# 11<sup>th</sup> East Symposium Conference

Advances in Harmful Algal Bloom Research, Monitoring, and Management



December 11-13 2019
Microtel Inn & Suites, Puerto Princesa City, Palawan,
Philippines



## 11th EASTHAB Symposium

ecurrent large scale Harmful Algal Blooms (HABs) have been experienced in Asian waters particularly during the last decades. The potential harmful effects have stimulated concerns from the adjacent countries. National and regional studies towards improved HAB understanding, monitoring and management have been increasing and collaborating representatives from China, Japan and Korea agreed in August 2004 to have regular symposium and meetings on the phenomenon and to have the 1st East HAB meeting in Jeju, Korea in December 2004. The 2nd, 3rd, 4th and 5th meetings/symposia were held in the Qingdao China, Nagasaki, Japan, Changwon Korea and Hangzhou, China, respectively, and focused on the dynamics of targeted species in East Asian waters. The Philippines was an invited observer during the 2nd meeting and became a new East HAB member country upon unanimous agreement during the 6th meeting in Tokyo, Japan in 2008. The members decided to have the meeting/symposium biannually from the 7th meeting in Bohol, Philippines in 2011. The 8th, 9th and 10th meetings were hosted by Korea, China and Japan in 2013, 2015 and 2017 respectively. The main objective of the East HAB is to share information and discuss recent advances in research, monitoring and management of HABs among scientists in the region.

### **4th National HAB Conference**

ABs that are commonly or collectively called by many Filipinos as "Red Tides" were first reported in the Samar-Leyte areas in 1983-1984. These toxic *Pyrodinium* blooms and other HABs were then experienced in other areas. The 1st, 2nd and 3rd National HAB Conference were hosted by University of the Philippines-Marine Science Institute (UPMSI) in 2002, 2008 and 2011, respectively in order to stimulate studies and enhance monitoring and management of the apparently increasing HABs in the Philippines, a country whose territory is 70% water (30% land). Despite the decrease or absence of poisoning cases in some areas where the main causative organisms still bloom, the number of areas affected by this and other type of toxic blooms has increased. Likewise, areas affected by Fish kills associated with HABs have escalated recently and new causative organisms identified. Participants have been representatives from government agencies, local government units, private organizations, and members of the academe.

URL: https://www.philhabs.net



Hon. Jose CH. Alvarez
Governor

**Province of Palawan, Philippines** 



I would like to convey my warmest greetings to all the esteemed guests, participants, and officials of this year's 4<sup>th</sup> National HABs and 11<sup>th</sup> East Asian HABs Conference—an international symposium organized by the UP Marine Science Institute and National Academy of Science & Technology.

Over the years, the occurrence of harmful algal blooms (HABs) has created major threats to our coastal areas resulting in the poisoning and killing of our marine organisms. The adverse impact of HABs resonates not only to fish, marine mammals, and bird species, but also to humans when consumed. The perennial occurrence of HABs outbreak across the country indeed necessitates for an extensive research and partnership to mitigate its long-term effects.

As an island province whose primary commodity are marine resources, Palawan can benefit from this prestigious symposium that aims to gather brilliant minds across East Asia and create cognitive discussions on issues relating to HABs. Likewise, this event is a great opportunity for stakeholders to create a policy discourse on how to improve and develop various HAB research practices.

Moreover, I would like to commend the organizers for taking the lead in this noble endeavor. On behalf of the Provincial Government of Palawan, I would like to express my sincerest regards to all the guests, participants, and organizers of this year's occasion. May this symposium continue to inspire our experts and researchers on the significant role of their studies in creating pragmatic solutions for our environment!

Congratulations and mabuhay!

OSE CH. ALVAREZ



Hon. Maria Nancy M. Socrates

Vice Mayor Puerto Princesa City, Palawan, Philippines



Greetings and welcome to Puerto Princesa City!

It is our great pleasure to be the host-city for this gathering of esteemed scientists and researchers from all over East Asia and the Philippines for the 4th National HABs and 11th East Asia HABs Conference. We therefore extend our gratitude to the organizers of this event. We are grateful as well to participate in this conference of experts, more so that it is co-hosted by our very own **City Agriculture Office** and our own excellent institutions, the **Palawan State University** and **Western Philippines University**. In recent years, our coasts have experienced cases of harmful algal blooms or "red tides", which have affected the surrounding communities and have posed as threats to public health and food safety. The danger of rising sea temperatures brought about by climate change prompts us to act swiftly and think about remedies which may be used to mitigate and end occurrences that places our ecology in danger. We consequently look forward to continue this partnership among the academe, private and public sectors as we all take part in this momentous occasion.

On behalf of the City Government of Puerto Princesa, I wish to congratulate all of you on the success of this event!

Welcome once again to Puerto Princesa, the city where nature begins and never ends.

MARIA NANCY M. SOCRATES



Rhodora V. Azanza

Professor Emeritus
The Marine Science Institute,
University of the Philippines Diliman,
Quezon City, Philippines



It is with great pleasure and honor that I welcome our speakers, participants and guests to the 11<sup>th</sup> EastHAB and 4<sup>th</sup> National HAB to be held on 11-13 December 2019 at Puerto, Princesa, Palawan.

The 3<sup>rd</sup> National Harmful Algal Bloom, conference was last held in Bohol Island on November 2010 and the 10<sup>th</sup> EastHAB conference was hosted by Japan in Hokkaido Prefecture in year 2017.

This year's back to back national and regional conferences on Harmful Algal Blooms will highlight the significance of harnessing Science, Technology and Innovation (STI) in the enhanced management of Harmful Algal Blooms in the country and the region.

These conferences also underline the fact that HABs like many other phenomena in the oceans/seas are without complete boundaries and could happen within a country's territorial waters and potentially move or have the same effects in other waters of the Region. Thus emphasizing the need for national and regional cooperation in Research and Development and initiatives in this field.

I wish everyone a productive engagement in this national and regional conferences. May you also enjoy the landscape and seascape of Palawan Island.

MABUHAY!

RHODORA V. AZANZA



Hon. Fortunato Dela Peña
Secretary
Department of Science and Technology



On behalf of the Department of Science and Technology (DOST), I would like to congratulate the organizers of the 11th International EASTHAB Symposium and the 4th National HAB Conference for leading the task of creating a professional venue for bringing together scientists, managers, and communities to share new developments, improvements, and efforts in the science and management of Harmful Algal Blooms (HABs) in the country with the presence of various stakeholders as well as experts from our East and Southeast Asian neighbors.

The DOST is honored to be a part of these symposium and conference which will bring together the scientific communities to exchange and share the experiences on HAB occurrence, monitoring, and management within the country and the region; and provide a transdisciplinary platform for researchers, practitioners, educators, managers, policy makers, and communities to present and discuss the recent trends, innovations and challenges in relation to HABs. Apart from these, this three-day event will also host a forum for the COASTS (Community Alliance for the Sustainability of our Threatened Seas) partnership launched by the program that will highlight the perspectives of our partner fisherfolks from the program's pilot sites.

Hand in hand, with research, development and extension, we will incessantly aim to present RDE results and science-based solutions to mitigate the threats emanating from HAB's occurrence and thereby place a great deal of emphasis on their monitoring and management. Rest assured that DOST will continue to support these initiatives in bringing together the scientific communities to share their knowledge and practices on HAB management, and set directions on how to enhance education, research, and innovations towards HAB mitigation.

May the exchange of knowledge in these activities be an avenue to surface out issues, challenges, and best practices that may advance the mitigation tools for algal blooms in the region and in a global scale, and deliver lasting impacts. Again, on behalf of DOST, let me extend my sincerest appreciation to all of your endeavors, and I wish you all a productive and fruitful three-day event. More power to all!

FORTUNATO T. DE LA PEÑA



#### Advances in Harmful Algal Bloom Research, Monitoring, and Management

11–13 December 2019 Microtel Inn & Suites, Puerto Princesa City, Palawan, Philippines

Time		DAY 1 (DEC 11)	Opening Program	
900	National Anth	nem		
905	Welcome from the Province		Governor Jose Alvarez	
915	Welcome fror	n the City	Vice-Mayor Maria Nancy M.	
			Socrates	
925	Conference R	eview	Professor Rhodora Azanza	
935	Opening Rem	arks	DOST Secretary Fortunato dela	
			Peña	
945	KEYNOTE		HAB Research Collaboration	
			Professor Yasuwo Fukuyo	
1030	BREAK			
Time	DAY 1	Talk Title	Presentor/Authors	
	(DEC 11)			
		cent HAB Research and Develop	oment Initiatives in the	
	Philippines			
	Session Chair:	Dr. Lilibeth Salvador-Reyes		
1045	PLENARY	PCAARRD's R&D Initiatives on	Dr. Mari-Ann M. Acedera	
		Harmful Algal Bloom (HAB)		
1115		Chemical basis of the toxicity	Batucan, JD, Davis, KB, Malto,	
		of Gambierdiscus carpenteri	ZL, Azanza, R, Salvador-Reyes,	
			L	
1130		Ciguatoxins detection using	Awatin, AG, Romero, MLJ,	
		radioligand receptor binding	Regidor, SE	
		assay in reef fishes	g, 01	
1145	Open Forun	•		
		•		
1155	LUNCH			
1300 -	SESSION: Managing Harmful Algal Blooms			
1455	Session Chair:	Dr. Marc Lawrence Romero		
1300	PLENARY	Managing HABs by Addressing	Dr. Ma. Lourdes San Dieao-	
.500	,	Eutrophication	McGlone	
		zati opinication	ricatoric	
1330		Analytic methods in the	Romero, MLJ, Saba, MYS,	
1330		context of Paralytic Shellfish	Lucban, AW, Awatin, AG,	
		Toxins monitoring in the	Porlaje, RP, Dayap, NA, Bato,	
		Philippines	LR, Avorque, LM, Oñate, JR,	
		· ·····ppiiies	Mora, IB, Cuyugan, RC, Juan, L	
			mora, is, cayagan, ice, raan, E	
1345		HAB concerns for a Philippine	Rodolfo, RS, Cabria, HB, Rosell	
13 13		oyster hatchery	NTII B, Lapus, MR, Ubial, EF	
		dyster nateriery	TVTII D, Lapas, IVIII, Obiai, Li	
1400		Harmful algal bloom impacts	Carigma, JLB, <u>Beringuela, RT</u> ,	
		on the livelihood of fisherfolks	Azanza, RV	
		in selected areas in the		
		iii selected aleas iii tile		
		Philippines		
1415			Olano, DEB, Salvador-Reyes LA	
1415		Philippines	Olano, DEB, Salvador-Reyes LA Montano MNE, Azanza RV	
1415		Philippines Sorption of Paralytic Shellfish		
1415 1430	Open Forum	Philippines Sorption of Paralytic Shellfish Toxins (PSTs) in algal		
	Open Forum	Philippines Sorption of Paralytic Shellfish Toxins (PSTs) in algal		

1500			
1500 - 1700		naging Harmful Algal Blooms Dr. Ma. Lourdes San Diego-McGlo	one
1500	PLENARY	Review and New Directions for Harmful Algal Bloom Monitoring in the Philippines	Drusila Esther E. Bayate, BFAR Assistant Director for Technical Services
1530		The Progression of HAB Vulnerability and Safety: Developing Community Risk Knowledge for HAB in Jiabong, Samar; Sapian, Capiz; and Bolinao, Pangasinan	Lagos, D., Gasmen, H., Eco, R.N., Gopez, J., Dungong, R., Novelero, J.M., Bacay, J.L., Yñiguez, A.T.
1545		Research groups on improving management strategies on marine ecosystem disturbance and harmful organisms: with emphasis on HAB species	Park J. Hwang I, Hyung JH, Kim EJ, Rho J-R.
1600		Current status of HAB studies and events in Indonesia: the use of Excel Dashboard to hasten data processing and visualization	<u>Intan, MDB</u> , Thoha, H, Rachman, A
1615		Monitoring Harmul Algal Blooms in Singapore waters using advanced techniques	Leong, SCY
1630		Baseline Assessment of the Shellfish Growing Areas in Batan Bay, Ivisan and Panay, Capiz, Philippines	<u>Dayap, NA</u> , Chua RJ, Pador EL
1645	Rapid Fire 1	Variation in paralytic shellfish toxin contamination in selected shellfish from coastal waters of Tagbilaran and Dauis in Bohol	<u>Alacida, WV,</u> Tenio GE, Bato, LR
1650	Open Forum		
1700	Opening of th	e POSTER SESSION	
1700 - 1830		ION: COASTS Forum Devralin Lagos and Harianne Gas	smen
1700		Introduction of COASTS (Community Alliance for the Sustainability of our Threatened Seas)	Aletta T. Yñiguez, Devralin Lagos
1710		Sapian Bay, Capiz: Communities' assessment of Vulnerability and Safety to HABs	Regina Barried, Gloriosa Peñaroyo
1725		Jiabong, Samar: Participatory assessment of effects and responses to the 2019 HAB event in Jiabong, Samar: Developing People-centered Early Warning System for Harmful Algal Bloom (HAB- EWS)	Melvin Labendia, Aida Quilloza
1740		Bolinao-Anda, Pangasinan	Roger Corbillon, Lily Corbillon
1755		Open Forum	
1830	Travel to Dinne	er venue	
1930	WELCOME DIN	INER	

Time	DAY 2 (DEC 12)	Talk Title	Presentor/Authors	Talk Title	Presentor/Authors		
800	Registration						
0830 - 1005	SESSION: HAB Dynamics Session Chair: Dr. Cesar Villanoy						
830	PLENARY				Strategies Activating Diatom		
900		Transition to PARALLEL SESSION					
		HAB Dynamics 1 Session Chair: Dr. Leni Yap		HAB Dynamics 2 Session Chair: Dr. Cesar Villanoy	,		
910		The diversity of phytoplankton in Bolinao, Pangasinan: a shotgun metagenomics approach	<u>Cadorna, KE</u> , Burgos, LA, Onda, DFL, Yñiguez, AT, Lluisma, AO	Distribution of dinoflagellate cysts in the coastal bottom sediment of Pangkajene, South Sulawesi, Indonesia	Rachman, A, Thoha, H, Intan, MDB, Sianturi, OR, Witasari, Y, Wibowo, SPA		
925		Genetic diversity and co- occurrence of Amoebophrya spp. (Syndiniales) with host dinoflagellates in mariculture- impacted areas	<u>De La Cruz, MAM</u> , Onda, DFL	Nutrient dynamics controlling the intensity of dinoflagellate red-tides occurring in the southern Sea of Korea: Overview	<u>Kim, G</u> , Kwon, HK, Lim, WA, Park, JW		
940		Viability of HABs-causing species on floating plastics debris in vitro	<u>Tolentino, MPS,</u> Onda, DFL	Spatial and temporal distribution of HAB species in Sapian Bay, Western Philippines	<u>Castro, FMC</u> , Campos, WL, Yniguez, AT		
955	Open Forun	า					
005	BREAK						
1020 - 1210	SESSION: HA	<b>B Dynamics</b> Professor Floredel Galon					
1020	PLENARY	A Retrospective Look at O	ur Evolving Understanding of <i>Py</i>	rodinium bahamense Bloom Dyn	amics, <i>Dr. Aletta T. Yñiguez</i>		
1050		Transition to PARALLEL SESSIO	N				
		HAB Dynamics 1 Session Chair: Dr. Po Teen Lim		HAB Dynamics 2 Session Chair: Professor Floredet	' Galon		
1100		Revealing the harmul algal species community shift along the Johor Strait using metabarcoding approach	<u>Hii, KS</u> , Lim, PT, Mohd-Din, M, Tan, SN, Lim, ZF, Lee, LK, Luo, Z, Gu, H, Leaw, CP	Study on hydrological characteristics and drifting trajectories of green algae in western Yellow Sea during the spring and summer	<u>Bao, M</u> , Guan, W, Cao, Z, Chen Q		
1115		Abnormal Cochlodinium polykrikoides bloom at high- water temperature in southern coastal water of Korea; comparison of physiological and genetical characteristics between different strains	<u>Lim, YK</u> , Baek, SH, Park, BS, Kim, JH	Trends of phytoplankton occurrence in the bays of Puerto Princesa City, Palawa with emphasis on HABs- causing species	Galon, FD, <u>Paladan, MM</u> , Yñiguez, AT		
1130		Microbial interactions during Cochlodinium polykrikoides bloom in South Sea, Korea	Ahn, C, Cui, Y, Chun, S, Baek, SH, Son, M, Oh, H	Temperature increasing pattern affect succession between Prorocentrum donghaiense and Karenia mikimotoi in East China Sea	Dai, X, <u>Hemed, AM</u> , Wang, P, Guo, R, Lu, D		
1145				Seasonal distribution and abundance of four Alexandrium species in Jinhae Bay Korea, 2011~2019	Park, TG, Kim, JJ, Song, SY		
1200	Open Forun	1					
1210	LUNCH						
	POSTER SESSI	ON					

300 - 650	SESSION: HAI	B Monitoring Tools and Approa Dr. Deo Onda	aches
300	PLENARY		g Tools and Approaches for Common HAB-Causative Organisms in East Asia  Dr. Rhodora V. Azanza
330		Microbial community composition in harmful algal bloom (HAB)- impacted coastal waters of Bolinao and Anda, Pangasinan using metagenomic and 16s rRNA metabarcoding approaches	<u>Aguinaldo, ZA</u> , Yñiguez, AT , Lluisma, AO
345			Malto, ZBL, Subong, BJJ, Azanza RV, Salvador-Reyes, LA
400		Harmful algal bloom occurrences in waters of Palawan, Philippines	Sumeldan, J, Avillanosa, A, Delgado, J, Cabungcal, F, Creencia, L
415		Assessment of algal bloom using remote sensing reflectance data of Aqua- Modis satellite in the Jakarta Bay, Indonesia	Sidabutar, T, Rahman, A, Wouthuyzen, S, Syahailatua, A, Arifin, Z, Iwataki, M, Fukuyo, Y
430		Spatial assessment of nutrient	Yñiguez, AT, Bacay, JLT
445	BREAK		
		Session Chair: Dr. Lota Creencia	
500	Rapid Fire 3	Spatial and temporal behaviour of <i>Pyrodinium bahamense</i> var. <i>compressum</i> in Puerto Princesa Bay, Puerto Princesa City, Philippines	Delgado, J., Creencia, L.
505	Rapid Fire 4		<u>Calalang, PC</u> , Roleda, MY, Yñiguez, AT
510	Rapid Fire 5	Possible link of Biecheleriopsis	Gernato, EGM, Malto, ZB, Salvador-Reyes, LA, Jacinto, GS, Santos, SD, Onda, DFL
515		The potentially harmful benthic dinoflagellates in seagrass and macroalgae of the Pari Island, Indonesia	
530		Remote identification of in- water <i>Noctiluca scintillans</i> blooms in the East China Sea using MODIS measurements	<u>Tao, B</u> , Jiang, Z, Wang, P, Wu, C, Mao, Z
545		Spatial dependencies between shellfish bans in adjacent embayments inferred through time series information	<u>Punongbayan, A.,</u> Yñiguez, A.T.
600		Paralytic shellfish toxins monitoring in Balite Bay, Davao Oriental	Avorque, MLM, Canobas, SM, Quiap, ES

1615	Critical Reflection of J., E	<u>men, H.,</u> Lagos, D., Gopez, co, R.N., Dungong, R., juez, A.T.
1630	Open Forum	
1645	POSTER SESSION	
1700	WESTPAC HAB Meeting	

Time	DAY 3 (DEC 13)	Talk Title	Presentor/Authors	Talk Title	Presentor/Authors
800	Registration				
830	KEYNOTE	E	Expansion of Harmful Species in Professor	East Asia, What Can Be Linke Douding Lu	d To?
		w HAB Species from East Asia			
1115	Session Chair:	Dr. Pengbin Wang			
915	PLENARY	Morphology and Phylogeny o		e Amphidomataceae (Azadin ori Iwataki	ium and Amphidoma) from Japan,
945		Benthic dinoflagellates in the coast of Jeju Island	<u>Lee, J,</u> Kang, S, Horiguchi, T		
1000		Rediscovery of <i>Ostreopsis</i> siamensis (Dinophyceae) from the inner gulf of Thailand and surrounding waters	<u>Gu, H.</u> , Luo, Z, Derrien, A, Wang, N, Lim, PT, Leaw, CP, Pransilpa, M		
1015		Taxonomy of <i>Prorocentrum</i> (Dinophyceae) in East Asian waters	Wang, P, Guo, R, Dai, X, Lu, D		
1030	BREAK				
1045		Ultrastructure and phylogeny of a harmful micropredatory species of <i>Karlodinium</i> (Kareniaceae, Dinophyceae) found in Manila Bay, Philippines	<u>Benico, G,</u> Takahashi, K, Lum, WM, Iwataki, M		
1100		Morphology and pigment composition of an unarmored dinoflagellate resembling <i>Gertia stigmatica</i> (Kareniaceae, Dinophyceae) isolated from Japan	<u>Takahashi, K</u> , lwataki, M		
1115 - 1230		untry Reports Dr. Kazumi Wakita			
1115		Japan: HAB Country Report of Japan (2017 - 2019)	Dr. Goh Onitsuka		
1130		South Korea: Outbreak and Disappearance of Cochlodinium Blooms under Influence of Typhoon and Rain in 2019	Dr. Weol-ae Lim		
1145		China: Status and Researches of Harmful Algal Blooms in China	Dr. Rencheng Yu		
1200		Philippines: Country Report: Harmful Algal Blooms in the Philippines	Ms. Elsa Furio		
1215	Open Forum	n			
1230	LUNCH				
	POSTER SESSI	ION			

	urrent HAB Monitoring and Res ir: Dr. Rhodora Azanza					
PLENARY						
			ghui Lyu			
	Transition to PARALLEL SESSION					
	Current HAB Monitoring and		<b>Current HAB Monitoring and</b>	Research 2		
	Session Chair: Dr. Rhodora Aza	nza	Session Chair: Dr. Goh Onitsuka			
	Identification and rapid detection of harmful algal species in Malaysian waters using molecular techniques	<u>Lim, PT,</u> Hii, KS, Tan, SN, Law, IK, Lau, WLS, Lim, ZF, Gu, H, Luo, Z, Leaw, CP	Recent researches on epiphytic dinoflagellates in Korean Seas	_		
	Molecular understandings of the Harmul Algal Blooms (HABs): our recent progress	<u>Ki, JS</u> , Guo, R, Wang, H, Abassi, S, Kim, H	The fate of massive floating green algae in the Yellow Sea	Geng, H, Yu, R, Zhou, M		
	Characteristics and causes of algal blooms in the southern coastal waters of Korea occurred during the summer	<u>Lee, MO</u> , Kim, JK	Field application of a red-tide acoustic sensing system (RASS) for monitoring of fish killing dinoflagellate <i>Cochlodinium polykrikoide</i> s in Korean coastal waters	<u>Baek, SH</u> , Kim, JH, Lim, YK, Ki H, Kang, D		
	Metagenomic analysis of Harmul Algal species in Jiaozhou Bay	<u>Liu, S</u> , Huang, T, Cui, Z, Chen, Y Chen, N	Algicidal effects on <i>Chattonella marina</i> and planktonic community by yellow clay and thiazolidinedione derivative TD49 in a mesocosm	<u>Lee, M</u> , Son, M, Baek, SH		
			Benthic harmul dinoflagellate assemblages and community dynamics in relation to benthic microhabitats and environmental variability: a case study from the Perhentian Islands Malaysia	<u>Leaw, CP</u> , Yong, HL, Mustapa NI, Lim, ZF, Lee, LK, Hii, KS, T TH, Luo, Z, Gu, H, Lim PT		
BREAK						
EASTHAB W	orkshop Session Networking and	Collaboration for HAB Research, I	Monitoring and Management			
Report of EA	ASTHAB Workshop outcomes					
CLOSING DI	NNER FOR ALL PARTICIPANTS					

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#### Yasuwo Fukuyo

Professor Emeritus Asian Natural Environmental Science Center, University of Tokyo, Tokyo, Japan



Professor Yasuwo Fukuyo's interest in dinoflagellates was sparked by PSP events in Ofunato Bay in the 1960s and 1970s, which had been tentatively attributed to a 'Gonyaulax' species. All the important HAB discoveries of the late 1970's in Japan required teamwork by chemists, taxonomists, toxicologists and Yasuwo was the taxonomist who guaranteed correct morphotaxonomy of the causative organisms, including the first identification of Dinophysis fortii as the causative agent of DSP, and description of the new species Gambierdiscus toxicus as the causative agent of ciguatera. Other HAB species such as Ostreopsis ovata, O. lenticularis, Alexandrium affine, A. hiranoi; A. satoanum, Prorocentrum concavum, P. emarginatum, P. vietnamensis were all first described by him.

Yasuwo has been a key figure in the organization of many training courses, conferences, and workshops, at the IOC-WESTPAC Scientific Symposia on Marine Science, the International Conferences on Molluscan Shellfish Safety, and International Conferences on Harmful Microalgae. He served an advisor to the Japanese Delegation to the Marine Environmental Protection Committee (MEPC) of the International Maritime Organization (IMO) for the development of the International Convention for the Control and Management of Ship's Ballast water and Sediments, and the International Measures for Minimizing the Transfer of Aquatic Invasive Aquatic Species through Biofouling of Ships. He played a leading role in the establishment of IOC-HAB Programme from its early beginnings and supported its activities as Japan's delegate to the Intergovernmental Panel meetings on Harmful Algal Blooms (IPHAB), the IOC Executive Committee, and IOC General Assembly. He also served on the Science Steering Committee of the IOC SCOR Programme Development for Harmful Algal Blooms and the Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB). At a regional level, in 1992 he took over the leadership from Professor Okaichi on the HAB project of IOC/WESTPAC and served as the coordinator for nearly 20 years. He is also one of the focal points of Japan on the HAB Working Group of North Pacific Action Plan (NOWPAP))/UNEP and endeavoured to create a HAB network within PICES (North Pacific Marine Science Organization) and a HAB Project of IOC/WESTPAC.

There is no one in the harmful algal bloom research community who has not benefited greatly from his legendary generosity, beautifully presented images of algae and his pedagogy and public service sustained over three decade.

#### **HAB Research Collaboration**

#### Yasuwo Fukuyo

Asian Natural Environmental Science Center, University of Tokyo, Tokyo, Japan

AB Research aims to understand the biology and ecophysiology of HAB species, toxicology and chemistry of bioactive harmful substances. It also aims to reduce harmful effects through providing scientific knowledge for wise management and biology of affected organisms, and applied science. In East Asia HAB problems becomes obvious after 1980s, and then HAB types and affected area are gradually expanding, along with development of HAB research. It means that HAB existed from long time before, but people could not recognize real figure of the problem, *i.e.* source of trouble and its mitigation ways, for a long time.

Now we know wide variety of scientific facts on HABs, and can explain cause and process of HAB occurrence. This is a great development achieved by many scientists. But there are many scientific areas that we need to study intensively and extensively. Nature and occurring process of each HAB event is specific by type of causative organisms, causative harmful substances, oceanographic condition of occurring area, management scheme of fisheries products, etc. It means that, based on scientific knowledge, scientist need to advise wise management methods to reduce harmful events to people in politicians, medical doctors and people in public.

For the purpose we need to develop further collaboration among scientists in various fields. For example, we know variety of toxins and their analytical methods. But we do not know how to reduce toxins in living invertebrates, and how to differentiate highly toxic ones from others in wild. We know life cycle of harmful plankton, and their resting stages. But we do not know how to reduce amount of resting cells in natural environment. There are many things we still cannot clarify yet. I am sure that international collaboration among scientists is the only way we can move forward to solve HAB problems.



#### Douding Lu

Professor/Senior Scientist
The Second Institute of Oceanography,
State Oceanic Administration,
Hangzhou, China



Prof. Douding Lu is a Senior Scientist in the Lab of Marine Ecosystem and Biogeochemistry at the Second Institute of Oceanography (SIO) of the Ministry of Nature Resources located in Hangzhou, China. He is Cochairman of the Section on Ecology of Harmful Algal Blooms in The North Pacific (PICES), an executive member of Chinese Society of Phycology, and founding member of EASTHAB.

Douding graduated from China Ocean University in 1979. He worked as visiting scientist at Florida Institute of Technology, USA with Prof. Dean R. Norris in 1985 and at Trondheim Biological Station, Norwegian University of Science and Technology under Prof. Egil Sakshaug in 1995-1996. Douding' research interests mainly focus on taxonomy and bloom dynamics of marine harmful algae particularly toxic dinoflagellates. He has authored over 100 scientific papers including 28 SCI publications.

Douding has been involved in a number of national projects such as national basic research program, high tech projects, Chinese Ecology and Oceanography on harmful Algal Blooms (CEOHAB), national science foundation of China as well as regional cooperative initiatives such as EASTHAB and GEOHAB Asia. He was a Chinese partner of bilateral cooperative project with Dr. Goebel in the State Agency of Nature and Environment, Schleswig- Holstein and Dr. Horstmann at Institute of Marine Science in Kiel, Germany. He has participated a number of international scientific symposiums as invited speakers including PICES 2007 Annual Meeting held in Vectoria, Canada. Together with Dr. Vera Trainer (USA), he served as a convener for a workshop on "Contrasting conditions for success of fish-killing flagellate species in the western and eastern Pacific –A comparative ecosystem approach" during the 2015 Annual Meeting. He was one of the conveners of several EASTHAB Meetings (Qingdao 2005, Hangzhou 2008, Guangzhou 2015) held in China.

# Expansion of Harmful Species in East Asia, What Can Be Linked To?

#### **Douding Lu**

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armful species, *Cochlodinium*, have been expanded in their geographic distribution and forming harmful algal blooms with fisheries losses in the coastal waters of East Asia particularly in South Korea in the past three decades. The HABs caused by *C. polykrikoides* were rare in China before 2000' and only a few blooms were recorded in the South China Sea. With the change of marine environment, the record of *C. polykrikoides* and its blooms show an increasing trend in the coastal waters of China. Biogeographic distribution pattern of this species shows that it has been expending from the South China Sea to the almost whole Chinese coastal waters including the Bohai Sea in the last two decades. In the meantime, sea surface temperature in China Seas has remarkably increased in the past five decades. Since *C. polykrikoides* is a warm water species, whether the expending mechanism of the species is linked to warm ocean current transportation and the signal of climate change remains to be further explored.



Mari-Ann M. Acedera

Director Marine Resources Research Division, PCAARRD, Department of Science and Technology



Dr. Mari-Ann M. Acedera is the Director of the Marine Resources Research Division (MRRD) of the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) of the Department of Science and Technology (DOST). As the Director of MRRD, she has provided dedicated leadership in directing the identification, planning, monitoring and evaluation of the R&D programs/projects, and in establishing relevant linkages for the management and conservation of the marine resources. She represents DOST-PCAARRD in various Committees and Technical Working Groups related to the marine resources sector. Dr. Acedera is a graduate of BS Fisheries from the University of the Philippines Diliman; and MS in Environmental Studies with minor in Resource Economics and Ph.D. in Environmental Science with cognate in Community Development both from the University of the Philippines Los Baños, School of Environmental Science and Management (SESAM). In 2018, she was awarded as one of the Distinguished Alumni Awardees of the University of the Philippines Los Baños as SESAM's Outstanding Alumna for Research and Development.

#### **PCAARRD's R&D Initiatives on Harmful Algal Bloom (HAB)**

#### Mari-Ann M. Acedera

Marine Resources Research Division, Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development, Los Baños, Laguna

he Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) is one of the sectoral councils of the Department of Science and Technology (DOST) responsible in formulating policies, plans, projects and strategies for science and technology (S&T) development in the agriculture, aquatic and natural resources (AANR) sectors. The Harmonized National Research and Development Agenda for Agriculture, Aquatic and Natural Resources Sector (HNRDA) 2017-2022 was crafted to ensure that results of S&T endeavors are geared towards and are utilized in areas of maximum economic and social benefit for the people. The HNRDA for the AANR sector is a product of a multi-sectoral consultation. It is an integration of the existing R&D agenda of government agencies conducting R&D in the AANR sector and inputs from various stakeholders. Over the years, the Council has put forth efforts in understanding the challenges faced by the industries and translate them to R&D initiatives. As the apex organization for S&T in AANR, the Council works with other agencies to focus their R&D initiatives towards achieving the desired S&T outcome. These R&D initiatives have been incorporated in the Industry Strategic Science and Technology Program or ISP which is the Council's blueprint in attaining its vision. This paper will feature the R&D initiatives on Harmful Algal Bloom (HAB) which is one of the ISPs under the marine environmental services of the aquatic sector.



Ma. Lourdes San Diego-Mcglone

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Dr. San Diego-McGlone is a Professor and former Director of the UP Marine Science Institute. Her research interests include nutrient biogeochemistry of coastal systems, estuarine chemistry, sediment-water interaction, and marine pollution. Her current research involvement includes coastal ecosystem response to anthropogenic stressors, ocean acidification, blue carbon, and harmful algal bloom mitigation. She has been interested in issues such as fish kills and the environmental consequences of mariculture and aquaculture activities. From her research works she has published 70 papers in international and local journals. Dr. San Diego-McGlone has also been involved in various environmental impact assessments, resource and ecological assessments, and was a resource person and regional mentor for an international initiative to examine land-ocean interaction in the coastal zone. She has a PhD in Chemical Oceanography from Old Dominion University in Norfolk, Virginia, USA.

#### **Managing HABs by Addressing Eutrophication**

Maria Lourdes San Diego-McGlone

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utrophication is defined as the nutrient enrichment of a water body leading to enhanced organic production. Sources of enrichment include nutrients released to freshwater and coastal areas from agriculture, aquaculture, and domestic wastes. In the Philippines, mariculture - culture of fish in marine waters - is an industry that aims to increase food supply and provide alternative livelihood for fisherfolks. However, as seen in the adjacent municipalities of Bolinao and Anda in Pangasinan, poorly regulated mariculture activities or the excessive expansion of fish farming has been linked to eutrophic waters, HABs, and fish kills. After a massive fish kill associated with an algal bloom in 2002, the occurrence of HABs and fish kills have continued. The reasons for sustained eutrophication are mainly decomposition of uneaten and undigested feeds (feces and excretion) and the increase of fish structures in. The excess phosphorus from feeds and fish byproducts has resulted in nitrogen-limitation (low N/P ratio) which is relieved by nitrogen supplied from rivers during the onset of the wet season. This often leads to recurring blooms of dinoflagellates and diatoms that often proliferate with change in N/P ratio conditions. Management efforts should include fish structure regulation (carrying capacity in number of fish pens and cages) for Anda, and perhaps revisit that for Bolinao; following proper feeding practice, improve feed quality, improve FCR (feed conversion ratio); and conduct of regular monitoring. Strong political will in leadership is also needed especially for municipalities sharing common waters. Mariculture to be sustainable requires a balance of economic gain and health of the environment.

#### Review and New Directions for Harmful Algal Bloom Monitoring in the Philippines

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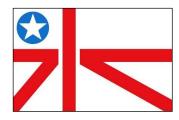
he Philippines is characterized by recurring harmful algal bloom events particularly that of Pyrodinium bahamense, a number of which was characterized by poisoning cases. In order to mitigate its negative impacts, a monitoring program is implemented by BFAR which follows a cyclic paradigm of activities such as sample collection and analysis, results evaluation, and information dissemination. Sample collection was jointly conducted by BFAR Central and Regional Offices in collaboration with the various local government units. The analysis of water samples for the presence of causative organisms and paralytic shellfish toxins (PSTs) in shellfish at the start of HAB occurrence were done at the BFAR Central Office. The laboratory results were evaluated by the National Red Tide Task Force (NRTTF) whose members are from the various Departments of the Government. The NRTTF disseminates information thru Red Tide Updates issued on a regular basis and when necessary. This centralized approach is by default for technical expertise required for analysis at remote sites is limited. Considering the geographic characteristics of the Philippines, the necessity to decentralize the monitoring system was recognized. Provinces where PSTs were prevalent were selected for the decentralized monitoring and capacity of the local government unit was established through IICA. The NRTTF developed the accreditation process for these provincial laboratories. However results of these initiatives were of low success in terms of sustaining the operation of the required laboratories. Out of 11 of provinces, six (6) were able to maintain phytoplankton analysis and out of these six (6), only three (3) are capable of PSTs analysis. None of these laboratories were accredited. In order to continually address adverse effects of algal blooms and PSTs in shellfish, BFAR took over the responsibility of results evaluation and information dissemination and to put in place the legal basis through Fisheries Administrative Orders. BFAR Regional Offices started to develop the capability for monitoring HABs and PSTs analysis using various analytical techniques. At this point, among the Regions with history of HABs and PSTs occurrence, nine (9) out of twelve (12) are capable of phytoplankton identification and six (6) of these are capable of toxin detection. The approach however was still centralized for the Shellfish Bulletins and Advisories which serve as the official documents for regulatory purposes originating from BFAR Central Office. The last few years were characterized by recurring blooms of toxic species in what can be considered hotspots. For a more effective way of responding to these blooms, a move to decentralize the monitoring activities and issuance of shellfish bulletins is underway but this time through the BFAR Regional Fisheries Laboratories (RFLs). Enhancement of the RFLs' capability for PSTs detection using immunoassays was already proposed including confirmatory testing for select laboratories. Regulations to further support of this endeavor were reviewed and revised.

Keywords: HAB monitoring, decentralized monitoring strategy



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Professor Imai is a Professor Emeritus at the Hokkaido University and was formerly a Professor in the Plankton Laboratory, Division of Marine Bioresource and Environmental Science. His research interests lie in the fields of Planktology, Biology, Ecology and Life Cycle of harmful algae, Mitigation strategies of HABs. He has been awarded by the Japanese Society of Fisheries Science, the Japanese Society of Scientific Fisheries and during the 3rd Asian Marine Biology symposium for his many scientific contributions.

#### Occurrence Mechanisms of Fish-killing Raphidophyte Red Tides and Proposal of Prevention Strategies Activating Diatom Resting Stage Cells in Bottom Sediments

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ather many species of harmful raphidophytes and dinoflagellates have cyst stages in their life cycles. Cysts settle to sea bottom to overwinter and thereby ensure persistent existence in the same area. Cyst germination provides primary populations for red tides. It is empirically known that flagellate blooms have been observed when diatoms are scarce in water. Diatoms form resting stage cells under nutrient-deficient environments, and rapidly sink to sea bottom. Cysts of Chattonella and Heterosigma can germinate in the dark, whereas diatom resting stage cells (DRSCs) require light for germination. Thus predominance of harmful raphidophytes can be attributed to disappearance of diatoms in water and subsequent failure of germination of DRSCs under low light environments at sea bottom. Selective germination of cysts at sea bottom is presumably a significant factor for the initiation and success in Chattonella red tides. Giving enough light to the abundant DRSCs (usually >10<sup>5</sup>/g wet sediment) at bottom was expected to enhance DRSCs germination and resultant vegetative cells are thought to proliferate and to overwhelm raphidophyte populations by consumption of nutrients. Hence we propose bottom sediment perturbation to lift DRSCs into euphotic layer in coastal areas as environment-friendly and practical HAB strategy. This trial was made using dragnet trawling in the Seto Inland Sea in July 2016. Densities of planktonic diatoms totally increased in water column after the trawling (maximum 1383 cells/mL) and Chattonella spp. decreased. The water sample after sediment perturbation was collected and bottle-incubation experiments were conducted in combination with inoculation of Chattonella antiqua (200 cells/mL) and strengthening of culture medium. Diatoms always successfully increased and Chattonella decreased in all the experimental bottles. We could demonstrate a feasibility of preventive control of Chattonella with sediment perturbation in coastal sea through artificial induction of phytoplankton flora to diatom dominant communities.



Aletta T. Yñiguez

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Dr. Aletta T. Yñiguez is an Assistant Professor at the Marine Science Institute, University of the Philippines in Diliman where she heads the lab Biological Oceanography and Modeling of Ecosystems or BiOME. She obtained her Ph.D. from the Rosenstiel School of Marine and Atmospheric Science, University of Miami in Florida with the support of Fulbright and Maytag Scholarships. She uses an arsenal of field, laboratory and computer modelling approaches to investigate the potential effects of environmental conditions including anthropogenic activities and climate change on the base of the marine food web and how these can impact Philippine fisheries including the occurrence of harmful algal blooms or red tide. She is a member of the Scientific Steering Committee of the IOC/WESTPAC HAB and the EASTHAB. Her research efforts have been locally and internationally recognized. She was the recipient of the 2012 L'Oreal -UNESCO For Women in Science Fellowship, and was awarded as an Outstanding Young Scientist by the Philippine National Academy of Science and Technology in 2017. In 2018 as well, she was one of the 8 Filipinos who were part of the Asian Scientist 100 list. The Senate of the Philippines honored her and the other 7 scientists by adopting Senate Resolution No. 141 on May 2019. Beyond research, Dr. Yñiguez advocates education and critical thinking through experiential learning. She has been the Camp Director of the DOST SEI-UPMSI Marine Science and Climate Change Summer Camp for public high school students for eleven years. Just recently, she became one of the four teachers honored by the Bato-Balani Foundation and Diwa Learning Systems in The Many Faces of the Teacher award.

#### A Retrospective Look at Our Evolving Understanding of Pyrodinium bahamense Bloom Dynamics

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yrodinium bahamense is a tropical dinoflagellate that has caused illnesses and deaths more than any other Paralytic Shellfish Toxin producing species. In this review, I assess the progress towards understanding its bloom dynamics within the context of its life cycle and the factors affecting the stages, as well as the relationship of the *Pyrodinium* population to shellfish toxicity. The life cycle of Pyrodinium has been mapped in detail though particular phases are understood more than others, especially in relation to influencing environmental conditions. As a cyst-forming dinoflagellate, the re-suspension of the hypnozygote cyst from sediments is a key aspect of initiating blooms, followed by excystment into a vegetative cell. This vegetative cell can rapidly divide depending on the prevailing conditions and consequently form blooms. These cells can switch to a temporary cyst, presumably allowing for smaller bloom cycles. Eventually, the vegetative cells enter the sexual phase and form the hypnozygote cysts thereby closing the cycle and leading to bloom decline. For some of these processes, previous research has shed light on the environmental cues such as light, temperature and salinity. However, until now the role of nutrients is still not well-understood though it is likely important. The processes involving cysts also need to be further investigated, together with the potential influence of competition and mortality on bloom decline. Beyond the life history of Pyrodinium, the link between its population and toxicity is an important aspect of HAB dynamics that needs expounding since the latter is of primary concern in food safety and human health. The patterns of shellfish toxicity do not necessarily follow the trends of cell abundances, and is likely due the interplay of various physical (e.g., circulation and patchiness) and biological (e.g., Pyrodinium stage and shellfish physiology) processes.



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Dr. Rhodora V. Azanza, is a Professor Emeritus of the Marine Science Institute, University of the Philippines (UP) – Diliman and President/Academician, National Academy of Science and Technology, Philippines (NAST-PHL). She has extended her expertise to the University in various positions including being the first woman dean of the College of Science – Diliman; government agencies and international organizations. Concurrently she was vice-chairman of UNESCO-Intergovernmental Oceanographic Commission (IOC) Panel on Harmful Algal Blooms (IPHAB) and leader of IOC's Harmful Algal Blooms in Southeast Asia (HABSEA) Portal and elearning for 7 years. The ASEAN Red Tide Network, was coordinated by Dr. Azanza for more than 10 years.

Dr. Azanza has more than 80 publications in international books and journals on the biology and culture of economically important seaweeds and in the biology and management of Harmful Algal Blooms (HABs). Most recently, Prof. Azanza is actively involved in valuing and promoting sustainable Blue Economy that highlight the importance of the Philippine marine and coastal domains. Dr. Azanza received numerous scientific awards which include the 2013 UPAA lifetime Distinguished Achievement Awards from the University of the Philippines Alumni Association. Recently she has been named as the 2015 Department of Science and Technology-Philippine Council for Agriculture, Aquatic and Natural Resources and Research (DOST-PCAARRD) Pantas Awardee for Most Outstanding Researcher/Scientist.

Early this year she was elected as one of the lifetime members of the International Society for the Study of Harmful Algae (ISSHA), a distinction of being the only Filipino and one of the five Asians in its list of 25 HAB Trailblazers.

#### Monitoring Tools and Approaches for Common HAB-Causative Organisms in East Asia

Rhodora V. Azanza

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Ithough the occurrences of Harmful Algal Blooms have been experienced/reported in the region and the rest of the world several decades before, as early as 2001 the Asia-Pacific Economic Cooperation (APEC) and United Nations Educational, Scientific and Cultural Organization—Intergovernmental Oceanographic Commission (UNESCO-IOC) came up with a compilation of existing and improvements on monitoring and management of Harmful Algal Blooms. The country representatives of East HAB since its inception in Jeju, Korea on December 2004 have likewise regularly discussed the HAB events and management systems during the East HAB Meetings. HAB International Meetings have likewise emphasized the need to initiate and/or improve each country's HAB monitoring and management systems.

HAB monitoring tools have been designed to be species-specific. The biology and ecology of the species have to be studied and understood to be able to conceptualize and implement an effective and efficient monitoring and management system. Likewise, the physico-chemical features of the affected/potentially affected areas need to be considered. In many cases therefore, the monitoring tools and approaches have to be area-specific. Modelling of available long term data sets of bio-physical parameters has been very useful in this regard.

Monitoring tools an approaches of the more common HAB species, e.g. *Pyrodinium*, *Cochlodinium* in East Asia will be highlighted and discussed in this presentation/paper.



#### Mitsunori Iwataki

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Dr. Iwataki is an Associate Professor at the Asian Natural Environmental Science Center of the University of Tokyo. His specialty lies in the taxonomy and phylogeny of microalgae. He is the Project Leader of the IOC/WESTPAC-HAB and serves as Associate Editor for Sorui and Phycological Research as well as the Editorial Board of the Harmful Algae journal. Dr. Iwataki is a member of the IP-HAB task team on algal taxonomy.

#### Morphology and Phylogeny of Armored Dinoflagellates in the Amphidomataceae (*Azadinium* and *Amphidoma*) from Japan

<u>Iwataki, M.¹</u>, Takahashi, K¹., Lum W.M.², Benico, G.², Ozawa, M.³, ⁴, Uchida, H.³, Oikawa, H.³, Suzuki, T.³

he armored dinoflagellates in the Amphidomataceae, Azadinium and Amphidoma, accommodate azaspiracid producers responsible for the shellfish poisoning. In Asian Pacific, three Azadinium species, A. poporum, A. dalianense and A. zhuanum, have so far been reported particularly from Chinese coasts, however, the species diversity and distribution in Asian coasts is not fully understood. In the present study, Azadinium and Amphidoma spp. were collected from Japanese coasts, and identified based on their morphology and phylogenetic positions. Morphology was examined by light and scanning electron microscopy, and phylogenetic positions were inferred from ITS and partial LSU (D1–D3) sequences. On the basis of their phylogeny, six Azadinium species in different phylogenetic position, and Amphidoma cf. languida were identified. The current results suggested; (1) The ribotypes B and C of A. poporum are distributed in Japan, which is consistent with the previous reports from Chinese waters. (2) A. cf. trinitatum is sister to A. trinitatum, but a small difference in the small antapical spine was also found. (3) Azadinium sp. 1 is similar to A. polongum and A. spinosum in the presence of the antapical spine and the ventral pore located at the lower left of the first apical plate (1'), but the phylogenetic position was distant from them. (4) A. cf. languida was morphologically consistent with A. languida.

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Prof. Songhui Lu is a Chinese phycologist, working particularly with the classification and ecology of algae, focusing on dinoflagellates. He works at the Institute of Marine Biology and Harmful Algae at the Jinan University. He has published over 200 scientific papers.

Dr. Lu has his doctorate in ecology, the University of Hong Kong, and has worked in the Woods Hole Oceanographic Institution as an visiting scholarship. His major areas of research include the taxonomy of harmful algae, toxic dinoflagellates, and ecology of the planktonic and benthic algae.

# The Occurrences of Brown Tide in China: Mechanisms and Effects

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he picoplanktonic pelagophyte Aureococcus anophagefferens could trigger harmful algal blooms (HABs) to discolor water in brown, known as brown tide. Since 2009, large-scale brown tides, caused by A. anophagefferens, had been occurred in early summer for three consecutive years in the coastal waters of Qinhuangdao, China and resulted considerable deleterious effects on the scallop mariculture industry. The causes for the occurrence of brown tides were not fully understood. Therefore, we conducted studies on the seasonal succession of the phytoplankton community, including A. anophagefferens and its relationship with environmental variables in the area. The results revealed that the population dynamics of the phytoplankton community were significant variation with seasonal succession, in which A. anophagefferens played an important role during the entire year. The trend of the whole diversity index indicated that the community structure became more stable in winter. The results of principle component analysis (PCA) applied to the environmental factors indicated that the dissolved organic nitrogen is the key nutrient in the bloom occurrence. In addition, a few another environmental factors commonly contributed to the winter waterbody, indicated that the aquatic environment is more complex in the cold season. The result revealed that the phytoplankton community structure and its variation were mainly affected by the hydrological factors, by using the redundancy analysis (RDA) for the relationship between dominant species and the environment. The effects of A. anophagefferens on zooplankton and shellfish have also been studied.

#### **HAB Country Report of Japan (2017-2019)**

Onitsuka, G., Nakayama, N., Sakamoto, S.

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AB events have occurred mainly in the Seto Inland Sea and around Kyushu area located in western Japan. In these areas, there were 71 and 80 bloom events respectively in 2017, and 82 and 69 events in 2018. Major HAB species in Japan are composed of Chattonella spp. (C. antiqua, C. marina, and C. ovata), Heterosigma akashiwo, Karenia mikimotoi, Heterocapsa circularisquama, and Cochlodinium polykrikoides. These HABs caused massive fish and shellfish kills with economic losses during 2017-2019. In summer 2017, the massive bloom of K. mikimotoi occurred in the Imari Bay located in northern Kyushu and the total amount of fishery damage (mainly for puffer fish) exceeded 600 million JPY. In summer 2019, the bloom of *Chattonella* spp. brought severe damage for aquaculture vellowtail (>100 million JPY) in the Yatsushiro Sea (Inland Sea in Kyushu). Other harmful species of Karenia digitata, Alexandrium leei, Dictyocha spp. and Prorocentrum spp. formed blooms and caused fishery damages in western Japan. Blooms of diatoms, Skeletonema spp., Chaetoceros spp. and Thalassiosira spp. occurred in winter in the Ariake Sea (Inland Sea in Kyushu) with damages to cultured Nori (Pyropia) farming. Paralytic Shellfish Poisoning caused by Alexandrium spp. (A. catenella and A. pacificum) and Gymnodinium catenetum frequently occurred in western and northern Japan. From winter to spring in 2018, large-scale blooms of A. catenella (Group I of the A. tamarense species complex) occurred in the eastern Seto Inland Sea and the Tohoku area (northern Japan), and the blooms caused the prolonged shellfish bans in the extensive areas of these regions. In the eastern Seto Inland Sea, fishery damage for fishes and cephalopods due to the bloom of the species was also reported.

# Outbreak and Disappearance of *Cochlodinium* Blooms Under Influence of Typhoon and Rain in 2019

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ochlodinium blooms in Korea in 2019 caused 3.6 billion won (about 4 million USD) in fish aquaculture. The blooms started in the middle of southern coast on August 20, and spread the whole south coast and lasted thirty-nine days. The outbreak, migration and spread of Cochlodinium blooms this year is thought to be related to typhoons and rainfall. Thirty-three days of long rain season (26 June - 28 July) and two typhoons that passed through Korea on July 20 (the fifth typhoon DANAS) and August 6 (the eighth typhoon FRANCISCO) caused diatom blooms in coastal waters until mid-August. The typhoon on September 7 (the thirteenth typhoon LINGLING) caused a strong south wind, which brought free living Cochlodinium cells, which was distributed in off the south coast, into the coast, and these Cochlodinium cells increased rapidly. On September 22, the typhoon (the seventheenth typhoon TAPAH) was a continuous strong north wind dispersing Cochlodinium blooms mass and drop in water temperature. Rainfall accompanying typhoons also caused diatom blooms. Within a week, eventually, the Cochlodinium cells disappeared around coastal waters. This result supports the previous our survey that the outbreak of Cochlodinium red tides is associated with the limited growth of diatoms under depleted DIP or DIN conditions. When the Cochlodnium blooms occur, inorganic nutrients show very low concentrations but organic nutrients show high concentrations. The disappearance of diatoms is an important factor that is supplied to the growth of Cochlodinium cells.

#### Status and Researches of Harmful Algal Blooms in China

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armful algal bloom (HAB) has been considered as a typical marine ecological disaster, which lead to significant negative impacts on sustainable development of mariculture industry, and health of human-beings and natural marine ecosystems. In the coastal waters of China, large-scale HAB events have been reported for a long time. Brown tides, which have been consecutively recorded in the Bohai Sea from the year 2009, had a decreasing trend recently. In the Yellow Sea, macroalgal blooms caused by green alga Ulva prolifera and brown alga Sargassaum horneri became the most dominant HAB events. In the East China Sea, the red tides of harmful or toxic dinoflagellate have been recorded for nearly two decades, and the continuous occurrence of large-scale blooms led to deleterious impacts on the health of marine ecosystems and societal development. In the South China Sea, large-scale bloom of haptophyte Phaeocystis globosa became the dominant type of HABs, particularly in the Beibu Bay. Besides, seafood contamination and poisoning incidents were recorded recently at several locations, reflecting the increasing threats of toxic algal blooms. Several national level key R&D programs have been implemented, and made significant progresses in understanding the mechanisms of different HABs, and in the development of monitoring, prediction and mitigation measures against HABs. However, there is still a long way to go to effectively control the disastrous impacts of HABs. In face of the requirement of UN Decade of Ocean Science to develop a safe ocean, it's suggested to promote the collaborations in Asia countries on a joint program focusing monitoring and early-warning of HABs in this region.

#### **Country Report: Harmful Algal Blooms In The Philippines**

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ince 1983 up to 2005, a total of 2,161 reported cases of paralytic shellfish poisoning (PSP) and 123 deaths due to the consumption of shellfish (mostly green mussels and oysters) caused by the blooms of toxic dinoflagellates *Pyrodinium bahamense* var. *compressum* (Bajarias & Relox 2006). HABs of *Pyrodinium* have expanded both in time and space, as *Pyrodinium* occurred in more than 40 different coastal waters of the country between 1983 and 2018. Other PSP-causative species that have been recorded in few affected coastal waters are *Gymnodinium catenatum*, *Alexandrium tamiyavanichii*, *Alexandrium* spp. PSP has been recorded primarily in filter-feeding bivalves, various species of clams, oysters, scallops, and the guts of crabs. BFAR has documented that PSP incorporates the most prevalent biotoxins affecting shellfish growing areas on the country's coasts. PSP is responsible for numerous seasonal shellfish closures in affected coastal waters in the country. To address the growing problem on PSP a strategy to mitigate the impact has been implemented. PSP monitoring program has been in placed for more than three decades now.

Large bloom of *Alexandrium* spp. occurred in Bolinao Bay, Pangasinan, northern Philippines, with a particularly widespread and intense event recorded in January 2010 to May 2011 (Azanza and Benico, 2013), wherein its abundance peaked in May 2010 that caused also high paralytic shellfish toxicity (Cruz et al. 2011).

In most aquaculture sites, other type of HAB was observed. A massive fish kill and water discoloration were reported off the western coast of Puerto Princesa, Palawan, Philippines in March 2005. Phytoplankton analysis revealed a near monospecific bloom of the dinoflagellate, *Cochlodinium polykrikoides*, with cell concentrations ranging from  $2.5 \times 105$  to  $3.2 \times 106$  cells per liter. On the other hand, fish growers in Balingasag Bay, Misamis Oriental, Northern Mindanao region have been losing millions of pesos in investment as massive fish kill brought by the blooms of *C. polykrikoides* since it was first reported on March 26, 2014. Balingasag town has been known for growing caged saltwater milkfish ('bangus') supplying not only northern Mindanao but also the other areas of the country.

Although *Noctiluca scintillans* species was observed to bloom in the past years particularly in Manila Bay, by forming foams and contributing to the distinctive decaying smell in the bay, it did not cause any fisheries concern. However, the species caught the researchers' attention in 1997 to 1998, when green *Noctiluca* were observed to co-exist with the declining cell numbers of *Pyrodinium* in Manila Bay (Azanza et al. 2001). From 1999 until 2013, green *N. scintillans* formed blooms and became more recurrent, prevalent and eventually succeeded *Pyrodinium*. Red *N. scintillans* bloom took place for the first time in the bay between the last week of January and the first week of February 2014 (Gatdula et al. 2017). Fishermen operating in the bay reported to the authors the unusual tomato red water discoloration in the western portion of the bay. Although red *N. scintillans* were present during the months of January to February from 2015 to 2018, density ranges of 21–35 cells/L did not produce water discoloration

This paper summarizes the current status of occurrence and historical perspective of HABs in the Philippines and collaborative efforts done to improve the monitoring system.

Keywords: Harmful Algal Blooms, Paralytic Shellfish Poisoning, *Pyrodinium bahamense* var. *compressum*, Philippine coastal waters and estuarines

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#### RRD-1

### Chemical Basis of the Toxicity of *Gambierdiscus* carpenteri (GAM1BOL080513)

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he Dinophyceae *Gambierdiscus carpenteri* (GAM1BOL080513) was recently described from Bolinao using morphological and phylogenetic characterization. Toxin production in this Dinophyceae was assessed using Receptor Binding Assay (RBA) however, there was no identification of the putative toxin. Our study focused on the determination of the chemical basis for the toxicity of *G. carpenteri*. *G. carpenteri* biomass was obtained from a 6 L culture by centrifugation. The biomass was extracted with acetone and the resulting extract was fractionated using a Solid Phase Extraction (SPE) Florisil cartridge with stepwise gradient elution of 4:1 Hexane:Acetone (F1), 9:1 Acetone:MeOH (F2), and 1:1 Acetone:MeOH (F3). Subsequent analysis of the fractions using Low Resolution Mass Spectrometry (LRMS) revealed the presence of peak at m/z 1,039.10 in F2. This fraction was further purified using reversed phase High Performance Liquid Chromatography (HPLC) with a linear gradient of 30%-100% aq. MeOH for 20 min. HPLC fractions were analyzed by High Resolution MS (HRMS). Based on the high-resolution molecular weight and fragmentation analysis, the toxin compound responsible for toxicity in *G. carpenteri* GAM1BOL080513 was identified to be the gambierone derivative maitotoxin-3 (MTX-3). Molecular Networking analysis was also done to assess the presence of putative derivatives of MTX/gambierone, as well as other compounds that might play an important role in the toxicity of *G. carpenteri*.

#### RRD-2

## Ciguatoxins Detection Using Radioligand Receptor Binding Assay in Reef Fishes

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iguatoxins (CTXs) are potent, heat stable and lipo-soluble polyether molecules produced by benthic dinoflagellates of the Genera *Gambierdiscus* and *Fukuyoa*. CTXs can accumulate and concentrate in fish tissues including the edible parts and can cause neurologic symptoms in humans when consumed. The Philippines as part of the Pacific is one of the identified geographical distribution of *Gambierdiscus* and *Fukuyoa* species rendering reef fishes susceptible for CTXs contamination. Since the Bureau of Fisheries and Aquatic Resources (BFAR) is a regulatory agency, one of the mandates is to evaluate methods that can be used for monitoring strategies. In addition, BFAR also caters to industry samples in compliance with RA10611 and RA10654. The method investigated in this study is radioligand receptor binding assay and these were applied from reef fishes collected from the coastal waters of Zamboanga and MIMAROPA Regions. CTXs were extracted from edible fish tissues via liquid partitioning using acetone, n-hexane and dichloromethane. The method described IAEA TechDoc No-1729 was used for the assay procedures. Results showed as high as 125.55 ng/g CTX-equivalent to below detection limit. Low limits of CTX can be detected. Although the method needs further optimization, results indicate that the method is a suitable replacement to the mouse bioassay that is currently used by BFAR.

## Analytical Methods in the Context of Paralytic Shellfish Toxins Monitoring in the Philippines

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he Philippines has a long history of algal blooms particularly that of Pyrodinium bahamense, a causative agent of paralytic shellfish toxins (PSTs). For more than three decades, management strategies follows a centralized approach but is now geared towards decentralization thru the BFAR Regional Offices. Selection of toxin detection methodologies would therefore play an important role for establishing a monitoring system. Mouse bioassay has been adapted as the standard method for PST detection for monitoring purposes with majority of results effectively serving as basis for shellfish bulletins issuance used for information campaigns. However, changing times demand for improved sensitivity, selectivity and operations cost of the method and address ethical concerns of live animal testing. Alternative methods were evaluated to address MBA issues. Immunoassay in lateral flow immunochromatography format (LIC) and enzyme-linked immunosorbent assay (ELISA) were evaluated. Assays in LIC format is a qualitative test but proves to be advantageous for regional laboratories for less technical skills are required. ELISA requires more advanced technical skills and equipment but provides estimation of shellfish toxicity. Both immunoassay methods are effective in establishing toxicity in shellfish but affected by crossreactivities of the saxitoxin congeners and would entail confirmatory tests. HPLC and receptor binding assay were evaluated at BFAR. The former was costly in terms of toxin standards and long turn around time while the latter requires only a one time equipment investment. Although considered as ex-vivo assay, RBA was the choice for the results provided for direct toxicity measurement unlike HPLC which provides for concentrations that requires toxicity equivalency factors. Confirmatory tests are required in compliance to regulatory requirements. Immunoassay in combination or independent of confirmatory tests has been used effectively for toxin detection. The lessons learned in the application of these methods can serve as basis and benchmark.

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#### **HAB Concerns for a Philippine Oyster Hatchery**

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griculture Sustainability Initiatives for Nature (ASIN), Inc. is a small startup company whose mission is to provide high quality, traceable and sustainable agricultural produce using environmental friendly sound practices. At present, its focus is to aid in the development of the oyster industry in the Philippines through project TALABA-TALABEST. An essential component of this project is the operation of a commercial oyster hatchery in San Juan, Batangas.

The hatchery's goal is to regularly supply oyster seeds to partner communities of oyster growers and reduce their dependence on the natural spat fall cycle which determines the frequency of harvest seasons.

Harmful Algal Blooms (HABs) greatly affect shellfish aquaculture especially in traditional oyster growing areas. The hatchery and grow-out areas are within Tayabas Bay, a body of water in the southern part of Luzon with no recorded occurrence of Harmful Algal Blooms (HABs). To prevent the occurrence of HABs in the area, the ASIN Oyster Hatchery has established two basic in-house protocols: 1) Only oyster seeds from broodstocks coming from Tayabas Bay will be distributed to partner growers. 2) Waste water from hatchery operations undergoes natural sand filtration. However, there is a need for industry, academe and government institutions to collaborate and develop standard guidelines and certifications for the following: 1) HAB Free Broodstock. 2) HAB Free Oyster Seeds. 3) HAB Free Oyster Transplantation to Non-Traditional Growing Areas. 4) HAB Free Oyster Hatchery Operations.

These activities will be able to help communities of oyster growers which are usually composed of fisher folks and informal settler that are among the vulnerable sectors of the country. It will also promote a sustainable oyster industry by making the necessary interventions throughout the supply/value chain anchored on science and technology, community involvement and collaboration among the various stakeholders and institutions.

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## Harmful Algal Bloom Impacts on the Livelihood of Fisherfolks in Selected Areas in the Philippines

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armful algal blooms have caused several poisoning cases and economic losses particularly in the aquaculture industries in the Philippines in the last three decades. Areas reported for HAB events in Pangasinan, Samar, Leyte, Iloilo and Bohol have been visited to determine the challenges and issues the fisherfolks in the coastal communities face in terms of livelihood and to describe their coping strategies during a "red tide" event. Key Informant Interviews (KII) were conducted using an interview guide and indexed using MaxQDA 12. Framework analysis has been employed which yielded recurrent themes in the interviews conducted. Fishing and mussel farming have been the two major livelihood of the respondents. Common challenges identified were typhoon, oversupply of shellfish products and "red tide" events. During "red tide" events, shellfish bans are imposed which cut off their major source of income when they can neither sell nor consume any shellfish products. To cope with lower income during these events, respondents opt for other alternative livelihood like hired manual labor (construction, farm, domestic work, etc.), others would get loan from cooperatives or from their employer. Management of shellfish aquaculture/harvest based on science/knowledge on bloom events could help alleviate these HAB associated socio-economic problems.

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## Sorption of Paralytic Shellfish Toxins (PSTs) in Algal Polysaccharide Gels

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orption mechanics of paralytic shellfish toxins (PSTs), saxitoxin (STX) and neo-saxitoxin (neo-STX) on algal polysaccharide gels was characterized using surface chemistry models. Refined (RC), semi-refined (SRC) carrageenan and alginate showed sorption of STX and neo-STX. The sorption of PSTs on RC, SRC and alginate was affected by contact time and in part, temperature. From surface chemistry models, alginate followed a spontaneous endothermic physical monolayer sorption of STX and neo-STX. SRC and RC favored the concurrence of physical and chemical monolayer sorption, being endothermic for SRC and exothermic for RC.

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#### The Progression of HAB Vulnerability and Safety: Developing Community Risk Knowledge for HABs in Jiabong, Samar; Sapian, Capiz; and Bolinao, Pangasinan

<u>Lagos, D.</u><sup>1</sup>, Gasmen, H.<sup>1,2</sup>, Eco, R.N.<sup>3</sup>, Gopez, J.<sup>3</sup>, Dungong, R.<sup>3</sup>, Novelero, J.M.<sup>3</sup>, Bacay, J.L.<sup>3</sup>, Yñiguez, A.T.<sup>3</sup>

major concern for Harmful Algal Blooms (HABs) in the Philippines is its impact on the livelihood and economic well-being of local communities. However, there is limited information on these impacts and the experiences of the communities even as HABs keep recurring around the country. This study presents insights from engaging communities as part of the development

of a people-centered early warning system for HABs in three localities in the Philippines: Jiabong, Sapian, and Bolinao. Through the use of participatory rapid appraisal tools, this study assessed the HAB risks in the three areas by a) examining people's knowledge on HAB hazards and risks; b) articulating community capacities and vulnerabilities to HABs, and c) identifying ways to reduce the HAB risk. The people-centered approach gives emphasis on people's capacity to understand risks, monitor relevant parameters, communicate information, and implement necessary responses to protect communities from the negative impacts of HABs.

To articulate communities' knowledge on HAB, tools such as historical timeline, hazard assessment table, and seasonal calendar were used. The community assessed their capacities using community mapping, and integrated the elements of HAB risk using the *progression of vulnerability* approach. Community members also came up with recommended actions to address their vulnerabilities through the *progression of safety* approach.

Progressing HAB risk in the communities are related to the lack of knowledge for alternative livelihoods, low educational attainment, lack of access to productive resources, crowded fish cages and over-feeding of fish in the fish cages, inadequate support to the shellfish growers, and the lack of poor people's control in decision making. Consequently, recommended actions by the community include the provision of alternative livelihood, provision of technology such as depuration chamber, assistance and access to calamity funds during the shellfish ban, and effective red tide disaster preparedness. Other significant recommended actions involve the allocation of resources for the post-processing facility of shellfish products, enforcement of ordinances against illegal activities in the coastal areas, eradication of corruption and the enhancement of poor people's control in the decision-making concerning coastal resource management.

This process is important in generating HAB risk knowledge that explores not only the biophysical and technological aspects but also the social, political and economic dimensions of HAB risk. The participatory risk assessment is also the foundation for building the early warning system for HABs. Moreover, it contributes to an empowering process by building on people's capacities, affirming people's role in knowledge creation and providing an impetus for collective planning and action.

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## Research Groups on Improving Management Strategies on Marine Ecosystem Disturbance and Harmful Organisms: With Emphasis on HABs Species

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arine ecosystem in local environment have successfully settled and formed solid relationships by eliminating unnecessary elements. However, increasing of water temperature due to global warming, marine pollutions, and introducing invasive alien species due to ballast waters can disturbing marine ecosystems. Introduction of some invasive alien organisms can influence of collapse existing system, and also can cause destruction of food web, or harm to human directly or indirectly through food web. Recently, the number of harmful and toxic species, their density, and distribution is increasing caused by increase of temperature of seawater, trades by ship between nations, and marine pollutions.

The purpose of this research group is to distinguish marine disturbances (disruption of marine ecosystems balance) and harmful organisms (marines organisms that damage to human life or property), and establish a standardized database by securing their distribution and ecological information data, and developing management techniques based on scientific research and to establish management system that can utilize policy.

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## Current Status of HAB Studies and Events in Indonesia: The Use of Excel Dashboard to Hasten Data Processing and Visualization

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here is an urgent need to process and visualize data of HAB monitoring in a fastest and automated way to assist the mitigation plans and to minimize the impact of HAB events. The increasing occurrence of HABs organisms in Indonesia is a significant and expanding threat to human and ecosystem health, to the fisheries and shellfish industries, and to ecotourism. To minimize the impact, collecting data to monitor HAB events is not enough without an efficient catalogue and database system. A quick data processing and visualizing is essential to make a quick response to the occurred HAB event. In this case, Excel Dashboard is proposed as an option to manage HABs data, mainly because of its ease of use and it has been widely used for data input, processing and visualization. Excel Dashboard is a spreadsheet in Excel that contains tables, charts, maps, and graphs which could be used to effectively visualize any type of data. The data input using Excel Dashboard is simple, fill the template form that already created and then the Excel Dashboard automatically process and visualize the data. In this case, we used Excel Dashboard as a platform to process and visualize HABs data in Indonesia that has been recorded since 1979. About 1400 data were gathered and analyzed from 57 publications in this study as samples. About 30 HAB species that commonly occurred and 77 species that potentially cause HAB event in Indonesia was successfully summarized and visualized with Trichosdemium, Pyrodinium, Cochlodinium, and Dinophysis as frequent causative species. From this study database, the common impacts caused by HAB event in Indonesia are fishkills (37%), discolouration (10%), and human health (8%). The data also shows that the frequency of HAB events increase gradually from 2003 to 2019, and mostly occurred in west and east Indonesia.

## Monitoring Harmful Algal Blooms in Singapore Waters Using Advanced Techniques

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lobal coastal environments are increasingly affected by regular harmful algal blooms (HABs). Many coastal regions in Southeast Asia (SEA) are also experiencing regular HAB outbreaks which have significant impact on coastal resources. In Singapore, several HABs have caused massive fish kills and great economical losses along the northern coastal region. In 2016, blooms of dinoflagellate Karenia mikimotoi were observed for an extended period of more than three months. The increase in frequencies of HABs have led to enhanced interests in monitoring and detecting such blooms. To better protect coastal economies and human health, improved HABs monitoring and prediction are necessary. Currently, there is an array of tools ranging from autonomous vehicles, portable instruments, optical sensors to remote sensing platforms that could be utilized for generating high-resolution data. These advanced technologies and tools contribute to new and high-resolution data, which could be incorporated in models for predicting and forecasting bloom events. In the present study, an integrated approach involving laboratory analysis, advanced technique and tools was utilized to study and monitor HABs. High-resolution surface maps of blooms were generated using the measurements collected from an autonomous surface vehicle (ASV). Distinct biological and environmental patterns were observed from the ASV measurements. Molecular technique was used for monitoring HAB species and phytoplankton compositional variability. In addition, we have successfully used a portable molecular instrument to detect HAB species in both laboratory and field conditions. For better management, it is essential to use rapid and reliable tools which mitigate the harmful effects of HABs.

## Baseline Assessment of the Shellfish Growing Areas in Batan Bay, Ivisan and Panay, Capiz, Philippines

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hellfish aquaculture is among the most promising fisheries industries in the country with economic potentials in local and international markets. However, product quality is generally affected by the environment where it is cultured, hence it is important that shellfish production areas must be free from harmful microorganisms, chemical contaminants and marine toxins to ensure that products harvested are safe for human consumption. This study aimed at generating baseline information of areas in the province of Capiz where shellfish, especially oysters and mussel, are commercially cultured. The results can be a useful input in determining and planning areas for possible commercial shellfish production. Physicochemical water quality parameters were determined on-site using a multiparameter water quality analyzer, while shellfish samples were collected and immediately brought to the regional fisheries laboratory of BFAR Region 6 for microbiological and heavy metals analysis.

Results showed that the physicochemical characteristics of the seawater in the shellfish areas studied are within ranges suitable for shellfish culture. However, seven (7) of the eight (8) water samples collected and analyzed for fecal coliform failed to meet the standards for Class SB waters based on DENR DAO 2016-08. On the other hand, the analysis results of the shellfish meat collected and analyzed for *E. coli* showed that one (1) area in Ivisan (Cabugao B) initially met the requirements for a Class A growing area while the other seven (7) sampled areas passed the criteria for Class B growing areas for shellfish, based on current EU regulations for the classification of bivalve mollusc harvesting areas as cited in the BFAR 2017 Philippine National Shellfish Sanitation Manual for the Production of Safe Bivalve Molluscs. The heavy metal mercury (Hg) was not detected in any of the shellfish meat samples collected and analyzed.

Keywords: shellfish, shellfish culture, shellfish product quality, water quality

## The Diversity of Phytoplankton in Bolinao, Pangasinan: A Shotgun Metagenomics Approach

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hytoplankton communities in coastal waters simultaneously respond to both natural forces and environmental impacts of human activities, such as aquaculture. As part of a long-term study to investigate the effects of aquaculture activities on phytoplankton communities and occurrence of harmful algal blooms, the diversity of the phytoplankton community in Guiguiwanen Channel (Bolinao, Pangasinan, Philippines), a site characterized by seasonally intensive aquaculture operations, was investigated using shotgun metagenomics. From water samples collected at two stations in February 2019 along the Guiguiwanen channel, DNA was extracted and sequenced using the MiniSeq platform (Illumina). The sequences were then characterized taxonomically based on sequence similarity to known sequences in a database of chloroplast and mitochondrial sequences obtained from NCBI. Although the taxonomic coverage of the database is incomplete, the shotgun sequence data recovered 33 species of dinoflagellates and 63 species of diatoms from the two stations. The dominant dinoflagellate species included Kryptoperidinium foliaceum, Durinskia baltica, and Karlodinium veneficum; these species have not been previously reported to occur in Bolinao. The dominant diatom species observed were Guinardia striata, Leptocylindrus danicus, Rhizosolenia setigera, and Pseudo-nitzschia multiseries. Also observed were nine HAB-forming species: Dinophysis acuminta, Dinophysis acuta, Phalacroma mitra, Azadinium spinosum, Gonyaulaceae, Karenia brevis, Karlodinium veneficum, Nitzchia spp. and Pseudo-nitzchia spp. The diversity of dinoflagellates observed in this study also captured parasitic (Hematodinium sp.) and symbiotic (Symbiodinium sp.) species which are difficult to identify using morphological criteria. The data obtained thus far in this continuing study, though limited by taxonomic coverage of the database and sequencing depth, provide new information on the diversity of phytoplankton species in Bolinao, Pangasinan.

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# Growth Change in Two Harmful Phytoplankton Blooming Species by Co-cultivation with Bacterial Communities Exposed to Dissolved Organic Matter Derived from *Heterosigma akashiwo* (Raphidophyceae)

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hytoplankton blooms lead to increase in dissolved organic matter (DOM) concentration and change in its composition in marine environments. Due to difference in DOM consumption depending on bacterial taxa, increase in DOM level and change in DOM composition can lead to variation in assemblage of bacterial community (BC). It is recently suggested that variation in BC may affect phytoplankton growth and composition. However, the effect of this variation on phytoplankton community is greatly unknown. Thus, in this study, we investigated variation in BC induced by the addition of DOM released from Heterosigma akashiwo (raphidophyte), and then examined effects of variation in BC on the growth of toxic dinoflagellate Prorocentrum minimum and diatom Skeletonema costatum. The BC was isolated from natural sample (Masan Bay, South Korea) when blooms of H. akashiwo were generated, and incubated for 3 days after addition of DOM H. akashiwo culture at a final concentration of 10 mg L-1. Several bacterial genera derived from DOM represented statistical difference. Particularly, relative ratios of Sphingobacteriia and Verrumicobiae increase than control. Then, the treated BC was inoculated into P. minimum and S. costatum cultures two phytoplankton species. As a result, the growth of P. minimum (76 to 220%) was clearly enhanced when DOMtreated BC was inoculated, whereas, the growth of S. costatum was suppressed (-24 to -28%). Several bacterial genera in BC which co-cultivated with two phytoplankton species represent statistical difference compared with control, particularly, Verrumicobiae and Sphingobateriia. Ratio of these two classes and humic acid like carbon (HL) fraction in H. akashiwo released DOM have shown high correlation coefficience. Our results showed that specific composition of DOM can change bacterial community composition that promote the growth of different phytoplankton taxa.

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### Viability of HABs-Causing Species on Floating Plastics Debris *in vitro*

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loating marine plastics provide a durable and persistent substrate for the colonization and survival of microorganisms, which is initiated by the formation of bacterial biofilms. Among these communities are potentially harmful algal blooms (HABs)-causing species, indicating the potential role of plastics in the increased global occurrences of HABs. Here, we tested the role of biofilm priming on low density polyethylene (LDPE) plastic fragments and how it can facilitate attachment and support the survival of *Alexandrium catenella* cells in vitro. Results showed that *Alexandrium* cells attached more to the biofilm especially when nutrients were very limited. Further, the attached cells were still viable, being able revert back to actively dividing motile vegetative forms when exposed to more favorable conditions. These results demonstrate that biofilms on plastic fragments can indeed sustain the survival of hitchhiking cells, possibly contributing to the success of dispersal of HABs-causing species to new environments. This study is a significant contribution to the growing knowledge on the ecological implications of floating marine plastic debris as a new environment in marine ecosystems.

#### Genetic Diversity and Co-occurrence of *Amoebophrya spp*. (Syndiniales) with Host Dinoflagellates in Mariculture-Impacted Areas

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moebophrya (Syndiniales), a group of marine eukaryotic parasites known to infect dinoflagellates including those of harmful algal species, has been gaining interest for its natural potential to control dinoflagellate blooms. However, our understanding of their diversity and host specificity in terms of infections remains incomplete since many of their hosts are uncultivable. In addition, recent environmental surveys using high throughput sequencing has steadily reported new sequences of the parasite, but with no reports in Southeast Asia including the Philippines. Here, we investigated the genetic diversity of Amoebophrya as well as their co-occurrence with their potential host dinoflagellates in mariculture-impacted areas where HABs frequently occur. We used high throughput amplicon sequencing and applied network analysis to an 18S rRNA gene dataset containing 23,664 picoeukaryotic sequences from 8 environmental samples collected from mariculture sites in Bolinao, Pangasinan and Masinloc, Zambales from January to April 2019. Results confirmed the presence of Amoebophrya in mariculture areas and potential dinoflagellate hosts include Gyrodinium, Heterocapsa, Protoperidinium and Ceratium. This study provides baseline information on the ecology and diversity of Amoebophrya in tropical waters.

## Revealing the Harmful Algal Species Community Shift Along the Johor Strait Using Metabarcoding Approach

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tudies of Harmful algal blooms (HABs) and phytoplankton community shift have relied heavily on classical microscopy approach that subjected to a high degree of bias and inconsistency in species identification. In this study, a genetics distinction approach, metabarcoding was adopted to characterize phytoplankton community and HABs species compositions in the Johor Strait - the Strait is important for finfish and shellfish mariculture industries in Malaysia and Singapore. Subsurface water samples (<150L) were collected monthly between May 2018 and January 2019 from 18 stations in the eastern and western Johor Strait. The plankton samples were undergone DNA isolation, and ampliconbased sequencing on the 18S rDNA V9 region was performed using an Illumina Miseq platform. A total of 14 HAB species were discovered from the operation taxonomic units (OTUs) taxonomic assignment. Pseudonitzschia australis and Karenia mikimotoi were found in dry season (May 2019), while Gymnodinium catenatum, P. cuspidata/lineola/turgidula and Chattonella subsalsa were detected during the inter-monsoon (Aug-Oct 2018). Alexandrium tamiyavanichii, A. pseudogonyaulax/hiranoi, C. minima, P. brasiliana and P. americana were abundant in wet season (Nov 2018 - Jan 2019). Conversely, Dinophysis miles/caudata/norvegica, Pseudo-nitzschia multiseries/pungens and Heterosigma akashiwo were detected throughout the sampling period. Alexandrium affine, A. minutum/ostefeldii/ insuetum and A. leei appeared sporadically throughout the season. Environmental variability in macronutrients, temperature, and salinity were likely the main drivers that influenced the composition shifts in the OTUs relative abundances and HABs assemblages. The finding of this study provides a valuable baseline information and species inventory for future HAB monitoring and early warning in the Strait.

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# Abnormal *Cochlodinium polykrikoides* Bloom at High-Water Temperature in Southern Coastal Water of Korea; Comparison of Physiological and Genetical Characteristics Between Different Strains

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ver the past three decades, the southern coastal area of Korea, as one of the seriously damaged area in the world, has been subjected to increased frequency of the ichthyotoxic dinoflagellate Cochlodinium polykrikoides. To investigate how the C. polykrikoides bloom appear in relation to environmental factors, we have examined the oceanographic characteristics in field and their grow characteristics in laboratory. In field, C. polykrikoides has shown optimum growth in moderate temperature (around 25°C). In 2018, however, blooms of this dinoflagellate executively occurred (maximum cell densities: 1,990 cells mL-1) under higher water temperature (above 29°C) in the southern coastal area of Korea, due to which is characterized by abnormal atmosphere temperature condition such as namely "heat dome effect" over one month between July and August in East Asian. Thus, to understand this, we have established C. polykrikoides culture isolated from this dinoflagellate blooms of 2018, and investigated physiological and genetic difference of cultures of 2018, compare to another C. polykrikoides culture which previously isolated in 2013. As a result of the growth experiments, 2018 strain grew under high water temperature (above 28°C), but on the first day of inoculation of 2013 strain, the cell density decreased significantly, and on the second day the cell density was less than 10 cells mL-1, most of which died. In addition, sequences in large sub-unit ribosomal RNA (LSU rRNA) and mitochondrial cox1 and cob gene were analyzed to find whether or not there is genetic difference between the two cultures. Based on our result, two cultures have identical sequences in LSU rRNA, cox1, and cob gene. Taken together, the culture of 2018 has clearly different physiological characteristic (particularly in water temperature) even though there was no genetic difference with other C. polykrikoides culture which was isolated from relatively low water temperature. This physiological difference might be capable of occurring C. polykrikoides in Korean coastal water even though water temperature in 2018 was relatively higher than previous years.

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## Microbial Interactions During *Cochlodinium polykrikoides* Bloom in South Sea, Korea

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armful algal blooms (HABs) by Cochlodinium polykrikoides cause huge economic damages frequently in Korea. Many previous studies revealed that the rise and fall of HABs are closely related to their associated microbes, including bacteria, archaea, and phytoplankton. However, their exact roles in the formation of HABs have not been elucidated. To explore the potential links between C. polykrikoides and other microbes, water samples in different size-fractions were collected during C. polykrikoides bloom in South Sea, Korea. Bacteria, archaea, and phytoplankton community structures were analyzed using next-generation sequencing. Marine group I (archaea), Micrococcaceae and Piscirickettsiaceae (bacteria), and Syndiniales group I (phytoplankton) were significantly enriched in C. polykrikoides bloom samples, compared with the non-bloom ones. Network analysis further showed that microbial compositions were quite different between C. polykrikoides—included module and others. It means that specific microbial clusters were more closely related to C. polykrikoides. Marine group I is supposed to supply vitamin B<sub>12</sub>, while the potential fish pathogens (Micrococcaceae and Piscirickettsiaceae) could partially contribute to massive fish death. Syndiniales group I, a parasite to dinoflagellates, is presumed to induce a sudden collapse of C. polykrikoides blooms. In conclusion, a new insight into the microbiological mechanisms of HABs could be derived through the network analysis of microbial interactions.

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## Distribution of Dinoflagellate Cysts in the Coastal Bottom Sediment of Pangkajene, South Sulawesi, Indonesia

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inoflagellate blooms are one of the rising problems in Indonesian coastal waters. One key component of the recurrent dinoflagellate blooms is the cyst bank which could act as a bloom timebomb that might initiate the future outbreak. Thus, this study aimed to describe and find the potential dinoflagellate bloom source, or cyst banks, and their relationship to the distribution of sediment particles and composition in the coastal of Pangkajene, South Sulawesi. Samples for this study were collected in 2017 at Northern, Middle, and Southern coastal areas of Pangkajene, with a southward increase in anthropogenic activities. Phytoplankton communities in the water column of Pangkajene showed a high diatom dominance, with a southward decrease in its cell density. In contrast, southward increase in dinoflagellate cell and cyst density was observed. Dinoflagellate communities in the water column were dominated by Ceratium spp., with two potentially harmful genera, Dinophysis spp. and Alexandrium spp., that were also found. Cysts of Protoperidinium spp., Scrippsiella spp., Pheopolykrikos hartmannii were common and abundant in the sediment of the study sites. Cysts of harmful and toxin producer dinoflagellates, such as Margalefdinium polykrikoides, Pyrodinium bahamense, and Gymnodinium catenatum were also found in the sediment. Sand, silt, and clay are the dominant grain size in the study sites, whereas detrital material was the most common sediment component. A potential cyst bank, with cyst density of 240 cyst.g-1 sediment wet weight was found at PK-19, a Southern sampling site in proximity with a large harbour complex. In general, higher cyst density seems to correlate with the high percentage of gravel and low percentage of pebble. In contrast, diversity of cyst seems to be affected by higher percentage of fine sediments, such as silt and clay. None of sediment composition was found strongly and significantly affecting both cyst density and diversity.

## Nutrient Dynamics Controlling the Intensity of Dinoflagellate Red-Tides Occurring in the Southern Sea of Korea: Overview

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e measured dissolved inorganic and organic nutrients, photosynthetic pigments, and short-lived radium isotopes (223Ra and 224Ra) in waters off the southern coast of Korea almost every summer since 2002. In general, the intensity of red tides correlated well with the activity of 224Ra (half-life: 3.66 d) in inshore waters. Thus, Ra isotopes could be successfully used as a tracer of the nutrients from inshore sources in offshore red-tide stations since they are conservative. The occurrence of red-tides was episodic and happened under conditions of depleted inorganic nutrients (nitrogen or phosphorus) which are favorable for the growth of dinoflagellates in competition with diatoms. The timing of red-tide occurrence was also revealed by real-time monitoring of changes in nutrients and the red-tide index using a buoy. In general, the intensities of red tides were proportional to the level of total nutrients as traced by Ra isotopes. Based on the mass balance of Ra isotopes, we found that the main source of nutrients is submarine groundwater discharge (SGD) in this region, rather than other sources previously suggested (i.e., the Yangtze River diluted water or the Kuroshio Current). Although inorganic nitrogen is introduced from SGD originally, it seems to be transformed to organic nitrogen as it is transported from the inner bay to the outer bay where red tide initiates. Therefore, the transport timing of high-nutrient inner-bay waters to the outer bay by winds seem to be critical to trigger red tides.

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## Spatial and Temporal Distribution of HAB Species in Sapian Bay, Western Philippines

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apian Bay is an important water body located in the Western Visayas region. The fishery of the bay provides livelihood to more than 5000 registered fisherfolk in coastal communities and the region itself is among the top shellfish producers in the country. Utilizing HAB monitoring records, the spatial and temporal distribution of harmful algal bloom species in Sapian Bay were analyzed. Results showed that there were a total of 14 HAB species recorded in Sapian Bay from 2013-2019. Most HAB species occurred in the eastern portion of the bay (Stations 7-9), followed by the stations located near the mouth of Sapian Bay (Stations 10-11), while a few were found in north western portion of the bay (Stations 13-14). The most frequently occurring HAB species were *Prorocentrum micans*, *Nitzschia spp.*, *Dinophysis caudata*, *Noctiluca scintillians*, *Akashiwo sanguinea and Pseudonitzschia spp.* Common fish kill-associated species were reported in the months of August-October. Blooms of *Pyrodinium bahamense* occurred during August-November, typically after warmer months of summer and start of rainy season. This study provides baseline information useful for policy making and management of HABs in Sapian Bay, as well as basis for future work in the region.

Key words: Sapian Bay, HAB, fish kill species

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patches.

#### Study on Hydrological Characteristics and Drifting Trajectories of Green Algae in the Western Yellow Sea During the Spring and Summer

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assive green tides caused by Ulva prolifera in the Yellow Sea have occurred every summer since 2007 and have caused huge economic losses for local governments. The Subei (North Jiangsu Province, China) Shoal, with its large-scale Porphyra aquaculture, has been regarded as the most important source of U. prolifera for green tides. A regional 3-D numerical model was built to reveal related hydrodynamic processes. The resolution was fine enough to capture the special topography of the radial sand ridges. One result showed that the prevailing southerly wind during spring and summer and the Changjiang diluted water blocked the way of Ulva prolifera in the Subei Shoal from moving to the Donghai Sea. Occasional northerly wind may help the algae moving southward but finally they tend to travel with the northeastward Changjiang diluted water. Seawater in the Subei Shoal (south of 34.6°N) with high turbidity limited photosynthesis to a certain extent for the young Ulva prolifera there. The ratio of PAR 10 cm under the water surface to that at the surface was about 30%, but in the other clear seawater, it could be above 90%. Ulva prolifera cannot grow well in such turbid waters. This is the major reason that no green tide happens there. Drift bottles and satellite-tracked Argos drifters provide solid direct dynamic evidence that *Ulva prolifera* could drift from the Subei Shoal to Qingdao coastal area and even further north. On average, the drifters drifted at a speed of 11.1 cm s<sup>-1</sup> (288.8 km month-1), which is approximately equal to the speed of floating *Ulva prolifera* 

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## Trends of Phytoplankton Occurrence in the Bays of Puerto Princesa City, Palawan with Emphasis on HAB's-Causing Species

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his study presents the trends on species composition and densities of phytoplankters in Honda and Puerto Princesa Bays, the two major bays of Puerto Princesa City in Palawan, in the west and east portions of the Province, respectively. These bays were sampled from December 2018 to August 2019. A total of 53 species of phytoplankters was recorded from Puerto Bay of which 22, species were dinoflagellates and 31 were diatoms, while in Honda bay a total of 47 species of phytoplankters was recorded, 20 species were dinoflagellates and 37 were diatoms. The occurrence of four toxic dinoflagellates, the Pyrodinium bahamense var. compressum (Böhm) Steidinger, Alexandrium tamarense (Lebour) Balech, 1995, Dinophysis caudata (Saville-Kent, 1881) and Dinophsys miles (Cleve, 1900), were observed on December 2018, January to May 2019 and June to August 2019 in Puerto Princesa Bay. Only two toxic species were observed to bloom at Honda Bay, namely P. bahamense and D. caudata on December 2018, January to March 2019 and July to August 2019. Average monthly species densities ranged from 10 - 3, 200 cells.L-1 (P. bahamense), 10 - 40 cells.L-1 (A. tamarense), 10 - 700 cell.L<sup>-1</sup>(D. caudata), and 3-7 cells.L<sup>-1</sup> (D. miles). At least three physico-chemical parameters, the salinity, temperature, and pH correlated well with densities of toxic dinoflagellates. Particularly, the P. bahamense's densities correlated well with water salinity (r<sup>2</sup>=0.7) in Honda Bay while in Puerto Princesa Bay, P. bahamense with water temperature (r=0.9), and salinity (r=0.86). This study will continue until 2021 to generate more comprehensive data and information concerning the trends of Harmful Algal Blooms (HABs) in the two bays, which have incidences of HABs since 2013 in Honda Bay and 2017 in Puerto Princesa Bay. This project is part of the program, "Hazard Detection and Mitigation Tools for Algal Bloom in Changing Marine Environment' supported by DOST-PCAARRD through the leadership of UP-Marine Science Institute, Diliman, Quezon, City.

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## Seasonal Distribution and Abundance of Four *Alexandrium* Species in Jinhae Bay Korea, 2011~2019

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ver the past four decades, paralytic shellfish poisoning (PSP) has occurred almost every year in Jinhae Bay Korea. Despite the long history of PSP events in Korea, little is known about the dominant PSP-causing Alexandrium species in Jinhae Bay and their seasonal variations. In order to find out which Alexandrium species are the dominant PSP-causing species, 35 Aleandrium cultures were isolated in Jinhae Bay during 2011~2017 and the LSU rDNA sequences of the cultures were analyzed. Four Alexandrium species were identified in Jinhae Bay: A. catenella (Group I), A. pacificum (Group IV), A. affine and A. fraterculus. Based on the sequence information, quantitative real-time PCR (qPCR) using TaqMan was developed for detection and quantification of the four Alexandrium species. Assay specificity and sensitivity of the TaqMan qPCR were confirmed by testing against related organisms and comparing TaqMan qPCR results with microscopic counts. The TaqMan qPCR was used to investigate temporal changes in abundances of the four Alexandrium species in Jinhae Bay. Environmental DNA samples were collected from Jinhae Bay at 1-month internals from March 2011 to July 2019. The 9-year field survey showed that A. catenella appeared in March to May at water temperature of  $6\sim17^{\circ}$ C, A. pacificum appeared in April to July at water temperature of  $12\sim24^{\circ}$ C, A. affine appeared in July to September at water temperature of 18~24°C and A. fraterculus appeared in July to September at water temperature of 18~24°C. PSP in Jinhae Bay mostly occurs in March to May which corresponds to the appearing period of A. catenella. These results indicate that A. catenella is the major PSPcausing species and A. pacificum is partly responsible for PSP in Jinhae Bay.

#### Temperature Increasing Pattern Affect Succession Between *Prorocentrum donghaiense* and *Karenia mikimotoi* in East China Sea

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oth Prorocentrum donghaiense and Karenia mikimotoi are two common harmful algae bloom causative species in the East China Sea. Especially for the latter, its blooms have caused huge economic losses. However, little is known about K. mikimotoi bloom formation and succession in East China Sea. An experiment with two temperature increasing patterns (increasing at 0.5 and 1°C per day from 16 to 28°C) was conducted. Also K. mikimotoi lethal effect was studied using Artemia salina shrimp bioassay. The maximum growth rate and maximum photosynthesis efficiency (Fv/Fm) of P. donghaiense was observed at 19.5 – 21°C whereas to K. mikimotoi occurred at 23 -24 °C. The growth rate and photosynthesis efficiency in K. mikimotoi increased with the increase of temperature above 22°C while P. donghaiense had maximum physiological changes when temperature was below 22°C. K. mikimotoi could take more advantage than P. donghaiense from fast temperature increasing pattern as the latter reached a lower highest abundance than in slow increasing pattern. It implied that the temperature of 22°C, no matter how fast it reached, can trigger K. mikimotoi bloom formation and succession from P. donghaiense blooms. Lethal effect of K. mikimotoi to A. salina shrimp rose from 45 % to 72 % when cell concentration was 500 and 1000 cell ml-1 respectively after 48 hours suggesting that marine organisms can be highly susceptible to death with the increase of toxic algal blooms. Generally, variation and physiological adaptation of the two species in relation to temperature changes simulates the Prorocentrum-Karenia bloom formation, occurrence and succession in East China Seas.

# Microbial Community Composition in Harmful Algal Bloom (HAB)-Impacted Coastal Waters of Bolinao and Anda, Pangasinan Using Metagenomic and 16s rRNA Metabarcoding Approaches

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icrobes and phytoplankton are engaged in a complex symbiotic relationship where both are dependent on each other for growth and development. These algae-bacteria interactions influence the development and decline of harmful algal blooms (HABs) especially in nutrientrich coastal waters. In this study, we characterized the natural assemblage of marine microbial communities in Bolinao-Anda, Pangasinan – a site impacted by anthropogenic events, primarily mariculture – where harmful algal blooms (HABs) have been reported. Comparative analysis of shotgun metagenomic and 16S rRNA gene sequencing data was employed to profile HAB-associated microbial taxa and their relative abundances. Our results revealed high and consistent microbial diversity and abundance in the Bolinao-Anda complex. Representatives of the bacterial groups Alphaproteobacteria, Betaproteobacteria, Cyanobacteria, Gammaproteobacteria, Flavobacteria, and Actinobacteria dominated the water columns from three sampling locations. Genera from these groups were reported to be associated with HAB development. Archeal sequences from Euryarchaeota and Thaumarchaeota were also detected, but at a relatively low frequency. Viral taxa were also present at low abundance. The results further revealed that shotgun metagenomic data provided more accurate taxonomic assignments and abundance estimates of the community taxa to the species level compared to 16S rRNA gene sequences. This was also supported by the lower percentage of unassigned taxa resulting from shotgun metagenomic analysis. Moreover, shotgun metagenomic sequencing also made possible the prediction of potential functional roles carried out by microbial communities (metabolic activity, cellular processes and signaling, energy conversion) to understand the underlying mechanisms of harmful algal bloom (HAB) formation and termination in aquatic ecosystems.

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## Biochemical Profiling of *Pyrodinium bahamense* var. compressum from Masinloc Bay, Zambales

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yrodinium bahamense var. compressum is a HAB-causative dinoflagellate that affects subtropical and tropical coastal environments. It remains to be the major paralytic shellfish poisoning-causative organism in the Philippines. Biochemical analysis can be used as a tool to understand the behavior of an organism to changes in its environment, as well as to identify possible chemotaxonomic markers. Here, we characterized the lipid, carbohydrate, and protein content of P. bahamense. Lipid analysis was done by converting the fatty acids to fatty acid methyl esters (FAMEs), subsequently analyzed using gas chromatography with flame ionization detector (GC-FID). Carbohydrate content was determined using liquid chromatography coupled with mass spectrometry (LC-MS) and compared to monosaccharide standards. Global proteome profiling was performed through gel-free shotgun proteomics approach. The results will serve as the baseline biochemical profile for vegetative P. bahamense in the study of different stages of its life cycle.

## Harmful Algal Bloom Occurrences in Waters of Palawan, Philippines

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armful algal bloom (HAB) specifically the dinoflagellate *Pyrodinium bahamense var compressum* has been occurring in three important bodies of water in Palawan, namely: Inner Malampaya Sound (IMS), Honda Bay (HB), and Puerto Princesa Bay (PPB). These bodies of water are major fisheries and aquaculture area, tourism and recreation area, and commercial with port area, respectively. To characterize the HAB occurrence in these areas, this study presents year-round data of *P. bahamense var compressum* cell density from designated sites within the three bodies of water, with 12-month data for IMS and 72-month data for both HB and PPB.

The highest recorded density of *P. bahamense var compressum* in IMS was 22,669 cells/L (August 2017), in PPB was 10,532 cells/L (June 2018), and in HB was 5,092 cells/L (April 2018). From 2017 to 2018, the three areas had been declared positive for paralytic shellfish poison (PSP) by the Bureau of Fisheries and Aquatic Resources (BFAR) from 03 August – 08 December 2017 for IMS, 03 August – 17 November 2017 and 18 June – 28 December 2018 for PPB, and 05 - 28 December 2017and 12 January – 21 September 2018 for HB. The HAB period in IMS coincided with high precipitation, indicating influx of nutrient load from upland and surrounding agricultural land. The HAB occurrence in IMS was shorter in duration with higher cell density than in HB and PPB. On the other hand, the HAB occurrence in HB and PPB was longer in duration with lower cell density than in IMS. This study provides relevant information for the education of local communities on management of shellfish fisheries and aquaculture affected by PSP as well as the management of land activities as major source of nutrients for these bodies of water.

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## Assessment of Algal Bloom Using Remote Sensing Reflectance Data of Aqua-Modis Satellite in the Jakarta Bay, Indonesia

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he Jakarta Bay has been experienced severe eutrophication over 3 decades due to very high input of nutrients from 13 rivers, which originated from human activities in the Jakarta metropolitan city and its hinterland cities called Jabodetabek (Jakarta-Bogor-Depok-Tanggerang-Bekasi) areas with a population of nearly 30 million. The study have been conducted to develop an empirical model to estimate phytoplankton abundance (cell/m³) using remote sensing reflectance data (Rrs) of Aqua-MODIS satellite that obtained from Giovanni NASA's web, and field phytoplankton sampling data of 2008-2011, 2013 and 2015. The empirical model shows that the average ratio of Rrs all Green/Rrs all Red bands correlated strongly with phytoplankton abundance (R2=0.74). This empirical model was used to map the phytoplankton abundance and then was used to explain the massive fish kills in Jakarta Bay. The algae blooms (HAB), which dominated by diatoms during two big fish kill events of 2004 and 2015 have a very high phytoplankton abundance of 2 and 3 million cell/L, respectively compared to normal condition. The massive fish kills were triggered by drastically the depletion of dissolved oxygen (DO) of <2 mg/L (hypoxic) or even to 0 (zero) mg/L (anoxic) and followed by a weak exchange of water masses after HAB. This finding shows that the empirical model developed using Rrs data of Aqua-MODIS satellite can be applied to estimate phytoplankton abundance or algal bloom and then to describe the dynamics of eutrophication in Jakarta Bay and its relation to massive fish kills. Nevertheless, this study still requires a lot of validation.

Keywords: Phytoplankton abundance, Rrs, Aqua-Modis, Fish kills, Jakarta Bay

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## Spatial Assessment of Nutrient Levels and Phytoplankton Communities in Puerto Princesa Bay, Palawan, Philippines

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xamining the dynamic processes that allow for the prevalence of Harmful Algal Blooms (HABs) is vital for the management of aquatic systems, as it reflects the impact of anthropogenic stressors and the health of existing ecosystems. Two weeks after a reported shellfish ban in Puerto Princesa Bay, a field survey was conducted to gather baseline data on nutrients and cell counts of phytoplankton species from several sampling stations within and outside the bay. We assessed the spatial distribution of the nutrient parameters (nitrate, nitrite, ammonium, phosphate, and silicate) in relation to the structure and abundance of phytoplankton communities present in the bay and compared these with other HAB-affected sites in the Philippines. The community structure was dominated by diatom species, accompanied by low concentrations of nitrate (0.39 - 1.12 μM), nitrite (0.05-0.39 μM), and orthophosphate (0.24-0.74 μM). Levels of ammonium varied across the bay, ranging from 1.22 to 14.72 µM while silicate was relatively high with values within the range of 5.83 to 50.66 µM. Generally, higher nutrient concentrations, with the exception of nitrate, occurred at the northern part of the bay. Nitrate and nitrite peaks were observed at one sampling station situated outside the bay. Meanwhile, high cell counts were found at sampling sites near the center and mouth of the bay. Identified toxin producing species occurred at very low counts, which included Pseudo-nitzschia sp., Pyrodinium bahamense, and Dinophysis sp.. Insights from the data obtained can be used for the planning and the conduct of more effective future monitoring activities in the area.

## The Potentially Harmful Benthic Dinoflagellates in Seagrass and Macroalgae of the Pari Island, Indonesia

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ari Island is part of Seribu Island archipelago located northern of Jakarta, the capital city of Indonesia. The island has undergone drastic changes due to rapid development of tourism which increases the anthropogenic pressure to its various ecosystems, such as seagrass bed and macroalgae patches. The previous study on those ecosystems suggests that there was potential harmful benthic dinoflagellate bloom, which could threaten the health and economy of the island community. Thus, this study aims to study the benthic dinoflagellates in seagrass and macroalgae, which focused on the harmful genera of Amphidinium, Coolia, Gambierdiscus, Ostreopsis, and Prorocentrum. Samples for this study were collected in June 2019 at Pari Island, Indonesia, from the seagrass beds and macroalgae patches using an artificial substrate. The artificial substrates were made from plastic with a size of 10 x 15 cm (surface area of 162.64 cm<sup>2</sup>) and were deployed for 24 hours next to the seagrass bed and macroalgae patches. Water samples were also collected and then analyzed using a spectrophotometer to determine orthophosphate, nitrate, and silicate concentration. The Bray-Curtis clustering analysis (simple average link) showed low similarity (31,6%) of harmful benthic dinoflagellates compositions between seagrass and macroalgae stations. In general, the density of harmful benthic dinoflagellates in the seagrass (3.840 cells/100cm<sup>2</sup>) was higher than the macroalgae (917 cells/100cm<sup>2</sup>). Coolia, Gambierdiscus, and Prorocentrum were present at all stations in both seagrass and macroalgae areas. Gambierdiscus and Prorocentrum were the most abundant genus at all stations, with were average 885 cells/100cm<sup>2</sup> and 864 cells/100cm<sup>2</sup>. Therefore, this study suggests further monitoring of harmful benthic dinoflagellate populations within the seagrass bed, in consideration with increasing level of anthropogenic pressure from tourism and domestic activities, which in turn, could lead to a benthic dinoflagellate blooms in the future.

## Remote Identification of In-Water *Noctiluca scintillans*Blooms in the East China Sea Using MODIS Measurements

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River Estuary and the adjacent area of the East China Sea. However, blooms of diatoms, dinoflagellate of *Prorocentrum donghaiense* (*P. donghaiense*) also frequently occurred in the same region. Unlike the *N. scintillans* bloom in floating state, which has extremely high cell abundance on the surface and shows high reflectance in red and near-infrared bands, the in-water *N. scintillans* bloom does not show similar spectral features due to not high enough cell abundance. Thus, discrimination of in-water *N. scintillans* blooms from diatom and *P. donghaiense* ones is currently a challenge work. According the comparison of spectral absorption between *N. scintillans* and other bloom species, we found *N. scintillans* have unique spectral behavior from green to red bands. A multispectral method was developed by combining our previously defined PDI, DI indices, and a simple green-blue band ratio, to discriminate *N. scintillans* from diatom and *P. donghaiense* blooms in a 3D feature space. The validation using observations collected during near-con-current field surveys showed that the classification technique for *N. scintillans* bloom is considered successful, and confirmed that specific MODIS green and red bands have more potential in the bloom type identification.

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#### Spatial Dependencies Between Shellfish Bans in Adjacent Embayments Inferred Through Time Series Information

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etecting dependency relations among sites with recurrent harmful algal blooms is relevant to risk management. Bayesian network and information theoretic approaches were used to analyze discrete -valued time series data (1991-2019) collected by the Bureau of Fisheries and Aquatic Resources on shellfish toxicity in different embayments in Samar and Leyte, Philippines. The optimal Bayesian network structure (BNS) revealed some dependencies between adjacent sites, where observing the state (e.g., clear, shellfish ban start, ban continuation) of one site decreases the uncertainty about the state of the other site(s). High values for mutual information, a measure of the amount of shared information between two variables, were consistent with the BNS. Moreover, information about one site at a given time could provide information about other sites at later time points, as measured by transfer entropy. Information can flow to non-adjacent sites, especially with time lags of more than a month. This study proposes an approach for enhanced understanding of bloom patterns in potentially connected systems in the absence of fine-scale hydrodynamic models.

### Paralytic Shellfish Toxins Monitoring in Balite Bay, Davao Oriental

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looms of *Pyrodinium bahamense* and paralytic shellfish toxin (PST) contamination in in shellfish in been from Balite Bay in Davao Oriental has been reported several times. To mitigate toxic algal blooms's negative impact in human health, BFAR Region XI implements a monitoring program. Sample collection for shellfish and water are regularly conducted. Shellfish are analyzed for PSTs using immunoassay in lateral flow immuno-chromatography format and *Pyrodinium bahamense* are counted and reported in terms of cells per liter. The recent bloom of *Pyrodinium bahamense* reported in Balite Bay was from March - July 2019. At the peak of the bloom *Pyrodinium bahamense* cell densities of 8047 cells-L-1 to 2360 cells-L-1 for stations 1 and 2, respectively. Shellfish consisting of *Anadara* sp. and *Katelysia* sp were positive or PSTs. Monitoring data showed that shellfish did not test positive at low *Pyrodinium bahamense* ranging from 7 cells-L-1 to 80 cells- L-1. Anadara sp and Katelysia sp are classified as benthic to which negative results can be attributed to for exposure to toxic organisms is less as compared to epifaunal species.

# Reclaiming Participation: A Critical Reflection of Community Organizing Dilemmas in The Context of People-Centered HAB Early Warning System

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articipation and equity in coastal resource management and development were fundamental principles that emerged in the 1970s and 1980s. It challenged top-down and centralized programs which exacerbated inequalities and undermined people's abilities to meet their needs. Participatory approaches are meant to build people's capacities to control and manage resources for increased productivity, sustainable coastal environment, and economic upliftment. Communities all over the country continue to carry the burden of environmental, health, and socio-economic impacts of various coastal challenges such as harmful algal blooms. The poorer sectors of the communities, particularly the small shellfish growers and subsistence fishers, experience loss of livelihoods and prolonged periods of hunger. Up to now, these marginalized sectors still cannot access and help shape policy discussions and decision-making regarding HAB mitigation and responses.

There is growing awareness that managing HAB risks and impacts cannot simply depend on HAB science and technology. It requires a broader and deeper understanding of underlying challenges such as people's lack of access to scientific information and their capacity to utilize and evaluate these types of information. Other challenges include barriers to collaboration between professional science workers and community people, the elite status of science, extractive research without giving back to the community, and the norm of patronizing local knowledge.

This is a critical reflection of the work of community development practitioners, environmental, disaster, and marine scientists with 3 coastal communities in Jiabong, Sapian, and Bolinao. Community organizing is a fundamental strategy in reclaiming participation, and creating social and environmental changes to reduce the risks associated with HABs.

The reflection begins with the following aspects: a) a critical view of science; b) understanding the coastal community, its people, development challenges and power structures; and c) an examination of the nature of participation and community organizing. These set the backdrop for analyzing the dilemmas in community organizing as experienced in working with fishers and shellfish farmers in the 3 communities. Finally, lessons are drawn to inform future directions of community organizing and community engagement for HAB early-warning system development, and the larger risk reduction and management efforts for HABs. For instance, scientists' and community's knowledge must both be valued in HAB science. In addition to that, development workers must ensure that engagement with communities do not reinforce power imbalances and the obstacles for collective decision-making. It is hoped that this process will affirm the interdisciplinary approach to solving problems, the adaptability to uncertainties and complexities in coastal ecosystems and communities, as well as the conservation of the marine environment.

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#### Benthic Dinoflagellates in the Coast of Jeju Island

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arine benthic (sand-dwelling and epiphytic) dinoflagellates consists of known potentially toxic species, which are harmful to marine organisms as well as human by consumption of sea food, alerting scientists, aquaculture industry, and government in mostly tropical and subtropical region. Occurrence and distribution of these species are less studied from temperate region. Occurrence and distribution of benthic dinoflagellates in the intertidal zone along the coasts of Jeju Island, Korea was determined by monthly collection of sand sediment and macroalgal samples from several sampling locations during the last 10 years. Identification and taxonomic observations were made of the benthic dinoflagellate samples using light and epifluorescence microscopy. More than forty dinoflagellate taxa were identified. Among them, twenty six were found only in sand sediment, seven in macroalgal samples and four were found in both sand and macroalgae samples. We have found five unrecorded species in Korean waters as follows, Sinophysis canaliculata, Bispidodinium angelaceum, Cabra aremorica, Prorocentrum bimaculatum, P. tsawwassenense. A tentatively new species belong to Heterocapsa will be reported. Recently Gambierdiscus jejuensis sp. nov. has been reported by a research group in Korea. Continually a few unrecorded and/or new species will be expected to be reported as well. The most frequently occurring species was Amphidinium carterae, followed by A. operculatum, Coolia malayensis, and Ostreopsis ovata, occurred at all sampling stations. G. yasumotoi and Gambierdiscus sp. were rarely occurred. Monthly variations in number of occurring benthic dinoflagellates showed that higher number of

- 34 psu) and low to moderate temperature ( $12 - 20^{\circ}$  C). Epiphytic dinoflagellates did not show specific preference for macroalgae species as host. Of the species, potentially toxic species were present throughout the

species could be found during early of spring to early summer (March to June) with moderate to high salinity (26

year, however, varied within the stations.

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## Rediscovery of *Ostreopsis siamensis* (Dinophyceae) from the Inner Gulf of Thailand and Surrounding Waters

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he toxigenic dinoflagellate genus Ostreopsis includes eleven morphologically similar species. Unfortunately, most of them do not have molecular sequences available, including the type species Ostreopsis siamensis. Ostreopsis ovata is morphologically similar to O. siamensis and differs only in size and shape. To fully understand the taxonomic relationship between O. siamensis and O. ovata, we established eight strains of O. siamensis from the type locality and surrounding waters. Their morphology was examined by light, scanning electron and transmission electron microscopy. All strains are broadly oval and indistinguishable in morphology. Cells of strain TIO897 from the type locality are 39.0-58.9 um in dorsoventral diameter deep and 29.6-50.3 µm wide. The thecal plate pattern is APC, 3', 7", 6c, 6s, 5", 2"", and the second apical plate (2') is narrow and elongated and contacts plate 4". Transmission electron microscopy revealed a mucus canal of 1.8–3.8 µm long and 0.4-0.5 µm wide which is surrounded by numerous mucocysts. Molecular phylogeny was inferred based on two independent regions of LSU rDNA (D1-D3 and D8-D10) and ITS rDNA sequences using maximum likelihood and Bayesian inference. Our results showed that O. siamensis comprises of three ribotypes (referred as ribotypes A, B and C), and each of them has a wide geographical distribution. The geographic range of these ribotypes is mapped. The results also suggest that O. wata might be a junior synonym of O. siamensis. Toxin analysis of four strains of O. siamensis ribotypes A and B was performed by LC-MS/MS but none of them produce detectable palytoxins (PLTXs) and ovatoxins (OVTXs), suggesting that toxin production might help to differentiate ribotypes.

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### Taxonomy of *Prorocentrum* (Dinophyceae) in East Asian Waters

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rorocentrum is a kind of dinoflagellate with cosmopolitan distribution. Many species of Prorocentrum can produce the diarrhetic shellfish poison (DSP), which can enter the food web through the prev of fish and shellfish. So far, there are many studies only on serval main species of *Prorocentrum*, such as Prorocentrum donghaiense (pelagic) and Prorocentrum lima (benthic) in China, even there still lack of deep studies in East Asian Waters. To this big genus with about 80 species, it is apparently that there is not sufficient on the study of Prorocentrum biodiversity and geographical distribution. Based on our previous work, samples will be taken by collecting the surface water and using vacuum collection, artificial substrates as well as other methods. The targeted species and strains will be isolated and cultured in lab condition. Normal light microscope, laser scanning confocal microscope, SEM and TEM will be used for morphological study. The molecular identification will be also performed by amplifying and sequencing for specific genes. The biogeographic distribution pattern of Prorocentrum in typical coastal habitats of East Asian Waters will be constructed, which will also be contributed to improving global distributive pattern of these important species. So far, we have collected more than 15 species, such as Prorocentrum clypeus, P. concavum, P. donghaiense, P. koreanum, P. lima, P. micans, P. minimum, P. triestinum, etc.. In addition, it will be beneficial for further study on deep research of marine biotic resources, maintaining marine ecosystem health and monitoring or early warning of HABs.

# Ultrastructure and Phylogeny of a Harmful, Micropredatory Species of *Karlodinium* (Kareniaceae, Dinophyceae) Found in Manila Bay, Philippines

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arlodinium is a small, marine, naked dinoflagellate belonging to the family Kareniaceae, which includes members forming harmful red tides. The morphology, ultrastructure and phylogeny of a Karlodinium species, isolated from Manila Bay, Philippines in June 2017, were examined by LM, SEM, TEM and molecular phylogeny inferred from ITS and LSU rDNA sequences. Cells possessed a straight apical structure complex and a ventral pore common in the genus, while the size was characteristically large (mean 25.7 µm long) in the genus. The longitudinally elongated nucleus was positioned at the center, and the yellowish-brown chloroplasts possessed an embedded pyrenoid. HPLC analysis revealed the presence of carotenoid pigments typical in the genus, i.e., fucoxanthin as major carotenoid with its derivatives (19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin). Cells had numerous reflective particles distributed peripherally, and accumulated abundant starch grains and lipid granules. Cells which were usually settling at the bottom of culture vessel was also characteristic. TEM revealed a portion of the flagellar apparatus with a long striated ventral connective, which is a first report in the Kareniaceae, and electron-dense vesicles associated with the peduncular apparatus. Under SEM, a slit-like ventral pore and a peduncle connected with the ventral ridge were observed. Molecular phylogeny showed the affinity of the species to K. australe and K. armiger. These morphological and phylogenetic data suggest that it is an undescribed species in Karlodinium. Exposure of brine shrimp, Artemia franciscana, to these cells resulted to Artemia mortality after 48 h. It also exhibited a micropredatory feeding, probably by myzocytosis using the developed peduncle, on individuals of A. franciscana and other zooplankton, larger than the dinoflagellate.

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## Morphology and Pigment Composition of an Unarmored Dinoflagellate Resembling *Gertia stigmatica* (Kareniaceae, Dinophyceae) Isolated from Japan

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he harmful unarmored dinoflagellates, Karenia, Karlodinium and Takayama species in the Kareniaceae, possess the unique chloroplasts containing fucoxanthin and its derivatives, instead of peridinin typical in dinoflagellates. It is recognized that the unique chloroplast is derived from the haptophyte, and the peridinin-type chloroplast is replaced with this in the common ancestor of Kareniaceae. Gertia stigmatica, a recently described species possessing the peridinin-type chloroplast, is phylogenetically positioned in the Kareniaceae; this attracts more attention to the unique chloroplast replacement in the family. In the present study, morphology, ultrastructure and pigment composition of a marine unarmored dinoflagellate morphologically similar to G. stigmatica, isolated from Japanese coast, was investigated by LM, TEM and HPLC, and its phylogenetic position was inferred from SSU, ITS and LSU rDNA sequences. Cells of the species were larger than G. stignatica (17.1 µm vs 8.0 µm), ovoid with the smaller epicone than the hypocone, and having a straight apical structure complex at the cell apex. Chloroplasts were yellowish brown, highly blanching and abundant in periphery of the cell. Under TEM, three stacked thylakoids were observed as in typical dinoflagellates of the peridinin-type. An eyespot, of which type-A was reported from G. stigmatica, was not observed under LM and TEM. HPLC revealed that its chloroplast is of the peridinin-type; the major photosynthetic pigment was peridinin, without fucoxanthin and its derivatives. A phylogeny based on nucleusencoded rDNAs suggested a position of this species in the Kareniaceae, together with Gertia stigmatica.

#### Identification and Rapid Detection of Harmful Algae Species in Malaysian Waters Using Molecular Techniques

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armful Algal Blooms (HABs) have been increasingly reported over broader geographical regions, with no exception to Malaysian waters of the South China Sea, notably with the increased emergence of new or unrecorded harmful algal species. With the advancement of molecular technology, it becomes more cost-effective-and technically feasible in identification and detection of HABs species, and to replace microscopy techniques that is generally time consuming and lack of reliability and sensitivity. In this presentation, molecular techniques applied in HABs research including single cell and quantitative real time PCR (qPCR) and metebarcoding of environmental DNA will be shared. Single-cell PCR has been demonstrated to be useful in rapid confirmation of new morphotypes and uncultivable species (mostly mixotrophic dinoflagellate species). Quantitative qPCR methods developed for several HAB species have been proven to be reliable and efficient in rapid and early detection of HABs (e.g. Alexandrium tamiyavanichii, Karlodinium australe). This method is particularly useful to aid identification of small dinofagellate species that is microscopically challenging. Metagenomics approach was adopted to establish distribution and baseline data of HABs species and phytoplankton community compositions along the east coast of Peninsular Malaysia. At least 70 taxa of phytoplankton species were identified from the V9 metagenome data. The paralytic shellfish toxin producer, Alexandrium tamiyavanichii, was found to be widely distributed in the water. Our results also revealed the distribution of 10 Alexandrium species (i.e. A. affine, A. andersonii, A. fraterculus, A. leei, A. margalefii, A. monilatum, A. ostenfeldii, A. insuetum and. A. tamerense). Several ichthyotoxic species Margalefidinium polykrikoides, Karlodinium veneficum and Chattonella spp. were identified genetically. Molecular techniques shared in this presentation has been shown to be reliable tools in identification and rapid detection, that aids in risk assessment of HABs occurrence along the east coast of Peninsular Malaysia.

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## Molecular Understandings of the Harmful Algal Blooms (HABs): Our Recent Progress

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he marine dinoflagellates are eukaryotic protists which exhibit a great diversity of forms, and are the most important primary producer in aquatic environments. However, some species can form harmful algal blooms (HABs; referred to red tide), and even contain biotoxins that affect humans and many other organisms. As genomic aspects, they have distinct genomic characters (e.g., permanently condensed and liquid-crystalline chromosomes, huge nuclear genome sizes, low amounts of histones, etc.). So far, possible mechanisms of HABs have been mainly explained by environmental factors such as sun light, temperature, nutrients, etc. In this study, we investigated certain internal factors for cellular mechanisms of HABs, considering gene and genome response in cells exposed to different environmental conditions. Particularly, we examined antioxidant genes and proteins (e.g., glutathione Stransferase, superoxide dismutase, lipid peroxidation, catalase-peroxidase, etc.) under environmental stress conditions. In addition, we investigated genome-scale transcriptional responses under treatments of some algicidal agents. This provides molecular understanding cellular and genomics mechanisms for the harmful algal blooms.

## Characteristics and Causes of Algal Blooms in the Southern Coastal Waters of Korea Occurred During the Summer

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his paper aims to provide an overview on the characteristics and cause of the algal blooms that have been occurred in the southern coastal waters of Korea since the last three decades. Firstly, we discuss the discriminant function introduced to understand the cause and establish the prediction method of algal blooms occurring in Jinhae Bay which is one of the most contaminated bays of Korea. In particular, two semi-enclosed inlets of the bay, Masan and Haengam areas, and Dangdong and Wonmun bays had different types of causes and patterns for algal blooms. In Masan and Haengam areas, algal blooms occurred in June in relation to the stratification and increase in planktons. On the contrary, in Dangdong and Wonmun areas, algal blooms mainly occurred between July and October in relation to meteorological and physical factors or water quality environmental factors appeared to contribute to the occurrence of algal blooms. Secondly, we examine marine environments of Masan and Jinhae bays because algal blooms had been not only first but also frequently occurred in those bays in the history of Korea. The result showed that algal blooms were closely related to the development of anoxic water masses. In addition, Skeletonema costatum, Heterosigma akashiwo and Prorocentrum sp. commonly appeared as the dominant organisms. In particular, algal blooms were more likely to occur when precipitation ten days in prior to the blooms was greater than the long-term mean values. Thirdly, we investigated marine environments when algal blooms occurred in Gamak Bay from 1984, the first occurrence year, to 2006. We identified 23 causal species such as *Prorocentrum* sp., Cochlodinium polykrikoides, Chaetoceros sp., Skeletonema costatum, and Heterosigma akashiwo. In addition, a principal component analysis (PCA) indicated that algal blooms by those causal species appeared to develop under different conditions in terms of water temperature, salinity, precipitation, and insolation. On the other hand, field data proved that Cochlodinium polykrikoides to be favored by high temperatures and to be euryhaline, whereas Skeletonema costatum appeared to be eurythermal and euryhaline. Prorocentrum sp. and Chaetoceros sp. appeared to be stenothermal and stenohaline. Furthermore, Heterosigma akashiwo appeared at the lowest temperature and highest salinity of the five species and was classified here as stenothermal and stenohaline. Finally, we conducted field observations to investigate the cause of Cochlodinium polykrikoides blooms in the Korean southern coastal waters (KSCW) and also analyzed information on marine environmental factors obtained from the National Fisheries and Development Institute(NFRDI) from 1995 to 2008. Long-term tidal currents revealed a two-layered flow with the upper current flowing toward Boddolbada located between Narodo and Nammyeon, and the lower current out of the bay. Water temperatures were higher at the north that at the south of the bay, whereas salinities exhibited the reverse trend. C. polikrikoides blooms occurred at a water temperature of approximately 25-26.0°C and a salinity of approximately 31.00 in the seas neighboring Narodo Islands. The outbreak of C. polykrikoides blooms in the seas neighboring Narodo Islands in summer was influenced by thermohaline fronts observed between the KSCW and Tsushima Warm Current (TWC).

**Keywords**: Discriminant function, algal blooms, Jinhae Bay, Water quality environmental factors, Causative organisms, *Chaetoceros* sp., *Cochlodinium polykrikoides*, *Heterosigma akashiwo*, PCA, *Prorocentrum* sp., *Skeletonema costatum*, *Cochlodinium polykrikoides*, Thermohaline fronts

## Metagenomic Analysis of Harmful Algal Species in Jiaozhou Bay

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ccurate detection of the composition and dynamics of harmful algal bloom (HAB) species is critical for studying mechanisms of HAB formation, and for developing means for predicting the occurrences of HABs. Jiaozhou Bay is an epitome of China coastal ecosystem and an ideal site for HAB research with the accumulation of decades of historical investigation records. Nevertheless, most of these earlier studies on phytoplankton communities applied primarily morphology-based approaches with limited resolution in phytoplankton species identification, especially for those with small-sized cells and cryptic species. Through analyzing samples collected at 12 spatially isolated locations using metagenomic methods, 110 phytoplankton species, including 40 Bacillariophyta, 22 Dinoflagellata, 15 Cryptophyta, 13 Chlorophyta, 13 Ochrophyta and 6 Haptophyta species were detected. Of those, 93 species had never been reported in the previous voyage investigations in Jiaozhou Bay, demonstrating strength of the metagenomic approach. Many algal species demonstrated unique distribution patterns, which were likely influenced by interactions among phytoplankton species, or by predation of Ciliophora and Cercozoa species. Among these algal species, 28 HAB species were annotated, 13 of which were reported for the very first time in Jiaozhou Bay, including a mixtotrophic dianoflagellate Heterocapsa rotundata, a chain-forming diatom Skeletonema marinoi, and a cryptophyte Teleaulax acuta. The present study represents a first attempt to study HAB species and other phytoplankton species in Jiaozhou Bay using the metagenomic approach, which revealed substantially more algal species in Jiaozhou Bay than previously identified, and sets a foundation for further research on mechanisms of HAB formation.

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### Recent Researches on Epiphytic Dinoflagellates in Korean Seas

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he occurrence of epiphytic dinoflagellates (EPDFs) in Korean seas was firstly reported in 2011 with samples from Jeju coastal waters, which is far behind the developments in china and Japan. Early reports on new EPDF genus by European researchers precedes Asian works by 110 years. During last 8 years of Korean EPDFs exploration more than 25 papers in peer-reviewed journals along with 2 MS thesis and several project reports were published, which covers taxonomy, distribution, growth response, feeding of EPDF species as well as bioactive MNPs from lab strains. Quantitative monitoring of EPDF species at some 20 station in Korean coastal waters for more than 5 years might hint on the issue of the potential northward shift of the northern boundary for the overwintering EPDF species in Korean seas. Long-term and gradual increase in population densities of each EPDF species on each of the different kinds of attaching substrates in each fixed monitoring station needs to be documented before the northward shifting velocity of the overwintering boundary could be estimated in the "remarkably" climate-change-sensitive Korean seas. Further, long-term investigation on the marine ecosystem including EPDFs-associated communities is of great importance for environmental safety and progressive oceanography.

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### The Fate of Massive Floating Green Algae in the Yellow Sea

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ince 2007, large-scale green tides formed by green alga Ulva prolifera have been recorded consecutively in the southern Yellow Sea. The green tides in the Yellow Sea, which are different from green-tide events in other regions around the world, have huge biomass of green algae about several million tonnes, and impact an extensive area up to 50,000 km<sup>2</sup>. Green tides in the Yellow Sea will change the distribution, concentration, and composition of nutrients, and probably affect the structure of the phytoplankton community, or even lead to derived ecological disasters (hypoxia and acidification) in a specific region. However, there is still little knowledge on the settlement region of massive floating green algae in the southern Yellow Sea, which limits the understandings on the ecological effects of green tides. To track the settlement region of massive floating green algae, 28-isofucosterol was proposed to be feasible sedimentary biomarker of green algae in genus Ulva. The methods for the extraction and determination of sterols in algae and sediments, including 28-isofucosterol, were established using gas chromatography-mass spectrometry (GC-MS). Based on laboratory simulation experiments, the specificity and stability of 28isofucosterol were examined, it was found that 28-isofucosterol in sediment was positively correlated with the amount of decomposed *U. prolifera*, and it remained stable for at least one month in the sediment. The seasonal and inter-annual distribution of 28-isofucosterol content in surface sediment samples collected from the Yellow Sea and Bohai Sea was then analyzed. It was found that massive floating green algae was mainly settled in the region (121-124°E, 36-36.5°N) southeast to Shandong Peninsula. The findings will help to further understand the ecological consequences of green tides and promote our knowledge on the ecosystem changes and their driving forces.

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### Field Application of a Red-tide Acoustic Sensing System (RASS) for Monitoring of Fish Killing Dinoflagellate Cochlodinium polykrikoides in Korean Coastal Waters

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armful algal blooms (HABs) caused by marine phytoplankton pose a significant threat to human health and result in enormous economic loss. In Korea, economic and ecological damage caused by Cochlodinium polykrikoides occurs annually, and great human and financial efforts are being made to detect and manage their impacts. Many strategies have been implemented for their detection, management and control. One such instrument is the Red-tide Acoustic Sensing System (RASS) which utilizes a backscattered acoustic signal to detect microalgal cells in the water column, proving to be a cheap and easy tool for the C. polykrikoides detection. In the present study, five RASSs were deployed at fish-farms located in C. polykrikoides -occurring areas of South Korea during the summer of 2018, and their performance examined for the C. polykrikoides detection. From July 26th to 6th August 2018, the dinoflagellate C. polykrikoides was the most abundant HAB species across all locations. A standard curve between the relative received level (RRL) of acoustic intensity as measured by RASS and cell densities of C. polykrikoides species was constructed ( $R^2 = 0.846$ ) and was successfully applied to quantify the HAB cell density. The RRL values noticeably increased from 25th July 2018 and decreased from 1st August. The RRL values corresponding to warning level of HABs (≥ 100 cells · mL-1) were not measured after 5th August. The highest 22 RRL was recorded on 29th July, which corresponded to 2,782 cells mL-1. To evaluate the efficacy and accuracy of the RASS, a significant correlation ( $R^2 = 0.997$ ) between HAB cell abundance and backscattered acoustic signals was confirmed. Our field data strongly suggests that RASS is an accurate and efficient tool for monitoring and alerting for HABs. Future work involves further fine tuning and optimization in the natural environment under a range of conditions, with the aim of reducing the background noise and more accurately quantifying the biomass of target HABs.

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# Algicidal Effects on *Chattonella marina* and Planktonic Community by Yellow Clay and Thiazolidinedione Derivative TD49 in a Mesocosm

Lee, M.1, Son, M.2, Baek, S. H.1

esocosm system (1000 L) was used to evaluate the potential of the algicide thiazolidinedione (TD49) and yellow clay to control *Chattonella marina* and to assessed their ecological risk for the planktonic community. Exposure experiments was measured at 12h and 24h intervals for a period of 9 days, based on *in vivo* fluorescence. TD49 controlled the growth of *C. marina* at ≥0.4 μM, but large amount of yellow clay treatments at 1 kg t<sup>-1</sup> could not inhibit enough. Especially, the algicidal activity of the TD49 (0.4 μM) + yellow clay (0.4 kg t<sup>-1</sup>) treatment was highest at 72.5 % to *C. marina*, but fluorescence increased after time. In all treatments, depletion of silicate in initial stages played a key role in species succession from diatoms to cryptophytes species. Although, the yellow clay treatments not affect to zooplankton community, TD49 substance had a negative effect to zooplankton abundance in initial stages. However, nauplii gradually increased in the final stages. Therefore, the initial concentration of substances and nutrients concentration were critical in determining the timing of shifts in the planktonic species composition. In conclusion, this study using mesocosm found that the toxic effects on natural planktonic communities are limited and that TD49 may be safe to apply to the selective regulation of *C. marina* blooms.

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#### Benthic Harmful Dinoflagellate Assemblages and Community Dynamics in Relation to Benthic Microhabitats and Environmental Variability: A Case Study from the Perhentian Islands Malaysia

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armful algal blooms in the benthic system (BHABs) are a major environmental issue that increased globally under the backgrounds of global climate changes. The detrimental effects of BHABs on marine coral ecosystems and human health make it one of the research priorities in HAB science nowadays. From ecological perspective, the following key questions need to be answered: What are the distribution patterns of BHABs in space and time? How BHABs respond to the environmental changes? What are the possible relationships with the seasonal benthic community composition? What is the status of BHAB threat to coral ecosystems? In this presentation, we explored the BHAB assemblages at spatial and temporal scales on a shallow reef flat of the fringing reef of Rawa Island, Malaysia, and examined the functional role of benthic microhabitat characteristics and its environmental variability in driving the community dynamics. A field-based survey was undertaken between 2015 and 2017. Sampling of benthic harmful dinoflagellates was carried out using an artificial substrate sampling method. Benthic microhabitats surrounding the sampling screens were characterized simultaneously by photo-quadrat method. Five taxonomic groups of BHABs: Ostreopsis, Gambierdiscus, Prorocentrum, Amphidinium, and Coolia were identified and enumerated microscopically. The primary finding revealed that microhabitat heterogeneity as a key factor governing the benthic harmful dinoflagellate assemblages and may account for much of the observed variability in dominant taxa. This finding has significant implications for the role of variability in the BHAB outbreaks and the potential in identifying BHAB-related toxin transfer pathways and the key vectors in the food webs. The application and datasets could serve as a fundamental basis of consideration in BHAB monitoring strategies in potential CFP prone areas.

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#### **COAST-1**

#### Mga Karanasan at Kaalaman ng mga Taga-Sapian Tungkol sa Red Tide o Harmful Algal Bloom (Experiences and Knowledge on HABs of the Sapian Community)

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Lonoy Agrarian Reform Cooperative, Sapian, Capiz

aong 1996, nagkaroon ng unang red tide o harmful algal bloom (HAB) sa Sapian Bay, Capiz. Pagkatapos ng halos dalawang dekada, nagkaroon ulit ng HAB sa Sapian noong 2014. Dahil dito, naapektuhan ng shellfish ban ang kabuhayan ng mga mangingisda lalo na ang mga magtatanong, magtatalaba, at nangongolekta ng mga panginhason (shells). Noong nagkaroon ng HAB, nakapagobserba kami ng pagbabago sa kalidad ng tubig: nagbago ang kulay, naging makati sa balat, at mabaho ang tubig. Sa kasalukuyan, kahit walang HAB sa Sapian, alam naming posible pa rin na magkaroon nito. Kung kaya, kinakailangan ang patuloy na pag-aaral at pag-iimbestiga upang mas lalong maunawan ang HAB at makapaghanda ng nararapat na aksyon upang mabawasan epekto nito.

Nagsagawa kami ng mga talakayan at pananaliksik para maunawaan ang risk sa HAB. Mula sa mga pinag-sama-samang kaalaman ng mga mangingisda sa Sapian, nakabuo kami ng community map, seasonal calendar, at hazard assessment table (HAT). Sa pagsasagawa ng community map, nalaman namin ang mga sitio, mga lowland at coastal area, maging ang mga lugar kung saan nakapwesto ang mga talabahan at tahungan. Sa seasonal calendar, nakita namin ang panahon ng iba't ibang kabuhayan, at panahon at pangyayari kung kailan pwedeng magkaroon ng HAB. Sa HAT, nalaman namin ang katangian ng HAB sa pamamagitan ng pag-alam sa mga senyales na maaring obserbahan sa paligid, bilis ng pagdating, pagpapasa ng impormasyon mula sa monitoring hanggang advisory, at ang mga aksyon na aming ginagawa. Nagsagawa rin stakeholder analysis upang mas makilala ang mga tao at grupong may tungkulin at responsiblidad sa mga gawain kaugnay ng HAB. Mahalaga ang tungkulin ng mga mangingisda, lalo na ng mga shellfish gatherer. Mahalaga rin ang aming pakikipagtulungan sa LGU, asosasyon at organisasyon, at ahensya ng gobyerno tulad ng BFAR.

Sa pamagitan ng sama-samang paggawa nito, nagsasama-sama rin ang mga kaalaman ng mga miyembro ng komunidad. Sa pagsasagawa ng ganitong pag-aaral, malaki ang papel naming mga tao sa komunidad. Kami pala ay may kakayahan sa pananaliksik. Matagal na pala kaming researcher. At kami rin ay mga eksperto sa buhay sa dagat. Ang mga kaalaman namin tungkol sa HAB ay nagagamit namin sa pagsasagawa ng aksyon upang mapaunlad ang aming pamumuhay, mapangalagaan ang kapaligiran at makapaghanda sa posibleng epekto ng HAB.

Gamit ang mga natutunan sa mga pag-aaral at pananaliksik, nakapagbuo kami ng mga rekomendasyon. Kinakailangan pang mapataas ang kaalaman ng mga fisherfolks, BLGU, asosasyon at komunidad tungkol sa HAB, sustenableng pamamaraan sa pagtatalaba at pagtatahong sa pamagitan ng pagpapakalat ng impormasyon. Kinakailangang ulitin pa ang mga pananaliksik at pag-aaral na isinagawa namin upang makadagdag pa sa aming kaalaman. At kami, bilang mga community researchers ay padayon lang sa pananaliksik sa pakikipagtuwangan sa komunidad, asosasyon, LGU, BFAR at UP.

#### **COAST-2**

#### Ang Kwento ng Aming Karanasan at Pagtugon sa Red Tide Dito sa Jiabong, Samar (Participatory Assessment of Experiences and Responses to the 2019 HAB Event in Jiabong, Samar)

Quilloza, A., Ladendia, M., Godin, A., Jabon, A., Opiasa, G., Jabien, V., Labendia, L., Labrague, C., Labrague, M., Abanes, B.

Community members of Brgy. Maligaya and Brgy. Malobago Jiabong, Samar

to ang kwento ng aming buhay sa Jiabong at ang aming mga karanasan sa panahon ng red tide. Kami ang mga taga-Jiabong, nakatira sa tabing dagat. Kami ay mga mangingisda at magtatahong, kami rin ay nagbibintol, at nanghuhuli ng hipon at pusit. Iyan ang aming kabuhayan. Mahalaga ang dagat sa amin dahil diyan kami umaasa at nabubuhay. Panawagan naming ingatan natin ang ating karagatan at huwag natin pagtapunan ng ating basura para maibsan din ang pagkakaroon ng red tide. Noong nakaraang Hulyo, huli naming naranasan ang ang red tide, marami ang nagutom sa amin. Mahirap ang aming kalagayan, hindi namin nabibili ang aming pangangailangan lalo na sa aming mga anak na nag-aaral dahil sa kakulangan ng kita. Ang iba sa amin ay nagkakasakit na, lalo na ang aming mga anak, kaya maraming tao ang nahihirapan. Dahil sa gutom, naranasan naming kumain ng mga pagkaing hindi pangkaraniwan sa amin. Naghanap din kami ng iba't ibang trabaho, tulad ng construction at iba pang pagkakakitaang hanapbuhay tulad ng pagpe-pedicab at pagbabenta ng kakanin at iba pa.

Hinarap naming ang problema sa pamamagitan ng pagkakaisa. Nagkaroon kami ng bayanihang paglilinis sa kapaligiran, at ibang alternatibong hanapbuhay. Sumangguni kami sa mataas na mga departamento o ahensya ng gobyerno tulad ng LGU, DA, BFAR, DSWD.

Nagplano kaming lumapit sa brgy officials. Nagsagawa kami ng household listing sa apektadong mga pamilya at nagsumite ng report sa DSWD at DA upang mabigyan kami ng tulong. Dahil dito'y binigyan kami ng DSWD ng relief goods bilang pansamantalang pantawid sa gutom. Patuloy kaming nagpaplano na magkaroon ng asosasyon para hindi masyadong mahirapan sa darating pang mga red tide.

Mula sa aming pagpaplano nakapagtukoy kami ng mga rekomendasyon na gagawin: Pintakasi at patuloy na kooperasyon ng mga tao; Pagbubo ng samahan at pagpapatatag ng samahan; Pakikpag-ugnayan sa LGU at BFAR tungkol sa shellfish ban at epekto nito sa kabuhayan, at upang magkaroon suporta sa kabuhayan ng mga apektadong pamilya; pagkakaroon ng plano at polisiya sa pagde-deklara ng "state of calamity" kapag may HAB; at, pagkakaroon ng mga dagdag na training at pananaliksik tungkol sa HAB, monitoring at warning advisory.

Mahalaga na ipaalam namin ang aming kaalaman dahil kami ang may karanasan sa panahon ng red tide. Alam din namin kung paano masolusyunan ang hinaharap naming ng mga problema. Ang kaalaman ding ito at hangad naming maihatid sa aming mga kababayan.

#### **COAST-3**

#### Ang Kalikasan ay Buhay: Mga Kaalaman at Karanasan ng Samahan ng Maka-kalikasang Mangingisda ng Victory (SMMV) sa Pangangalaga sa Kalikasan, Environmental Monitoring at Early Warning System

## (The Environment is life: Knowledge and Experiences of the Victory Association of Fisheries in Environmental Monitoring and Early-Warning Systems)

Corbillon, R., Corbillon, L., Caasi, P.

Samahan ng Maka-kalikasang Mangingisda ng Victory (SMMV)

ayaman ang dagat na primaryang pinagkukuhanan ng kabuhayan ang mga taga-Bolinao, Pangasinan. Subalit, ang kabuhayan namin ay naapektuhan ng pangyayari tulad ng red tide o harmful algal bloom (HAB). Sa aming pagkaaalam, ang HAB ay ang pagdami ng plankton na nagdadala ng lason o toxin. Kinakain ng mga tahong, talaba at iba pang shellfish ang plankton. Kapag kinain ang shellfish sa panahon na may HAB, maaring magdulot ng epekto sa kalusugan. Nagkakaroon ng HAB dahil sa kondisyon ng tubig tulad ng pagdami ng nutrients o abono, temperature, salinity, turbidity, dissolved oxygen at iba pang salik. Kapag nagkakaroon ng HAB, nakakaapekto ito sa kabuhayan

abono, temperature, salinity, turbidity, dissolved oxygen at iba pang salik. Kapag nagkakaroon ng HAB, nakakaapekto ito sa kabuhayan ng mga mangingisda.

Sapagkat sa kalikasan tulad ng dagat namin kinukuha ang aming kabuhayan, gusto naming itong protektahan at pangalagaan. Ito ang layunin ng organisasyon na Samahan ng mga Maka-kalikasang Mangingisda sa Victory. Kaming mga mangingisda ay may kaalaman at kakayahan sa pangangalanga ng kalikasan. Ang mga kaalamang ito ay galing sa aming mga karanasan sa buhay sa tabing dagat, maging mga pagsananay na ibinigay sa amin. Alam naming tuwing may "aramag" o malakas na hangin, o "dagudog" o hanging galing sa hilaga at silangan, ay walang huling isda. Nagkaroon kami ng pagsasanay sa pagpapalaki at pagpaparami ng mga yamang dagat tulad ng balatan, mangrove, at iba pa. Mayroon din kaming kakayahan sa pag-monitor ng kalidad ng tubig. Sa pamamagitan ng pagmo-monitor, nakakapagbigay kami ng babala kung ang kalagayan ng tubig ay maaring magdulot ng "gataw". Isinusulat namin ito sa mga bulletin board upang maipaalam nsa lahat at makapagsagawa ng aksyon ang mga tao, mga mangingisda, ang local government unit, at iba pa. Dahil sa mga kaalamang ito, madali para sa aming mangingisda na makita ang problema sa dagat.

Bukod sa environmental monitoring, gawain din ng samahan at mga tao ang pagpapataas ng kaalaman para mapanatili at mapayabong ang karagatan, coastal clean-up ang mga kababaihan, pagbubuo ng mga community organizers para magturo din sa iba, pagsasagawa ng public hearing para sa mga proyekto, at iba pa. Sa pagsasagawa ng mga ito, mayroon din kaming kinaharap na hamon tulad ng mga problema sa samahan at miyembro at kawalan ng mga resources para ipagpapatuloy ng mga gawain kapag tapos na ang mga proyekto . Ang mga aral na ito ay magagamit din para mapalawak ang kaalaman sa HAB early warning system.

Sa kabila ng mga hamon, nananatili pa rin ang aming pagnanais na mapangalagaan ang ating kalikasan at magkaroon ng nararapat na aksyon sa HAB. Nais naming maging patuloy ang pagmumulat sa mga tao, pagkalap ng kaalaman, at paghiyat na makibahagi sa gawaing environmental monitoring. Kailangan din matutunan pa na gumamit ng makabagong teknolohiya sa pagmo-monitor. Kasabay nito, ang aming lokal na kaalaman at makabagong teknolohiya ay masusing pagsamahin para mapalakas pa ang siyensya ng pangangalaga ng kalikasan at HAB early warning system. Kinakailangan din ng suporta ng LGU. Nakikita din naming mahalaga ang partsisipasyon ng mga mangingisda sa pagde-desisyon sa mga aksyon tulad ng monitoring, warning, advisory, and pagbababala sa mga tao, paghahanda, at pagtugon kung may HAB.

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# Overall Transition Patterns of Microbial Modules During Succession of *Microcystis* Genotypes Using Network Analysis

Ahn, C.-Y<sup>1,2</sup>, Chun, S.-J.<sup>1,2</sup>, Cui, Y.<sup>1</sup>, Lee, J.J.<sup>3</sup>, Choi, I.-C.<sup>3</sup>, Oh, H.-M.<sup>1,2</sup>

o far, at least a dozen diatom virus species have been isolated based on a culture method. These studies have contributed to reveal biological features of diatom viruses. The culture-based method, however, is time consuming and laborious, furthermore only able to isolate the most dominant virus in a sample. Recent studies have suggested that diatom virus infections are inter-species. For understanding relationships between a host diatom and its infectious viruses, more efficient methodologies to determine these systems are necessary. In this study, we challenged a new protocol to identify previously unrecognized host-virus relationships. The host cell cultures after inoculations of natural sediment pore water samples were obtained before complete lysis, and the proliferated viral genomes in the host cells were amplified using degenerate primers targeted for replication proteins of single-stranded RNA (ssRNA) and single-stranded DNA (ssDNA) viruses, and then sequenced. The results showed that diverse ssRNA and ssDNA virus groups were detected from inoculated *Chaetoceros* cells. Although we should recognize the possibilities of protocol biases affecting the results, for example non-specific adsorptions of virions onto the host cells, the present method would be effective for understanding diversities of viruses infectious to single host species. Further improvements of this protocols including degenerate primer designs might reveal unexpected diversities of diatom-virus ecological relationships.

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## Spatial and Temporal Variability of Phytoplankton Assemblages in Bolinao and Anda, Philippines

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ssessment of the spatial and temporal variability of phytoplankton assemblages in relation to natural and human-induced environmental conditions is essential to understanding the dynamics of algal blooms. In this study, phytoplankton composition and abundance at four aquaculture-impacted sites in Bolinao and Anda (Pangasinan, Philippines) were investigated. Water samples were collected from these sites from March 2019 up to April 2020 and examined using microscopy. Environmental parameters (pH, salinity, dissolved oxygen and temperature) were also recorded. A total of 48 species belonging to 25 genera were recorded in one of the stations (Station 2), representing preliminary results for the months of March 2019 to May 2019. Of these, 27 species were members of Bacillariophyceae and 21 species were members of Dinophyceae. The highest density was recorded in April 2019 at a total of 1.1 x 104 ind./L; the dominant species were Coscinodiscus spp. (50.5%), followed by Chaetoceros spp. (20.3%) and Hemialus bauckii (19.7%). However, on average, the most abundant diatom species for the period was Leptocylindrus spp. (34.8 %). Dinoflagellates were much less abundant, with Scriepsiella spinifera as the most common representative of the group accounting for 2.2% of the total counts. Harmful-algal bloom species were also observed, including Alexandrium catenella, Alexandrium tamarense, Dinophysis acuminata, Dinophysis fortii, Gambierdiscus spp., Pseudo-nitzchia spp., and Gonyaulax spinifera, but were observed less frequently (<1%).

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# Effects of Acidification on the Growth and Physiology of *Alexandrium minutum* and *Chaetoceros calcitrans* in Laboratory Cultures

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Increasing CO<sub>2</sub> leads to a consequent decrease in seawater pH in a process called ocean acidification (OA). Under these changing conditions, studies have shown a potential shift in the dominant phytoplankton species within a community. For this study, *Alexandrium minutum* represents a harmful algal bloom (HAB) dinoflagellate species that was seen to dominate in conditions with low pH, while *Chaetoceros calcitrans* represents a non-toxic diatom that has been found to be more abundant in conditions with relatively ambient pH. The study was done in a temperature and light controlled environment for a duration of 4 weeks with pH manipulation achieved through the use of a mass flow controller unit (MFCU). Daily cell counts were collected for analysis of growth rates while toxin analysis was accomplished on a weekly basis to show how each species will respond to the CO<sub>2</sub>-induced decrease in pH. Growth rates from both *A. minutum* and *C. calcitrans* showed negative initial responses due to acidification, but recovered in the later weeks. For toxin production, *A. minutum* exhibited increased toxin production in acidified conditions during week 1 and 2 but decreased for all treatments in the later weeks. The results showed that both species adjusted during the first two weeks of exposure to lowered pH but eventually adjusted to the change in conditions towards the end of the experiment.

## Toxic *Pyrodinium* Blooms in Sapian Bay, Western Philippines

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fter more than two decades, *Pyrodinium bahamense* bloomed once again in 2015-2016 resulting in closures of shellfish harvest in Sapian Bay, which affected the coastal provinces of Aklan and Capiz in Western Visayas. Phytoplankton samples were collected by vertical net hauls from 2015 August to 2016 February at seven (7) fixed monitoring stations in Sapian Bay. Shellfish meat samples were analyzed by mouse bioassay. *P. bahamense* recorded a cell maximum of 3.9 x 10<sup>4</sup> L<sup>-1</sup> (accounting for 72.3% of the overall HAB species population) during November. Toxicity in shellfish peaked at 1.27 x 10<sup>3</sup> ugSTX equivalent/100g meat sample in 12 November 2015. Water temperatures during the bloom ranged between 26-33.5°C, salinity between 19-37 ppt, dissolved oxygen levels between 2.12- 6.90 mg/L and water transparency between 60-150 cm. Densest *Pyrodinium* concentrations of up to 1.0 x 10<sup>4</sup> cells L<sup>-1</sup> were recorded from the eastern portion of the bay. Correlation analysis showed that abundance of *P. bahamense* cells was very strongly correlated with toxicity levels in oysters (r= 0.881, n= 14, p< .001) and mussels (r= 0.866, n= 16, p< .001). A decline in bloom was observed in December and subsequently *P. bahamense* cells dropped to <10 L<sup>-1</sup> in 2016 January. This is the first documented toxicity of *Pyrodinium* in Sapian Bay coastal waters since its first occurrence in 1988.

**Key words:** Pyrodinium bahamense, phytoplankton, mouse bioassay, STX,

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## Positive Effect of Ultraviolet A (UVA) in Benthic Dinoflagellate

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enthic dinoflagellate growing in the intertide zone endured acute light change and exposure of ultraviolet radiation (UVR). However, less is known about effect of UVR on metabolism and photoprotection mechanisms of benthic dinoflagellate. Consequencely, the objective of this study was to evaluate the effect of ultraviolet A (UVA, λ=320-400 nm) and ultraviolet B (UVB, λ=280-320 nm) treatments through artificial light source on growth, photosynthesis, pigments and ultraviolet absorbing compounds (UVACs) of four benthic dinoflagellate species from different genera (*Gambierdiscus pacificus, Coolia tropicalis, Ostreopsis ovata* and *Prorocentrum lima*) under low light (LL) and high light (HL) condition. There are significant enhancement of oxygen release and growth of all species with UVA addition under light limited condition. Meanwhile, these species also have the ability of UVA to excite chlorophyll (Chl) a fluorescence directly, which means UVA may enter into photosynthetic pathway and be utilized by benthic dinoflagellate. Otherwise, the growth and photosynthesis of *G. pacificus*, *O. ovata* and *C. tropicalis* were greatly inhibited by UVB after 96 h exposures whereas *P. lima* showed less inhibition and photodamage resulted from higher UVACs content and ratio of carotenoid to Chl a. This study clarifies that benthic dinoflagellate can utilized UVA waveband commonly, especially gaining positive effect at light limited condition, and their tolerances to UVR were species-specific which depend on their contents of photoprotection compounds.

## Possible Link of *Biecheleriopsis adriatica* (Suessiaceae) to the Fishkill in Obando, Bulacan, Philippines

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ish kills due to Harmful Algal Blooms (HABs) are increasing spatially and temporally in the Philippines. In 2017, a massive fish kill event took place in Obando, Bulacan affecting over 100 hectares of fishponds with estimated economic losses amounting to Php 29M. The Bureau of Fisheries and Aquatic Resources (BFAR) suggested that the fishkills were due to decrease in DO, but the factors that led to such decrease remain unknown. Notably, the event was accompanied by a dense bloom of a phytoplankton species and extreme sea surface temperature in Manila Bay. However, its possible contribution to the fish kill remain uncertain. This study then aims to identify the potential factors that contributed to the demise of the fish by combining hindcasting, field-based survey, and lab-based experiments. We hypothesize that the extreme temperature condition could have led to the collapse of the phytoplankton populations, which then resulted in low DO conditions favoring the fish kill. Here, water samples were collected on the day of the fish kill, and the abundant phytoplankton was isolated and later identified using morphological and molecular analyses. Batch cultures of the purified strain were tested for their responses to varying temperature. Morphological and phylogenetic analyses revealed the organism to be Biecheleriopsis adriatica, and closest to those isolated from South China Sea regions. Live and lysed cultures of the organism showed no toxicity against Artemia salina nauplii and no hemolytic activity against mice blood. Hindcasting of temperature and chlorophyll-a concentrations in the area support the hypothesis. Implications of the study are further discussed.

#### **Metabarcoding Analysis of Red-Tide Water Bloomed with Cochlodinium** in Southern Korean Waters

Knag H.E.<sup>1</sup>, Yoon, T.H.<sup>2</sup>, Park, J.W.<sup>3</sup>, Lim, W.A.<sup>3</sup>, Kim, H.W.<sup>2,4</sup>

e collected sea water samples at the end of July, 2018. The relative amounts of microorganisms ranged from 11.56 to 471.94 (copy number/104). The relative amounts of heterotrophic bacteria ranged from 9.79 to 416.81 (copy number/104) and the relative phytoplankton ranged from 1.77 to 55.12 (copy number/104). Genomic analysis identified 252 microbial OTUs, which included 15 phyla: Actinobacteria, Bacillariophyta, Bacteroidetes, Crytophyta, Cyanobacteria, Euryarchaeota, Foraminifera, Haptophyta, Marinimicrobia, Ochrophyta, Planctomycetes, Proteobacteria, Tenericutebia, The most dominant taxon was Proteobacteria, but the proportion of Proteobacteria was decreased and the proportion of Cyanobacteria was increased in a sample which not found the cochlodinium. Based on the microbial and phytoplankton communities, samples were divided into four groups according to the sample, and among them, the sample where no cochlodinium was found and the sample where cochlodinium was found were clearly distinguished. 182 phytoplankton OTUs were identified, belonging to 11 phyla: Cyanobacteria, Bacillariophyta, Cerocozoa, Chlorophyta, Cryptophyta, Haptophyta, Miozoa,

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# Tracing Nutrient Sources Fueling *Cochlodinium*polykrikoides Blooms Occurring Along the Coast of Korea Using Radium Isotopes

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armful algal blooms caused by the dinoflagellate *Cochlodinium polykrikoides* often occur in coastal waters of Korea. However, nutrient sources fueling *C. polykrikoides* blooms are difficult to identify since *C. polykrikoides* blooms usually occur under very low inorganic nutrient conditions. In this study, we used two short-lived Ra isotopes, <sup>223</sup>Ra (half-life: 11.4 d) and <sup>224</sup>Ra (half-life: 3.66 d), as tracers to determine the nutrient sources fueling initiation and spread of *C. polykrikoides* blooms along the coast of Korea during the summers of 2014, 2016, and 2017. The offshore bloom areas were characterized by high <sup>224</sup>Ra activities and low-inorganic and high-organic nutrient concentrations, which are favorable conditions for blooming *C. polykrikoides* in competition with diatoms. Horizontal and vertical distributions of nutrient concentrations correlated well with the activities of <sup>224</sup>Ra in nutrient-source waters. The Ra tracers reveal that the main source of nutrients in the initiation region (southern coast of Korea) is transported horizontally from inner-shore waters. However, the nutrients in the spread region (eastern coast of Korea), approximately 200 km away from the initiation region, are supplied continuously from the subsurface waters by vertical mixing or upwelling. Thus, these results suggest that short-lived naturally occurring Ra isotopes are excellent tracers of major nutrient sources fueling harmful algal blooms in coastal waters.

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#### Preliminary Investigation in Linkage Between Hypoxia at the Lower Reach of the Peal River Estuary and High-Biomass Algae Blooms

Meng, Q.1, Zhou, F.1,2,3

ypoxia has been found both at the upper reach and the lower reach of the Pearl River Estuary (PRE). The hypoxic area at the lower reach in 2014 was larger than 300 km<sup>2</sup> (Dissolved Oxygen (DO)<2 mg/L) or 900 km<sup>2</sup> (DO<3 mg/L), which also extended off the PRE. The major contributor to the hypoxia at the upper reach was argued to be the sediment oxygen demand. The predominant reason for the hypoxia at the lower reach, however, is not clear yet. The hypoxia there is influenced by both the South China Sea (SCS) and the Pearl River discharge. We applied the coupled physical-biogeochemical model, ROMS-CoSiNE, to the SCS and took into account the runoff of the Pearl River. The SCS ROMS-CoSiNE model divided the phytoplankton community into two compartments, namely the diatom and non-diatom (small phytoplankton). Therefore, it could be used to investigate the dynamics of highbiomass algae blooms mainly composed of diatom. It has been found that in many areas the decomposition of the detritus in consequence of high-biomass algae blooms is the major DO sink. The model has qualitatively reproduced the hypoxia at the lower reach of and off the PRE in 2014. Then the nutrient discharge from the Pearl River was turned off in the model to address the relative contribution of the terrestrial discharge. It is also found from the model that the synoptic variation of the hypoxic area was remarkable, which was consistent with discoveries from two cruise legs in 2014. Via the numerical experiments and analysis, the linkage between highbiomass algae blooms and the hypoxia will be discussed in order to understand the formation, disappearance and variation of the hypoxia at the lower reach of the PRE.

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## Dinoflagellate Cysts in Surface Sediments from Lampung Bay, Indonesia

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armful Algal Bloom (HABs) has become a problem in Indonesia since the 1970s. Like other developing countries in the world, Indonesia has faced enormous aquatic environmental degradation. Consequently, those proliferation phenomena of harmful phytoplankton, Harmful Algal Bloom (HABs), has increased significantly since the last few decades. The increasing occurrence of HABs organisms is a significant and expanding threat to health and the fisheries and shellfish industries. To investigate the distribution of dinoflagellate cysts in relation to environmental condition from Lampung Bay, Indonesia, surface sediment samples were collected from 21 stations. There were 33 dinoflagellate cysts were observed, with the dominated cysts were Margalefidinium polykrikoides, Pyrodinium bahamense, and Protoperidinium spp. Total abundance of cysts that were observed ranged between seven to 667 cysts.g-1 dry weight sediment. The highest abundance found at the area that was high of anthropogenic activity. This study will discuss further the relation of environment condition to the dinoflagellate cysts.

### P-HD-11

# Challenges for Detecting Diverse Viruses Infecting Marine Planktonic Diatoms from Coastal Sediments

Tomaru, Y.1, Toyoda, K.2, Kimura, K.3

o far, at least a dozen diatom virus species have been isolated based on a culture method. These studies have contributed to reveal biological features of diatom viruses. The culture-based method, however, is time consuming and laborious, furthermore only able to isolate the most dominant virus in a sample. Recent studies have suggested that diatom virus infections are inter-species. For understanding relationships between a host diatom and its infectious viruses, more efficient methodologies to determine these systems are necessary. In this study, we challenged a new protocol to identify previously unrecognized host-virus relationships. The host cell cultures after inoculations of natural sediment pore water samples were obtained before complete lysis, and the proliferated viral genomes in the host cells were amplified using degenerate primers targeted for replication proteins of single-stranded RNA (ssRNA) and single-stranded DNA (ssDNA) viruses, and then sequenced. The results showed that diverse ssRNA and ssDNA virus groups were detected from inoculated *Chaetoceros* cells. Although we should recognize the possibilities of protocol biases affecting the results, for example non-specific adsorptions of virions onto the host cells, the present method would be effective for understanding diversities of viruses infectious to single host species. Further improvements of this protocols including degenerate primer designs might reveal unexpected diversities of diatom-virus ecological relationships.

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### **P-HD-12**

# Genomic Response of Metacaspase Genes in the Harmful Dinoflagellates *Cochlodinium polykrikoides* and *Prorocentrum minimum* During Cell Death

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etacaspases (MCAs) are cysteine proteases that share sequence homology with caspases, and may play roles in programmed cell death (PCD). In the present study, we identified the novel MCA genes (designated as *CpMCA* and *PmMCA*) from the red tide-causing dinoflagellates *Cochlodinium polykrikoides* and *Prorocentrum minimum*, and examined their molecular characteristics and gene expression in response to algicide-induced cell death. Both MCA genes contained the dinoflagellate spliced leader sequence (dinoSL) and a poly (A) tail. Putative CpMCA (293 aa, 32.4 kDa) and PmMCA (288 aa, 32.7 kDa) proteins had conserved MCA family motifs, and genomic comparison revealed that no intron present in *CpMCA*, but two introns were found in *PmMCA*. Phylogenetic analysis showed that *C. polykrikoides* and *P. minimum* may have acquired the *MCA* gene from bacteria by means of horizontal gene transfer (HGT). In addition, expressions of *CpMCA* significantly increased following exposure to the common algicides copper sulfate and oxidizing chlorine, which trigger cell death in dinoflagellates, suggesting that *CpMCA* may be involved in cell death. Further experiments (e.g., TUNEL apoptosis assay) are required to elucidate the exact role of *MCA* genes in the activation, regulation, and execution of PCD in response to environmental stressors and nutrient limitation.

### **P-HD-13**

### Numerical Modelling of the Circulation of Puerto Princesa Bay

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ver recent years, Puerto Princesa Bay has experienced seasonal red tide contamination, which has posed harsh consequences on the local community's livelihood and health. In an effort to further understand the dynamics of HABs in the area, a 3-dimensional model is created with Delft3D software to examine the varying hydrodynamic and meteorological factors that affect the circulation in the Puerto Princesa Bay. The model's major tidal constituents are extrapolated from recorded water level data to provide tidal forcing. Experimentally obtained water quality data, including temperature and salinity profiles from 2018 and 2019, and published wind data are also integrated into the model. The model's output water velocities at varying depths are compared with experimentally collected data for examination and verification of the model's driving variables. The circulation and residence time in the bay are visualized via the displacement of a conservative tracer uniformly distributed at the surface layer, with these results aiding in the analysis of the hydrodynamics that affect the HABs in the area.

### **P-MH-1**

### Variation in Paralytic Shellfish Toxin Contamination in Selected Shellfish from Coastal Waters of Tagbilaran and Dauis in Bohol

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hytoplanktons are photosynthetic microscopic organisms and known to proliferate to very significant number that they can be seen with the naked eye through the inherent pigment. Certain phytoplankton are associated with toxins and an example of which is Pyrodonium bahamense, a known producer of paralytic shellfish toxins (PSTs). Bloom of Pyrodinium bahamense was reported in the coastal waters of Tagbilaran and Dauis in Bohol and likewise, shellfish tested positive for PSTs. To ensure consumer safety BFAR regularly checked shellfish from the said area. This study is on monitoring paralytic shellfish toxins in shellfish from Tagbilaran and Dauis from 2015 - 2018. Four commonly available shellfish were monitored and these were, Anadara sp., Atrina sp., Modiolus sp and kuja. Shellfish were analyzed using a variety of methods which include mouse bioassay, immunoassays and receptor binding assays. Shellfish are considered positive if the regulatory limit stipulated in Fisheries Administrative Order 235 was exceeded. Monitoring data showed three (3) occurrences from June 2015 to end of 2018. Recorded duration of shellfish toxicity was from six (6) to 10 months. Majority of monthly occurrence is wet and cold months associated with monsoon seasons. Variation in toxin accumulation was also observed between shellfish types. Kuja was reported as positive for a total of sixteen months in contrast to Atrina sp. which was reported positive for 10 months. The highest toxin of level 440 ug-100-1g-1 was also detected in kuja and lowest in Anadara sp. The PSTs occurrence on June 2015 was the first report of contamination in Bohol and thus, the results served as basis for information campaign materials to mitigate its negative impacts. On the context of monitoring, the results can also serve as determinants for sentinel species as basis for management strategies.

### **P-MH-2**

# Water Quality Dynamics in Malampaya Sound, Palawan, Philippines in Relation to *Pyrodinium* Blooms: A Preliminary Study

Sumeldan, J.1, Avillanosa, A.1, Cabungcal, F.2, Creencia, L.1

his is an ongoing study of the Blue Communities Programme, which aims to determine the influence of water quality parameters with Pyrodinium blooms in Malampaya Sound, Taytay, Palawan, Philippines. Partial results of water samples collected from Malampaya Sound, particularly, Phosphorous, Ammonia and Lead were interpolated using free GIS software to produce initial gradient density map. This study is expected to run until May 2020 and will be correlated with the data on *Pyrodinium* density collected by the Provincial Agriculturist Office of Palawan in Malampaya Sound.

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### Harmful Algal Bloom (HAB) Monitoring in Region VI

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he occurrence of Harmful Algal Bloom (HAB) is known as one of the factors that is responsible for the economic losses in the fisheries sector of the country particularly in shellfish industry. The Bureau of Fisheries and Aquatic Resources (BFAR) through their Regional Fisheries Laboratories shall take the lead in the conduct of HAB monitoring activities in their areas of jurisdiction (FAO No. 235, s. 2010). In Western Visayas, this has significant economic impact for the shellfish growers and vendors considering that the region is one of the top shellfish producers in the country. Recent incidents recorded by BFAR from the period of 2015 – 2017 include Paralytic Shellfish Poisoning (PSP) - positive shellfish in Batan Bay, Aklan (2015), Sapian Bay, Capiz (2017) and Gigantes Island, Carles, Iloilo (2017) particularly that of Pyrodinium bahamense var. compressum, a causative agent for PSP. Hence, regular monthly HAB monitoring is being implemented throughout the region to ensure public consumers' safety. The HAB monitoring is being implemented by means of plankton and shellfish sampling for plankton identification and cell count and shellfish toxin detection using immunoassay in lateral flow immuno-chromatography (LIC) format, respectively. Assays in LIC format is used for Paralytic Shellfish Toxin (PST) detection due to the fact that it requires less technical skill. Thus, samples tested positive for PST using LIC format is/are then submitted to BFAR Central Office for confirmatory testing. Meanwhile, the University of the Philippines Marine Science Institute (UP MSI), in collaboration with BFAR, is implementing a DOST-PCCAARD-funded program entitled "Hazard Detection and Mitigation Tools for Algal Blooms in a Changing Marine Environment (HAB Program)" which will address the risks from HABs by providing ways to more rapidly detect the conditions and organisms leading to HAB occurrences and eventually develop an early-warning system that can be used by shellfish/fish farmers and government agencies.

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### **Diversity of Dinoflagellates in Davao Gulf, Philippines**

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he spatial and temporal distribution of dinoflagellates in Davao Gulf were studied for 4 months (February, May, September and November 2018). During the survey, a total of 175 taxa of dinoflagellates belonging to 27 genera were documented and identified. Among the dinoflagellates observed, *Ceratium spp.* were the most dominant followed by *Dinophysis spp.* and *Histioneis spp.* Dinoflagellates occurred throughout the 4 months survey with distinct temporal and spatial variations. In Davao Gulf particularly in Mati, Davao Oriental, toxic dinoflagellates *Pyrodinium bahamense* var. *compressum* were already reported as early August 1983 which is responsible for several paralytic shellfish poisoning (PSP) cases in the area shortly after ingesting green mussels. In recent surveys, *Alexandrium sp.* forming long chains was observed for the first time in Davao Gulf. In emergence of other HAB species, a continuous monitoring of phytoplankton in the coastal waters of Davao Gulf is indispensable for HAB management.

## Distribution and Abundance of *Ceratium furca* in Manila Bay, Philippines

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ne of the consistently included in top ten phytoplankton during the survey from January 2012 till November 2015 in Manila Bay, (14.5188° N, 120.7580° E), Philippines is the *Ceratium furca*. Next to *Protoperidium* spp., *C. furca* had been observed to be consistently present even in environments dominated by diatoms. The highest observed density of *C. furca* had been recorded in September 2012 (263 cells/l) at the southwestern portion, near the mouth of the bay. The species had been observed all throughout the year but mostly dense from the months of May to November, the distribution however, follows no particular pattern. Although the said species did not cause any alarm to sound off during the duration of the survey, its appearance had been reported to be the red-brown discoloration of water in September 2015 near the eastern portion of the bay where the city of Manila is also at. This species is a known toxic red tide causative organism which damages fish gills and depletes dissolved oxygen to anoxic conditions. Its bloom is associated with coastal eutrophication and as such, may be used as one of indicator species in determining the condition of marine waters.

**Keywords:** Harmful Algal Blooms, *Ceratium furca*, Philippine coastal waters

## Long-Term Deployment of Biotoxin Adsorption Tracking Technique (BATT) in Puerto Princesa Bay, Palawan

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ccurrences of harmful algal blooms have increased in frequency/number of affected areas and intensity during the past decades. In the Philippines, the aquaculture industry has been affected by this phenomenon resulting to great economic losses. Toxicity monitoring in the country still primarily relies on the mouse bioassay of shellfish tissue, which is inexpensive but is not as precise as other methods such as RBA and ELISA.

In this study, the efficiency of Biotoxin Adsorption Tracking Technique (BATT); monitor toxicity associated with HAB events in Puerto Princesa, Palawan was investigated. BATT set-ups were deployed in the study area for three consecutive months. Each set-up consisted of a set of samplers which contained two grams of Sepabeads SP700 for dissolved toxin adsorption. Resin adsorption and shellfish toxin uptake were monitored weekly while cell abundance was monitored in two-week intervals. All samples were analyzed by Lawrence and Mennard pre-chromatographic oxidation method using Shimadzu Prominence LC-20AD.

Presence of *Pyrodinium bahamense* and *Alexandrium sp.* were observed throughout the sampling period. Sepabeads SP700, known for its high adsorption capacity, was able to adsorb dissolved toxin as low as 9.68±0.61 ng STX eq./g resin. Highest adsorbed toxin recorded was obtained from the 7<sup>th</sup> week sampling with values ranging from 3320.04-9202.48±1454.18 ng STX eq./g resin. Toxin profile obtained from plankton samples were composed mainly of saxitoxin but unlike its typical toxin profile, gonyautoxin-5 (GTX5) was present instead of neosaxitoxin (NSTX). Moreover, presence of gonyautoxin-1,4 (GTX1,4) and gonyautoxin-2,3 (GTX2,3) in shellfish samples likely resulted from biotransformation within the shellfish itself. Linear relationship between resin adsorption and shellfish toxin uptake was also observed making BATT as a good candidate for HAB event early warning.

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## Phytoplankton Composition of Villareal Bay and Maqueda Bay, Western Samar, Philippines

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he first record of a Harmful Algal Bloom (Red Tide) event in the Philippines was in Samar. Villareal and Maqueda Bays are two bays in this area. Particularly, Maqueda Bay, was the site of that first recorded HAB event that caused 21 deaths and affected 278 people in June 1983. This study was done to compare the phytoplankton composition and diversity of these two (2) nearby bays during the four (4) month sampling from December 2019 to March 2019. The results showed that the diversity of Maqueda Bay was opposing trend to Villareal Bay where the former was dominated by the diatom groups under genus Chaetoceros (12.28x10³ cells/L) and dinoflagellates Gyrosigma (33.39x10³ cells/L) and Noctiluca (13.45x10³ cells/L) while Maqueda Bay was dominated mostly by diatom groups under genus Cylindrotheca (12.55x10⁴ cells/L), Rhizosolenia (41.60x10³ cells/L), and Chaetoceros (34.46x10³ cells/L). Both bays have increasing phytoplankton density over-time in which it was highest in March 2019 with 17.7 x10⁵cells/L in Maqueda Bay and 59 x10⁴cells/L in Villareal Bay.

Keywords: red tide, phytoplankton, density, composition

# Spatial and Temporal Behaviour of *Pyrodinium bahamense* var compressum in Honda Bay, Puerto Princesa City, Philippines

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he study visualized the spatial and temporal behaviour of *Pyrodinium bahamense var compressum* in Honda Bay. Using free available GIS software, interpolated images of monthly cell density of this dinoflagellate were created, which showed coastal waters where the dinoflagellate proliferated most (mean = 88 cells /L) and where it proliferated less (mean < 1 cell/L). This study presented only the mean density of *Pyrodinium bahamense var compressum* from 2013 to 2018. Further study on its correlation with other environmental factors is highly recommended.

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# Spatial and Temporal Behaviour of *Pyrodinium bahamense* var compressum in Puerto Princesa Bay, Puerto Princesa City, Philippines

Sumeldan, J.<sup>1</sup>, Avillanosa, A.<sup>1</sup>, Delgado, J.<sup>2</sup>, Creencia, L.<sup>1</sup>

n this study, map images showing bloom of *Pyrodinium bahamense var compressum* along Puerto Princesa Bay were generated using free GIS software. The map shows the months where the phytoplankton is seemingly inactive and active as well as its relative population abundance from 2013 to 2018. The study only shows mean density of the phytoplankton species and succeeding monitoring studies are further recommended to determine correlation for possible prediction of *Pyrodinium bahamense var compressum* bloom.

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# Development of Sandwich Hybridization Integrated with Nuclease Protection Assay (NPA-SH) Probes to Monitor Cochlodinium polykrikoides

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he occurrence of harmful algal blooms (HABs) worldwide has serious impacts on the marine environment and fishery resources. *Cochlodinium polykrikoides* is an important causative agent of HABs in Korea's seas, but the presence of this organism cannot be tested in large number of samples using light and electron microscopy in long-term monitoring. As such, an integrated sandwich hybridization and nuclease protection assay (NPA-SH) was established for the quantitative detection of *C. polykrikoides*. The specificity of the probes was verified with individual and mixed cultures as well as field collection from South sea, Korea, and the quantity of *C. polykrikoides* determined by NPA-SH analysis showed a good correlation with cell-counting with a light microscope. In addition a standard curve for *C. polykrikoides* was established to represent the correlation between optical absorbance in the NPA-SH assay and cell density. The results show that the NPA-SH method is reliable, specific, and accurate in the detection of *C. polykrikoides*; thus, this approach provides an efficient alternative for the rapid and quantification of harmful algae and could be used to monitor in field surveys.

### P-RRD-1

## Ultrastructure of *Chattonella subsalsa* (Raphidophyceae) Found in Philippine Water

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battonella species are notorious fish killers during their blooms, especially in Japan, but the identification is difficult due to their high morphological similarity. In this study, morphology and ultrastructure of Chattonella subsalsa found in Manila Bay and Bolinao, Philippines were examined by bright field and fluorescence microscopy, and transmission electron microscopy (TEM). Cell shape of C. subsalsa from Philippines was consistent with type species C. subsalsa by having a teardrop shape and a rounded posterior end. Cells were  $42.2 \pm 3.91 \,\mu\text{m}$  (n = 51) long and  $25.2 \pm 3.35 \,\mu\text{m}$  (n = 51) wide. Cell surface was covered by several large button-like granules and numerous small granules. Chloroplasts were ellipsoidal or irregular shaped with several lobes, and each with a pyrenoid. Ultrastructural examination by TEM showed the chloroplasts composed of stacks of three thylakoids. In the pyrenoid, only one of the three thylakoids penetrated into the matrix. The number of thylakoids in the pyrenoid was identical to C. subsalsa isolated from Mediterranean but is different from C. marina which had one to three penetrating thylakoids. LM revealed several oboe-shaped mucocysts with both tapered and pointed ends were released when the cell was compressed and when stained by neutral red. Ultrastructure of cells show mucocysts were found at the periphery. The ultrastructure of mucocyst was also observed by wholemount TEM and compared to previous study. Among Chattonella species, mucocysts have been reported only in C. subsalsa from Mediterranean and Adriatic Sea but not in C. subsalsa from US, Mexico and Singapore. However, the Philippines C. subsalsa possessing mucocysts are more phylogenetically related to the latter.

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# What are the Most Important Factors for Monitoring the Dynamics of Microbial Ecosystem Due to Microalgae Mass Cultivation Using a Floating Photobioreactor in Coastal Water of Yeongheung Island, Korea

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iodiesel production via ocean microalgae has great economic potential. Since 2012, microalgal mass cultivation using a floating photobioreactor has been piloted in Yeongheung Island, Incheon, Korea. Operation of algal mass cultivation in ocean could induce the disturbances of microbial food chain such as nutrient consumption and dissolved organic carbon excretion. However, the long-term operation of microalgal mass cultivation in ocean area has not been attempted globally, and no research has been conducted on how it affects microbial ecosystems in ocean area. Therefore, to operate algal mass cultivation sustainably, we identify whether the microbial ecosystem is disturbed by the operation of the algal mass cultivation and the environmental evaluation factors for monitoring the microbial ecosystem in the nearby mass cultivation site. For this study, we carried out monitoring of physiochemical and biological factors from 2013 to 2018 during the operation of algal mass cultivation site and control sites. As a result, environmental factors such as temperature (5.1-26.0°C) and dissolved oxygen (5.73-14.31mg/L) showed seasonal dynamics. However, changes in salinity (28.1-34.1psu) and pH (6.65-8.80) was hardly detected. In the case of nutrients, nitrite, nitrate, phosphate and silicate had been shown to maintain high concentrations since July, Ammonium nitrogen usually had been shown to high concentration from May to August. Dissolved organic carbon, annual fluctuations (0.60-4.82mg/L) were detected small and thus remained constant throughout a year. Effect of operating algal mass cultivation on biological factors, such as bacteria (40-3,755x103cells/mL), phytoplankton (33 -3,890cells/mL), HNF (31-2277cells/mL), ciliate (330-183,150cells/mL), zooplankton (1.09-33.33x103indiv./ ton), was not detected but seasonal dynamics of biological factor were observed. One-way ANOVA was performed to analyze the seasonal changes of physicochemical and biological factors. As a result, Temperature, Nutrients, Phytoplankton, HNF, Ciliate had evident seasonal dynamics. However, salinity, pH, dissolved oxygen, dissolved organic carbon, bacteria remained stable throughout the year regardless of season. These results enable to figure out the major monitoring factor to sustainable operation of algal mass cultivation. Research about disturbances of microbial ecosystem by figured out factor is speculated to be important.

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## Abundance of Epiphytic Dinoflagellates from Coastal Waters Off Jeju Island, South Korea in Autumn 2019

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pecies within the genera *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, and *Amphidinium* are known to be epiphytic dinoflagellates. Most of the epiphytic dinoflagellates is harmful to human as well as to marine organisms, so that scientists, the aquaculture industry and government are keenly attentive. The occurrence of epiphytic dinoflagellates in temperate waters has been reported as evidence of increasing water temperature. Kim *et al.*(2009) reported the presence of 5 species in the coastal waters of Jeju Island, southwestern area of South Korea. Recently, we identified dominant macro-algae species in Jeju Island and examined the amount of epiphytic dinoflagellate species attached to the macro-algae, and this results showed a suggestion of the possibility in regard with climate change. We have a plan to investigate the effect of the Kuroshio Current influenced on the appearance of epiphytic dinoflagellates in coastal water of South Korea.

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## Accumulation and Depuration of Paralytic Shellfish Toxins in Green Mussel *Perna viridis* Fed on *Alexandrium minutum*

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he accumulation and depuration of PSP toxins in green mussel *Perna viridis* fed on *A. minutum* was investigated under laboratory conditions. *A. minutum* used in this experiment was isolated from Chao Phraya River mouth and green mussel were collected from the mussel farm at Sriracha, Chonburi Province, the inner Gulf of Thailand. As for the feeding experiment, mussels have been designed to feed on *A. minutum* for 2 weeks and followed for 2 weeks of the depuration period by fed on the non-toxic algae *Isochrysis* sp. The changes in toxin content and composition in *A. minutum* and mussels were analyzed by HPLC with pre-chromatographic oxidation method. The results showed that the highest toxin content in *A. minutum* has been found at the late-exponential phase of growth (3.75-4.46 pgSTXeq./cells) and GTX1,4 was the major toxin component(>90%). Cells at this stage of growth were used for the feeding experiment and found that PSP toxin contents per one mussel have rapidly increased and reached the maximal amount of toxin (11.20 µg STXeq./ind.) after 14 days of the experiment, which approximately 14-folds higher than the regulatory limit. During the depuration period, the toxin content was gradually decreased and has fallen to safety concentrations within 3 days of which only 0.08% of the toxin was detected at the end of the experiment.

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## Characteristics of Harmful Algal Blooms in Coastal Water Since 2013, Korea

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he National Institute of Fisheries Science (NIFS) has monitored Harmful Algal Blooms (HABs) in the Korean coastal water since 1972. Since 1980s, HABs such as Prorocentrum spp. and Heterosigma akashiwo have been detected from the costal water. HAB by Cochlodinium polykrikoides, which is the most HABs in Korean fishery, has begun to occur at almost every summer since 1995. The characteristics of *C.polykrikoides* bloom had been changed since 2011, when didn't occurrence of C. polykrikoides bloom. In 2013 and 2014, C. polykrikoides bloom had shown the earliest and longest period in a record. In 2015, C.polykrikoides bloom was the first to spread out on the south-western coastal water, where is the biggest aquaculture of abalone. In 2016 and 2017, we observed unusual phenomena: exceptionally high water temperature and no C.polykrikoides bloom. In 2018, C.polykrikoides bloom was occurred at 23th August, that is the latest date since 2011. To explain the annual change of C.polykrikoides bloom, we analyzed the meteorological and environmental data. As a result, we found out that the North Pacific high pressure had strong impact on the Korean peninsula, and these results in no typhoon and strong radiant heat which constantly increased water temperature and retained wind direction. To define of the blooming mechanism, we have monitored the free living cells of C.polykrikoides from the East China Sea to the Korean costal water from June to September bimonthly. Most of *C.polykrikoides* seed was introduced by the Tsushima warm current. Therefore, unusual strong Tsushima warm current is not invaded into the southern coastal water. A view of ecological phenomena, nonoccurrence of the C.polykrikoides bloom was attributed to diatom-dominated environment or was replaced by others such as Karenia mikimotoi, Alexandrium affine, Ceratium spp. etc.

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### P-NHS-1

# Fishery Damage Due to a Bloom of *Alexandrium spp*. in Nomi Bay, Japan: Taxonomy and Growth Physiology of the Causative Species

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n early April 2017, the dinoflagellate *Alexandrium* spp. appeared relative high density in Nomi Bay, Kochi Prefecture, Japan, and the cultured fish died. We identified the causative species as *Alexandrium leei* by the morphological and genetical analysis. Although this species has been reported to occur in Southeast Asia, Northeast Asia, North Pacific, Mexico, and the Mediterranean, it was the first report of the bloom of *A. leei* with fisheries damages in Japan. In molecular phylogenetic analysis based on rDNA LSU partial sequence, *A. leei* was divided into two clades, Northeast Asian and Southeast Asian clades. A clone culture strain (Al\_KOCHI) obtained from the red tide seawater of Nomi Bay, in April 2017, was located in the Northeast Asian clade. In order to investigate the growth characteristics of this species, the strain was incubated under different water temperatures (10, 15, 20, 25, 27.5, and 30°C), salinity (10, 15, 20, 25, 30, and 35) and light intensities (125 and 500 µmol m² s¹ with 12hL: 12hD cycles). The growth was monitored by the in vivo chlorophyll fluorescence and the growth rate was calculated. The culture strain was able to grow at 10-27.5 °C and salinity 10-35. The maximum growth rate (0.47 div. days¹) under low light intensity was obtained at 20 °C and salinity 20, while it (0.53 div. day⁻¹) under high light intensity was obtained at 25 °C and salinity 15. These results show that this species can be adapted wide range of temperature and salinity conditions.

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