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Temporal Variation of the Phytoplankton Community in the Coastal Waters of Kota Kinabalu

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Abstract

Seasonality of phytoplankton community in temperate coastal waters is well documented due to distinct and predictable seasonal cycle of environmental parameters. However, phytoplankton succession in tropical regions are still poorly understood. Although changes in physical parameters are not as distinct seasonally as in temperate waters, tropical regions experience two seasons: the Northeast monsoon (NEM) and the Southwest monsoon (SWM), which are distinguished by the variation in rainfall. The objectives of this study were: (1) to examine the diversity of the phytoplankton species, and (2) to examine the changes in their community composition in relation to the seasonal changes in environmental parameters. Samples were collected off the coast of Kota Kinabalu at bi-weekly intervals from August 2017 to April 2018, covering the end of the SWM (August to September), the NEM (November to March) and the inter-monsoon periods (IMPs) in October and April. A total of 30 diatom taxa from 18 genera, 20 taxa of dinoflagellates from 12 genera and one species of silicoflagellate were identified. Mean cell densities for diatoms and dinoflagellates during the SWM were 342 cells L⁻¹ and 8,011 cells L⁻¹, respectively, while they were 2,949 cells L⁻¹ and 1,552 cells L⁻¹, respectively during the NEM. Mean cell densities between the monsoons of diatoms were not significantly different ($p = 0.302$, one-way ANOVA) whereas that of dinoflagellates were significantly different ($p = 0.002$, one-way ANOVA). There was a shift from a dinoflagellate-dominant community observed between the end of August 2017 and early December 2017 (coinciding with a period of higher rainfall) to a diatom-dominant community observed between mid-December 2017 and April 2018 (coinciding with a period of lower rainfall). Principal component analysis (PCA) identified diatom abundance was positively associated with salinity whereas dinoflagellate abundance was positively associated with light intensity and temperature. Cluster analysis of the phytoplankton composition further revealed two characteristic community patterns: a dinoflagellate-dominant and a diatom-dominant community. The results of this study implicate that the changes in environmental parameters during the transition of the monsoons brought about the shift in the phytoplankton community composition.

Keywords--- Phytoplankton community, monsoonal variation, Sabah

Preliminary Test of Emergent and Submerged plant to Treat Real Wastewater from Al-Daura refinery in Iraq

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Abstract

Plant species play important role in physical, chemical and biological wastewater purification. Phytoremediation that depends on the plant and their associated rhizobacteria in the removal of pollutants in water and it is more efficient and cost-effective technology. In this study, the ability of emergent plant (*Phragmites australis*) and submerged plant (*Ceratophyllum*) to tolerant real wastewater from Al-Daura refinery contains hydrocarbons were investigated. This work was conducted in the University of Baghdad in the outdoor conditions for an observation period of 14 days. First, the two species of plants were exposed to different concentrations of 0, 10, 25, 50, and 100% (Vrefinery wastewater /Vwater). After 14 days of exposure, *Phragmites australis* was survived for all wastewater concentrations. While *Ceratophyllum* shown inhibition with concentrations of 100%.

Keywords--- Phytoremediation, preliminary test, hydrocarbons, *Phragmites australis*, and *Ceratophyllum*

Marine Cyanobacteria in Tropical Waters

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Abstract

Cyanobacteria are oxygenic photosynthetic organisms which play a crucial role in marine ecosystems. They are important for nutrient cycling as some species are able to fix atmospheric nitrogen for growth. Cyanobacteria are diverse organisms that are present in coccoid and filamentous forms. They are found in a wide range of environments, from the desert to the Antarctic. Some genera of cyanobacteria such as *Trichodesmium*, *Lyngbya* and *Aphanizomenon* are able to form blooms that are a cause of concern to human health. Filamentous cyanobacteria such as *Lyngbya majuscula* produce toxic metabolites such as Curacin A and Barbamide, which cause severe dermatitis and blistering upon skin contact. However, these cyanobacteria also produce secondary metabolites with therapeutic properties. To date, Singapore has had two blooms of cyanobacteria, *Trichodesmium* (1998) and *Planktothrix* (2018). Given the possible toxicity of such harmful algal blooms (HABs), it is necessary to identify and monitor the species of cyanobacteria present in Singapore waters. In this study, cyanobacterial communities along coastal regions of Singapore were studied using morphological, genetic and ecological characteristics, leading to the discovery of new species from the filamentous genera *Lyngbya* and *Spirulina* and a new heterocystous genus *Lanniella*. The portable sequencing device MinION™ by Oxford Nanopore Technologies was used to profile marine cyanobacterial communities down to the species level. A total of 46 genera and 40 species were identified from two Southeast Asian countries. This is the first study that employed amplicon sequencing using the next generation MinION™ sequencer to study marine cyanobacterial communities. This allows the real-time monitoring of coastal cyanobacteria for species detection and identification in order to rapidly respond to HABs.

Keywords--- Algae, microorganisms, genomic, biodiversity

Life-History Strategies of Tropical *Heterosigma akashiwo* (Raphidophyceae)

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Abstract

Heterosigma akashiwo is a raphidophyte species which has bloomed multiple times in Southeast Asia. Information about the species is however largely based upon temperate studies. The paucity of tropical studies poses a challenge, particularly since *H. akashiwo* blooms tend to associate with seasonal cycles. Specifically, cyst-forming strategies during winter provide for an accumulation of dormant cells which can rapidly activate during the more conducive spring and summer seasons. The conditions necessary for encystment include temperatures < 10 °C, which are typically not present for tropical waters. The question was therefore raised—how does tropical *H. akashiwo* form blooms within such environments? Specifically, the objectives of this study were (1) to identify the effect of growth phases upon dark incubation at 25 °C or 5 °C; (2) to identify long-term growth responses under replete nutrients and light cycles; and (3) to investigate the factors which may regulate cell-decline. Results identified transient, short-lived cysts under dark-incubation at 25 °C. Furthermore, cells incubated under light and warm conditions displayed self-regulatory mechanisms that reduced cell-density, but allowed for subsequent recovery. This process of decline was furthermore driven both by cell-density and chemical exudates within the culture. Cells also had the same recovery period despite an immediate growth period when inoculated into freshly-made media. The process of decline and recovery is therefore tightly-regulated. Conditions tested in this study may represent tropical coastal environments which experience nutrient-pollution and are frequently eutrophic. Ultimately, the results indicate a potential for *H. akashiwo* to frequently recur within such environments. They may also pose bloom threats even if present at low cell densities. Monitoring efforts should account for such potential by examining declining cells at depth which may be able to rapidly recover if suitable growth conditions are provided.

Keywords--- *Heterosigma akashiwo*, self-regulation, recovery, tropical

Harmful Dinoflagellate *Takayama acrotrocha* (Kareniaceae): Physiological Responses to Nitrogen Enrichment

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Abstract

Harmful dinoflagellate species, *Takayama acrotrocha*, has been observed to bloom in Singapore's tropical waters. Dinoflagellate blooms typically depend on high nutrient conditions for their occurrence and maintenance. However, species of the *Takayama* genus share a common paucity in knowledge of their physiological responses to environmental conditions, such as nutrient availability. The present study aimed to elucidate the physiological responses of *T. acrotrocha* to different concentrations of ammonium (NH₄⁺) and nitrate (NO₃⁻). For this purpose, cells acclimated to low nitrogen (N) conditions were exposed to a sudden increase in N concentration, and the cellular responses were examined. Choice of NH₄⁺ and NO₃⁻ presented a comparison between reduced and oxidised inorganic N forms, respectively. Both forms of N were recently reported to have a dominant presence in marine environments. This is contrary to previously accepted views that NO₃⁻ was the dominant inorganic nitrogen form in coastal environments. Ammonium has showed increasing prevalence in coastal waters due to changes in anthropogenically-linked effluents as well as climate change. Dinoflagellates are also known to have different uptake pathways for NH₄⁺ and NO₃⁻. The prior is known to follow passive uptake, while energy costs are incurred in active NO₃⁻ uptake. Upon cell entry, NO₃⁻ undergoes intracellular reduction before utilisation. While the present study showed that *T. acrotrocha* could grow on either N forms, the cells responded differently to N type and their respective concentrations. Distinct growth patterns were observed between the two N sources. Moreover, the pigment composition of *T. acrotrocha* was reported for the first time. This study provided insight regarding the physiological responses of *Takayama* species, furthering the understanding of this genus. The ability to utilise different types of N sources is an advantage for the survival of *T. acrotrocha* in competitive coastal ecosystems. Its potential for forming blooms in eutrophic environments is suggested.

Keywords--- Harmful algae, Kareniaceae, physiology, nitrogen

Growth and Nutrient Uptake Characteristics of Brown Seaweed *Sargassum macrocarpum* Cultivated with Bio-Filtered Wastewater

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Abstract

Recently, degraded seaweed beds have expanded due to multiple factors such as global warming and oligotrophication caused by decrease of nutrient loading from the land. In the coastal areas of East China Sea, phosphate depletion is considered as a possible reason causing degraded seaweed beds. As one of the effective methods for restoring seaweed beds, wastewater fertilization may be applicable to oligotrophic environments. In order to apply wastewater as a nutrient fertilizer, the influence on the physiological response of seaweeds should be carefully examined. This study aimed to evaluate the growth and nutrient uptake characteristics of brown algae *Sargassum macrocarpum* supplied with phosphate-replete wastewater. Bio-filtered wastewater (BFW), which passed nitrogen removal processes (i.e. nitrification and denitrification) in a wastewater treatment plant, contains relatively high phosphate concentrations and was used for cultivating *S. macrocarpum*. Semi-continuous cultivation of *S. macrocarpum* was conducted in the laboratory, and nutrients were supplied using BFW or synthetic nutrient medium (SNM) once every other day for 10 days under the following five treatments: 0.5% and 5.0% (v/v) BFW, 0.5% and 5.0% (v/v) SNM, and no nutrient addition (control). Specific growth rate was calculated using the wet weight of *S. macrocarpum*. Nutrient uptake rate was evaluated by the batch cultivation of *S. macrocarpum* for three hours under the different conditions of temperature (10-30°C) and phosphate concentration (0.2-5.0% of BFW). Specific growth rates of *S. macrocarpum* supplied with 0.5% and 5.0% BFW were approximately the same levels (0.015-0.025 day⁻¹) as those with 0.5% and 5.0% SNM (0.016-0.023 day⁻¹) and significantly higher than that of control (0.006 day⁻¹). This result suggests no positive and negative effect of BFW on *S. macrocarpum* growth. Phosphate uptake rate showed saturable kinetics against medium concentration. Maximum uptake rate (V_{max}) at temperature ranging from 15°C to 25°C maintained the same levels (0.40-0.45 $\mu\text{mol g}^{-1}\text{-DW h}^{-1}$), and that at 30°C increased (0.69 $\mu\text{mol g}^{-1}\text{-DW h}^{-1}$) probably due to their high phosphate demand induced by high respiration rate. These results indicate that the addition of phosphate is effective for summer season.

Keywords--- *Sargassum macrocarpum*, wastewater fertilization, specific growth rate, nutrient uptake rate

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Bioaugmentation of *Vibrio alginolyticus* for Aluminium Contaminated Soil Remediation

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Abstract

As a result of the increasing interest on aluminium recycling industry, its wastewater offers challenges to be managed, particularly when dealing with the surrounding environment. This aluminium contamination has caused the degradation of agricultural soil fertility as mainly reported at close proximity to the waste disposal area. The addition of bacteria, known as bioaugmentation, is widely applied to remediate metal contaminated soil. *Vibrio alginolyticus* is one of the bacterial species reported to have a good capability in reducing aluminium pollution. Bioaugmentation of *V. alginolyticus* was carried out by inoculating 2% v/v of bacteria to 3 kg of aluminium contaminated soil for 14 days of test period. Concentration of 0, 37.5 and 75 mg/kg were used to simulate the load of aluminium contaminated soil tested in this research. Results showed that *V. alginolyticus* were capable of reducing up to 4.59% of 37.5 mg/kg initial aluminium concentration and 5.48% of 75 mg/kg initial aluminium concentration during the test period.

Keywords--- Al, bacteria, bioremediation, metal, pollution, *V. alginolyticus*

Effect of Lipid Component on Egg Production of a Calanoid Copepod *Acartia steueri*

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Abstract

Lipids, composed of fatty acids, provide energy for copepods to reproduce and metabolize. Egg production rate, which is the most important factor for secondary production of copepods, might be related to the lipid component in eggs and females. In order to clarify the effect of lipid component in egg on egg production rate, egg production experiments of *Acartia steueri* was conducted, and lipid component of eggs and females were measured. *A. steueri* were obtained from March to May 2018 at Manazuru Port in Sagami Bay, Japan. Three females were held in a 200 mL incubation chamber at 20°C for 6 days. In order to evaluate the effect of different lipid components of microalgae as diets, *Thalassiosira weissflogii* and *Chaetoceros gracilis* were used for the experiments. Each microalga was fed at 1500 µg C L⁻¹ every day for six days. Egg production rate was measured for 24 h. Egg production rates of females fed on *T. weissflogii* and *C. gracialis* were 2.61±1.97 and 1.10±0.74 eggs female⁻¹ day⁻¹, respectively. The egg production rate of females fed on *T. weissflogii* was higher than that on *C. gracialis* throughout the incubation period. The amount of lipid in females fed on *T. weissflogii* and *C. gracialis* were 1.19 and 3.34 µg female⁻¹, respectively, while the amount of lipid in *in situ* female was 1.21 µg female⁻¹. The amount of lipid in female fed on *C. gracialis*, especially triacylglycerols (TAG), was extremely higher than those of *in situ* female and female fed on *T. weissflogii*. Female fed on *C. gracialis* might accumulate TAG as an energy source with low egg production for six days.

Keywords--- Egg production, diets, lipid, Triacylglycerols (TAG), *Acartia steueri*

Water Quality Bioassessment of Sampean River in East Java Using Macroinvertebrates

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Abstract

Sampean River that flows through Bondowoso and Situbondo receives pollutants from agricultural, domestic and industrial waste. Bioassessment is a widely used method in many countries but has not been commonly used in Indonesia. River water quality assessment is frequently conducted by physical-chemical methods. Both of these methods can be complementary used together in order to get more convincing and comprehensive water quality assessment results. This study aims to compare the quality of Sampean River using physical-chemical and bioassessment methods using macroinvertebrates. Sampling was conducted at 10 locations with 2 replications. Temperature, pH, BOD, ammonium, nitrite, nitrate, and phosphate were measured in March and April 2018. Sampling and analysis of physical-chemical water quality were conducted according to Standard Methods for Water and Wastewater Analysis. Macroinvertebrates were collected using a standard handnet with 500 µm pore size, in effective 5-minute collection time. The macroinvertebrate samples were separated from the sediment and preserved with alcohol of 70% for further identification. Water quality assessment by physical-chemical method using Dutch Score and LISEC Score. The biological quality of the river was measured according to several biotic indices which were developed based on macroinvertebrates. These biotic indices were: Biological Monitoring Working Party (BMWP), modified BMWP and BMWP Thai. Dissolved oxygen were in the range of 2.95-7.81 mg/L, pH values were 6.29-8.78, and BOD levels were 2.55-43.23 mg/L. The concentrations of ammonium, nitrite, nitrate, and phosphate were 0.002-0.08 mg/L, 0.05-0.2 mg/L, 2.11-29.49 mg/L, and 0.05-0.26 mg/L respectively. According to LISEC Score the river water was of "very good" to "medium" quality. Whereas the DUTCH Score method resulted in "very good" to "slightly contaminated" quality. Meanwhile the all bioassessment methods resulted in "moderately impacted" to "heavily polluted quality". The most polluted site is located in upper part, which is polluted by wastewater from tofu industry.

Keywords --- Bioassessment, river water quality, macroinvertebrates, pollution

Reproduction Strategy of *Acartia japonica* using Three Types of Eggs in Sagami Bay, Japan

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Abstract

49 species of marine copepods produce subitaneous eggs which hatch within a few days and resting eggs. According to hatching pattern, resting eggs are categorized as diapausing or delayed-hatching eggs. Diapausing eggs go through a long refractory period during which hatching is not possible even if the environmental conditions are suitable. Delayed-hatching eggs hatch over a broad time span, and do not belong to either of subitaneous and diapausing eggs, because the hatching is not fast enough to be considered as subitaneous and refractory period is not long enough to be categorized as diapausing eggs. *Acartia japonica* has appeared in water column at Manazuru Port in Sagami Bay, Japan only from summer to autumn. The species has disappeared in the late autumn, and has appeared again in the next summer. In order to clarify the resting egg production of *A. japonica* at Manazuru Port in Sagami Bay, *in-situ* egg production was examined in 2015. *A. japonica* occurred from July to November 2015. Eggs produced in the laboratory were classified into three types of eggs subitaneous, diapausing and delayed-hatching. Subitaneous eggs were mainly produced from August to September. Delayed-hatching eggs were produced from September to November and hatched within 30 days. Diapausing eggs were produced from September to November and did not hatch for more than a year. This study revealed for the first time that three types of eggs are produced by the same female. Subitaneous eggs hatch immediately and increase the individual numbers. Delayed-hatching eggs could be a bet-hedging strategy which extends offspring's abilities to utilize resource and reduces mortality of the offspring in unexpected fluctuation of environmental conditions. Diapausing eggs may play a role to connect the temporally divided populations in each year. The production of three types of eggs may allow the species to be well adapted to largely fluctuated environments in the temperate embayment. (308 words)

Keywords--- *Acartia* copepod, resting egg, delayed-hatching egg, temperate embayment, bet-hedging strategy

Bacterial Growth and Grazing Pressure on Bacteria in the Euphotic and Disphotic Layers of Temperate Coastal Waters

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Abstract

In the ocean, heterotrophic microbial communities drive microbial loop, which begins with the utilization of dissolved organic carbon (DOC) by heterotrophic bacteria. Thus, the variations in bacterial growth and loss are major concerns in the study of microbial food web. Bacterial growth is mainly regulated by water temperature and the concentrations of inorganic nutrients and DOC. On the other hand, bacterial loss is mainly caused by predatory grazing by heterotrophic nanoflagellates (HNF). Bacterial growth and grazing pressure on bacteria vary temporally and vertically based on the distribution of bacterial resources and predators. The present study aimed to clarify the vertical differences in bacterial growth and grazing pressure on bacteria by HNF and to identify the controlling factors of bacterial growth in temperate coastal waters. This study was conducted monthly from May 2012 to May 2013 at Station M (120 m depth) in Sagami Bay, Japan. Water temperature, salinity, the concentrations of ammonium, phosphate, chlorophyll *a* (chl. *a*), and DOC, bacterial abundance (BA) and production (BP), and HNF abundance (HNFA) at 0–100 m depth were measured. Bacterial growth was evaluated by BP/BA as bacterial growth rate (BGR) whereas grazing pressure on bacteria by HNF was evaluated by BA/HNFA. BGR and BA/HNFA showed significant vertical differences between the euphotic and disphotic layers during the stratified period from May to October 2012 and from April to May 2013. BGR indicated significantly stronger limitation of bacterial growth ($p < 0.05$) while BA/HNFA indicated significantly higher grazing pressure on bacteria by HNF in the euphotic layer compared to the disphotic layer ($p < 0.05$). However, vertical differences in BGR and BA/HNFA were not observed during the mixed period between November 2012 to March 2013 ($p = 0.69$ and $p = 0.66$, respectively). Multiple regression analyses suggest that bacterial growth was most controlled by chl. *a* concentration in the euphotic layer and DOC concentration in the disphotic layer.

Keywords--- Heterotrophic bacteria, growth rate, grazing pressure, euphotic and disphotic layers

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Variations in Egg Production and Egg Lipid Content of *Neocalanus cristatus* in the Oyashio Region

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Abstract

A calanoid copepod *Neocalanus cristatus* is a dominant oceanic species in the subarctic Pacific Ocean and the important prey of pelagic fishes. This species accumulates a large amount of lipid to survive the starvation period which lasts about six months. This species produces eggs for approximately three months using large amount of storage lipids without feeding. The present study aimed to investigate the variation of egg production and egg lipid content of *N. cristatus* through spawning period. *N. cristatus*, together with *N. plumchrus* and *N. flemingeri* as comparison species, were collected in the subarctic Pacific Ocean by a plankton net with 330 µm mesh in October 2017. Females incubated at a laboratory produced one to 11 clutches with the interval of five to 29 days. Fecundity, the number of eggs produced by an individual throughout the spawning period, was 18 to 581 eggs female⁻¹, and was 419 ± 32 eggs female⁻¹ on average. Clutch size varied from 153 eggs clutch⁻¹ at early spawning period to one egg clutch⁻¹ at late spawning period and was 49.3 ± 4.1 eggs clutch⁻¹ on average. Egg hatching success varied from 100% at early spawning period to 0% at late spawning period and was 46.7 ± 3.3% on average. Egg lipid content varied from 2.7 µg egg⁻¹ at early spawning period to 1.1 µg egg⁻¹ at late spawning period and was 1.8 ± 0.2 µg egg⁻¹ on average. The present study firstly reported that the egg hatching success decreased with decreasing the clutch size from the early spawning period to the late spawning period. Egg lipid content was significantly low at small clutch size ($p < 0.05$). Therefore, the low egg hatching success may be induced by inadequate egg lipid content. While egg hatching successes of *N. flemingeri* and *N. plumchrus* maintained high values throughout spawning period, the egg lipid contents of these species should be measured to clarify whether egg lipid content affects egg hatching success in *Neocalanus* species, or not.

Keywords--- egg lipid content, egg hatching success, egg production, *Neocalanus cristatus*

