied to screen estrogenic, anti- and pro-estrogenic substances; and rapid monitor the estrogenic activity of environment samples, foods, drugs and so on. Because of the great practical potential, we devoted much effort to promote the transfer of this technology into commercial use. (1) We patented this technology; (2) participated in international exhibition, won Gold Prize from Korea International Women's Invention Exhibition and Special Prize from the Federation of Korea Industries; (3) elected by HK public to exhibit at Science News Corner of Hong Kong Science Museum for 5 months (Mar to Aug 2010); (4) invited to give a talk at HKIE (Hong Kong Institute of Engineering), which has attracted an extra 40 HKIE members to visit our laboratory as well as BCH department at CityU; (5) and educated three CityU students use this technology to participate in local and global business plan competition and won the Champion at HSBC Young Entrepreneur Competition, the Best of the Best at HSBC Asia Young Entrepreneur Competition, and the 2nd Runner Up of Lee Kuan Yew Global Business Plan Competition, all these have raised high attention from society and have been covered by more than 20 media, and successful raised the needed start-up fund to transfer this technology into commercial application.

Development of a high-throughput embryo-chip for quantitative molecular and histological profiling of chorionated medaka eggs and eleutheroembryos

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Medaka embryos have been proven useful experimental models for early stages toxicological studies. They are transparent and easy available in high quantity under laboratory condition. However, the very small size embryo for organ isolation is technically challenging, forbidding multiple molecular analyses (protein and DNA/RNA) on individual tissue in each embryo. This difficulty can be circumvented by immunohistochemistry (IHC) and *in situ* hybridization (ISH) on embryo tissue sections. However, two problematic prerequisites were encountered: (1) Orientation. Setting the orientation of a sphere-like chorionated embryo is not technically practical. Earlier reports on successful orientation of hatched/dechorionated embryos for sectioning through classical agarose embedding method relied on the access to high-end milling systems for making tailor-made moulds for precise fitting of embryo in each well, which is technically difficult, inflexible and expensive. (2) Dechorionation. Medaka chorion is impermeable to routine embedding media and O.C.T. compounds. Hence, chorionated medaka embryos have to be dechorionated prior to processing and sectioning. Despite standard dechorionation procedures are adaptable, the procedures are often time consuming and usually devastate to early stage embryos.

In this study, an easy-to-make, inexpensive and high-throughput embryo-chip has been designed and constructed to allow simultaneous molecular and histological profiling of chorionated embryos (i.e. no dechorionation is required) and eleutheroembryos (a.k.a hatched embryos). Each embryo-chip holds > 23 aligned embryos per cm^2 according to user-defined orientation(s). Multiple groups of control and treated embryos can be incorporated into the same chip for simultaneous molecular analysis. Each chip can give rise to ~100 embryo sections (at 5 μm thickness) for multiple quantitative protein profiling (by IHC) and nucleic acids localization (by ISH). Tissue specific genes/proteins perturbations can be directly related to significant histopathological alterations in the target tissue. Fine alignment and user-defined orientation not only allow locating organs on a planar section with ease, but also facilitate direct comparison and quantification (aided by stereological tools, florescence and colour deconvolution) of the structures/molecules-of-interest within comparable region across all individuals.

We have successfully employed this embryo-chip design to investigate chronic effects of hypoxia on early liver development in medaka. Tissue turnover was determined by quantification of proliferating (PCNA-positive) and apoptotic (TUNEL-positive) nuclei within the same individual for large number of embryos. Results indicate cell proliferation was suppressed in the hypoxia-exposed livers and whole embryos, whereas apoptosis was not affected. PCNA/TUNEL ratio indicated that hypoxia-exposed embryos (PCNA/TUNEL > 1) remained proliferative whereas their liver (PCNA/TUNEL < 1) were likely to have a reduction in cell mass. The results suggest embryonic liver of medaka is highly sensitive to chronic hypoxia.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Genomics and proteomics characterization of biomarkers in marine medaka (*Oryzias melastigma*) exposed to BDE-47

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The objective of this project is to carry out genomic and proteomic studies for identification of biomarkers of marine medaka (*Oryzias melastigma*) exposed to PBDEs. *Oryzias melastigma* cDNA libraries from gonad, brain and liver were constructed and 2370 clones amplified for the libraries were used to fabricate the cDNA microarray for transcriptional profiling. The sequences and potential functions of the 2370 clones were identified using the NCBI BLAST search, and further categorized in association with different biological processes using AmiGO against the Gene Ontology database. Four types of organs, e.g. liver, gonad, brain, and spleen from *Oryzias melastigma* exposed to BDE-47 were collected for genomics and proteomics analysis, respectively. The genomics signatures of liver and gonad samples were characterized and analyzed, and the differentially expressed genes under PBDE exposure were identified. Moreover, we have optimized the proteomics analysis protocol using the isobaric tags for relative and absolute quantification (iTRAQ) labeling method and mass spectrometry (LC–MALDI-TOF/ TOF), and the proteomics characterization of the liver proteins differentially expressed due to BDE-47 exposure were carried out. The information obtained from the study is useful to unravel the molecular mechanisms leading to the biological responses of marine medaka to PBDEs.

Heat shock protein expression profiles in liver and muscle of PBDE47 fed marine medaka

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Polybrominated diphenyl ethers (PBDEs) are persistent environmental pollutants that have been detected in tissues of both terrestrial and aquatic animals. To examine the impact of environmentally important chemicals on the stress response, in fish, a host of assays can be used including studies pertaining to the expression profiles of a range of heat shock protein (HSP) families. Towards this and to investigate whether dietary sources of PBDE could modulate HSP expression profiles an experiment was designed such that three month old marine medaka were fed PBDE 47 via bioencapsulation into *Artemia* at two doses (low or high) for 21 days. For analysis, liver and muscle tissue were taken from male and female fish separately and immunoassays for the major HSP families namely HSP60, HSP70 and HSP90 were performed. In male marine medaka HSP60 levels were elevated in liver tissue from fish fed high doses of PBDE 47 but remained unchanged in females and in muscle tissue taken from both males and females. For HSP70 expression studies it was found that elevated amounts were detected in liver and muscle of female marine medaka that were fed low and high doses of PDBE 47 but remained unchanged in males. A similar finding was found when HSP90 amounts were studied as expression levels were increased only in liver and muscle tissues taken from female marine medaka. These preliminary findings indicate the possibility of sex related responsiveness of marine medaka to dietary accumulation of certain environmentally important chemicals. Further work on other cellular processes associated with stress such as apoptosis will be undertaken in order to better understand the stress response of marine medaka.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Identification of Immune-relevant Genes and Characterization of Two Hepcidin Genes in the Marine Medaka *Oryzias melastigma* upon Bacterial Challenge

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The immune system of fish has generated increasing interest in recent years as it is of key importance in primary defence against diseases. In this study, suppression subtractive hybridization (SSH) technique was used to identify differentially expressed immune genes in liver of the marine medaka *Oryzias melastigma* infected with *Vibrio parahaemolyticus*. A forward subtracted cDNA library (ESTs expressed in the infected fish compared with the control fish) and a reverse library (ESTs expressed in the control fish compared with the infected fish) were generated, which were enriched with up- and down-regulated gene transcripts. A total of 1279 clones were sequenced, and among the clones, 397 potentially functional genes were identified using the NCBI BLAST search. Of the identified genes, 38 were involved in the immune system. Besides, genes involved in other important functions such as biological regulations, cellular metabolic process, response to stimulus, cellular component organization, signal transduction, transport process were also obtained.
Hepcidin is a very important antimicrobial peptide in the innate immune system. Two hepcidin genes (OM-hep1 and OM-hep2) were identified and characterized in the *O. melastigma*. The transcript levels of both hepcidin genes (by Real-time PCR) were highest in liver, moderate in spleen and lowest in other non-immune related tissues in normal adult medaka. Upon bacterial challenge, induction of hepatic OM-hep1 mRNA was most rapid (within 6h) and remarkable (42-fold), and that the level remained high (24-fold) at 12h. Hepatic OM-hep2 mRNA was significantly up-regulated at 12h (19-fold). In spleen, a relatively slower response time (at 12h) and a lower induction level for OM-hep1 (2-fold) and OM-hep2 mRNA (7-fold) were observed, however, the induction response last till 24h. Our results indicate that the two hepcidins may play a tissue-specific role in the innate immunity of *O. melastigma*.
Overall, findings of this study have established a genomic platform for studying immune genes responses using the *O. melastigma*. The potential of using the small size *O. melastigma* as a marine fish model for studying innate immune function and immuno-suppression effects of environmental contaminants in vertebrates will be further investigated and evaluated.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Immuno-modulation effects of PBDEs in the marine medaka (*Oryzias melastigma*)

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Immunotoxicology is rapidly advancing in the field of ecotoxicology because of its great impact on the health and survival fitness of organisms. Some immune function endpoints were found to be highly sensitive to physical and chemical stresses. Novel endpoints for assessing immune function impairments in vertebrates are continually being developed and evaluated.

PBDEs (Polybrominated diphenyl ethers), important flame retardant additives, were produced in large quantity since 1970s. The family of PBDEs consists of 209 possible congeners. Low molecular weight PBDE congeners can be easily accumulated in biota. Of all the congeners, BDE-47 is one of the major components in commercial PBDEs product and predominantly accumulated in marine animals. Recently, PBDEs have been shown harmful to fish growth, but the potential effects of PBDEs on fish immune function are virtually unknown.

The objectives of this study are to investigate the immune-modulation effects of PBDEs (BDE-47) in marine fish using the marine medaka (*Oryzias melastigma*) as a model animal. Three useful *O. melastigma* platforms have been developed for this study: i) 38 immune genes have been identified in our SSH cDNA library for *O. melastigma* upon bacteria challenged; ii) a cDNA microarray has been constructed for the marine medaka, and iii) a whole medaka adult histo-array platform and a medaka embryo chip have been developed, which allow localization and quantification of immune genes (by ISH) and proteins (by IHC) simultaneously in multiple immune organs of a single fish.

In this study, three months old marine medaka was subject to short term (5 days) and long term (21 days) food-borne exposure to two concentrations of BDE-47, by feeding BDE-47 contaminated artemia. The complement system plays a critical role in fish innate immune function. Using the cDNA microarray, two key complement genes, *c8* and *properdin*, were found down-regulated in liver of medaka exposed to BDE-47 treatments, whereas the other complement genes, *c3*, *masp-3* and *factor b*, were differentially regulated under different BDE-47 treatments. The preliminary results indicate that BDE-47 exhibits potential immuno-modulation effects in the *O. melastigma*. Dose dependent, tissue- and gender- specific effects of BDE-47 on the complement systems and other innate immune function of marine medaka will be further investigated. The potential effects of BDE-47 on liver histopathology and other key immune organs: thymus (site for T-cell proliferation and maturation), kidney (site for B cell proliferation and antibody production) and spleen (site for interaction between immune cells) will be also examined. Alterations of immune cells composition, the number and distribution of melano-macrophage centre (MMC) are the potential endpoints employed for detection of significant histological change in BDE-47 exposed fish.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Can bromophenol metabolites in human urine be used as population exposure markers for PBDEs?

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Polybrominated diphenyl ethers (PBDEs) are commonly used brominated flame retardant additives in polymers and textiles and are present in many household appliances and furnishings. Because of their wide application, these compounds are entering our global ecosystem, and humans, at an alarmingly rapid rate, leading them to be formally recognized as persistent organic pollutants (POPs) by the Stockholm Convention. Monitoring human exposure to this class of pollutant is, therefore, very important to the assessment of their risk to society. Although numerous studies have been carried out to trace and assess population exposure to PBDEs in different parts of the world, it can be argued that the reliability of some of these results is limited, mainly due to the difficulty in the acquisition of adequate human tissue samples for measurement, and the bias inherent in limited sample sizes and sampling schemes. Blood and breast milk are the most frequently used human tissues for exposure quantification. Nevertheless, sampling of blood is an intrusive operation and large-scale sampling is usually difficult to achieve. Although breast milk sampling can be regarded as a non-intrusive process, samples are restricted to lactating women within a relatively narrow age distribution, and therefore these results may not reflect population-level exposure. Sampling human urine, on the other hand, is a truly non-intrusive process and it is much easier to obtain a large number of urine samples from voluntary donors for a large-scale population survey. Thus, it would be desirable if metabolites of PBDEs in human urines can be used as exposure markers. We have synthesized and purified glucuoride and sulfate conjugates of 2,4-dibromophenol and 2,4,6-tribromophenol to be LC-MS standards for their monitoring in human urine samples. Analytical protocol and preservation methodology have also been developed for the determination of these four bromophenol metabolites in human urine. Parallel blood plasma and urine samples from 100 voluntary donors (50 males, 50 females) from Hong Kong were analyzed for their levels of selected BDE congeners, OH-BDEs, MeO-BDEs, bromophenols and bromophenol glucuoride and sulfate metabolites. Data obtained were analyzed by multiple regression to reveal any correlation between the brominated compounds in human blood plasma and bromophenol metabolites in urine. In this presentation, we will report our preliminary results that suggest urinary bromophenol metabolites are potential biomarkers for the estimation of population exposure to PBDEs.

The marine medaka, *oryzias melastigma*: a model species for marine molecular ecotoxicology

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The marine medaka, *Oryzias melastigma,* has been considered as a promising model species for developmental biology, evolution, and environmental genomics. In an attempt to accelerate the molecular work on *O. melastigma*, I sequenced its genomic DNA using the genome sequencer GS-FLX-Titanium and SOLEXA, and subsequently obtained 647 Mb (average read length 329.7 bp) and 46.224 Gb, respectively. Also I assembled those sequences after we read 5 kb mate pair sequences, and finally obtained 132,623 contigs including approximately 737 Mb (average read length 5,556 bp) with N50 value of 10,004 bp. In this presentation, I summarized the genomic DNA sequences of *O. melastigma* and discussed their potential use in reproductive biology, endocrinology, environmental genomics, and ecotoxicological studies in order to uncover mechanisms, such in the formation of eggs, antioxidant stress defense, and xenobiotic metabolism in this species.

PRESENTATION

BY

TASK TEAM 2

Does eutrophication reduce the effect of top-down control on phytoplankton in Hong Kong waters?

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Rates of microzooplankton grazing on phytoplankton were estimated monthly in 2007 and 2009 using the dilution technique at two stations in Hong Kong waters. The western estuarine station (WE) in the Pearl River estuary was strongly influenced by freshwater discharge, while the eastern oceanic station (EO) was mostly affected by oceanic influences of the South China Sea. Growth rates of phytoplankton were often limited by nutrients at EO, while nutrient limitation of phytoplankton growth seldom occurred at WE due to the high level of nutrients delivered by the Pearl River, especially in the summer rainy season. Higher chlorophyll *a* and microzooplankton biomass were found at the eutrophic WE than at the mesotrophic EO, while the rate increases of phytoplankton growth and microzooplankton grazing at WE were modest compared with biomass increases. Compared with the oligotrophic South China Sea where the increase of phytoplankton growth rate is much greater than that of phytoplankton biomass in response to nutrient enrichment, the top-down control on phytoplankton biomass exerted mostly by microzooplankton seems been weakened in eutrophic Hong Kong waters. The increased grazer (microzooplankton) biomass cannot be fully transformed into grazing impacts in eutrophic waters owing to combined effects of grazing saturation, changes in grazer community composition and size structure, and changes in prey (phytoplankton) community structure. The weakened top-down controls on phytoplankton biomass in eutrophic marine systems may intensify the eutrophication effect and lead to outbreaks of algal blooms and coastal water hypoxia.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Utilization of dissolved organic carbon by bacteria at two contrasting sites in Hong Kong waters

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Bacterial activity in marine environments is mainly regulated by the quantity and quality of dissolved organic carbon (DOC). However, no studies have previously been conducted to compare the bioavailability of low molecular weight (LMW) DOC with that of high molecular weight (HMW) DOC among a variety of Hong Kong environments. Across Hong Kong waters, there is a gradient in environmental conditions from western waters (estuarine site) that are influenced by Pearl River discharge, to eastern waters (coastal site) that are mainly dominated by coastal/oceanic water during summer. Hence, the origin of DOC is quiet different at both sites.

In late summer 2010, a series of bioassays were conducted in order to determine the bioavailability of size-fractionated DOC to natural bacterial populations, as well as the functional response of the bacterial community to DOC with different origins. DOC was separated into two fractions: 0.2 μm filtrate (Tot-DOC) and <30 kDa (LMW-DOC). Eight treatments were conducted: coastal bacteria (CB) + coastal Tot-DOC; CB + coastal LMW-DOC; CB + estuarine Tot-DOC; CB + estuarine LMW-DOC; estuarine bacteria (EB) + coastal Tot-DOC; EB + coastal LMW-DOC; EB + estuarine Tot-DOC; EB + estuarine LMW-DOC.

Our results revealed that although the concentrations of inorganic nutrients (NO_3 and PO_4) in the estuarine site were much higher than the coastal site, Tot-DOC concentrations did not exhibit a significant difference at both sites where > 60% of the total DOC was LMW-DOC. In all experiments, bacterial growth rates (2.0-3.9 d^{-1}) in the LMW-DOC treatment were significantly higher than those (0.12-1.8 d^{-1}) in the Tot-DOC treatment, suggesting that LMW-DOC is highly bioavailable. In contrast, bacterial growth efficiency (BGE) was generally higher (0.47-0.58) in the Tot-DOC treatments than (0.28-0.54) the LMW-DOC treatments. We speculated that viruses which were present in the Tot-DOC treatments were responsible for the lower growth rate and respiration rate of bacteria due to viral-induced mortality in the Tot-DOC treatments.

In addition, bacteria grown on estuarine DOC had higher growth rates (1.8-3.9 d^{-1}) and BGE (0.28-0.58) than those utilizing coastal DOC (0.12-2.4 d^{-1}; BGE 0.06-0.47). The highest growth rate (3.9 d^{-1}) and BGE (0.58) occurred in the CB + estuarine LMW-DOC treatment and the CB + estuarine Tot-DOC treatment, respectively, and the lowest (0.12 d^{-1} and 0.06) in the EB + coastal Tot-DOC treatment. This was likely attributed to high bioavailability of DOC in estuarine waters accompanied by high concentrations of inorganic nutrients.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Mortality and oxygen consumption of polychaete *Hydroides elegans* under hypoxic conditions

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Mortality of *Hydroides elegans* was examined at 10 oxygen concentrations (0.14, 0.25, 0.35, 0.5, 0.75. 1.0, 1.5, 2.0, 4.0 and 6.3 mg O_2 l^{-1}) for 48 hours. No mortality was observed at 2.0 mg O_2 l^{-1} or above. For oxygen concentrations < 2.0 mg O_2 l^{-1} mortality increased as oxygen level was reduced. LC_{50} at 24 and 48 hrs were estimated at 0.32 and 0.45 mg O_2 l^{-1}, respectively.

Oxygen consumption rate was investigated at 2.0 and 6.3 mg O_2 l^{-1} for five days. Individuals from the control group were exposed to 6.3 mg O_2 l^{-1} throughout the experiment. For the hypoxic group, individuals were exposed to 6.3 mg O_2 l^{-1} on the first day but the oxygen level was reduced to 2.0 mg O_2 l^{-1} on Day 2 and Day 3. Oxygen level was then returned to normoxia on Day 4 and Day 5 to study recovery from hypoxic stress. Oxygen consumption remained constant throughout the experiment for the control group. For the hypoxic group, oxygen consumption was reduced by 40 – 50% when dissolved oxygen concentration was reduced on Day 2 and Day 3. When normoxic condition was resumed, oxygen consumption increased gradually but was still 20% lower than the control on Day 5. The present findings indicated that hypoxic stress reduced metabolic rate of *H. elegans* of which more than 2 days were required for complete recovery. As a filter feeder, slow recovery from hypoxia stress may reduce its competitiveness in a hypoxic environment.

Polybrominated diphenylethers (PBDEs) alter larval settlement of marine benthic polychaetes

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Polybrominated diphenylethers (PBDEs) are found ubiquitously in marine environments worldwide. Sediment is the major sink of PBDEs, with the congener BDE-47 being most abundant. In this study, laboratory experiments were carried out to test the hypothesis that contamination of BDE-47 at environmentally realistic sediment concentrations can change bacterial community on sediment surface qualitatively and quantitatively and hence alter polychaete larval settlement. Using multiple-choice experiment, settlement of three polychaete species (*Pseudopolydora vexillosa*, *Polydora cornuta*, and *Capitella* sp. I) on different types of spiked sediment was studied and compared: (i) low BDE-47 concentration (0.5 ng g^{-1} dry weight); (ii) high BDE-47 concentration (3.0 ng g^{-1} dry weight), (iii) hexane (solvent control), and (iv) natural sediment (control). Our results showed that settlement of *P. vexillosa* and *Capitella* sp. I larvae was significantly promoted, while settlement of *P. cornuta* reduced, at high BDE-47 concentration in sediment compared with the respective controls under both short- (24-h) and long-term (4-week) exposures. After 4 weeks, body burden of BDE-47 in all polychaete species was directly related to the spike concentration, and body length of settled juveniles of *P. vexillosa* and *Capitella* sp. I at the high-concentration treatment was significantly longer compared with that of other treatments and controls. Bacterial community compositions and abundance in sediment at high BDE-47 concentration were statistically the same as with the respective controls within the first 24 h, which is critical for larval surface exploitation and substratum choice for permanent settlement. For the first time, we demonstrated that environmentally realistic concentrations of BDE-47 in sediment can affect polychaete settlement in species-specific and dose-dependent manners. Given the global contamination of PBDE in marine sediment, BDE 47 may potentially alter the settlement pattern of marine polychaetes and hence their benthic composition over large areas.

Hypoxia alters larval settlement of marine benthic polychaetes

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Hypoxia is generally defined as dissolved oxygen levels below 2.8 mg O_2 l^{-1} in aquatic systems. Frequent occurrences and the extent of hypoxia pose a major threat to costal marine ecosystems worldwide. In this study, larvae of two polychaete species (*Pseudopolydora vexillosa* and *Capitella* sp. I) were individually exposed to seawater at different dissolved oxygen (DO) levels over 24 h for larval mortality examination; whilst sediment was simultaneously exposed to severe hypoxia (1.5 mg O_2 l^{-1}) and normoxia (6.5 mg O_2 l^{-1}) over 28 days for larval settlement and bacterial community analysis. Larval settlement of *P. vexillosa* and *Capitella* sp. I on hypoxic and normoxic sediment was studied and compared using multiple-choice experiment, while bacterial community on sediment surface was analyzed with oligonucleotide probes selectively targeting the *Bacteroidetes* and sulfate-reducing bacteria (SRB) and quantified using epifluorescent microscopy. Our results showed that LD_{50} of *P. vexillosa* and *Capitella* sp. I was 4.89 and 2.26 mg O_2 l^{-1} respectively, indicating *P. vexillosa* is less tolerant to hypoxia than *Capitella* sp. I. Settlement of *P. vexillosa* larvae was significantly reduced, while settlement of *Capitella* sp. I promoted, in hypoxic sediment compared with normoxic sediment. The bacterial community composition of the *Bacteroidetes* in hypoxic sediment was qualitatively and quantitatively different from normoxic sediment. In particular, SRB was greatly promoted in hypoxic sediment under 28 days of exposure. This study not only demonstrated that hypoxia can impair larval mortality and alter polychaete larval settlement directly in a species-specific manner, but also indirectly affect polychaete larval settlement through alteration of bacterial community composition and abundance on sediment surface. Given hypoxia becomes a global problem due to expanded eutrophication from industrialization and coastal urbanization, severe hypoxic events may potentially alter the settlement pattern and recruitment of polychates, one of the major components of benthic structure in soft-bottom sediments in the marine environment.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Effects of hypoxia on Darwanian fitness traits and physiologies of larvae of the marine invertebrate *Crepidula onyx*

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Crepidula onyx is found in great abundance in intertidal and subtidal waters over large geographic areas along the Pacific coast of North and South America, China, Japan and Hong Kong. Using *C. onyx* as a model species, we investigated how hypoxia affected the Darwinian fitness traits of marine invertebrate larvae, including survival, growth and metamorphosis as well as their physiologies, including filtration and respiration. The filtration rate of this filter feeder was not affected by a dissolved oxygen level as low as 3 mg O_2 l^{-1}. Nevertheless, the respiratory rate was significantly reduced even by a very weak hypoxic condition of 5 mg O_2 l^{-1}. Furthermore, we found that 3 mg O_2 l^{-1} treatment significantly decreased larval survivorship and growth rate. By the time the control larvae reared under normoxic condition reached metamorphic competency with 100% metamorphic rate, the larvae in this hypoxic treatment had a significantly reduced body size and only *ca.* 60% of them successfully metamorphosed into juveniles. A weaker hypoxic condition of 4 mg O_2 l^{-1} also decreased larval growth rate and percentage of metamorphosis. Since Darwinian fitness traits are of utmost importance in determining the sustainability of a species, the adverse effects of hypoxia on the fitness of this important gastropod species may implicate major ecological consequences over large coastal areas.

Effects of polybrominated diphenyl ethers on Darwinian fitness traits of marine invertebrates

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Using model species of three different taxonomic groups, *Balanus amphitrite*, *Hydroides elegans* and *Crepidula onyx*, we investigated the effects of PBDEs on the Darwinian fitness traits (survival, growth and metamorphosis) of marine invertebrate larvae. Our experiments with *B. amphitrite*, *H. elegans* and *C. onyx* larvae showed that the acute toxicity of PBDEs is low. A 24 hours exposure to 10,000 μg BDE47 l^{-1} did not cause mortality, and this result is consistent with studies using human, mammal and fish models which also suggested a low acute toxicity of PBDEs. However, chronic exposure at environmentally relevant concentration impaired the Darwinian fitness of marine invertebrates. *C. onyx* larvae that had been exposed to 1 μg BDE47 l^{-1} since hatching failed to metamorphose into juveniles, but their body size was not affected. Since metamorphosis is guided by species-specific chemical cues produced by the microbes in biofilms, future research will investigate if PBDEs can alter the composition of microbial communities in biofilms, hence affecting the chemical cues for larvae and metamorphosis.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

High-CO_2 is likely to exacerbrate the negative impact of hypoxia on larval metamorphosis in edible oysters: a proteomic perspective

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Oysters are an important ecological and commercial shellfish resource. They have a complex life cycle, during which the swimming (pelagic) larvae must select a suitable substrate, attach to it, and then metamorphose into benthic adults. One of the important objectives of the MERIT-AoE is to understand the impact of hypoxia on the highly demanding larval metamorphic process at proteomics level. In nature, especially in Hong Kong's oyster culture zone, the Deep Bay, the metamorphosing oyster larvae are likely to be exposed not only to hypoxia but also to high-CO_2. The rising anthropogenic CO_2 emission already started decreasing carbonate ion concentration and pH in the Deep Bay area, called ocean acidification (OA). Our studies on the effects of OA on larval growth and protein expression clearly demonstrate (1) that larval metamorphosis is more sensitive to OA than other larval traits, and (2) that proteins related to metabolism and biomineralization appear to be overexpressed at OA. The hypoxia is predicted to exacerbate these observed negative effects of OA on larval metamorphosis, calcification and metabolic activities through altered protein expression pattern or proteome structure. This multidisciplinary (larval biology and proteomics) collaborative project is, therefore, designed to test the above hypothesis using the commercial oyster (*Crassostrea hongkongensis*) by exposing their competent (to metamorphose; the pediviliger stage) larvae to several treatments mimicking natural and projected carbonate chemistry and hypoxia regimes in Hong Kong's oyster culture zone. After 24 h of exposure, larval ability to metamorphose (on biofilms) was significantly reduced at moderately elevated CO_2 (700 ppm or pH 7.6) and reduced oxygen (3 to 4 mg L^{-1}) levels when compared to control pH (8.1) and oxygen (8 to 8.5 mg L^{-1}). Strikingly, oyster larvae were almost completely lost their capacity to attach on suitable substrate when they were exposed as little as 24 h to hypoxia at high-CO_2 environment. Our results illustrate the importance of the synergic effect of these two climate change stressors on larval metamorphosis. Now, we are studying larval global protein expression patterns that make them to or not to attach on suitable substrate using a gel-based (2-DE) quantitative proteomic approach. Our preliminary proteomic results suggest that our approach could be promising in identifying protein expression signatures (PES's) responsive to hypoxia in this non-model sentinel species. If functional roles of the identified PES's are established, the sustainability of various non-model species in hypoxic oceans may be more accurately predicted.

Adaptive protome plasticity during larval metamorphosis: response of barnacle larvae to high-co_2 and hypoxia

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Barnacles are an important biofouling species and their larval attachment on man-made substrate cause huge economic loss to marine industries. After developing into a competent (to attach and metamorphose) stage, the barnacle larvae ("cyprids") exhibit a lower and an upper limit to metamorphic success (i.e. variation in time of metamorphosis) and body size at metamorphosis. Within these limits, cyprids could exhibit an extreme plasticity in response to environmental stressors such as hypoxia and high-CO_2. Using our current interdisciplinary MERIT-AoE team, we are interested to examine the larval proteome plasticity to emerging twine threats, the hypoxia and high-CO_2. The decreasing carbonate ion concentration in coastal waters due to anthropogenic CO_2 emission (high-CO_2), called ocean acidification (OA), is predicted to affect larval metamorphosis, calcification and metabolic activities through altered protein expression pattern or proteome structure. Our work further suggests that physiological quality, settlement rate, expression of several metabolic enzymes and metamorphic success are reduced, within their tolerable limits, in high-CO_2 seawater. Using a proteomic (the large-scale study of proteins expressed by a genome) approach, we have tested the hypothesis that elevated CO_2 could alter the expression of calcification-related proteins. Using the same approach, we have also examined, for the first time, cyprids response to high-CO_2 and hypoxia stress. The larval proteome is plastic and capable of change in response to CO_2 stress. Twelve proteins have been tentatively identified as high-CO_2-responsive, effectively creating unique protein expression signatures (PES) for CO_2 stress. We are currently examining the hypothesis that decreasing oxygen (hypoxia) is exacerbate the negative effects of high-CO_2 in cyprids, i.e. the lower limit of the cyprids metamorphic capacity (lower end of the plasticity) may be pushed below the threshold line. This interdisciplinary collaboration will enable us to study, for the first time, larval metamorphic plasticity at proteome level in a biofouling species in natural as well as conditions projected to be imposed on them by global upcoming environmental changes (hypoxia) and CO_2 emissions.

Characterizing the dynamic nature of biofilm formation, metabolome and proteome of *Vibrio harveyi* M4 isolated from marine biofilms in response to a bioactive poly-ether from a sponge-associated bacterium

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Vibrios constitute a considerable part of halophilic bacterial population in the marine environment and usually form symbiotic or pathogenic relationships with eukaryotic hosts. They colonize diverse environmental habitats and are able to persist and grow even under nutrient-poor and hostile conditions. One key factor for environmental survival and transmission is their ability to form biofilm. The aim of this study was to understand the effects of a potential antifouling candidate, poly-ether, on biofilm formation, metabolome and proteome of *Vibrio harveyi*. Confocal microscopy revealed that the control biofilms were well developed and four times thicker than the biofilms treated with poly-ether. Flow cytometry analysis showed that the percentage of dead cells in the treated biofilms was 14% when compared to 3% in the control biofilms. Since cell viability could not completely explain the differential biofilm formation, we explored the biochemical changes occurring during the process based on metabolome and proteome profiling. The metabolites in control and treated cells were profiled by UPLC- MS analysis followed by unsupervised PCA analysis which statistically narrowed down the differentially expressed metabolites to tri-peptides, fatty acids like oleamide and quorum sensing molecules. Poly-ether exposure also depleted cellular levels of reduced glutathione and other thiols to a greater extent than in the control cells. The 2D-gel based proteome analysis showed 50 up-regulated proteins and 9 down-regulated proteins in the treated biofilms. MALDI TOF/TOF MS analysis of the differentially expressed protein spots revealed the up-regulation of superoxide dismutase and periplasmic proteins and the down-regulation of ferrous trafficking proteins in the treated cells, which may play a role in biofilm formation.

Transcriptome profiling and gene characterization in the settlement of the barnacle *Balanus amphitrite*

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The barnacle *Balanus amphitrite* is a major marine fouling organism in most tropical and subtropical areas around the world. An understanding on the underlying molecular mechanisms of larval settlement is important for developing an environmental friendly antifouling technology. However, limited information hinders us to gain a comprehensive understanding of the gene expression pattern and signal transduction pathway during larval settlement. In this study, we conducted a comparative transcriptome analysis on the larvae and adult of *B. amphitrite* by using 454 pyrosequencing and identified highly expressed genes in cyprids, which are competent larvae ready to attach on a substrate and settle. A total of 215,308 high quality reads were obtained from the larval pool and 194,223 of which could be assembled into 15,690 contigs with an average length of 646 bp; whereas in the adult pool, 362,879 reads of a total 415,537 formed 28,043 contigs of an average length of 707 bp. Among those annotated best hits, 255 and 1,051 putative genes specific for the larval and adult stages, respectively, were isolated, providing a rough estimate of the number of differentially expressed genes in these two pools. Some of the putative larval specific genes were related with cement proteins for attachment or some receptor tyrosine kinases for metamorphosis. They may play a key role during *B. amphitrite* settlement. Further studies on some other gene candidates are still ongoing to elucidate their functions in larval settlement.

Multiplexed proteomic approach to investigate the effects of the anti-fouling agent butenolide on the development of the barnacle *Balanus amphitrite*

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The barnacle *Balanus* (=*Amphibalanus*) *amphitrite* is a major marine biofouling invertebrate worldwide. It has a complex life cycle during which the swimming nauplius larva molts six times before transforming into a swimming cyprid. The cyprid stage in *B. amphitrite* is critical for the larvae to decide where to attach and metamorphose to begin a sessile life. Even with the longstanding interest in the attachment and metamorphosis of this marine invertebrate, the molecular mechanisms that control these biological processes remain unclear. In this study, proteome and phosphoproteome alterations during cyprid development/aging and upon treatment with the antifouling agent butenolide were examined with a two-dimensional electrophoresis (2-DE) multiplexed fluorescent staining approach. Our results showed that the differential regulation of target proteins was highly dynamic on the levels of both protein expression and posttranslational modification. Two groups of proteins, stress-associated and energy metabolism-related proteins, were differentially expressed during cyprid development. A comparison of protein expression level between the control and treatment cyprids suggested that butenolide exerted its effects by sustaining the expression levels of these proteins. Altogether, our data suggested that the proteins involved in stress regulation and energy metabolism play crucial roles in regulating larval attachment and metamorphosis of *B. amphitrite*.

Quantitative proteomics identify molecular targets that are crucial in metamorphosis of the marine bryozoan *Bugula neritina*

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Metamorphosis of the marine bryozoan *Bugula neritina* transforms a ball-shaped swimming larva into a tubular sessile juvenile within 48 hours. The metamorphosis consists of various complex processes such as the morphogenetic rearrangement of larval tissues and the development of juvenile tissues from primordial cells. Understanding the metamorphosis of *B. neritina* can provide insights into their colonization, as well as have implications on bryozoan anti-fouling methods. Despite many studies on metamorphosis of this species, little is known about the molecular mechanism of these processes. In this study, we firstly established a transcriptome database for *B. neritina* using 454 pyrosequencing. This equipped us with a customized *B. neritina* protein database for our subsequent comparative study on swimming larvae and metamorphosing individuals at 4 h and 24 h post-attachment using label-free quantitative proteomics. We identified more than 1,100 proteins at each developmental stage, 61 of which were differentially expressed. Specifically, proteins involved in energy metabolism and structural molecules were generally down-regulated, whereas those related with transcription, translation, extracellular matrix, and calcification were strongly up-regulated during metamorphosis. Many tightly regulated novel proteins were also identified. Subsequent analysis of the temporal and spatial expressions of some of the proteins by qRT-PCR and *in-situ* hybridization, and bioassays assessing their functions indicated that they may play key roles in the metamorphosis of *B. neritina*.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Molecular analysis of settlement of the marine fouling polychaete *Hydorides elegans*

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The polychaete *Hydroides elegans* is a common marine fouling organism in most tropical and subtropical waters. The life cycle of *H. elegans* includes a planktonic larval stage in which the swimming larvae normally take 4 to 5 days to become competent to settle (attachment and completion of metamorphosis), which marks the onset of their benthic life. However, the endogenous molecular mechanism that regulates settlement remains largely unknown. Using a conventional gene isolation technique - suppressive subtractive hybridization (SSH) – we isolated the genes that were differently expressed between the competent larvae and metamorphosing juvenile. Ninety-seven differently expressed genes were found in the forward hybridization library (i.e. competent larvae), while 121 were isolated from the reversed hybridization library (i.e. metamorphosing juvenile). Follow-up real-time PCR and bio-assay experiments further confirmed the possible involvement of these genes in the larval attachment and metamorphosis. In addition, pyrosequencing was used to profile the overall transcriptome of *H. elegans*. The cDNA samples from four developmental stages of *H. elegans*, including pre-competent larvae, competent larvae, male and female adults, were sequenced by using a 454 pyrosequencing platform. After *de novo* gene assembly, 136,490 open-reading frames were predicted, 38,259 of them could be matched to known genes on NCBI database whereas no homolog was found for the reamining 98,231. The pyrosequencing revealed the existence of putative spliced-leader in *H. elegans*. Furthermore, a large number of genes and pathways involved in *H. elegans* development were identified.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Bacterial community succession and chemical profiles of subtidal biofilms in relation to larval settlement of the polychaete *Hydroides elegans*

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Earlier studies have shown that biofilms can mediate the larval settlement of the polychaete *Hydroides elegans* and that changes in the bacterial community structure and density of biofilms often alter the larval settlement response. However, the chemical cues that mediate this response remain unknown. In this study, both successional changes in the bacterial community structure and the chemical profiles of subtidal biofilms are described and related to the larval settlement response. Multispecies biofilms were developed on polystyrene Petri dishes and granite rock in the subtidal zone over a period of 20 days. The effects of the substratum and age on the bacterial community structure and chemical profiles of the biofilms were evaluated with two molecular methods (microarray (PhyloChip) and denaturing gradient gel electrophoresis) and with gas chromatography–mass spectrometry, respectively. Both age and substratum altered the bacterial community structures and chemical profiles of the biofilms. Age had a greater effect in shaping the bacterial community structure than did the substratum. In contrast, the type of substratum more strongly affected the chemical profile. Extracts of biofilms of different ages, which developed on different substrata, were tested for the settlement of H. elegans larvae. The extracts induced larval settlement in a biofilm-age-dependent manner, and extracts originating from different substrata of the same age showed no differences in larval settlement. Our results suggest that the larval settlement response cannot be predicted by the overall chemical composition of the biofilm alone.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th Januarv – 7th Januarv 2011

Highly diverse microbial communities in natural marine biofilms and the relationship with larval settlement of the barnacle *Balanus amphitrite*

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Marine biofilms are aggregates of a mixed population of microbes embedded in a matrix of extracellular polymeric substances. Previous studies have shown that settlement of many marine invertebrate larvae is mediated by marine biofilms. However, the underlying mechanism of the mediation is still unresolved mainly because of the uncertainty in characterizing members in the communities using existing 16S rRNA gene-based molecular methods. In this study, a newly developed method – barcoded 16S rRNA gene pyrosequencing – was employed to provide a detailed characterization of the bacterial communities in intertidal and subtidal marine biofilms developed in two seasons. This method has high sensitivity to detect low-abundance taxa and is able to generate a large amount of highly reliable data in a very short period of time. Our results revealed highly diverse biofilm bacterial communities varied with season and tidal level. Over 6,000 OTUs with species estimates of up to 15,000 were recovered in a biofilm sample, which is by far the highest record in sub-tropical marine biofilms. Nineteen phyla were found in the biofilm samples, for which Cyanobacteria and Proteobacteria were the most abundant phylum dominating the intertidal and subtidal biofilms, respectively. Apart from these two phyla, Actinobacteria, Bacteroidetes, and Planctomycetes were found to be the major groups recovered in both intertidal and subtidal biofilms, yet their relative abundance varied among samples. Full-length 16S rRNA gene clone library was constructed for the 4 biofilm samples and recovered similar abundant phyla as shown by pyrosequencing. The relationship of bacterial community and settlement of larvae of a marine invertebrate is discussed.

Changes in the proteome and phosphoproteome expression in the bryozoan *Bugula neritina* larvae in response to the antifouling agent butenolide

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Larval attachment and metamorphosis, commonly referred to as larval settlement, of marine sessile invertebrates can be triggered or blocked by chemical cues and affected by changes in overall protein expression pattern and phosphorylation dynamics. This study focused on the effects of butenolide, an effective larval settlement inhibitor, on larval settlement of the bryozoan *Bugula neritina* at the proteome level. Liquid-phase IEF sample prefractionation combined with 2-DE and MALDI-TOF MS were used to identify the differentially expressed proteins. Substantial changes occurred in both protein abundance and phosphorylation status during larval settlement and when settling larvae were challenged with butenolide. The proteins that responded to the butenolide treatment were identified as structural proteins, molecular chaperones, mitochondrial peptidases and calcium-binding proteins. Compared with our earlier results, both genistein and butenolide inhibited larval settlement of *B. neritina* primarily by changes in protein abundance and phosphorylation status but they have different protein targets in the same species. Clearly, to design potent antifouling strategy and to understand the mode of action of antifouling compounds, more studies on the effects of different compounds on proteome and phosphoproteome of different larval species are required.

Effect of tidal height on chemical profile of biofilms and subsequent larval settlement pattern of the barnacle *Balanus amphitrite*

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The gregarious settlement of barnacles in intertidal shoreline is a common field observation. Earlier studies have shown that biofilm attributes such as bacterial density and community structure mediate the larval settlement and habitat selection ability of barnacles. However, the link between metabolites of biofilm and larval settlement has not been fully understood. In this study, we observed clear preference for the cyprids of the barnacle *Balanus amphitrite* settling on intertidal biofilms than on subtidal biofilms when the two choices were provided and this observation was independent of the bacterial density or biomass of the biofilms. Moreover, we demonstrated that the preferential settlement of cyprids was a chemically-mediated process because they could distinguish biofilm extracts originating from different tidal levels in a chemical extract choice assay. Chemical profiles of biofilms from intertidal and subtidal zones were analyzed by ultra performance liquid chromatography-mass spectrometry (UPLC-MS) for comparison. The chemical profile data were subjected to principal component analysis (PCA) to seek for the differences in metabolites that likely lead to settlement preference of cyprids. Results suggested an amino acid related compound and a fatty acid ester may be the cues leading to the settlement preference.

Developing 2-DE proteome and phosphoproteome maps: a new insight into the molecular mechanism of larval settlement and metamorphosis

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There is a long history in larval biology of descriptive work to characterize behavioural and morphological changes during settlement and metamorphosis; and until recently, proteomics technologies are being applied to gain an in-depth understanding of the molecular processes regulating larval settlement and metamorphosis in individual marine invertebrates. Because the behavioural, morphological, and molecular changes associated with settlement and metamorphosis vary between marine invertebrate species, here we used proteomics-based technologies to develop and compare proteome and phosphoproteome maps during settlement and metamorphosis of a wide range of marine invertebrate species including mollusc, crustacean, bryozoan, and polychaete. Specifically, we constructed 2D protein maps and identified differentially expressed proteins with the goal of identifying common molecular patterns across multiple species. Larval protein lysates from selected marine invertebrate species were subjected to 2D gel electrophoresis, and multiplex fluorescence staining. The preliminary results indicated that many isoforms of structural proteins and proteins related to energy metabolism, apoptosis and stress were commonly and differentially expressed in different developmental stages across the marine invertebrates. Expression patterns of some of the identified proteins were confirmed by western blot and real-time PCR analysis. This study provides a framework for the identification of important molecular processes and pathways involved in the settlement and metamorphosis of different marine invertebrates.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Bacterial population dynamics during degradation of copepod fecal pellets

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Population dynamics of bacteria associated with copepod fecal pellets and free-living bacteria in seawater throughout a 10-day laboratory fecal pellets degradation experiment were investigated by denaturing gradient gel electrophoresis (DGGE). Generally, copepod fecal pellets containing bacteria were composed of different phylogenetic groups from those living free in seawater. Bacterial in fecal pellets were dominated by *γ-Proteobacteria* and *α-Pro-Sulfitobacter*, while bacteria in seawater were more diversified and mainly consisted of *α-Proteobacteria*. Remarkable bacterial community shifts occurred in the first two days of the experiment along with a sharp increase of dissolved organic carbon (DOC) and a sharp decrease of dissolved oxygen (DO) in the incubation bottles. Indeed, RDA analysis revealed that population dynamics of free living bacteria in the incubation bottles during the degradation experiments could be explained mainly by changes in DO and DOC concentrations. Interactions between bacteria that were originally associated with fecal pellets and those in surrounding seawater occurred during the degradation process as indicated by the increased similarity between the bacterial community composition in fecal pellets and in seawater towards the end of the incubation. However, it is evident that the colonization of free-living bacteria, rather than bacteria that are intrinsic to fecal pellets, were mainly responsible for the degradation of fecal pellets. This was supported by: 1) the community composition of both free living and attached bacteria was resembled more those in the seawater control than those in fecal pellet initials; 2) fecal pellet specific bacteria groups, such as *α-Pro-Sulfitobacter* and *γ-Pro-Vibrio*, were never detected in the seawater throughout the incubation.

PRESENTATION

BY

TASK TEAM 3

Development of Chronic Life-Cycle Toxicity Tests for Emerging Chemical Contaminants Using the Marine Copepod *Tigriopus japonicus*

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Ecological risk assessment of emerging chemical contaminants (ECC) is at the core focus of the extended AoE. Our proposed study aims at developing and evaluating the tiered risk assessment protocols using our well established model organism — the marine copepod *Tigropus japonicus* to illuminate the relationship of the toxicity endpoints between lower tier screening with molecular biomarkers and higher tier full-life cycle toxicity test for selected ECC. In this presentation, we will update our latest development of both chronic life-cycle and multigenerational tests using the copepod. Double-walled carbon nanotubes (DWNTs) are used as an ECC example, as they widely exist in a variety of consumer products with very little information on their toxicity towards marine organisms. A full-life cycle chronic toxicity test was developed and conducted for comparing the toxicity of DWNTs generated by two different dispersal methods, namely sonication (so-DWNTs) and stirring (st-DWNTs). The median aggregation size (0.89 μm^2) of so-DWNTs was smaller than that of st-DWNTs (21.8 μm^2). Population growth of *T. japonicus* was reduced to 0.1 mg/l for so-DWNTs and 10 mg/l for st-DWNTs, suggesting that so-DWNTs are significantly more toxic than st-DWNTs and thus dispersion method and size of aggregations should be considered in DWNT toxicity testing.

Furthermore, we developed a multigenerational test using copper (Cu) as a model chemical, and successfully tested whether Cu resistance can be developed through multi-generation acclimation to elevated Cu levels and verify if there is a fitness cost of such adaptation. *T. japonicus* (F0) were acclimated to three Cu concentrations (0 [control], 10 and 100 ppb) and offspring (F1 or F2) of each treatment were subsequently acclimated at these three concentrations, respectively. Our results indicated that Cu resistance of the copepod was increased even after just one generation of acclimation to 100 ppb, resulting in a 20% reduction in mortality compared to the control when exposed to 1000 ppb for 96 h. Such a Cu resistance also gradually increased throughout the three generations. However, the acquired Cu resistance had an associated fitness cost, as the intrinsic population growth rate of this Cu resistant lineage was significantly lower than that of those raised under control conditions. After reverting back to the control conditions, Cu resistance of the offspring from these Cu resistant copepods returned to a level comparable to the control. The acquired Cu resistance is, thus, regarded as a physiological adaptation which is also phenotypically plastic.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Transport and Removal of Nanoparticles in Porous Media

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There are growing concerns about the ecological impact and health risk of emerging man-made nano-materials that are introduced into the environment during their production and application. The fate and transport of nanoparticles in saturated porous media, e.g., the subsurface environment and water and wastewater treatment facilities, is of great importance to the assessment of environmental impact of nanoparticles. In this study, laboratory experiments were carried out to investigate the transport behaviour and removal efficiency of nanoparticles in porous media during granular filtration. Standard latex particles of different sizes - 50 nm, 100 nm and 1 μm were used to make particle suspensions for the filtration tests. Packed in glass filter columns (10 cm long and 1.05 cm in diameter), the filter materials tested included glass beads (0.225 mm), sand grains (0.25 mm) and granular activated carbon (GAC, 0.9 mm). The results show that for particles of different sizes through different filter media the removal efficiency increased as the filtration velocity decreased. With glass and sand, in agreement with filtration theory, the particle removal efficiency decreased as the particle size decreased. Thus, conventional granular filters apparently are not effective to remove nanoparticles from water. In contrast, the particle removal efficiency increased for GAC filtration as the particles decreased in size. Thus, GAC appears to be a more effective filter medium for nanoparticle removal. It is argued that a lower removal efficiency by glass beads than by sand is likely due to the higher hydrophilicity and smoothness of the glass surface. Brownian diffusion, which becomes more significant for the transport of smaller particles, is believed to be responsible for the greater removal of nanoparticles by the GAC column. Further examination with scanning electron microscopy (SEM) provides a direct evidence of the capture of nanoparticles within the GAC pores. In addition the standard latex particles, several types of engineered nanoparticles, including alumina, silver, titania and silica nanoparticles, were also investigated on their transport behaviour and removal during granular medium filtration. Silica nanoparticles display the highest mobility, followed by titania, silver and alumina nanoparticles.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Determination of Hazardous Concentration for Species Densities from Field-Based Community Sensitivity Distributions

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In this study, the field sediment data in the Norwegian Oil Industrial Association database, which were collected between 1996 and 2001, were used to derive the field-based community sensitivity distribution (f-CSD) which reflected the impact of the toxic chemicals on the species density. This database covered the abundance data for 1,944 species and 182,201 individuals in 1,902 stations which located in the Norwegian continental shelf. Effect concentrations of each installation (oil platform) at a given reduction in species density were derived by fitting a logistic regression function of the benthic species density and the chemical concentration in sediment. These effect concentrations were further improved by the nonparametric empirical Bayes method which incorporated information of the other installations. The distribution of these improved effect concentrations of installations was determined non-parametrically by the Kernel method. The median and the mode concentrations based on the estimated density were considered as the measure of hazardous concentration HC(γ) at a given reduction in species density by proportion. Therefore, the hazardous concentrations here can describe the effects of various concentrations of chemicals on the percentages of affected species density in the area. The f-CSD was then constructed from HC(γ)'s over the range of $\gamma \in (0, 1)$. As examples, barium, cadmium, chromium, copper, mercury, lead, tetrahydrocannabinol and zinc were selected to derive their HC(γ) values using this novel method. An alternative interpretation of derived HC(γ) values was also provided as the concentration under which (100 - γ)% of species density in Norwegian continental shelf was protected. Furthermore, the safety levels, that are, the lower bounds of confidence intervals, of HC(γ) values of toxic chemicals can be derived for all installations in Norwegian continental shelf. Since the number of observations was small to derive the confidence intervals by conventional bootstrap method, the implementation of smoothed bootstrap method can tackle this problem and obtain an accurate estimate of the lower confidence limits. Surprisingly, our results showed that the lower confidence limits of derived HC(0.05) were comparable with those critical HC values derived from species abundance data using the field-based species sensitivity distribution approach. In contrast to previous approaches where the linear adjustment is necessary for the derived hazardous concentration, the lower confidence limits of the HC(γ) values can be adapted as the threshold effects level (TEL) directly without further adjustment. More importantly, this data-driven approach can be used as supplemental information for ecological risk assessment in terms of biodiversity protection.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Numerical Investigation of Circulation and Nutrient Transport in the Mirs Bay

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Many years after the devastating red tide that originated from the Mirs Bay and swapped across the Hong Kong waters in 1998, the mechanism that led to this marine environment catastrophe remains unclear. The Mirs Bay is located to the east of Hong Kong Island and occupies about 50 % of total sea area of Hong Kong. Since nutrient rich Pearl River water is potentially limited by light and phosphate, most of red tides or algal blooms are originated in the eastern part of Hong Kong waters. Study of Mirs Bay circulation is crucial to scientifically understand the interactive dynamics of the coupled bay-shelf system as well as the associated biogeochemical response in Hong Kong waters. A coupled three-dimensional physical model and nitrogen-phosphate (NP)-based dissolved inorganic nitrogen, phosphate, phytoplankton, zooplankton, and detritus (NPPZD) ecosystem model was used to study the ecosystem responses to the circulation in the Mirs Bay. The circulation in the Mirs Bay is not isolated from the adjacent shelf processes. With a deep central channel in the bay and unique shelf/coastline topography in the adjacent shelf waters, Mirs Bay is closely influenced by the intrusion of dense and nutrient rich deep shelf waters, induced by the frictional bottom Ekman layer and by the amplified cross-isobath shoreward transport at the lee of the coastal promontory due to Hong Kong Island. The intrusive nutrient rich deep waters surface in the bay and subsequently transport to the rest part of Hong Kong waters by the shelf circulation. Both shelf and bay circulations are highly time- and space-dependent governed by variable wind and tidal forcing as well as by the local intrinsic hydrodynamics.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

A Time-series Study on Marine Water Quality Monitoring Data in Hong Kong: Implications of the Effectiveness of Environmental Policy and Management

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This study makes use of the past, yet limited, marine water monitoring data to objectively identify and evaluate the effectiveness of implementation of various environmental policy and measures (e.g. establishment of Water Pollution Control Ordinance, WPCO and Harbour Area Treatment Scheme, HATS). In 1989, the Hong Kong Government enacted the White Paper to fight against pollution in Hong Kong. Through implementation and enforcement of WPCO, there were some marked improvements of both inland and coastal water quality across Hong Kong over the last 20 years. Since 1994, the Hong Kong SAR Government had commenced the HATS construction, trying to further improve the water quality of Victoria Habour. The first stage of HATS was completed and launched in December 2001. One of the purposes of this study is to investigate whether or not the first stage of HATS is effective in improving the water quality of Victoria Harbour. Advanced time-series analyses and intervention analyses are employed to characterise and identify the critical time points in terms of water quality improvement over the past. So far, we have completed the analyses for ammonia and dissolved oxygen. The results are highly encouraging and able to objectively show the critical time points coincided with the major environmental policy being implemented.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Real-time Forecasting of Beach Water Quality by Data Assimilation Model Based on Bacterial Load

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A novel data-assimilation method for real time forecasting of daily bacterial concentration at a coastal marine beach is presented. The beach water quality during the bathing season is strongly affected by storm water runoff from the upstream catchment. Conventional data driven models suffer from the lack of adequate water quality data to capture the range of expected concentrations, and often predict unreasonably high concentrations during rainfall events – leading to "false alarms". We propose a data-assimilation model in which the daily *E.coli* level is predicted as a function of different hydro-meteorological inputs (e.g. solar radiation, tide, rainfall, water temperature, wind) as well the catchment microbial load using an Artificial Neural Network (ANN) model. The catchment microbial load is predicted in turn by integrating a physically-based distributed hydrological model (MIKE-SHE) to predict the runoff based on real time rainfall data, and a watershed water quality model (HSPF) to predict bacterial concentration. Numerical predictions of the deterministic hydrologic and water quality models serve as numerical data to calibrate a ANN sub-model for forecasting of bacterial load. The model has been validated against field data and shown to be superior than pure data driven models such as Multiple Linear Regression (MLR) models; the approach can be readily coupled with online systems of water quality forecast.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Adsorption of selected EDCs and antibiotics on marine sediment in relation to sediment organic diagenesis

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Adsorption is one of the most important environmental phenomena at the sediment-water interface. Owing to the quick and strong chemical adsorption, the fate and transport of organic contaminants in marine waters are greatly related to the organic matter in sediment. The present study was carried out to investigate the diagenesis of sediment organic matter (SOM) and its influence on the dynamics of adsorption of selected emerging pollutants. Artificial sediment that was made up of fine sand and clay and contained starch as the SOM was used for the experimental study. The SOM-containing sediment was incubated to simulate the natural diagenesis process. During a period of 5 month incubation, a portion of the sediment was sampled every week, and the batch adsorption experiment was conducted on the sediment sample. Two endocrine disrupting chemical (EDC) compounds, bisphenol A (BPA) and nonylphenol (NP), were selected as the model pollutants for the adsorption study. The results show that the SOM initially loaded into the sediment was reduced continuously during the incubation, indicating biodegradation and SOM diagenesis. However, the organic normalized partition coefficient, K_{oc}, of BPA on the sediment more than doubled from around 100 L/kg to 225 L/kg. Experiments with NP also confirmed the same trend of K_{oc} increase. The experimental results suggest that during the dynamic process of sediment diagenesis and biomass growth, the remaining SOM increases in its affinity with the organic pollutants. The research finding is of importance to a comprehensive assessment of the fate and transport of BPA, NP, other EDCs and similar environmental contaminants in water-sediment systems.

Biokinetics of dioxin in marine planktonic food chain

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In this study, uptake, assimilation efficiency, and elimination of dioxin were measured in marine phytoplankton, copepods (*Acartia spinicauda*) and fish (*Acanthopagrus schlegeli*), using radiotracer methodology. Because of the significant sorption, uptake rate constant of dioxin from water could not be determined in marine phytoplankton, but decreased with increasing trophic level. The dietary assimilation efficiency was 24%~53% in copepods, and varied considerably with different food concentrations and food diets. The elimination rate constants of dioxin in the copepods were comparable following uptake from the diets and from the water. Both aqueous and dietary uptake were equally important for dioxin in the copepods.

PRESENTATION

BY

TASK TEAM 4

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

The significance of Fe plaque formation on mangrove plants in the removal of wastewater-borne nutrients, heavy metals and persistent organic pollutants

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Mangrove sediments have been suggested as good sinks for nutrients, heavy metals and persistent organic pollutants (POPs). Mangrove plants were able to release oxygen from root to the rhizosphere to oxidize ferric/ferrous ions and create iron-rich root coatings, generally called iron (Fe) plaque. Our recent studies demonstrated that mangrove plants have the ability to form Fe plaque on the root surface. However, the significance of Fe plaque formation on mangrove plants in the removal of wastewater-borne nutrients, heavy metals and POPs is less reported. How Fe plaque formation is related to the removal of these pollutants is also unclear. The present study aims to investigate the difference in the formation of Fe plaque on roots of three mangrove species, namely *Bruguiera gymnorrhiza*, *Excoecaria agallocha* and *Acanthus ilicifolius*, when receiving different strengths of wastewater, and its effects on treatment performance. For *B. gymnorrhiza*, the total amounts of Fe plaque formed on the whole root were 97.49, 150.26 and 167.81 mg at Day 75 in treatments of control (fresh water, FW), medium wastewater (5 times the strength of the primary settled municipal sewage, 5SW) and strong wastewater (10SW, double the strength of 5SW), respectively; and the respective amounts for *E. agallocha* were 16.88, 79.56 and 100.45 mg. For *A. ilicifolius*, the amount of Fe plaque formed at Day 75 was 14.45 mg when receiving 5SW, but in the 10SW treatment, all seedlings were dead. Mn and Cu were the most effective metal ions immobilized on roots of *B. gymnorrhiza* when receiving wastewater, with removal percentages of 13.17% and 10.25%, respectively, followed by Zn, Ni, Pb, Cd and Cr, and the least effectively immobilized element was P, with a removal percentage less than 1%. For both *E. agallocha* and *A. ilicifolius*, Mn was the most effective element being immobilized on roots when receiving wastewater, followed by Zn, Cu, Ni, Pb, Cd and Cr. The immobilization of P in Fe plaque on roots of *A. ilicifolius* was much more effective than that of *E. agallocha*, ranking the second most effective element immobilized in Fe plaque for *A. ilicifolius*. The removal of heavy metals from soils was positively correlated to the formation of Fe plaque, irrespective of plant species and wastewater strengths. In addition to nutrients and heavy metals, the formation of Fe plaque also had a positive effect on the immobilization of POPs, including polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs). These results suggested that the formation of Fe plaque on mangrove plant roots contributed to the removal of wastewater-borne toxic pollutants, and such contribution was the greatest for *B. gymnorrhiza*, followed by *E. agallocha*, and the least for *A. ilicifolius*, which was due to that the production of root biomass was the highest for *B. gymnorrhiza* but the least for *A. ilicifolius*.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Tolerance and toxicity of estradiol and ethinylestradiol in green microalgae

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Estrogens are a group of steroid hormones and their pollution is a serious environmental concern due to their persistence, ubiquity in the environment and endocrine-disrupting toxicity. The tolerance of six microalgal species, including three local isolates of *Chlorella minata* (WW1), *Chlorella* sp. (2f5aia) and *Chlorella* sp. (ivo5ai) and three commercially available species of *Scenedesmus quadricauda* (SQ), *Chlorella vulgaris* (CV) and *Selenastrum capricornutam* (SC) to the toxicity of natural and synthetic estrogens, namely estradiol (E2) and ethinylestradiol (EE2), respectively, was compared. Growth responses of algal cells, including changes in cell number, cell volume, cell autofluorescence, cell complexity, cell division and cell viability, to the exposure to mixed E2 and EE2 at equal concentrations, ranging from 0 to 20 mg L^{-1}, at different time intervals of up to seven days were monitored.

The sensitivity of microalgae to estrogen toxicity was highly dose-, incubation time-, and species- dependent. The percentages of inhibition at 96 h of exposure to E2 & EE2 suggested that the sensitivity of the six microalgal species followed the order of SQ > CV = WW1 = 2f5aia ≥ ivo5ai = SC. The increase in cell number of SQ at the end of 7-day incubation reduced significantly with increasing E2 & EE2 concentrations. Two-way MANOVA results also showed that the changes in cell number of SQ were significant with time. Accompanied with the changes in cell number, other growth responses, including increases in cell volume and degrees of cell complexity and decreases in cell division, were also observed with increasing E2 & EE2 concentrations. The other five species presented different degrees of adaptations to E2 & EE2. For species of 2f5aia and CV, E2 & EE2 had an adverse effect on the increase in cell number, but such response was poorly correlated with concentrations. For species of SC and WW1, E2 & EE2 had no (1 mg L^{-1}) or even stimulating (4-20 mg L^{-1}) effect on the cell number. No significant differences were found in the cell number of ivo5ai when exposed to different E2 & EE2 concentrations (Two-way MANOVA, $P = 0.349$). Not only cell number, other growth parameters in the five species also showed significant changes in response to estrogen toxicity. The accompanied reduction in chlorophyll a fluorescence suggested that these adaptive changes might be due to a shift from the autotrophic to heterotrophic mode of growth under increased stress of E2 & EE2.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Combined TiO_2 photocatalysis and phytoremediation for efficient removal of flame retardants (PBDEs)

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Brominated flame retardants have been widely used in industry. There is a rapidly growing public concern about their ubiquity in the environment. This project is aimed at investigating the possible removal treatments of polybrominated diphenyl ethers (PBDEs) using two treatment methods: (I) photocatalysis by TiO_2 and (II) phytoremediation by *Oryza sativa*. Advanced oxidation process (AOP) is a promising technology for removing emerging chemicals due to its efficiency. In this case, nano-scaled titanium (IV) oxide was applied to evaluate its capability in the degradation of emerging chemicals. The residual PBDE congeners after photocatalytical degradation of BDE-209 by TiO_2 were analysed by gas chromatography-mass spectrometry (GC-MS). It was found that the degradability of TiO_2 was attributed to its photocatalytic activity, and not attributed to the small size of the particles, even though the nano-size might have increased the effective reaction areas. The photocatalytical degradation of BDE-209 performed the best at pH 12 (93 % ± 1 %), and in 10-20 mg/L (91.6 % ± 3.21 %; 91.9 % ± 0.952 %) of humic acid using anatase/rutile TiO_2 (82 % ± 3 %). Hence, the efficiency of PBDEs removal can be promoted under these operation conditions. Incomplete removal of PBDEs by water treatment plants and point-source contamination may lead to their discharge into water bodies and ultimately into soils. Consequently, the second part of the project will be phytoremediation of PBDEs and experiments are currently underway. Plants play a significant role in restoring contaminated soils by removing contaminants from soil and then carrying out metabolic processes and assimilation after uptake. More importantly, the symbiosis between the host plants and rhizospheric organisms play an essential role in removing persistent organic pollutants. Hence, arbuscular mycorrhizal (AM) fungi will be added into the soil to build up a symbiotic relationship with the host plant and thereby allowing the investigation of rhizodegradation of the contaminants.

Keywords: PBDEs, TiO_2, photocatalysis, phytoremediation

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Photodegradation and phytoremediation of perfluorooctanoic acid Part I: Photodegradation

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Degradation of perfluorooctanoic acid (PFOA) is of prime importance since it is a persistent organic pollutant which is widespread in the environment and in biota. It is classified as a likely carcinogen by EPA. Due to its toxicity to humans and to the environment, it is necessary to develop effective methods for degrading PFOA into harmless form. In this research, optimum physio-chemical conditions for degradation of PFOA by titanium dioxide (TiO_2) and their linkage to radical oxygen species (ROS) production were investigated. The feasibility of phytoremediation of PFOA by rice will also be examined to serve as a less expensive and more cost effective method when compared to chemical treatments.

For Part I: Photodegradation, results obtained showed that UV at 254 nm significantly ($p<0.05$) degraded PFOA. PFOA was decomposed into shorter carbon chain length intermediates and fluoride ions. The degradation rate and defluorination rate were calculated to evaluate the treatment efficiency. With UV and TiO_2 combined treatment, the degradation rate reached 30.6%, 18.0% and 30.8% and the defluorination rate 1.9%,1.0 % and 6.3% under pH conditions of 4, 7 and 11, respectively. pH 4 and pH 11 provided a better environment for TiO_2 to degrade PFOA. The degradation efficiency of UV treatment was effective over time. UV at 254 nm was an effective treatment method only when treatment time of 1 week was employed, with 14% degradation, compared with 0% degradation for a 3 day treatment time. The defluorination rate was increase with increasing concentrations of TiO_2 added, 0.03% to 9.2% for 0.25, 0.5, 0.66, 0.75 and 1 g/L TiO_2 added.
Experiments to investigate the time profiles of PFOA treated with different concentrations of TiO_2 and their linkage to ROS production from 0 to 1 week is currently ongoing.

For Part II: Phytoremediation, experiments are underway to investigate the feasibility of using plant to absorb PFOA in water.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Photocatalytic Activity for Degradation of Organic Pollutants under Visible Light

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The presence of organic pollutants in water has become a serious problem and is threatening the natural environment as well as human health. Removal of these pollutants by photocatalytic degradation using solar light has attracted great interest in recent years. In this report, nitrogen doped $K_2Nb_4O_{11}$ ($K_2Nb_4O_{11}$-N) has been prepared and then used for the photodegradation of various organic pollutants in water, including Orange G (OG), Bisphenol A (BPA) and pentachlorophenol (PCP). $K_2Nb_4O_{11}$ was prepared by heating a stoichiometric mixture of Nb_2O_5 and K_2CO_3 at 900 ^oC for 24 h. Nitrogen doping of $K_2Nb_4O_{11}$ was achieved by a solid state reaction method using urea as the nitrogen source. $K_2Nb_4O_{11}$-N has been characterized by XRD, SEM, XPS, UV/Vis diffuse reflectance and photoluminescence. The photocatalytic activity of the materials was evaluated by OG, BPA and PCP photodegradation under visible light irradiation. The results show that the visible light photocatalytic activity of $K_2Nb_4O_{11}$-N is higher than those of $K_2Nb_4O_{11}$ and Degussa TiO_2 P25, indicating the positive effect of nitrogen doping. A mechanism for the photodegradation of OG and BPA by the $K_2Nb_4O_{11}$-N photocatalyst under visible light is proposed.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Application of nanomaterials on removal of natural organic matter (NOM) in drinking water and the association with formation of disinfection by-products (DBPs)

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Disinfection by-products (DBPs) are toxic substances formed by the chlorination of organic matter in the treatment of drinking water. The chlorination of natural organic matter (NOM) leads to the formation of trihalomethanes (THMs) and haloacetic acids (HAAs), the two major classes of DBPs. Due to its toxicity to humans and to the environment, it is necessary to reduce the production of DBPs. In this research, effective treatments using different nanomaterials for altering the structures or reducing the amounts of NOM, and hence minimizing the DBP formation and its associated toxicity were investigated.

Micro green algae (*Chlamydomonadales*) and humic acid, the major NOM sources in the raw water for drinking water in Hong Kong and other countries, respectively, were studied. Various nanomaterials (0.1%) were used as photo-chemical treatment and reservoir water (1 %) was served as the microbial treatment, were added into dissolved NOM water. Total organic carbon was monitored at different intervals throughout the treatment period (0, 2, 6, 24 and 72 h). Samples then underwent chlorination. DBPs concentration was determined by gas chromatography (GC). *Salmonella* mutagenicity test was also conducted.

Results showed that treatment of NOM using nanomaterials significantly ($p<0.05$) reduced the amount of DBPs formed per milligram carbon. Zinc oxide (ZnO) was the most effective treatment, followed by silicon dioxide (SiO_2) and titantium dioxide (TiO_2). The chemical structure of NOM was altered or degraded by the oxidation or reduction power and the size of nanomaterials. Microbial treatment was less effective and took a longer inoculation period, whereby three days was needed for treatments with nanomaterials compared to 30 days for microbial treatment. For the analysis of humic acid as the NOM source, the mutagenicity test showed that samples treated with nanomaterials were more mutagenic than the untreated or biologically treated samples.

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Generation of reactive oxygen species and oxidative stress in *Escherichia coli* and *Staphylococcus aureus* by a novel semiconductor catalyst

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The antimicrobial mechanisms of a new catalytic material (charge transfer auto oxidation-reduction type catalyst, CT catalyst), which may have great potential for application in water/wastewater treatment, were investigated. The generation of reactive oxygen species (ROS) in both bacteria-free and bacteria solution (*E. coli* and *S. aureus*) by CT catalyst were examined by using two fluorescent probes (Dihydroethidium and 2',7'-dichlorofluorescin diacetate). Oxidative stress in bacteria was also investigated in terms of lipid peroxidation and protein carbonyl production by examining two biomarkers (malondialdehyde and thiobarbituric acid reactive substance). The results showed that a significantly higher ($p < 0.05$, via t-test) amount of hydroxyl radicals was generated by the CT catalyst compared with the control, particularly after 6 h of contact time in which more than twice the amount of the control was produced. The generation of ROS in the bacteria increased with higher pH and temperature levels, and was closely related with the oxidative damage in cells. The results indicated that CT catalyst-induced oxidative damage in the bacteria might serve as an important mechanism contributing to the anti-microbial function of the CT catalyst.

Keywords: Superoxide anions, hydroxyl radicals, lipid peroxidation, protein oxidation, charge transfer auto oxidation–reduction type catalyst

Centre for Marine Environmental Research & Innovative Technology (MERIT)
Annual Symposium
6th January – 7th January 2011

Removal and biodegradation of nonylphenol by immobilized *Chlorella vulgaris*

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Microalgae have been used to remove organic matter and inorganic nutrients from wastewater in tertiary or quaternary treatment units for several decades due to its low capital investment and operating costs and high efficiency. Many laboratory- and pilot- scale studies have proved that immobilization of microalgae enhances the removal efficiency of inorganic nutrients and heavy metals. However, only limited studies have been carried out to investigate the feasibility of using immobilized microalgae to remove and biodegrade toxic organic pollutants, especially nonylphenol (NP), which is environmentally persistent and is highly toxic to aquatic organisms. In the present study, the removal and biodegradation of NP by Ca-alginate immobilized *C. vulgaris* cells were investigated. Effects of biological factors such as cell density per algal bead and algal bead density per unit of culture on the removal and biodegradation of NP were also evaluated. The immobilization of *C. vulgaris* led to a significant decrease in growth rate while a significant increase in chlorophyll content as compared to its counterpart of free cells. The removal efficiency of NP increased in a short incubation period in immobilized *C. vulgaris*, while decreased with longer incubation time, probably due to the decrease in growth rate and the reduction of NP bioavailability, as some NP was adsorbed on the alginate matrix. The mechanisms of NP removal involved in immobilized cells was similar to those in free cells, which included adsorption onto algal cells and alginate matrix, absorption within cells and cellular biodegradation. The increase in algal bead density but not the increase in cell density in algal beads had a positive effect on the removal of NP in a short incubation period. On the other hand, increasing both cell density per algal bead and algal bead density with extended incubation time considerably enhanced the NP biodegradation efficiency. The spiked NP at an initial NP concentration of 1 mg l^{-1} was almost completely removed (>98%) under an optimal algal bead density (4 beads ml^{-1}) after 96 hours of exposure. These results clearly demonstrated that immobilized *C. vulgaris* cells could be potentially used in semi-continuous systems to remove and biodegrade wastewater-borne NP under photo-autotrophic conditions.