



28 – 30 November, 2022 Ara Convention Hall, Jeju National University,

# Program & Extended Abstracts

# Asia-Pacific Network (APN) Workshop on Key Indicator Species and Habitats for Marine Biodiversity Change in East Asia

# **Program & Extended Abstracts**

Organized by

Asia-Pacific Network for Global Change Research Northwest Pacific Action Plan (NOWPAP) Jeju National University, Korea

### Welcome Messages



# Prof. Eel-Hwan Kim President of Jeju National University

On behalf of the Jeju National University, I warmly welcome you to the Asia-Pacific Network (APN) workshop on Key Indicator Species and Habitats for Marine Biodiversity Change in East Asia hosted by Jeju National University. It is indeed a privilege and pleasure to deliver this message as a President of the Jeju National University on the occasion of the APN workshop scheduled to be held on 28th, 29th, and 30th November 2022. I appreciate the project team for choosing a timely topic that suits the current situation, where marine biodiversity is affected by intensive urbanization combined with climate change impacts resulting in substantial changes. Jeju Island is rich in marine biodiversity, including finfish, shellfish, seagrass, corals, and other marine creatures. However, recent climatic changes influence marine diversity all over the region, including Jeju Island. Therefore, understanding the key indicator species will play a vital role in characterizing ongoing marine and coastal biodiversity changes, including standardization and new approaches to studying and conserving marine biodiversity changes. Especially, Jeju National University immensely contributes to monitoring and studying marine organisms and plays an important role in preserving marine organisms around the region.

I found that the APN workshop includes a range of inspiring keynote addresses and invited talks from eminent scientists and scholars. Further, I believe the workshop aims to disseminate the research findings and exchange experiences with researchers, policymakers, economists, and with budding young scientists and provides new insights for new approaches to studying marine biodiversity. I congratulate the project collaborators, all the scientists, and the organizing committee of the APN workshop for organizing this landmark workshop and wish the workshop and its participants all success.

Prof. Eel-Hwan Kim

President of Jeju National University

# Welcome Messages



**Prof. Kwang-Sik Choi** Chair of Organizing Committee

Dear Colleagues,

On behalf of the organizing committee, I welcome you to the Asia-Pacific Network (APN) Workshop on Key Indicator Species and Habitats for Marine Biodiversity Change in East Asia in Jeju, Korea from 28 to 30 November 2022. The purpose of this workshop is to enhance capabilities to participate in research on global change and sustainability and to support science-based decision-making in the region and beyond to understand what are key indicator species, ecosystem types and major pathways resulting in marine biodiversity changes in the Northwest Pacific.

I hope you enjoy the scientific and social programs that we have prepared for you and have a great time in Jeju Island.

Prof. Kwang-Sik Choi

Jeju National University

# Welcome Messages



**Dr. Yegor Volovik** NOWPAP Coordinator

Dear Ladies and Gentlemen, Dear Colleagues,

It is a great pity that I cannot participate in this event in person. It would have been a unique opportunity to discuss in more detail the very important aspects of conserving biodiversity in the Northwest Pacific region and ensuring the development and sustainable likelihoods of local communities and countries' economies as a whole

I do believe the audience of this workshop can speak for the present and future of our environmental science and action. Inevitably, the involvement of young scientists and professionals in advancing the current global environmental agenda is vital for overall success.

Most of us live in the Northwest Pacific region. We know that our region is one of the most densely populated areas of the world, with significant anthropogenic pressure on the regional marine ecosystems. More and more, this pressure is intensified by the negative impacts of climate change. Coastal and marine ecosystems of the Northwest Pacific continue to suffer and are further threatened by unsustainable aquaculture practices, as well as over-fishing and destructive harvesting habits.

I want to inform you that in 2014, NOWPAP Member States agreed to adopt a suite of five Ecological Quality Objectives (EcoQOs) for the region, two of which are directly relevant to marine biodiversity:

- EcoQo1: Biological and habitat diversity are not changed significantly due to anthropogenic pressure;
- EcoQO2: alien species are at levels that do not adversely alter the ecosystems.

In 2019, NOWPAP, in cooperation with the Jeju National University, submitted a joint project proposal to the APN Secretariat and received a grant to implement the current project.

The project aims to enhance the region's capabilities to participate in research and specific studies on global change versus sustainability and to support science-based decision-making in the region and beyond. These capacities are critical for developing a good knowledge base of what key indicator species are for typical natural ecosystem types and major pathways resulting in the current changes in marine biodiversity in the Northwest Pacific.

Recommendations of this international workshop, entitled "Key Indicator Species and Habitats for Marine Biodiversity Change in East Asia", are expected to strengthen the evidence-based

science-policy dialogue and capacitate the process of supporting decision-makers and enhancing public awareness in the region about marine biodiversity loss and recent negative changes.

Furthermore, the outcomes of the workshop would provide information and the required inputs into the development of the NOWPAP Regional Action Plan on Marine and Coastal Biodiversity and contribute to the achievement of SDG 14 – Life below Water.

In conclusion, I want to assure you that NOWPAP is committed and looks forward to working closely with scientists and practitioners in the Northwest Pacific region and APN Secretariat to advance our joint action to strengthen the efforts on marine biodiversity conservation and management.

With this, I want to wish the workshop participants very fruitful discussions, which would lead to looking-ahead outcomes and for the workshop - to be a success.

Yegor Volovik, MCE, PhD NOWPAP Coordinator

# **Organizing Committee**

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# **Keynote Speakers**

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# **Invited speakers**

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Dr. Christophe Vieira	Jeju National University, Republic of Korea cvcarp@gmail.com	
Dr. Hongjun Li	National Marine Environmental Monitoring Center, China hjli@nmemc.org.cn	
Dr. Shingo Udagawa	University of the Ryukyus, Japan shingouda0117@gmail.com	
Dr. Hyun-Sung Yang	Jeju Research Institute, Korea Institute of Ocean Science and Technology, Republic of Korea hsyang@kiost.ac.kr	
Dr. Hee Yoon Kang	Chonnam National University, Korea heeyoun0809@naver.com	
Mr. Ronald G Noseworthy	Jeju National University, Republic of Korea rgnshells@yahoo.ca	

# **Workshop Schedule**

28 Novembe	er 2022 (Monday)
09:00 - 09:30	Workshop Registration
09:30 - 10:00	<b>Opening Address</b> Prof. Kwang-Sik Choi (Chair of the Organizing Committee)
	Welcome Address Prof. Eel-Hwan Kim, President of Jeju National University Dr. Do-Hyung Kang, Director of KIOST Jeju Center Dr. Yegor Volovik, NOWPAP Coordinator
	Group Photo
10:00 - 10:30	<b>Conserve Marine Biodiversity in the Northwest Pacific Region</b> Dr. Ning Liu NOWPAP/UNEP

# Session 1-1 : Marine Biodiversity Indicators I Chair : Dr. Ning Liu

10:30 - 11:00	[Keynote Speaker] Biodiversity and Ecology of High Latitude Scleractinian Corals in Jeju Island Dr. Kwang-Sik Choi Jeju National University, Republic of Korea
11:00 - 11:30	[Invited Speaker] Biogeography of Marine Mollusks on Jeju Island Mr. Ronald G Noseworthy Jeju National University, Republic of Korea
11:30 – 12:00	<b>[Invited Speaker]</b> <b>Distribution and Diversity of Corals in Korea</b> Dr. Hye-Won Moon National Marine Biodiversity Institute of Korea, Republic of Korea

12:00 – 13:30 Lunch & Break

# Session 1-2 : Marine Biodiversity Indicators II Chair : Dr. Tatsuya Kawakami

13:30 – 14:00	[Keynote Speaker] Climate-Change Driven Range Shifts of Exploitable Chub Mackerel (Scomber japonicus) Projected by Bio-physical Coupling Individual-based Model in the Western North Pacific Dr. Sukgeun Jung Jeju National University, Republic of Korea		
14:00 – 14:30	[Invited Speaker] An Integrated Phylogenomic Approach for Potential Host Associated Evolution of Monstrilloid Copepods Dr. Donggu Jeon Chung-Ang University, Republic of Korea		
14:30 – 15:00	[Invited Speaker] Seaweeds Biodiversity and Role in Globally Changing Environment Dr. Christophe Vieira Jeju National University, Republic of Korea		
15:00 - 15:10	Coffee Break		

# Session 2 : Marine Biodiversity Changes Chair : Dr. Benjamin Jr. Vallejo

15:10 - 15:40	[Keynote Speaker] Changes on the Plankton Functional Groups in Jiaozhou Bay, the Yellow Sea Dr. Xiaoxia Sun Chinese Academy of Sciences, China
15:40 – 16:10	[Invited Speaker] Overview of Marine Environment and Ecology Monitoring and Assessment in China Dr. Hongjun Li National Marine Environmental Monitoring Center, China
16:10 – 16:40	[Invited Speaker] Relationship Between Moonlight and Coral Reef Organisms in Okinawa Dr. Shingo Udagawa University of the Ryukyus, Japan
16:40 - 16:50	Coffee Break
16:50 - 17:30	Discussion
17:30	Session Closing
18:00	Networking Dinner

# 29 November 2022 (Tuesday)

# Session 3-1 : Marine Biodiversity Monitoring I Chair : Dr. Kwang-Sik Choi

10:00 - 10:30	[Keynote Speaker] Environmental DNA as a Powerful Tool to Detect Fish Biodiversity in the Open Ocean Dr. Tatsuya Kawakami Hokkaido University, Japan
10:30 – 11:00	[Invited Speaker] How to Monitor Changes in the Marine Biodiversity in Jeju, Korea Dr. Hyun-Sung Yang Korea Institute of Ocean Science and Technology (KIOST), Republic of Korea
11:00 – 11:30	[Invited Speaker] Identifying Patterns in the Multitrophic Community and Food-Web Structure of a Low-Turbidity Temperate Estuarine Bay Dr. Hee Yoon Kang Chonnam National University, Republic of Korea
11:30 - 13:30	Lunch & Break

# Session 3-2 : Marine Biodiversity Monitoring II Chair : Dr. Christophe Vieira

13:30 – 14:00	[Keynote Speaker] Using eDNA to Detect the Presence of Marine Invasive Species in Ports and Harbors Dr. Benjamin Jr. Vallejo University of the Philippines, Philippines
14:00 – 14:30	[Invited Speaker] Artificial Drivers of Jellyfish Blooms and Transport of Non- Native Species Dr. Jinho Chae Marine Environmental Research and Information Laboratory, Republic of Korea
14:30 - 15:00	[Keynote Speaker]
	Dr. Tatyana Orlova National Scientific Center of Marine Biology, Russia
15:00 - 15:10	Coffee Break
15:10 - 16:00	Discussion
16:00	Session Closing
18:00	Networking Dinner

# **30 November 2022 (Wednesday)**

10:00 – 12:00 Field Trip Sungsan Folk Village Intertidal Area

# **Biodiversity and Ecology of High Latitude, Scleractinian Corals in Jeju Island**

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Abstract: Many coastal benthic communities have undergone significant changes in response to climate change and rising sea-surface temperatures. However, the shallow subtidal hard bottoms surrounding Jeju Island in Korea are an exception, as they host thriving scleractinian corals. Recent research has revealed a more diverse biodiversity of six to seven species of scleractinian corals in the region than previously thought. Notable hard coral species in Jeju include Alveopora japonica, Oulastrea crispata, Psammocora profundacella, Psammocora sp., Montipora millepora, Acanthastrea sp., and Goniastrea deformis. These hard coral communities thrive in the high-latitude marginal zone of corals and their associated fauna, where shallow marine coral species live at the edge of their environmental tolerance range. These hard coral communities thrive in the high-latitude marginal zone of corals and their associated fauna, where shallow marine coral species live at the edge of their environmental tolerance range. The scleractinian coral A. *japonica* is thriving in shallow subtidal hard bottoms around Jeju Island, off the southern coast of Korea. The presence of these corals has negatively impacted subtidal kelp populations on Jeju Island. However, few studies have been done to document how the presence or absence of this coral relates to other benthic communities. This review summarises the biodiversity and ecology of scleractinian corals of Jeju Island and identifies crucial consequences that we believe should be addressed to understand and provide suggestions for future work.

Keywords: Alveopora japonica, benthic community, diversity, ecology, Jeju Island, Korea,

### Highlights

- Previously, the coastal region of Jeju Island rich in macroalgae population
- Scleractinian corals are present in the shallow subtidal hard bottoms surrounding Jeju Island.
- The presence of *A. japonica* has negatively impacted subtidal kelp populations on Jeju Island.

### **1. Introduction**

Scleractinian corals, also known as stony or hard corals, are a diverse group of marine animals that belong to the class Anthozoa, including soft corals, sea anemones, and sea pens (Sugihara et al. 2014; Kim and Kang 2022). Scleractinian corals are characterized by their hard, calcium carbonate skeletons secreted by the coral polyps that live inside them. Among them, Zooxanthellate scleractinian corals are a group of stony corals that form symbiotic relationships with tiny algae called zooxanthellae (Jones et al. 2008; Kang et al. 2020). The zooxanthellae live inside the coral polyps and provide the coral with nutrients through photosynthesis, while the coral provides the algae with a protected environment and access to sunlight. The symbiotic interaction between zooxanthellae and scleractinian corals is crucial for developing and surviving many hard corals ecosystems. The zooxanthellate scleractinian corals receive a lot of energy from the algae's photosynthesis process, enabling them to develop their dense calcium carbonate skeletons and create intricate reef formations. In exchange, the corals give algae nutrients like carbon dioxide and nitrates. Zooxanthellate scleractinian corals are known to precipitate calcium carbonate (CaCO<sub>3</sub>) much faster than their azooxanthellate corals. This faster rate of skeletal growth is due to the fact that zooxanthellate scleractinian corals are able to utilize the energy produced by the photosynthetic activity of the symbiotic zooxanthellae to fuel their metabolic processes, including the production of calcium carbonate. This allows them to create complex three-dimensional structures such as coral reefs much more quickly than azooxanthellate corals (Vieira et al. 2016; Innis et al. 2018; Kang et al. 2020).

Scleractinian corals are usually found in the tropical region. However, corals are not limited to these areas and can also be found in temperate regions (Harii et al. 2001; Denis et al. 2015; Sugihara et al. 2014; Park et al. 2020). Studies conducted in recent years have indicated that certain scleractinian corals may be capable of migrating toward higher-latitude environments, which could serve as a refuge for these organisms to cope with the impacts of climate change (Yamano et al. 2011). The warming of ocean waters and other environmental stressors linked to climate change threaten the health and survival of coral, leading scientists to explore physiological and ecological influences in marine ecosystems. The potential migration of corals towards higher latitudes is an area of growing interest and research in the scientific community (Sugihara et al. 2014; Denis et al. 2015; Vieira et al. 2016; Lee et al. 2022). This process could allow corals to escape the most severe effects of warming waters and other climate-related stressors, such as coral bleaching, disease outbreaks, and ocean acidification (Yamano et al. 2011). The availability of suitable habitats, access to nutrients, and other factors could determine the success of coral migration and the ability of these ecosystems

to adapt to changing environmental conditions. However, migrating corals toward higher latitudes is not a straightforward process, and it is not yet clear which coral species are most capable of adapting to these new environments. Other factors, such as competition with existing organisms, changes in ocean currents and weather patterns, and the availability of suitable habitats, could also influence the success of coral migration (Yamano et al. 2011; Vieira et al. 2016; Kang et al. 2020; Lee et al. 2022).

Unlike in tropical regions, where corals form massive reef structures, they do not usually form coral reefs in temperate areas. Instead, they tend to grow as solitary individuals or in small colonies. One major factor affecting the distribution of corals in temperate regions is the impact of warm poleward currents. These currents transport warm water from tropical regions to temperate areas, providing suitable conditions for corals to grow. With the increasing effects of climate change, these warm currents are becoming more common and intense, allowing corals to expand their range and establish new colonies in previously uninhabitable areas (Yamano et al. 2011; Denis et al. 2015). The increase in Surface Seawater Temperature (SST) has been a major factor in the poleward movement of corals. SST has increased by 1.6 to 2.1 Celsius over the past century around Jeju Island, which is expected to continue (Takatsuki et al. 2007). This increase in temperature is leading to changes in the distribution and abundance of many marine species, including corals (Yamano et al. 2011). The expansion of coral populations into temperate regions has significant implications for the biodiversity of these ecosystems. Fish, crustaceans, and mollusks also show range expansion with the corals, which changes temperate marine ecosystems' food webs and trophic interactions (Vergés et al. 2014; Vergés et al. 2016). While the expansion of corals into temperate regions is a fascinating phenomenon, it is also a cause for concern. The warming of the oceans is a clear sign of climate change, and the impacts of this warming on marine ecosystems are still not fully understood. Coral populations in temperate regions may be at risk if SST continues to increase, as the corals may not be able to adapt quickly enough to these changes (Denis et al. 2015; Vieira et al. 2016).

Jeju Island is the largest island, approximately 90 km south of the Korean Peninsula. Geographically, Jeju Island is ellipsoidal, with its long and short axes measuring 74 and 32 km, respectively (Figure 1), with an annual seawater temperature ranging from 13.7 to 25°C (Park et al. 2020). The island's southern coast is influenced by the Yellow Sea Warm Current, which flows along the western coast of Japan and into the East China Sea, ultimately reaching the south coast of Jeju Island (Denis et al. 2015). Further, the island is located within a high-latitude region, which means it is not typically associated with the presence of coral reefs. However, recent studies have shown that Jeju Island is home to a healthy population of scleractinian corals not typically found in temperate regions. Therefore, in recent years, Jeju Island has become an important center for marine research, particularly in coral ecology. Scientists have been studying the distribution and abundance of scleractinian corals in the region and have found that the presence of these corals is closely linked to changes in the local benthic ecosystem (Denis et al. 2015; Veira et al. 2016; Park et al. 2020; Lee et al. 2022).

Underwater photography and image analysis have revolutionized the study of hardbottom ecosystems. The technique requires SCUBA diving skills to capture images of the hardbottom ecosystem. The images can then be analyzed using various software applications to extract useful data. One of the biggest advantages of underwater photography and image analysis is that it is non-destructive. Traditional methods of studying hard-bottom ecosystems often involve collecting samples, which can damage the ecosystem, affecting the data collected and having long-term effects on the ecosystem (Smith et al. 2005). On the other hand, underwater photography and image analysis do not require physical contact with the ecosystem, making it a truly non-destructive method. Underwater photography and image analysis are also very useful in providing visual data that can be both qualitative and quantitative (Foster et al. 1991; English et al. 1997; Leujak and Ormond 2007). Researchers can use images to identify different species, assess the coverage or density of a particular species, or count the number of individuals of a species. This information can then be used to make inferences about the health and diversity of the ecosystem. The data obtained from underwater photography and image analysis can be very diverse. Species images are one of the most common data types obtained from this method. These images can identify different species of flora and fauna in the hard-bottom ecosystem. Density data, such as coverage or counts, can also be obtained using this method. Researchers can use this data to determine the abundance of different species in the ecosystem and monitor changes in population sizes over time (Ninio et al. 2003; Lee et al. 2022).

The review article aims to provide a comprehensive overview of the biodiversity and ecology of high-latitude, scleractinian corals on Jeju Island. The scope of the review article will cover the different species of scleractinian corals found on Jeju Island, their distribution and abundance, and the factors that affect their ecology, including physical and chemical characteristics of the environment, reproduction and recruitment strategies, and interactions with other biotic and abiotic factors. The review article will also examine the threats to highlatitude scleractinian corals on Jeju Island, including climate change, and anthropogenic impacts.



Figure 1. Map showing the location of the Republic of Korea and a close-up view of Jeju Island

#### 2. Biodiversity of high latitude, scleractinian corals in Jeju Island

Jeju Island is known for its unique and diverse marine environment, with a rich biodiversity of at least 92 species of Anthozoa inhabiting Jeju waters. At the same time, few of them belong to the hard scleractinian coral group that can be found along the coastline of Jeju Island. Among the hard coral species found in Jeju, some of the most notable include Alveopora japonica, Oulastrea crispata, Psammocora profundacella, Psammocora sp., Montipora millepora, Acanthastrea sp., and Goniastrea deformis (Denis et al 2015; Veira et al. 2016). These hard coral communities are able to survive and thrive in the high-latitude marginal zone of corals and their associated fauna, where shallow marine coral species live at the edge of their environmental tolerance range. As a result, shallow marine coral species in this region live at the limits of their environmental tolerance range. Especially the harsh environment affects growth and reproduction, which are considerably reduced, particularly in the case of scleractinian corals of tropical origin (Kim and Kang, 2022). These high-latitude scleractinian corals on Jeju Island represent an important component of marine biodiversity in the region, and the study of biodiversity in high-latitude scleractinian corals in Jeju Island provides insight into the unique and diverse marine environment of this region and the importance of understanding and preserving it for future generations.

#### 2.1 Description of the different species of scleractinian corals found on Jeju Island

#### 2.1.1. Alveopora japonica

Alveopora japonica, a species of scleractinian coral belonging to the Acroporidae family, is a unique organism found exclusively in non-reefal temperate waters. This coral is endemic to the north-western Pacific and has been observed in only a few locations, including the Pacific Coast of Japan, Jeju Island in Korea, and Taiwan in China (Harii et al. 2001; Park et al. 2020). Despite its limited distribution, *A. japonica* is a fascinating coral that has captured the attention of researchers and conservationists alike. This species is known for its distinctive appearance, characterized by its long finger-like branches (Figure 2A). In addition to its aesthetic qualities, *A. japonica* plays an important ecological role in its habitat, providing shelter and habitat for a wide range of other marine organisms. Interestingly, an outbreak of *A. japonica* has been observed in the northern part of Jeju Island for the past 30 years, highlighting the dynamic nature of coral populations and their ability to adapt and change over time (Denis et al. 2013; Sugihara et al. 2014; Denis et al. 2015). Understanding their distribution, abundance, and ecological relationships can provide valuable insights into the functioning of marine ecosystems in high-latitude environments (Denis et al. 2015).

#### 2.1.2. Oulastrea crispata

*Oulastrea crispata* is a coral species found across a wide range of locations, spanning from the Great Barrier Reef in Australia to Japan (Veron 2000). This coral forms colonies that

exhibit various morphologies, including encrusting and sub-massive forms. The size of the colonies can range from just a few centimeters to over 10 centimeters in diameter (Figure 2B). *Oulastrea crispata* is also known as "Zebra coral" for its unique colony structure due to its black skeleton with white upper margins to the radiating septa (Sugihara et al. 2014; Park et al. 2020).

#### 2.1.3. Psammocora profundacella

*Psammocora profundacella* is a coral that can form encrusting or submassive colonies. In tropical and subtropical regions, the size of the colony is typically not more than 15-20 cm in diameter, although, in the temperate areas around Jeju and Japan, it can reach up to 30 cm. The edges of the colony are thin, undulated, and usually remain attached to the substrate. The surface of the colony appears rugged due to the presence of well-developed ridges or collines, with small nodular mounds scattered throughout. During the day, the coral's tentacles extend outward, and they are typically short and slightly tapered, ending in a rounded tip. The color of the polyps can range from light brown to dark green, and the appearance of the colony may vary depending on environmental factors such as light and water quality (Veron 2000; Benzoni et al. 2010; Sugihara et al. 2014).

#### 2.1.4. Psammocora sp.

The growth pattern of this coral species is predominantly encrusting, following the contours of the underlying substrate. The surface of the colony is characterized by small, knobby, or nodular mounds, which can be scattered irregularly across the colony. The size of these mounds can range from a few millimeters to several centimeters in diameter. The diameter of the colony itself can range from just a few centimeters to over a meter in size. It is not uncommon for the edges of the colony to be slightly raised, giving it a detached appearance from the underlying substrate. The colony's surface is typically smooth. Interestingly, the outlets of the siphons of boring bivalves may also be present on the surface, indicating the presence of other organisms that coexist with the coral. The color of this coral species varies from yellowish-green to dark brown, depending on the specific location and environmental conditions. The tentacles of this species are a defining feature, as they are short, even, and densely distributed across the surface of the colony (Benzoni et al. 2007; Sugihara et al. 2014; De Palmas et al. 2015). The tips of the tentacles are rounded; during the daytime, they extend outwards, creating a unique and striking appearance.



Figure 2. Notable scleractinian coral species are found around Jeju Island. A- Alveopra japonica; B- Oulastrea crispate; C- Montipora millepora

#### 2.1.5. Montipora millepora

*Montipora millepora* is a species of coral that exhibits an encrusting growth pattern, forming colonies with an average thickness of approximately 6mm. This type of coral is commonly found on rock substrates, typically at depths ranging from 5-10 meters, throughout the Indo-Pacific region, including Jeju Island. The colonies of *M. millepora* consist of smaller polyps, the individual animals that make up the colony. The polyps are interconnected through a network of tissue, and they work together to form the overall structure of the colony. The surface of the colony can appear green to brownish in color, depending on the specific location and environmental conditions (Figure 2C). *Montipora millepora* is a particularly important coral species in coral reef ecosystems, as it plays a key role in providing habitats and shelter for various marine organisms (Sugihara et al. 2014; Kim and Kang 2022).

#### 2.1.6. Acanthastrea sp.

The shape of colonies of this coral species is primarily encrusting to submassive, and in some instances, they can grow to an impressive size of approximately 20 cm across. When living, these colonies exhibit a fleshy and vibrant appearance, making them a fascinating sight to behold underwater. The polyps of this species extend during the day, showcasing an array of stunning colors, including shades of gray, red, purple, and green. In some cases, the color of the oral disc can differ from that of the other living parts, adding even more visual intrigue to the colony. Additionally, concentric wrinkles surrounding the oral discs may be observed, creating a unique texture and pattern on the polyp surfaces (Sugihara et al. 2014).

#### 2.1.7. Goniastrea deformis

Goniastrea deformis is a species of stony coral found in the Indo-Pacific region's shallow waters, including Australia, Fiji, and Indonesia. Colonies of this species typically take

on a hemispherical to massive shape, with a cerioid structure characterized by round to polygonal corallites that range from 8 to 17 mm in diameter (Sugihara et al. 2014). The occasional formation of valleys with up to three distinct centers creates an intricate and visually striking appearance.

#### 2.2. Factors affecting the distribution and abundance of corals on Jeju Island

A complex interplay of various biotic and abiotic factors influences the distribution and abundance of corals in a region. Some of the major factors affecting the distribution and abundance of corals in a region are as follows:

**Temperature:** Temperature is one of the most significant factors influencing the distribution and abundance of corals. Corals are highly sensitive to changes in temperature and require specific temperature ranges for growth and reproduction (Harii et al. 2001; Park et al. 2020). As a result, the distribution of corals is often limited by temperature, and their abundance is affected by temperature fluctuations.

**Water Quality:** The water quality of a region is another critical factor influencing the distribution and abundance of corals. Corals require clear and clean water with low nutrient levels to thrive. Water pollution, eutrophication, sedimentation, and other water quality factors can negatively impact coral communities (Littler et al. 2009).

**Light:** Light is a key factor that influences the distribution and abundance of corals. Corals require sunlight to grow and form symbiotic relationships with photosynthetic algae called zooxanthellae. Therefore, the distribution of corals is often limited to shallow areas with adequate light penetration (Innis et al. 2017). Usually, in the temperate region, light penetration is limited up to 20 - 30m (Vieira et al. 2015; Innis et al. 2017).

# **2.3.** Comparison of the biodiversity in Jeju Island with other high-latitude coral ecosystems

Similar coral species have already been reported in Japan (Harii et al. 2001; Sugihara et al. 2014; Isomura and Fukami 2018). The species composition of coral varies in the different areas of Japan, with a notable decrease in species numbers along the latitudinal gradient. In the Ryukyu Archipelago-Ogasawara Islands, the dominant genera of coral are *Acropora*, *Montipora*, *Pocillopora*, and *Porites*. However, the coral community in the temperate reefs of Iki and Tsushima Islands is dominated by *Dipsastraea*, *Echinophyllia*, and *Caulastrea*. The coral reefs in Japan are unique due to the occurrence of well-developed coral reefs in temperate areas, facilitated by warm currents such as the Kuroshio and Tsushima Currents (Nomura et al. 1994; Sugihara et al. 2009). The Iki-Tsushima region and the Oki Islands have been recognized as important sites for studying high-latitude coral ecosystems in Japan. It has been reported that all seven species of hard corals which are found on Jeju Island expected to be found in the

Iki-Tsushima region, and of these, *A. japonica*, *P. profundacella*, and *O. crispata* have been observed in the Oki Islands. Interestingly, the species composition of Jeju Island falls between those of the Iki-Tsushima region and the Oki Islands. However, Jeju and the Oki Islands corals are sparsely distributed and do not form large communities, indicating their marginal location at the end of the latitudinal gradient (Sugihara et al. 2009; Sugihara et al. 2014).

#### 3. Ecology of high latitude, scleractinian corals in Jeju Island

Several factors, including water temperature, light intensity, water movement, nutrient availability, and substrate type, influence the ecology of high-latitude corals. Due to the sparse distribution of corals on Jeju Island, competition for resources among corals is less intense. However, this also makes them vulnerable to environmental disturbances like pollution and climate change. Further, Jeju Island is located at the northern limit of the range for many coral species, making it a unique site for studying the impacts of environmental change on these organisms. The presence of a stable and thriving population of scleractinian corals in the waters surrounding Jeju Island suggests that significant changes have occurred in the coastal ecosystem, leading to alterations in benthic composition, competition, and biodiversity (Sugihara et al. 2014; Denis et al. 2015; Vieira et al. 2016; Lee et al. 2022). Recent reports of sub-tropical fish species and new scleractinian coral species (Takatsuki et al. 2007; Denis et al. 2013; Kim et al. 2015; Kang et al. 2020) suggest that these coral communities have either migrated from tropical regions or expanded due to rising sea surface temperatures. Consequently, there has been an increase in scleractinian coral populations in the coastal benthic ecosystem (Vieira et al. 2016), leading to a shift from macroalgae to dominant coral ecosystems in some parts of Jeju Island (Kang 2010; Denis et al. 2015; Vieira et al. 2016; Kim and Kang 2022). Despite their ecological significance, the high-latitude corals in Jeju Island are still poorly understood. More research is needed to understand their ecological roles, interactions, and responses to changing environmental conditions. The discovery and study of A. japonica have significantly increased our understanding of coral diversity and evolution. This coral's unique distribution and ecological requirements have shed light on the adaptations that enable corals to survive in various marine environments. Researchers continue to study A. japonica to gain insights into this fascinating species' biology, ecology, and conservation, which may hold important clues for preserving other corals and marine ecosystems worldwide. Some corals in Jeju Island may have unique genetic and morphological characteristics that have not been documented in other regions, which could have important implications for coral conservation and management (Kang et al. 2020).

Remarkably, the northern coast of Jeju has reported up to 75 percent coverage of scleractinian corals, despite being considered marginal and unable to form reefs at high latitudes (Vieira et al. 2016). Additionally, when keystone species disappear, new species may be introduced from tropical locations, leading to further changes. Studies have shown that these changes have affected the benthic community dynamics in Jeju Island, with the dominant coral species *A. japonica* experiencing high densities in some locations (Denis et al. 2013; Denis et

al. 2015; Vieira et al. 2016). Increased abundance of *A. japonica* may contribute to the decline of brown macroalgae in Jeju. Anecdotal evidence has shown that until the 1980s, brown macroalgae were dominant in shallow subtidal rocky bottoms in Jeju, playing a vital ecological and economic role (Kim 2006; Kang et al. 2012; Vieira et al. 2016). However, in the past few decades, macroalgal communities in high latitudes, including Jeju, have been decimated due to temperature and other factors, resulting in barren grounds with coralline algae and little else covering the rocks and has led to a coral takeover in many locations. Despite several studies on the benthic organisms in Jeju Island, few studies have examined the ecological association of *A. japonica* with macroalgae and other benthos at different locations and depths (Vieira et al. 2016; Lee et al. 2022).

#### 4. Conclusion

The impact of climate change on coastal benthic communities has been significant, with rising sea-surface temperatures causing major changes. However, Jeju Island in Korea has shown to be a unique exception with its thriving scleractinian coral populations. This review has highlighted the diversity of hard coral species found on Jeju Island, including Alveopora japonica, Oulastrea crispata, Psammocora profundacella, Psammocora sp., Montipora millepora, Acanthastrea sp., and Goniastrea deformis. Among these species, A. japonica is particularly noteworthy as it thrives in the shallow subtidal hard bottoms around Jeju Island, despite the climate changes. However, the presence of these corals has negatively impacted subtidal kelp populations on Jeju Island, and further studies are needed to understand how the presence or absence of these corals affects other benthic communities. Overall, the unique biodiversity and ecology of scleractinian corals in Jeju Island underscore the need for continued research and conservation efforts to ensure their long-term survival. Interestingly, M. millepora has been the subject of extensive research in recent years as scientists seek to understand better the mechanisms that underlie its remarkable resilience and adaptability in the face of environmental stressors, such as ocean acidification and rising sea temperatures. By studying these corals, scientists hope to gain valuable insights into how corals and other marine organisms may be able to adapt and survive in the face of ongoing climate change.

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## **Biogeography of the Mollusks of Jeju Island**

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Jeju Island is Korea's largest island with an area of about 1800 km<sup>2</sup>. It is located about 80 km south of the Korean Peninsula. The island has a subtropical climate with mild winters and hot, humid summers, and an average yearly temperature of 16°C. (Chen, 2009)). The warm Tsushima Current branches from the northeastward flowing Kuroshio Current and flows past Jeju Island through the Korea Strait. Cooler water currents, particularly from the eastern area of the Yellow Sea which flow past the western Korean coast, exert some influence, particularly to the north of Jeju Island, The area with the lowest water temperature is the north-eastern coast of Jeju: in February (11.0°C) (Ko J.-C. et al., 2003). However, the Tsushima Current predominates, with; the southern coastal area of the island having somewhat warmer sea temperatures, The blending of warm and cool currents has given Jeju Island a rich mollusk fauna, with over 1000 species presently recorded (Noseworthy et al., 2007).

A survey of mollusk distribution along the Jeju coastline reveals that most species occur on the south and east coasts, where the Tsushima Current exercises a stronger influence as it flows northeastward into the East Sea, and this influences the coastal distribution of mollusks. Gastropods, such as Cypraeidae, Conidae, and Ovulidae, which are mostly tropical in distribution occur mainly on the south coast, and most others are also found along the south and east coasts. Although many common bivalves are found on all coasts of the island, the largest number of species, including those with tropical distribution such as the Pteriidae, occurs on the south coast only, with a slightly smaller number found on both south and east coasts. (Lutaenko *et al.*, 2019, 2021) The Polyplacophora and Cephalopoda (mainly cuttlefish and argonautids), also have a mostly southerly island distribution. The Scaphopoda are divided evenly between the south and east coasts (Noseworthy, 2015)

Zonal-geographical groupings more clearly show the biogeographical affinities of mollusk species. The Jeju fauna is mainly a combination of subtropical (Korea and Japan to Taiwan) and tropical-subtropical species (southward to southeast Asia). There has been a large number of new species reported for the Jeju fauna. Since the first catalogue of Jeju Island mollusks was published in 2007 (Noseworthy *et al.*, 2007), which indicated that a subtropical influence predominated, approximately three dozen new species have been added (Table 1), almost all with a tropical-subtropical affinity (Fig. 1), giving the island a fauna, which is increasingly influenced by the warming ocean currents. The exception is the Polyplacophora, which is subtropical-low boreal in distribution, preferring cooler water (Noseworthy, 2015)

Most Jeju mollusk species are associated with rocky and sandy coastlines; mudflats are virtually nonexistent (Choi K-S. et al.,2000), and mudflat-associated mollusks, such as Rapana venosa and Meretrix spp. do not occur on Jeju Island. Most of its 92 species of hard and soft corals are found mainly on the south coast, and many species of nudibranchs, cypraeids, ovulids, and chamids inhabit the corals (Koh, 2006, 2011). Of particular interest is the scleractinian coral *Alveopora japonica*, which forms coral carpets at several localities around the island.

**Table 1.** New species records for Jeju Island showing zonal-geographical affinities (Since 2007). (From published records and field surveys)

#### Polyplacophora

Cryptoplax propior Is. & Iw. Taki, 1930

subtropical

#### Gastropoda

Ginebis crumpii (Pilsbry, 1893)	subtropical
Enida japonica A. Adams, 1860	tropical-subtropical
Canarium microurceus Kira, 1959	tropical-subtropical
Mauritia arabica asiatica Schilder & Schilder, 1939	tropical-subtropical
Monetaria moneta (Linnaeus, 1758)	tropical-subtropical
Gelagna succincta (Linnaeus, 1771)	tropical-subtropical
Turritriton labiosus (Wood, 1828)	tropical-subtropical
Bursa bufonia (Gmelin, 1791)	tropical-subtropical
Monophorus testaceus (Kosuge, 1963)	tropical-subtropical
Bouchetriphora conspersa (E. A. Smith, 1885)	tropical-subtropical
Semiricinula muricoides (Blainville, 1832)	tropical-subtropical
Vexillum zelotypum (Reeve, 1845)	tropical-subtropical
Vexillum roseum (Broderip, 1836)	tropical-subtropical
Conus sponsalis Hwass in Bruguière, 1792	tropical-subtropical
Ammonicera japonica Habe, 1972	tropical-subtropical
Bullina lineata (Gray, 1825)	tropical-subtropical

#### Bivalvia

	tropical-subtropical
06)	subtropical
	subtropical
	subtropical
	tropical-subtropical
	tropical-subtropical
tropica	l-subtropical
t	ropical-subtropical
	tropical-subtropical
	tropical-subtropical
	tropical-subtropical
	subtropical
	subtropical-lowboreal
	tropical-subtropical
	subtropical-lowboreal
	lowboreal
	tropical-subtropical.
	subtropical
	tropical-subtropical
	subtropical-lowboreal
	06) tropica t

#### Cephalopoda

Liocranchia reinhardti (Steenstrup, 1856)

tropical-subtropical

Several studies have revealed that 27 bivalves and gastropods have been identified, with bivalves predominating, which inhabit the base of the coral carpets The mollusk species in the coral may use it as a nursery for juvenile species, a food supply of organic detritus, and a refuge from predators (Noseworthy *et al.*, 2022)



Fig. 1: Zonal-geographical characteristics of all new species

Climate change caused by global warming has caused many marine organisms to expand the geographical range of their habitats and is a strong possibility for the addition of new mollusk species to Jeju Island's fauna. The region around Jeju Island has been reported as one of the fastest warming regions in the world, and the sea surface temperature has risen significantly over the last century. There has been a  $1.6^{\circ}$ C increase in average winter temperatures since the 1930s (Kim Y.*et al.*, 2012). Furthermore, sea temperature in the southern Yellow Sea area, including Jeju Island, has risen by  $1.3^{\circ}$  C in the past 100 years (Yamano *et al.* (2010).

Coastal development, especially port development, has caused a loss of habitat along some parts of Jeju Island's coastline. Overcollection of edible species is becoming a problem in certain areas; intensive collecting also disturbs the habitats of other species, resulting in a loss of biodiversity. At Ojo-ri, a large lagoon on the east coast, the populations of *Solen strictus* and *Venerupis (Ruditapes) philippinarum* have been significantly reduced (Lee *et al.*, 2014). A very important factor influencing the natural environment of Jeju Island is tourist pressure which has also had a detrimental effect. More than 12 million tourists visited the island in 2921 (Asia Gaming News, 2022), and this has resulted in the construction of resorts and other tourist facilities, also including the modification of beaches. The effects of this can be seen in the

hotspots" of mollusk diversity at Hwasoon, Yeongmeori, Yerae, and Seongsan, where, a decade ago, large numbers of species could be obtained, but now yield far fewer.

Thus, with climate change and coastal development, the biogeography of Jeju Island is dynamic, and future changes are to be expected.

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# **Diversity and distribution of corals in Korea with notes on key indicator species**

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**Abstract:** Total 170 species, including soft corals (80 species of the order Alcyonacea), hard corals (35 species of the order Scleractinia), sea anemones (30 species of the order Actiniaria), and black corals (12 species of the order Antipatharia) have been reported from Korean waters by examining the specimens collected in the coastal areas of Korea from 1965 to 2021. Geographical analyses were performed to determine the distribution of coral species and to compare the species composition between regions in Korean waters. The 170 recorded coral species have been distributed in the following four regions, Korea: East Sea (28 species), Yellow Sea (21 species), South Sea (68 species), and Jejudo Island (124 species). In particular, given that scleractinian corals are significantly influenced by climate change in various ways, including because of their symbiotic relationship with zooxanthellae, the species composition to predict future environmental change in Korean waters.

Keywords: Anthozoa, Montipora efflorescens, Scleractinia, Korean waters, climate change

## Highlights

- Jejudo Island has the highest diversity of coral species
- Most coral species in Korea are limitedly distributed along the Kuroshio Current
- Montipora effloresces is would be important as an indicator species

### **1. Introduction**

The class Anthozoa comprises approximately 7,500 species in the world. Among them 170 species, including soft corals (80 species of the order Alcyonacea), hard corals (35 species of the order Scleractinia) (Choi and Song, 2017), sea anemones (30 species of the order Actiniaria), and black corals (12 species of the order Antipatharia) (Moon and Song, 2008a; 2008b) have been reported from Korean waters by examining the specimens collected in the coastal areas of Korea from 1965 to 2021. Korea being located in the temperate zone is showing the world-widely rare habitat aspect forming the soft coral community belonging to Alcyonacea in the southern part of Jejudo Island. Also hard corals are being distributed mainly in JeJudo Island area, but they are not forming the coral reef (Song, 2004). This study aims to

clarify the distribution of coral species along the coastal waters of Korea and to identify indicator species for the long-term monitoring of the changes in the distribution of coral species in response to climate change.

### 2. Methodology

The specimens were collected from subtidal zones in the coastal regions Korea between by SCUBA or fishing nets from 1965 to 2021. The specimens were anesthetized with menthol for 6-8 hours, and then fixed in 4-5% seawater buffered formalin. After that fixed specimens were washed with tap water, and preserved in 70% ethyl alcohol, and identified on the basis of morphological characters such as growth form and skeletal structure with light and stereo microscopes. The specimens were deposited in the Natural History Museum of Ewha Womans University and National Marine Biodiversity Institute of Korea. Geographical analyses were also performed to determine the distribution of coral species and to compare the species composition between regions in Korean waters.

### 3. Results and Discussion

The 170 recorded coral species have been distributed in the following four regions, Korea: East Sea (28 species), Yellow Sea (21 species), South Sea (68 species), and Jejudo Island (124 species). As a results, it is shown that Jejudo Island has the highest diversity of coral species (73%) while the Yellow Sea has the lowest diversity of coral species (12%) (Fig. 1). The results reflect that most coral species in Korea are limitedly distributed along the Kuroshio warm Current with development of unique soft coral communities (Fig. 2)

In particular, given that scleractinian corals are significantly influenced by climate change in various ways, including because of their symbiotic relationship with zooxanthellae, the species composition and distribution of scleractinian corals as a key species provide critical baseline information to predict future environmental change in Korean waters (Fig. 3). Most scleractinian corals in the tropical oceans are zooxanthellate. Six species of 35 scleractinian corals recorded in temperate Korean waters are zooxanthellate. As sea temperatures increase, the distribution of tropical zooxanthellate scleractinian *Montipora efflorescens* Bernard, 1897 is dominantly expanding from the southern part to the northern part in Jejudo Island. In this respect, it is considered that this species would be important as an indicator species for monitoring environmental consequences induced by climate change.



Figure 1. Comparison of coral species composition between regions



Figure 2. Vertical distribution of coral communities in Jeju Is.


Figure 3. Species composition of Scleractinian corals by region

# 4. Conclusion

The species composition and distribution of scleractinian corals as a key species provide critical baseline information to predict future environmental change in Korean waters.

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# Session 1-2: Marine Biodiversity Indicators II

# Climate-change driven range shifts of exploitable chub mackerel (*Scomber japonicus*) projected by bio-physical coupling individual-based model in the western North Pacific

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Abstract: We projected the effects of warming ocean on the range shift of biomass of chub mackerel (Scomber japonicus) covering from the larval to the adult stages up to age 3 yr by developing and applying individual-based models (IBM) based on a regional ocean circulation model for the western North Pacific and two climate change scenarios. From laboratory experiments, we observed a diurnal cycle in the buoyancy of larval mackerel. Our IBMs tentatively suggested that the larval and juvenile mackerel in the Korea Strait, the Japanese coastal areas and the Kuroshio extension areas are mostly transported from the East China Sea where they were hatched. Despite the greater uncertainty, the preliminary results of our IBMs projected that, by the 2050s, the strengthened Tsushima warm current in the Korea Strait and the Japan/East Sea (JES), driven by global warming, will shift the young-of-the-year mackerel biomass distribution north to the JES, and adult mackerel biomass north, especially in the Yellow Sea. To improve the model performance, international cooperative research among the regional countries is required, especially for extensive ichthyoplankton surveys in the East China Sea.

**Keywords:** Tsushima current stock, temperature-dependent growth, sizedependent mortality, Lagrangian tracking

# Highlights

- Projection of the chub mackerel dispersion by the IBMs based on the global warming
- The larval mackerel hatched in the ECS are mostly transported by Kuroshio Current
- The mackerel habitats will shift north to the Japan/East Sea

# **1. Introduction**

Chub mackerel are widely distributed in the western North Pacific. During the past 40 years, the sea surface temperature around Korea have significantly increased by global warming (Jung, 2008). In the Korean waters, some species showed a significant relationship between the mean monthly-latitude of the catch distribution of fishes from and the water temperatures (Jung, Pang, Lee, Choi, & Cha, 2014). The mean latitude of chub mackerel has not shown a significant overall northward shift (Jung, Pang, Lee, Choi, & Cha, 2014), however, the catch distribution of chub mackerel has shifted from the Korea Strait to the Yellow Sea (YS) in recent years, with Korean fishermen historically catching mackerel in the Korea Strait before the early 1990s. Therefore, we tried to project the chub mackerel dispersion based on the climate changes in the ocean circulation for predicting shifts in the major fishing grounds of chub mackerel. For this purpose, we first developed a regional ocean circulation model for projecting oceanic conditions in the 2050s based on the IPCC climate change scenarios. Second we developed a bio-physical coupling, individual-based model (IBM) (Hinckley, Hermann, & Megrey, 1996, 2000; Megrey & Hinckley 2001) for predicting the spatial distributions of young-of-the-year and exploitable chub mackerel.

# 2. Methodology

## 2.1 Physiological model

We developed the regional ocean circulation model for our study area (western North Pacific; 118°00'-143°00'E and 20°00'-52°00'N) based on a numerical ocean model, the Regional Ocean Modeling System (ROMS) (Shchepetkin & McWilliams 2005). The model, based on the IPCC AR5 RCP 8.5, encompassed the entire Korean waters with a 1/12° horizontal resolution and 7 vertical layers (1, 10, 20, 30, 40, 50, and 75 m depth). We retrieved the daily-averaged water temperatures and velocities of the current in x, y, z directions from the model results to be used in the IBM.

## 2.2 Biological model

From literature, we collected information on the biology of chub mackerel, such as spawning grounds (Yukami, Ohshimo, Yoda, & Hiyama, 2009), growth (Go, Lee, & Jung, 2020), mortality (Go, Lee, & Jung, 2020), and swimming speed (Dickson, Donley, Sepulveda, & Bhoopat, 2002). We measured the daily specific gravity of chub mackerel larva in the laboratory at Pukyong National University in 2014 and 2015. We estimated the optimal temperature ranges of juvenile and adult mackerel using spatially-explicit catch and water-temperature data.

## 2.3 Bio-physical coupling

The larval mackerel drift passively with currents because of their limited swimming ability. Therefore, we assumed that their horizontal movement speed is same to the current speed of their ambient water. We applied Stokes' law to estimate their sinking speed by mass-density difference (Parada, Van Der Lingen, Mullon, & Penven, 2003). We calculated their

vertical movement speed by adding the sinking speed and the vertical current speed. We estimated their growth as a function of the ambient water temperature derived from the ROMS data. We assumed that, after metamorphosis, chub mackerel actively move towards a water mass of their optimal water temperature range at a speed below their maximum sustaining swimming speed.

# 3. Results and Discussion

Our IBM showed that the mackerel larvae spawned in the southern East China Sea are transported north and east to the YS, the Korea Strait and the Japan/East Sea (JES) (Fig. 1). The simulations showed that the potential catch of chub mackerel will increase substantially in the Yellow Sea in 50 years by global warming. To validate our model, we compared predicted data based on our IBM with observed catch data reported by Korean fishermen (Fig. 2). The predicted areas of mackerel catch embraced the observed catch areas. The Korean fishing vessels targeting mackerel seemed to stay in the waters around Jeju island and the YS from June to December. Although the Korean fishermen seemed to hesitate to trip far away to catch mackerel, they occasionally went to the Japanese coastal area, especially from January to March, when they could not catch mackerel in the YS.



Figure 1. Prediction of the young-of-the-year mackerel biomass using the individual based model of chub mackerel based on RCP 8.5



Figure 2. Comparison of the prediction based on the result of our individual based model for chub mackerel (above) and observed catch data reported by Korean fishermen (below)

# 4. Conclusion

We developed individual-based model (IBM) of chub mackerel for predicting spatial distributions of chub mackerel. Despite uncertainties, our model projected that the survival of larvae will be enhanced in overall, and the distribution of chub mackerel during juvenile/adult stages will be shifted northeast to the Japan/East Sea in the 2050s. The results are preliminary, and the model is still at the early stage of development. We hope further internationally-cooperative studies to reliably assess and project the climate-driven range shifts of chub mackerel.

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# Monstrilloid phylogeny and evolution

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**Abstract:** The order Monstrilloida is one of the most mysterious groups of Copepods with an unusually atrophied morphology and a complex semi-parasitic lifestyle. The lack of common diagnostic features from their morphological peculiarity and little information about the endoparasitic juveniles have caused many uncertainties and ambiguities in their taxonomy and phylogeny. To elucidate phylogenetic relationships and evolutionary significance of these organisms, we generated two genomes and three transcriptomes from four monstrilloid species and analyzed the 25 nuclear protein-coding genes from 40 arthropods. The molecular phylogenomic results revealed a monophyly of Monstrilloida and found that Monstrilloida is more closely related to Harpacticoida (Oligoarthra) than Siphonostomatoida. These phylogenomic relationships within Copepoda were confirmed by statistical tree topology tests and the previously known phylogenies were rejected. Given the new phylogeny, we investigated hypotheses about monstrilloid evolution by integrating the known morphological traits of four monstrilloid genera.

Keywords: copepod evolution, Monstrilloida, molecular phylogenomics.

# **1. Introduction**

Phylogenetic considerations into Copepoda including Monstrilloida were carried out mainly based on their morphological characteristics (Dahms, 2004; Ho, 1990; Ho, Dojiri, Hendler, & Deets, 2003; Huys & Boxshall, 1991). These previous studies indicated that the monophyletic Copepoda is divided into two infraclasses, Progymnoplea (order Platycopioida) and Neocopepoda, and the later infraclass was further divided into two superorders, Gymnoplea (order Calanoida) and Podoplea. In the podoplean phylogeny, Monstrilloida has frequently appeared as a sister group of another parasitic order, Siphonostomatoida, but this relationship had been questioned by Huys et al. (2007). These authors conducted molecular analyses using nearly complete sequences of the nuclear 18S ribosomal RNA gene from four copepod orders (Calanoida, Cyclopoida, Siphonostomatoida, and Monstrilloida) and suggested an alternative relationship that Monstrilloida would be a subgroup of Siphonostomatoida. On the other hand, similar molecular study using the same genetic information showed unresolved

polytomy between three orders, Harpacticoida, Siphonostomatoida, and Monstrilloida (Ki et al., 2009). Therefore, there is no ultimately agreed upon phylogenetic position for monstrilloids with either morphological or molecular data.

The purpose of this study is to clarify the phylogenetic uncertainty of Monstrilloida. For this, we used transcriptomic and/or genomic data from four monstrilloid species (*Monstrilla grandis*, *Caromiobenella ohtsukai*, *Maemonstrilla* sp., and *Monstrillopsis longilobata*). Further inclusion of the data from a wide range of arthropod taxa including four other copepod orders greatly enhances our understanding of the copepod phylogeny.

# 2. Methodology

Transcriptomic and genomic data of four monstrilloid species were used to investigate the molecular phylogenetic position of Monstrilloida. In addition, publicly available genomic and/or transcriptomic data of diverse arthropods (Hexapoda, Branchiopoda, Remipedia, Decapoda, and Thecostraca) including four major copepod orders (Calanoida, Cyclopoida, Harpacticoida, and Siphonostomatoida) were downloaded from GenBank and used for the downstream analyses. The next-generation sequencing data from 40 arthropod species were assembled using SPAdes (ver. 3.15.2) (Bushmanova, Antipov, Lapidus, & Priibelski, 2019) or Trinity (ver. 2.10.0) (Grabherr et al., 2011). Based on previous studies (Eyun, 2017; Regier et al., 2008), the orthologous sequences of 25 nuclear protein-coding genes were searched from the assemblies using the Basic Local Alignment Search Tool (BLAST, ver. 2.10.0+) (Camacho et al., 2009). The coding regions of the genomic sequences were inspected using genewise (ver. 2.2) (Birney, Clamp, & Durbin, 2004) to determine the open reading frames and the exon/intron boundaries. All resulting nucleotide sequences were then translated into amino acid sequences, and verified again by BLAST against the NCBI non-redundant protein database. Multiple sequence alignments for the 25 nuclear protein-coding gene sets were individually generated using MAFFT (ver. 7.475) (Katoh & Standley, 2013) with the L-INS-i algorithm. Additional alignment trimming was performed using trimAl (ver. 1.4.rev15) (Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009) for phylogenetic tree reconstruction. The best-fit substitution model search for each gene set was carried out based on the Bayesian Information Criterion using IQ-TREE2 (ver. 2.1.2) (Minh et al., 2020). Phylogenetic tree of maximum-likelihood (ML) were reconstructed using RAxML-NG (ver. 1.0.1) (Kozlov, Darriba, Flouri, Morel, & Stamatakis, 2019), and bootstrap support values were calculated with 3,000 replicates. The Bayesian inference (BI) analysis was run for  $3 \times 10^6$  generations, sampling every 100 generation using MrBayes (ver. 3.2.7a) (Ronquist et al., 2012). Trees were visualized using FigTree (ver. 1.4.4; http://tree.bio.ed.ac.uk/software/figtree). Statistical assessment of the loglikelihood, the Kishino-Hasegawa (KH) (Kishino & Hasegawa, 1989), Shimodaira-Hasegawa (SH) (Shimodaira & Hasegawa, 1999), and Approximately Unbiased (AU) (Shimodaira, 2002) tests for the present and previously phylogenies were performed with 10,000 re-samplings using the RELL method in IQ-TREE2.



**Figure 3.** Phylogenetic tree of Arthropoda reconstructed based on 25 nuclear protein-coding genes with two non-pancrustacean species, *Ixodes scapularis* and *Strigamia maritima* as outgroups. The numbers near the branching points indicate the maximum-likelihood bootstrap support values (BP, percentage) and Bayesian posterior probabilities (PP, probability) in order of BP/PP. The red dots at the nodes indicate that the MLB and BPP are 100% and 1.00, respectively.

## 3. Results and Discussion

The orthologous sequences of 25 nuclear protein-coding genes were obtained from 40 arthropod species. The concatenated sequence data were aligned in a length of 17,436 amino acids. The ML and BI phylogenetic trees supported the formation of a monophyletic Copepoda, and its subdivisions into two superorders in congruence with the previous studies (Fig. 1). On the other hand, the Monstrilloida clade has shown a new phylogenetic relationship. Monstrilloida has long been regarded as a sister group of Siphonostomatoida (Dahms, 2004; Ho, 1990; Ho et al., 2003; Huys & Boxshall, 1991). However, a close look at these early morphology-based phylogenetic relationships alludes that other parasitic (or parasitic taxon-

rich) groups are also closely related to Monstrilloida. We interpreted this typical flocking of parasitic groups as a biased results that may be caused by the use of similar morphological features resulting from the convergent evolution (Castiglione et al., 2019; Huang, Roy, Valentine, & Jablonski, 2015; Speed & Arbuckle, 2016). Indeed, the statistical tree topology tests suggested our phylogenetic hypothesis as the most likely one, while all previous phylogenies were rejected at the 0.001 significance level (Table 1). The previous molecular phylogeny of Huys et al. (2007) rendering the inclusion of Monstrilloida within Siphonostomatoida were also rejected. The choice of suitable sequence data with sufficient phylogenetic signal is probably one of the most important keys for the successful phylogenetic inference (Blanco-Bercial, Bradford-Grieve, & Bucklin, 2011; Eyun, 2017; Mikhailov & Ivanenko, 2021; Rosenberg & Kumar, 2001, 2003). In this respect, the previous analyses based on partial or short single gene sequences dealing with a wide range of taxa might have a limited power to reveal true relationships.

**Table 1.** Statistical comparisons among the best maximum-likelihood trees and alternative phylogenetic hypotheses within the Copepoda orders.

Tree topology <sup>a</sup>	logL	$\Delta L^{\rm b}$	<i>р-</i> КН <sup>с</sup>	p- SH <sup>d</sup>	<i>p</i> - AU <sup>e</sup>	References
(CAL,((HAR,MON),(CYC,SIP)))	-258924.0 6	Best	1	1	1	This study
(CAL,(HAR,(MON,(CYC,SIP))))	-259028.9 0	104. 8	0	< 0.001	< 0.001	Но (1990)
(CAL,(CYC,(HAR,(SIP,MON))))	-259059.0 1	135. 0	0	0	< 0.001	Huys and Boxshall (1991)
(CAL,(CYC,(nc-SIP,(MON,c- SIP))))	-244220.1 3	300. 5	0	0	< 0.001	Huys et al. (2007)

<sup>a</sup> CAL = Calanoida, CYC = Cyclopoida, HAR = Harpacticoida, SIP = Siphonostomatoida, nc-SIP = non-caligiform siphonostomatoid (*Tracheliastes polycolpus*), c-SIP = caligiform siphonostomatoids (*Lepeophtheirus salmonis* and *Caligus rogercresseyi*), MON = Monstrilloida

 ${}^{b}\Delta L$ : logL difference from the maximal logL in the set

<sup>c</sup> *p*-KH: *p*-value of the one-sided Kishino-Hasegawa test (Kishino & Hasegawa, 1989) <sup>d</sup> *p*-SH: *p*-value of the Shimodaira-Hasegawa test (Shimodaira & Hasegawa, 1999)

<sup>e</sup>*p*-AU: *p*-value of the approximately unbiased (AU) test (Shimodaira, 2002)

Our phylogenomic result strongly suggested that Monstrilloida is a monophyletic group with a close molecular affinity to Harpacticoida. Within-Monstrilloida, the present four genera have appeared to evolve in sequence *Monstrilla*, *Caromiobenella*, *Maemonstrilla*, and *Monstrillopsis*. It is known that the copepod evolution is accompanied with structural oligomerization, and reappearance of ancestral character state from once derived state in descendants is an extremely rare case (Huys & Boxshall, 1991). In the aspect of this concept, *M. longilobata* can be considered the most recently appeared group among the present monstrilloid genera in aspect of the reduced number of caudal setae and of scars (Suárez-Morales, Bello-Smith, & Palma, 2006). Likewise, *M. grandis* would be the most primitive taxon as it shows the most complex morphological structure of the female fifth legs armed with the largest number of setae. However, the evolutionary priority between *Caromiobenella* and

*Maemonstrilla* is difficult to determine due to some morphological characters with unknown origins. However, given our molecular results and the partial agreement with the morphological assessment support the present phylogenetic relationships and evolutionary pattern.

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# The use of seaweeds as bioindicators: a brief review and call for research

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Major environmental challenges associated to natural Abstract: and anthropogenic stressors are critically affecting marine ecosystems. Impact that anthropogenic pollution and climate change pose on marine ecosystems is particularly alarming. Efficient biological systems, such as species and communities, have been searched for to detect early on the effects of human activities on the quality of the environment, and to take early action to protect and restore ecosystems. Seaweeds represent valuable bioindicators because of their fundamental ecological roles, sensitivity and quick response to changes in environmental and biological factors, and ease to collect. Their responses vary from taxa to taxa, and species relevant to the specific stressors and environmental conditions being investigated need to be identified and selected as bioindicators. However, research efforts on seaweeds' value as bioindicators have been scanty and primarily focused on water quality assessment. I offer a brief summary of past efforts and a call for research to further investigate seaweeds potential as bioindicators of water quality, anthropogenic climate change, habitat loss, and invasive species.

*Keywords:* algae, bioindicators, anthropogenic climate change, marine ecosystems, pollution

# Highlights

- Seaweeds are potentially interesting bioindicators because of their ecological importance in marine ecosystems, sensitivity and quick response to environmental changes, and ease to collect.
- Seaweeds have been used primarily used as bioindicators of water quality, and to a lesser extent to monitor effects of climate change, habitat loss and invasive species.
- A research call is presently made to increase efforts in identifying seaweeds bioindicator candidates for monitoring marine ecosystems health.

# **1. Introduction**

Anthropogenic climate change is having significant impacts on marine ecosystems (Aronson, Thatje, McClintock, & Hughes, 2011; Hoegh-Guldberg & Bruno, 2010). Some of the ways in which climate change is affecting the marine environment include: (1) rising sea temperatures, (2) ocean acidification, (3) changes in ocean circulation, and (4) sea level rise. As the Earth's climate warms, sea temperatures are increasing. This can cause coral bleaching events, which occur when the symbiotic relationship between corals and their photosynthetic algae breaks down due to stress caused by high temperatures (McClanahan, Ateweberhan, Muhando, Maina, & Mohammed, 2007). It can also lead to changes in the distribution and abundance of marine species, as some species may not be able to tolerate the warmer water temperatures (Southward, Hawkins, & Burrows, 1995). The increasing amount of carbon dioxide in the atmosphere is being absorbed by the oceans, which is causing the pH of seawater to decrease. This can make it more difficult for organisms like corals, mollusks, and some planktonic species to build and maintain their calcium carbonate structures, which can have serious implications for the health of these organisms and the ecosystems they support (Findlay et al., 2011; Turley et al., 2006). Climate change can also lead to changes in ocean currents and circulation patterns (Munday et al., 2009; L. J. Wilson et al., 2016). This can affect the distribution of nutrients (Marinov et al., 2008; Schneider & Schmittner, 2006), which can in turn impact the distribution and abundance of marine species. As the Earth's temperature rises, the polar ice caps are melting, which is causing sea levels to rise. This can lead to coastal erosion, flooding, and saltwater intrusion into freshwater systems, which can have significant impacts on coastal ecosystems and the species that rely on them (Cai, Su, Liu, Li, & Lei, 2009; Poff, 2002). The impacts of climate change on marine ecosystems are overall complex and can have wide-ranging effects on the organisms and ecosystems that depend on them. Urgent action is needed to mitigate the impacts of climate change on marine environment.

Bioindicators can provide valuable information about the status of ecosystems and the impacts of human activities on the environment, and can thus be used to guide efforts to protect and restore ecosystems (Burger, 2006; Reagan, 2007). Bioindicators are organisms or biological systems that can be used to monitor and assess the quality or health of an environment (Holt & Miller, 2011). They can be used to detect the presence of pollutants or other harmful substances, as well as to monitor the overall health and diversity of an ecosystem. There are different types of bioindicators, including plants, animals, and microorganisms (Holt & Miller, 2011). For example, lichens are often used as bioindicators of air quality, as they are sensitive to air pollution and can absorb pollutants such as sulfur dioxide and heavy metals (Conti & Cecchetti, 2001). Similarly, certain species of fish can be used as bioindicators of water quality, as they are sensitive to changes in pH, dissolved oxygen levels, and the presence of contaminants (Manickavasagam, Sudhan, & Aanand, 2019). Bioindicators are important for (1) early detection of environmental problems, (2) cost-effective monitoring, (3) long-term monitoring, (4) integrated monitoring, (5) public awareness. Bioindicators can detect environmental problems early, before they become more widespread or more difficult to

address (Adams, Shugart, & Hinton, 2018). This can allow for timely management and conservation actions to be taken to prevent or mitigate further damage (Read & Andersen, 2000). They can be cost-effective monitoring tools, as they can provide information about environmental conditions and changes in ecosystem health with relatively low cost and effort (Griffiths et al., 2016). Bioindicators can be used for long-term monitoring of environmental conditions, as they can provide information about changes in ecosystems over time. This can help to identify trends and patterns in environmental conditions and assess the effectiveness of conservation and management measures (T. Cooper, Gilmour, & Fabricius, 2009). Bioindicators can be used to monitor multiple environmental stressors or pollutants simultaneously, providing a more integrated view of ecosystem health (Adams et al., 2018). They can also help to raise public awareness about environmental issues and the impacts of human activities on ecosystems (Kienzl, Riss, Vogel, Hackl, & Götz, 2003). By using bioindicators to monitor and communicate changes in ecosystem health, people can become more engaged and informed about environmental issues, and be more motivated to take action to protect the environment. Overall, bioindicators are an important tool for monitoring and managing environmental issues and ensuring the health and sustainability of ecosystems.

There are several bioindicators that are commonly used to assess the health and quality of marine ecosystems, including (1) phytoplankton, (2) seaweeds, (3) seagrasses, (4) zooplankton, (5) benthic macroinvertebrates, and (6) coral reefs. Microscopic (phytoplankton) and macroscopic (seaweeds) algae are at the base of the marine food chain and are sensitive to changes in nutrient levels, temperature, and light (Jakhar, 2013; Vasquez & Guerra, 1996). Monitoring changes in phytoplankton and seaweeds populations can provide insight into the overall health of marine ecosystems. Similarly seagrasses or marine plants provide important habitat for many marine species and are sensitive to changes in water quality and light availability (McMahon, Collier, & Lavery, 2013; Mishra & Farooq, 2022). Microscopic animals or zooplankton are also important indicators of changes in the marine food web (Ferdous & Muktadir, 2009). They are sensitive to changes in water quality, temperature, and other environmental factors. Larger organisms that live on or in the seabed (i.e., benthic macroinvertebrates), such as worms, clams, and crabs, are useful indicators of changes in sediment quality and water quality (Gazonato Neto, Silva, Saggio, & Rocha, 2014). Biological systems like coral reefs form complex ecosystems that are home to a wide variety of marine species and are sensitive to changes in water temperature, acidity, and other environmental factors (T. F. Cooper et al., 2008). The choice of bioindicators will depend on the specific goals of the monitoring program and the characteristics of the ecosystem being studied. It is important to select bioindicators that are relevant to the specific environmental conditions being assessed.

I aimed to provide here a brief overview of the value of seaweeds as bioindicators of marine ecosystems health, and call for research to further investigate seaweeds potential as biological indicators.

## 2. Methodology

I aimed to provide here a non-exhaustive summary of literature on the use of seaweeds as bioindicators of marine ecosystems. My search bas been conducted in the following scientific databases: Google Scholar, PubMad, web of Science, Scopus. The keywords I used were ("seaweeds" OR "macroalgae" OR "algae") AND ("bioindicator" OR "biomonitoring" OR "climate change" OR "pollution" OR "invasive species" OR "habitat loss"), paired with Boolean Operators. I made a first screening of relevant studies based on the resulting publications titles and abstract, and afterwards made a full text assessment.

## 3. Results and Discussion

Seaweeds, also known as macroalgae, are large, multicellular algae that grow in marine and freshwater environments (Lobban & Wynne, 1981). They display a wide range of shapes and sizes, from small, delicate filaments to large, robust kelps that can grow up to 60 meters (e.g., Macrocystis) in length (Lobban & Harrison, 1994). Some 10.000 species of seaweeds are currently known worldwide (Guiry, 2012). Seaweeds play an important role in marine ecosystems (Cotas, Gomes, Pacheco, & Pereira, 2023; Lobban & Harrison, 1994). They are primary producers, which means they convert sunlight into energy through photosynthesis and form the base of the food web. Many species of fish, invertebrates, and marine mammals rely on seaweeds directly or indirectly for food and habitat (Cotas et al., 2023). They also play an important role in the cycling of nutrients in marine ecosystems (Cotas et al., 2023). They take up nutrients such as nitrogen and phosphorus from the water, and when they die and decompose, these nutrients are released back into the environment, where they can be taken up by other organisms (Cotas et al., 2023; Klinger, 2015).

Environmental changes have already considerably affected seaweeds globally, and greatly reshaped benthic landscapes in many parts of the world (Bruno, Sweatman, Precht, Selig, & Schutte, 2009; Eger et al., 2022; Harley et al., 2012). Seaweed species are responding differently to environmental changes, thus leading to algal species composition shifts with some seaweeds becoming rare or disappearing, and others to bloom (Harley et al., 2012; Vieira, Payri, & De Clerck, 2016). Changes in seaweed populations can have cascading effects on other organisms in the ecosystem. Because they play critical ecological roles, are sensitive and respond quickly to changes in water quality, temperature, and other environmental factors, and are easy to collect, seaweeds represent valuable bioindicators (Harley et al., 2012; Vasquez & Guerra, 1996). Based on a brief review of selected literature, seaweeds have shown potential as bioindicators in the detection of impacts from several anthropogenic stressors including but not restricted to: (1) water quality assessment (e.g., trace elements and contaminants presence in waters), (2) climate change (e.g., changes in temperature and pH), (3) habitat loss and exploitation, and (4) invasive species.

### 3.1. Bioindicators for assessing water quality

Seaweeds are commonly used as bioindicators for assessing water quality, from the time when correlations between nutrient and trace metals contents in seaweeds tissue and in surrounding waters has been shown in some species in the 1960s (Bryan, 1969; Gutknecht, 1965). In the assessment of water quality, certain species of seaweeds are sensitive to changes in nutrient levels, such as nitrogen and phosphorus, and can be used to detect excess nutrients in coastal waters (Volterra & Conti, 2000). Some species are more tolerant of pollution than others and can therefore be used to identify areas of high pollution (Volterra & Conti, 2000). Nutrients in the water may favor the growth of generalist species of seaweeds (Volterra & Conti, 2000). Seaweeds are also sensitive to changes in water clarity (Pehlke & Bartsch, 2008), which can be an indicator of changes in water quality. In addition, some seaweeds are able to accumulate heavy metals, which makes them useful as bioindicators of metal pollution in marine environments (Chung & Lee, 1989; Shams El-Din, Mohamedein, & El-Moselhy, 2014).

#### **3.2. Bioindicators of climate change**

Because seaweeds are very sensitive to changes in environmental conditions, including temperature, salinity, pH, and nutrient availability, all of which can be affected by climate change, they are considered to be good bioindicators of climate change. Effects of climate change on marine ecosystems that translate for instance as changes in temperature and ocean chemistry can affect seaweeds growth and survival (Straub et al., 2019). Seaweeds have been used as bioindicators in a variety of studies around the world, including in the Arctic and Antarctic, where melting sea ice is affecting the distribution and abundance of seaweeds, and in coastal regions, where changes in water temperature and nutrient availability are affecting the growth and distribution of seaweed species (Jueterbock, Smolina, Coyer, & Hoarau, 2016; Marbà et al., 2017; Pellizzari et al., 2017; Shams El-Din et al., 2014; K. L. Wilson, Skinner, & Lotze, 2019).

#### 3.3. Bioindicators of habitat loss

Some species of seaweed are highly specialized and only grow in specific habitats, such as rocky shores or coral reefs. Changes in the abundance or diversity of these species can indicate shifts in the composition of the ecosystem and may suggest the presence of environmental stressors (Airoldi, Balata, & Beck, 2008; Mineur et al., 2015; Walker & Kendrick, 1998).

### 3.4. Bioindicators of invasive species

Invasive species can have a significant impact on marine ecosystems, often outcompeting native species and disrupting food webs and other ecological processes (Molnar, Gamboa, Revenga, & Spalding, 2008). Seaweeds are good indicators of invasive species for several reasons. Firstly, they are often the primary producers in marine ecosystems, meaning that changes in their abundance and distribution can have significant downstream effects on other organisms (Sempere-Valverde et al., 2021; Silva, Lemos, Gaspar, Gonçalves, & Neto,

2021). Secondly, many seaweed species have narrow ecological niches, and changes in their composition can indicate changes in the conditions that favor different species, including invasive species (Marcelino & Verbruggen, 2015; Sempere-Valverde et al., 2021). Finally, some invasive seaweed species, such as the notorious Asian seaweeds *Caulerpa taxifolia* and *Rugulopteryx okamurae*, are highly visible and easily identifiable, making them good candidates for monitoring efforts (Montefalcone, Morri, Parravicini, & Bianchi, 2015; Silva et al., 2021). Researchers can use seaweeds as bioindicators of invasive species by monitoring changes in their abundance and diversity over time, and by identifying the presence of invasive species within seaweed communities (Anderson, 2007). A notorious example is the invasion on the Bahamian coral reef of the lionfish (*Pterois volitans*), which led to a phase shift to algal dominated communities at mesophotic depths in the green and brown algal species *Halimeda copiosa* and *Lobophora schneideri*, as a result of herbivore fishes decline, i.e., top-down process (Lesser & Slattery, 2011).

#### 3.5. Commonly used seaweeds species as bioindicators

Several species of seaweeds are used as bioindicators in marine ecosystems, depending on the specific environmental conditions being assessed. Here are some examples of seaweeds commonly used as bioindicators: (1) the green seaweeds Ulva spp. (Ulvales, Chlorophyta) are commonly used as bioindicators of nutrient pollution in coastal waters (Areco, Salomone, & dos Santos Afonso, 2021; Boubonari, Malea, & Kevrekidis, 2008; Yokoyama & Ishihi, 2010). They are highly sensitive to excess nutrients, such as nitrogen and phosphorus, and can accumulate these nutrients (as well as trace metals) in their tissues; (2) the brown seaweeds Fucus spp. are commonly used as bioindicators of changes in water quality, temperature, and other environmental factors (Cairrão et al., 2007; Van der Ben et al., 1990). They are also sensitive to heavy metals and can accumulate these pollutants in their tissues; (3) the red seaweeds Corallina spp. (coralline algae; Corallinales, Rhodophyta) are used as bioindicators of ocean acidification, which can affect their ability to build and maintain their calcium carbonate skeletons (Egilsdottir, Noisette, Noël, Olafsson, & Martin, 2013; Gao & Zheng, 2010); (4) the brown seaweeds Padina spp. (Dictyotales, Phaeophyceae) are used as bioindicators of ocean acidification, heavy metal pollution, changes in water temperature and other environmental factors (Dulymamode, Sukhoo, & Bhugun, 2001; Gil-Díaz, Haroun, Tuya, Betancor, & Viera-Rodriguez, 2014; Herbert et al., 2016). They are also sensitive to heavy metals and can accumulate these pollutants in their tissues (Dulymamode et al., 2001).

## 4. Conclusion

Due to their fundamental ecological role, sensitivity and rapid responses to environmental changes, seaweeds present potential good bioindicators to assess the health of marine ecosystems and impact of pollution and anthropogenic climate change. The choice of seaweed bioindicator depends on the specific environmental conditions being assessed and the goals of the monitoring program. It is important to select seaweed bioindicators that are relevant to the specific environmental conditions being studied. Main efforts have been primarily focused on water quality assessment (e.g., pollutants monitoring). This is therefore a call for research on the use of seaweeds as bioindicators to assess the numerous impacts of anthropogenic pollution and climate change.

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# **Changes on the Plankton Functional Groups in Jiaozhou Bay, the Yellow Sea**

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**Abstract:** To reveal the long-term changes of Jiaozhou Bay ecosystem and identify the key drivers of coastal ecosystem, changes on plankton functional groups and key environmental factors were studied. Concentrations of dissolved inorganic nitrogen increased during 2006-2010, then decreased. Warm-water species of phytoplankton and zooplankton increased. Proportion of dinoflagellate abundances increased and gelatinous zooplankton increased as well. The size of zooplankton was smaller. The ecosystem of Jiaozhou Bay was in a good status based on ecosystem health assessment. Jiaozhou Bay is considered as a good demonstration of the coastal ecosystem health under multi-stressors, and can be a good demonstration of coastal sustainable development.

*Keywords:* longer-term change, phytoplankton, zooplankton, functional groups, Jiaozhou Bay, Yellow Sea

# Highlights

- Long-term changes on plankton functional groups in Jiaozhou Bay were studied.
- Dinoflagellates and gelatinous plankton increased in Jiaozhou Bay.
- The size of plankton was getting smaller.
- The health status of Jiaozhou Bay marine ecosystems was improving.

# **1. Introduction**

Plankton play crucial roles in marine ecosystem. The change on marine plankton functional group is a good indication for marine ecosystem health. To reveal the long-term changes on plankton, we chose Jiaozhou Bay as a case study. The purpose is: 1) to study the long-term changes of plankton functional groups in the Jiaozhou Bay; 2) to improve understanding of interactions and cumulative impacts of various local and global stressors; 3) to identify the key drivers of coastal ecosystem; 4) to target management interventions that are most effective from both environmental and economic perspectives.

# 2. Methodology

## 2.1 Study area

Jiaozhou Bay is located in the southern part of the Yellow Sea, a medium size of bay in the temperate sea area. It has been influenced by intensive human activities and climate change. It has good exchange with the open water, can be well representative of the coastal area, and was considered as a model bay for the coastal research.

## 2.2 Data sources and collection criterion

Most data were obtained during a long-term survey of Jiaozhou Bay at 14 stations monthly conducted by the Jiaozhou Bay National Marine Ecosystem Research Station. The variables we observed include Meteorological variables, Hydrological variables, Chemical variables, Microorganisms, Phytoplankton, Zooplankton, Benthos, sediments. The rest data were collected from published papers and books.

# 3. Results and Discussion

## 3.1 Long-term changes of physical chemical parameters in Jiaozhou Bay

Seawater temperature is increasing, coincide with the trend of global warming. Salinity shows a slight decreasing trend, might be affected by increase in precipitation. The main nutrients -Dissolved inorganic nitrogen and phosphates have been increasing by 2006-2010, then decreased continuously. Which are in accord with the environmental protection measures. The trend in concentration of Silicates is similar as dissolved inorganic nitrogen and phosphates. The dissolved oxygen status is good, no hypoxia occurred.

## 3.2 Long-term changes of plankton functional groups in Jiaozhou Bay

## 3.2.1 Changes on Warm-water plankton species

Warm-water phytoplankton species increased in both water columns and sediments. For instance, the cell abundance of *Lithodesmium undulatum* in Jiaozhou Bay increased significantly after the year of 2000 compared with before (Sun et al., 2011). In sediment core, almost all of the dominant species decreased sharply in abundance since 1980s, while *Cyclotella stylorum*, increased markedly in abundance in recent decades (Liu et al., 2008). The increasing appearance of warm-water species in Jiaozhou Bay is a response of the

phytoplankton community to the warming of water temperature. Besides, a warm-water zooplankton species *Dolioletta gegenbauri* was firstly recorded in Jiaozhou Bay during the autumn of 2019-2020 (Wang et al., 2022). The continuous warming trends both in the China coastal seas and Jiaozhou Bay likely expanded their biogeographic range.

### 3.2.2 Changes on composition of phytoplankton

Diatom and dinoflagellate were the major phytoplankton groups in Jiaozhou Bay (Sun et al., 2011). Although diatom was dominant, the abundance of dinoflagellate increased gradually, resulting in the increase of dinoflagellates/diatoms ratio. Dinoflagellate has a competitive advantage relative to diatom under the high N/P ratio, which may be one of the main reasons causing the increase in dinoflagellate. Another study on long-term changes of phytoplankton in Jiaozhou Bay in the past 30 years got similar results (Zhang et al., 2021).

## 3.2.3 Changes on gelatinous zooplankton

Gelatinous zooplankton increased in Jiaozhou Bay. Take small jellyfish for instance, the abundance of small jellyfish during 2001-2009 were almost 5 times higher than that during 1991-2000 (Sun et al., 2012). The frequency of blooms of small jellyfish increased after 2000. The dominant species of jellyfish has changed. Several new dominant species appeared after 2000, while some previous dominant species throughout the 1990s were no longer dominant after 2000.

### 3.2.4 Changes on size of zooplankton

The size of zooplankton become smaller. We observed year to year variability in the main groups of zooplankton, characterized by an overall decrease in biovolume for some groups from 2005 to 2012(Wang et al., 2020). This decrease was particularly evident in the populations of copepods, appendicularians, and medusae. The abundance of microzooplankton *Noctiluca scintillans* increased.

### 3.2.5 Long-term Change on ecosystem health in Jiaozhou Bay

Based on the trend of plankton and key environmental factors, we established Jiaozhou Bay Marine ecosystem health assessment systems. The overall health in the bay exhibited an increasing trend. The water quality in the bay has been improving while the health conditions of biota exhibited a fluctuating pattern, more research is necessary for further research.

## 4. Conclusion

The long-term changes on phytoplankton and zooplankton in Jiaozhou Bay under the influences of anthropogenic activities reveal the increase of warm-water plankton species, dinoflagellates and gelatinous zooplankton. The size of plankton community was smaller. The overall health in Jiaozhou Bay exhibited an increasing trend.

## Acknowledgement

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# **Overview of Marine Environment and Ecology Monitoring and Assessment in China**

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The coastal zone of China comprises an area of more than three million square kilometers, and possesses an 18000 km coastline stretching across tropical, subtropical and temperate zones. The Ministry of Ecology and Environment is responsible for supervising human activities in marine environment, and organizing the investigation, monitoring, surveillance and evaluation of the coastal environment. The Bulletin of Marine Ecology and Environment Status of China has been published annually for more than twenty years, and is responsible for directing, coordinating and supervising marine environmental protection work throughout the nation. In 2021, we monitored seawater quality in 1,350 national monitoring sites, 193 riverine sections flowing into the sea, 442 sewage outlets with daily discharge volume exceeding 100 tons, and 31 bathing beaches. We also monitored the marine sediment quality of 540 national monitoring sites and the ecological status of 24 typical marine ecosystems. The monitoring results showed that China's marine ecology and environment status remained stable in 2020. The overall quality of marine water was getting better, with 96.8% of the marine water under jurisdiction of China according with the Seawater Quality Standard Grade I. In the coastal area, 77.4% of these areas had Excellent or Good water quality, up by 0.8% compared with the previous year. The polluted areas were mainly located at Liaodong Bay, Yellow River Estuary, Jiangsu Coast, Yangtze River Estuary, Hangzhou Bay, Zhejiang Coast, and Pearl River Estuary. The dominant indicators failing the Seawater Quality Standard were inorganic nitrogen and active phosphate. The health status of typical marine ecosystems remained stable overall. The water quality of all the monitored sea-entering rivers is Slightly Polluted on the whole, with no significant change compared with the previous year. The marine environment quality in the ocean dumping zones and oil/gas exploration zones basically met the environmental protection requirements for marine functional zones. The environmental quality of marine fishery areas was generally in good status. Both the frequency and the cumulative area of recorded red tides have slightly decreased from the previous year.

# **Relationship between moonlight and coral reef organisms in Okinawa**

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Most of the organisms inhabiting a variety of environments on Earth synchronize their own ecological and physiological activities to their habitats. Periodic changes in the environment (e.g., circadian, circatidal, circalunar, and circannual) have a variety of effects on marine organisms. These are caused by the environment with cyclic changes in light and darkness (approximately 24 hours), tidal change (approximately 12.4 hours), moon phase (approximately one month), seasonal change (approximately one year), and so on. Most organisms have developed an endogenous clock that allows them to anticipate daily and seasonal changes and adapt their physiological, behavioral, and biochemical activity accordingly. Endogenous clocks are entrained to their local conditions by environmental cycles through input cues such as light or nutrition. In fish, entrainment of biological activity to habitat environmental factors is an important adaptive strategy and is essential for improving reproductive success and reducing predation risk. It is generally accepted that cyclic changes in the photoperiod and water temperature are potent environmental factors, and that daily and yearly periodicity in these factors is closely related to the initiation or termination and acceleration or deceleration of synchronous reproductive activity in fishes that inhabit temperate and higher latitudes. In the fishes inhibiting coral reefs, various reproductive events are known to be often synchronized with periodic changes associated with the Moon.

It is known that *siganidae* inhabiting tropical and subtropical area spawn in synchronized with a particular lunar phase. Melatonin is deeply involved in this lunar-synchronized reproduction, and Exposure to moonlight at midnight during the full and new moon phases showed an immediate and significant decrease in plasma melatonin levels during the two lunar phases. In addition, spawning did not occur in fish reared under constant light conditions. This suggests that nocturnal brightness may inhibit the initiation of gonadal development in this fish (Ikegami *et al.*, 2014; Takemura *et al.*, 2004) . It has been confirmed that blood melatonin levels change at full and new moons and melatonin administration suppresses the expression of reproduction-related genes in Honeycomb grouper *Epinephelus merra*. In addition to this, several clock genes (cry1, 2, and 3) are altered during the lunar phase. These reports suggesting that moonlight plays an important role in the induction and phase-setting of this transcription (Fukunaga *et al.*, 2019 and 2020.

In addition to the moon as a nighttime light, the effects of Artificial Light At Night (ALAN) on organisms have also been reported. In the territorial fish species Crownfish *Amphiprion ocellaris*, Treatment 12:12 day-ALAN light regimes, ALAN had dramatic effects on hatching (Fobert *et al.*, 2019). In the crustacean Crustacean: *Orchestoidea tuberculata*, ALAN suppressed activity, food consumption, and growth rate (Luarte *et al.*, 2016). In invertebrate *Acropora* corals, ALAN caused elevated differentially expressed genes and cluster into pathways regulating cell cycle and protein synthesis in *A. eurystoma* (Rosenberg *et al.*, 2019). ALAN of cold and white spectrum disrupted in reproduction in *A. digitifera* and *A. millepora* (Ayalon *et al.*, 2021).



Fig.1 Schematic diagram of ALAN's impact on marine organisms.

These reports suggest that nighttime light has a negative impact on the ecological and reproductive activities of some organisms with low mobility and those living in territorial areas. Therefore, conversations and policies developed to protect marine environments should include regulations to ensure wasted and unnecessary light does not make its way into these incredibly diverse and remarkable areas.

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## Session 3-1: Marine Biodiversity Monitoring I

# **Environmental DNA as a Powerful Tool to Detect Fish Biodiversity in The Open Ocean**

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Monitoring marine biodiversity on a global scale and revealing the distribution of indicator species will provide an essential baseline for detecting changes in marine ecosystems. Recent advances in the analysis of environmental DNA (eDNA), which is extra-organism DNA extracted from various environmental samples, have made it possible to obtain high-sensitivity information about fish diversity in aquatic ecosystems in a less laborious, non-invasive, and cost-effective way. Because of its advantages, eDNA has emerged as a promising tool for biomonitoring as an alternative or supplement to conventional methods such as net sampling.

The Arctic ecosystem is undergoing substantial changes due to ongoing warming and rapid sea ice reduction. Because sea ice provides a unique habitat for various species, including marine fish, sea birds, and marine mammals, its decline seriously threatens the Arctic ecosystem and the local communities that rely on it. Despite the importance of understanding the ecological changes in the Arctic, harsh weather and geographic inaccessibility have made comprehensive biomonitoring difficult. Therefore, it is expected that eDNA will improve our understanding of the Arctic ecosystem by reducing the constraints inherent to conventional surveys.

We present our recent progress in the eDNA study, which is part of the Arctic Challenge for Sustainability II (ArCS II) Project, an Arctic region research project initiated in Japan. Because our primary concern is the distributional shift of Arctic fish associated with recent warming, this study aimed to evaluate the ability of eDNA to characterize the distribution of pelagic fish and detect a transition in fish species composition in the open ocean across multiple biogeographic zones. To achieve this purpose, we analyzed eDNA samples collected across Japanese waters to the Arctic Chukchi Sea during the R/V Mirai (JAMSTEC) Arctic cruise held in the open-water season of 2020.

To reveal the compositional differences in fish communities among marine regions, the fish community structure in pelagic water was analyzed using eDNA metabarcoding, which can comprehensively identify species in a sample. Fish eDNA was detected at all 45 sites and assigned to 170 taxa. The results showed a clear latitudinal cline in taxonomic richness (2–48 taxa per site), with a notable boundary in the Kuroshio–Oyashio transition zone. Successive clustering and ordination analysis indicated that the fish communities in each region were

distinguishable from one another and closely matched the conventional biogeographic classification of pelagic waters. This result demonstrated that eDNA can depict latitudinal transitions in fish communities in the open ocean.

To explain the distribution of polar cod, *Boreogadus saida*, species-specific eDNA detection was performed on the eDNA samples using a newly developed assay. The polar cod is an ice-associated species considered a key species in the Arctic ecosystem. Polar cod eDNA was detected primarily in surface water from the central Chukchi Sea shelf and the northernmost observation area (75°N), including the marginal ice zone, while only trace amounts of eDNA were detected in the Bering Sea. The eDNA also suggested that the distribution of polar cod is most likely restricted to cold and low-salinity waters. This result suggests that eDNA can be a reliable tool for providing a baseline for tracking the range shift of Arctic fish associated with climate change.

Although eDNA could successfully provide information about fish species composition and distribution, the sampling protocol required further optimization to reduce the uncertainty in species detection. Therefore, we evaluated the sampling effort for the metabarcoding-based detection of fish eDNA in the subtropical and subarctic northwestern Pacific Ocean and Arctic Chukchi Sea based on replicated sampling with filters having different pore sizes (Kawakami et al., 2023). The species accumulation curves against filtration volume and the number of replicates did not saturate in most cases, indicating that the ordinal sampling effort was insufficient to fully assess species diversity in the open ocean. The species composition was highly dissimilar between the filter types and even between filtration replicates at any site, with the turnover component predominating. These results highlighted the difficulty of standardizing the sampling protocols across various water masses.

Our results demonstrate that eDNA is a powerful tool for detecting fish biodiversity in the open ocean and describing the geographical distribution of target species across a broad range of oceans. Although several technical difficulties need to be overcome (such as reducing contamination risk, preventing false negatives, and ensuring the accuracy of taxonomic assignment), eDNA can be a reliable tool for replacing or supplementing conventional approaches. In conjunction with oceanographic research, continual eDNA surveys will enhance our understanding of the marine ecosystem.

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# How to Monitor Changes in The Marine Biodiversity in Jeju, Korea

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Abstract: Increasing seawater temperatures driven by climate change have a negative impact on marine biodiversity. Jeju Island has been suggested as an area capable of supporting high biodiversity owing to its geographical position and the different water masses that influence the marine environment around the island. However, a major limitation of monitoring programs is the difficulty of comparing and quantifying the results generated by different methods. To overcome these problems, the MarineGEO program of the Smithsonian Institution's Tennenbaum Marine Observatories Network (TMON) developed a quantitative, standardized method for sampling monitoring data called Autonomous Reef Monitoring Structures (ARMS). Due to climate change, various subtropical/tropical organisms and new species have recently been found along the southern coast of Korea, including Jeju Island. We expect ARMS monitoring will provide further information to detect "climate refugees," i.e., newly extended species due to climate change, as well as new species in understudied taxa. These "climate refugees" would disturb marine ecosystems as they compete with indigenous marine organisms. In addition to investigating changes in marine ecosystems caused by climate change and detecting invasive marine species, this standardized monitoring method (ARMS) was applied to understand the role of biodiversity in sustaining resilient coastal marine ecosystems under climate threat.

*Keywords:* Climate change, Jeju Island, biodiversity, MarineGEO, Autonomous Reef Monitoring Structures (ARMS)

# Highlights

• Recently, a variety of subtropical/tropical organisms as well as new species, have been found along the southern coast of Korea, including Jeju Island, due to climate change

- We expect Autonomous Reef Monitoring Structures (ARMS) monitoring will provide further information to detect "climate refugees," i.e., newly extended species due to climate change
- This standardized monitoring method (ARMS) was applied to understand the role of biodiversity in sustaining resilient coastal marine ecosystems under climate threat

# 1. 'Autonomous Reef Monitoring Structures (ARMS)' development background

Coral reefs are known to have high marine biodiversity. Biodiversity studies in coral reefs have experienced problems, such as difficulties in collecting microbenthic organisms. Therefore, artificial reef matrix structures (former ARMS) were developed in 2004 to study the biodiversity of coral reef waters in a low-cost, non-destructive, and effective way. (Fig. 1A) (Zimmerman and Martin, 2004). Developers installed this structure in the Guana Island reef area in the eastern Caribbean Sea, analyzed the attached organisms a year later, and demonstrated higher biomass and diversity than organisms collected through SCUBA diving (Fig. 1B-C) (Felder and Martin, 2003; Martin 2002; Haney and Martin, 2004; Zimmerman and Martin, 2004).



Figure 1. Diagram of a completed ARMS in place (A). Photographs of two of our ARMS in the field. Deployed ARMS in place at Long Point, Guana Island (B). The ARMS at Bigelow Beach (Guana Island) after about 1 yr in the field (C) (sourced from Zimmerman and Martin (2004))

The Smithsonian Institution in the United States has developed the Autonomous Reef Monitoring Structures (ARMS) prototype, complementing the previously developed former ARMS (Fig. 2). ARMS has been used to quantitatively assess the diversity and ecosystem structure of coral reefs and rock ecosystem marine benthic organisms in various programs, such as NOAA's Pacific Reef Assessment and Monitoring Program, the Mo'orea Biocode Project, and the Devotes-project in Europe. ARMS is more environmentally friendly and quantitative than existing marine biodiversity research methods and can effectively monitor changes in marine ecosystems. Monitoring results between different waters can also be compared.



Figure 2. Assembly schematic and completed view of ARMS (A) and quantity, materials and description of ARMS (B) (Sourced from NOAA Fisheries. http://www.fisheries.noaa.gov)

Since the standardization of the ARMS in 2008, it has been installed in Samoa, Hawaii, and Guam. Since then, its research scope has expanded to Asian countries in South America and the Pacific coast to monitor coral reefs, ocean coral acidification, and climate change (Fig. 3).



Figure 3. Locations of current ARMS deployments (sourced from Smithsonian Institution. https://naturalhistory.si.edu/research/global-arms-program)
## 2. Marine Global Earth Observatory (MarineGEO) program

The Marine GEO program was initiated in 2013 under the auspices of the Smithsonian Tennebaum Marine Observatory Network (TMON). The MarineGEO project installed ARMS in the research area to investigate each country's marine biodiversity and ecosystem structure. The research contents were as follows: 1) listing marine biodiversity, 2) providing evidence of biodiversity changes due to climate change, and 3) determining the effects of changes in diversity due to pollution on ecosystem function and restoration through quantitative analysis of the attached organisms according to time and space by observing marine ecosystems for an extended period using ARMS. MarineGEO is constantly 1) clarifying the role of biodiversity and its function and restoration in the ecosystem, 2) developing the identification of the cluster structure of coastal submarine topography in which humans and biodiversity are concentrated according to low-quality composition, and 3) developing the rigorous use of standards, quantified observation methods, and internationalized experimental methods. In 2015, the University of Hong Kong became the first institution in an Asian country to participate in the Marine GEO network. To quantify the diversity of marine life in Hong Kong's waters and compare biodiversity according to the degree of contamination, the research team installed an ARMS in 2015 and collected and analyzed it two years later. More than 400 species of morphologically classified fish, arthropods, gastropods, bivalves, and sandworms were identified, and more than 1,000 biological samples were collected. In 2018, a Marine Biodiversity Observation Network for Genetic Monitoring of Hard-Bottom Communities (ARMS-MBON) was formed by installing ARMS near coastal stations, ports, anchorages, and long-term ecological research sites distributed in Europe and the polar regions (Fig. 4). ARMS-MBON has approximately 20 observatories, including 14 European countries, Greenland, and Antarctica, and monitors marine life from tropical waters to polar environments along the European coastline. A total of 134 ARMS were installed by 2020, and the 2018 test phase was arranged in short- and long-term manners according to the experimental conditions for each annual structure distribution cycle. The data collected by the ARMS-MBON program can be used to calculate biodiversity variables (EbV) that are essential for the distribution and abundance of benthic and non-native species and are designed for use in continental-scale ecological studies and biogeography of benthic invertebrates.

The International MarineGEO Symposium was hosted in Jeju, Korea, by KIOST in 2018. Researchers from the United States, Hong Kong, Japan, and South Korea attended the symposium. The results of this study on the introduction of the ARMS program, the MarineGEO Hong Kong project, the assessment of coral reef health in Okinawan waters, the monitoring method of marine benthic flora and fauna in Jeju waters using underwater photography techniques, the participation of the KIOST MarineGEO project, and the diversity, distribution, and utilization of Jeju marine habitats are discussed. After the symposium, KIOST installed an ARMS in Jeju and Ulleungdo waters in 2018 to study marine biodiversity fluctuations in Jeju and Ulleungdo waters affected by Kuroshio turbulence. (Fig. 5).



**Figure 4.** Deployed ARMS. Geographical overview (B, C) of currently established observatories (A); Numbers in parentheses (B, C) indicate the number of ARMS deployed in 2018, 2019, and 2020, respectively. Growth metrics of the network over time (D, for details, see Supplementary Table) (sourced from ARMS-MBON, A Marine Biodiversity Observation Network for Genetic Monitoring of Hard-Bottom Communities (Obst et al., Frontiers, 2020))



**Figure 5.** Deployed ARMS in Korean waters (A-B in Jeju Island, C-D in Ulleungdo Island) (sourced from Yang et al. (2023))

## 3. Value of monitoring

Technology for monitoring the natural environment facilitates a basic understanding of natural changes. It provides information for management decisions; however, developing technology is sometimes costly (Nygard et al. 2016). Therefore, we conclude that marine environment monitoring is essential for effective marine management because of the evaluation of the estimated cost and valuation of marine monitoring. A global project called the Census of Marine Life was started in London in 2010 and monitors marine biodiversity and distribution due to climate change, ocean acidification, and natural disasters. Marine life resources are being monitored in the Pacific coastal countries of Japan and Australia. (Ausubel 2001; Costello et al. 2010; O'Dor et al. 2010; Williams et al. 2010). New inflow organisms, which are byproducts of marine monitoring, can be utilized as a basis for research on new marine materials by developing mass production methods and materialization projects. (Ryu and Jeon 2018; Zamani et al. 2021). The Natural Geography in Shore Areas (NaGISA) project surveyed the marine biodiversity of 25 counties from 2000 to 2010 and reported that Korea had the highest biodiversity per unit area (10 km2) worldwide (Costello et al. 2010). In addition, 51% of marine organisms in Korea live in Jeju waters, and approximately 30% are native to Jeju Island. Recently, a variety of subtropical/tropical organisms as well as new species have been found along the southern coast of Korea, including Jeju Island, owing to climate change (Hettiarachchi et al. 2022; Reimer et al. 2018; Yang et al. 2018). Owing to the subtropicalization of Jeju waters, a result of climate change, the benthic ecosystem is changing from a seaweed-centered habitat to a coral-centered habitat (Hong et al. 2015; Kim et al. 2022; Vieira et al. 2016). As habitat changes can lead to changes in future fisheries resources, it is necessary to explore and prepare species of great value as fisheries resources by monitoring newly introduced organisms due to subtropicalization in Jeju waters. The ARMS is a suitable structure for monitoring benthic rock ecosystems. It can be used as an appropriate model to observe the long-term changes in marine ecosystems caused by climate change in the waters around Jeju, which have rocky ocean beds.

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# Identifying patterns in the multitrophic community and food-web structure of a low-turbidity temperate estuarine bay

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Abstract: Changes in community structure and food web dynamics attribute to complex ecosystem processes. To understand the temporal and spatial variations in community structure and predator-prey interactions, new approaches are required to capture the topological descriptions of food webs and quantify their trophic interactions. Here, I introduce a study applying Kohonen's self-organizing map algorithm and stable isotope-mixing models to identify food web patterns across a low-turbidity water channel of a temperate estuarine-coastal continuum. By combining both approaches, the study finds a spatial difference in the patterns of community compositions between the estuarine and deep-bay channels and a seasonal difference in the plankton pattern but less in the macrobenthos and nekton communities. Dietary mixing models of co-occurring dominant taxa reveal site-specific but unchanging food web topologies and the prominent role of phytoplankton in the trophic base of pelagic and prevalent-detrital benthic pathways. This approach would be a realistic framework for linking key nodes from producers to predators in trophic networks.

*Keywords:* Community structure, food web, estuarine ecosystem, self-organizing map, stable isotope, mixing model

## Highlights

- Self-organizing map and stable isotope approaches were used for the food web study
- Two community structures differentiate between the estuarine channel and deep bay
- Autochthonous production of phytoplankton serves as a principal basal resource
- Food-web topologies persist across seasons in respective areas

## **1. Introduction**

As a transitional ecosystem characterized by dynamic physical, biogeochemical, and biological processes, the coastal ecotones create distinct community patterns along the salinity gradient (Baird et al., 1991). The spatial and temporal community patterns develop a wide spectrum of variability in the structure and dynamics of estuarine food webs (Christian et al., 2005; Hagy and Kemp, 2013). The identification of patterns in structure and processes within food webs is fundamental for understanding function and further constructing quantitative food webs for more detailed predictions of ecosystem-level responses (Winemiller and Polis, 1996; Jordán et al., 2008). By simply clustering the highly complex composition of an ecosystem, the multitrophic community patterns can be compiled and compressed into lower-dimensional matrices. Once typological patterns and characteristics with time and space are determined, we can simulate the trophic relationships and the energy flow pattern through the food webs (Layman et al., 2012) to trace the trophic pathways of carbon/energy and the trophic connectivity between habitats within estuaries (Fry, 2002). Here, an artificial neural network algorithm and stable isotope techniques were combined to identify the food web structure in the water channel along the estuarine-coastal marine continuum of a temperate coastal embayment, Gwangyang Bay, Korea, subject to low-turbidity riverine discharge and a short water residence time.

## 2. Methodology

#### 2.1 Sample collection and data acquisition

Nine sampling stations were chosen along a main water-channel trajectory of the riverestuary-coastal sea continuum in the bay in February, May, and November 2015, and February, August, and November 2016. Data including physical and chemical parameters, abundances of phytoplankton, mesozooplankton, benthic invertebrates, and nektonic samples, and the potential sources of organic matter were collected at each location.

#### 2.2 Stable isotope analysis

For all faunal samples and organic matter sources, the  $\delta^{13}$ C and  $\delta^{15}$ N values were measured using a continuous flow isotope ratio mass spectrometer (Isoprime, GV Instrument, Manchester, UK) connected to an elemental analyzer (Eurovector 3000 Series, Milan, Italy). Isotopic values were expressed in the conventional delta as deviations from standards.

#### 2.3 Self-organizing map (SOM) approach

A self-organizing map (SOM) with Kohonen's competitive and unsupervised artificial neural network algorithm was used to characterize the distribution patterns of communities (Kohonen, 2001). The input layer includes 54 nodes for plankton and benthos and 18 nodes for nekton. Consequently, the output layer is composed of a grid of  $6 \times 6$  for plankton and benthos, and  $4 \times 4$  for nekton. After training, Ward's minimum variance method with the Euclidean distance measure was applied to the hierarchical cluster analysis of the SOM units (Legendre and Legendre, 2012).



**Figure 1.** Patterning communities on the self-organizing map (SOM). Ordination of samples for (a) plankton, (b) benthos, and (c) nekton on the SOM. The clustering of the trained SOM units is indicated below the SOM map. (Source: Kang et al., 2020)



**Figure 2.** Representative food web of (a) the estuarine channel and (b) the deep-bay community of Gwangyang Bay. Sizes of linkage arrows indicate the relative contribution of prey to consumer nutrition. Blue arrows indicate pelagic pathways and red benthic pathways. (Source: Kang et al., 2020)

#### 2.4 Isotope mixing model

The trophic interactions between co-occurring dominant taxa in each cluster and/or a combination of clusters were evaluated by employing a stable isotope mixing model. Two separate steps were applied to mixing model calculations. First, the contributions of putative sources of primary organic matter to the nutrition of primary consumers were evaluated using an IsoSource mixing model. Second, the relative contributions of benthic affinity prey to the

nutrition of higher-level consumers were calculated to identify their trophic links with benthic vs. pelagic pathways.

## 3. Results and Discussion

#### 3.1 Patterning communities

The SOM map of phytoplankton configuration supported clear segregation of seasonal samples from distributions in different areas on the map as well as spatial separation of the estuarine stations from the deep bay stations (Fig. 1a). The SOM map of macrobenthos assemblages showed the spatial distribution of the samples on the map revealing a longitudinal pattern from the estuarine-channel stations to the deep-bay stations (Fig. 1b). Community patterns of nekton assemblages revealed a clear spatial pattern in the nekton community by an array of estuarine channel stations and deep bay stations in the other regions (Fig. 1c).

#### 3.2 Isotope mixing model

A generalized food web structure that remains unchanged with the season in the estuarine community and the deep-bay ecosystem was illustrated by the overall combination of the mixing-model calculations (Fig. 2). The estuarine phytoplankton played a dominant role as the main nutritional contributor to estuarine channel consumers and deep-bay phytoplankton to the nutrition of deposit feeders and zooplankton. The general pattern of the  $\delta^{13}$ C alignment and  $\delta^{15}$ N increase between the benthic and nektonic consumers reveals their strong trophic linkages, emphasizing the prevalence of benthic pathways. A functional role of diverse benthic taxa as trophic mediators in the mid-trophic levels would attest to food-web stability in response to compositional changes in the lower trophic-level assemblages.

## 4. Conclusion

This study successfully differentiated two contrasting community structures between the estuarine channel and the deep bay. These empirical approaches enable us to synthesize multitrophic community patterns and calculate the trophic links and interactions of key nodes of food-web networks. Furthermore, it helps us to clarify the changes in food-web architecture under the influence of natural and/or anthropogenic forcing across the coastal ecotone.

## Acknowledgment

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# Using eDNA to detect the presence of marine invasive species in ports and harbors

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Abstract: With the global extent of maritime trade, the risks for introducing marine invasive species in ports and harbors worldwide is great. A majority of member states of the International Maritime Organization (IMO) have acceded to the Ballast Water Management Convention of 2004 (BWMC) and the Antifouling Systems Convention of 2001 (AFS) which aim to reduce the risks of introducing non-indigenous and invasive species in ports. The IMO has set certain standards of ballast water release compliance, the D1 and D2 standards. The installation of ballast water treatment systems on ships have reduced the risk but not eliminated risks of marine invasive introductions. The current ecological count methods of assessing compliance are time consuming and tedious that only 1% of global shipping is regularly scientifically assessed. Environmental DNA (eDNA) metabarcoding presents a promising approach for rapid assessment of marine biological invasion risks in ports but presents certain methodological difficulties especially in the lack of environmental baseline and genomic databases. I present initial results of our research on tropical ports on eDNA and in ballast water that show the practicalities and promising directions of using eDNA in ballast water compliance assessment and in port ecological baselines.

**Keywords:** ballast water, invasive aquatic species, molecular ecology diagnostics, International Maritime Organization

## Highlights

- Ballast water release and marine biological invasion.
- eDNA as a rapid assessment tool
- Usefulness and present limitations of eDNA for ballast water monitoring

## **1. Introduction**

Marine biological invasion via the maritime industry is one of the greatest threats to marine biodiversity. While biological invasion by natural means is an ecological process that structures marine biodiversity, biological invasion mediated by human activity is likely to stress ecological communities which may lead to localized extinctions as well as economic and public health impacts. In the global maritime industry, this is largely through ballast water discharge and biofouling of ship hulls. The ecological effects of maritime industry mediated biological invasion are well documented in the literature.

Studies of port ecological communities suggest that biofouling is more likely a vector of biological invasion than ballast water release. In Port Phillip Bay, Australia, up to 55% of non-indigenous marine species may have been introduced from ships' hulls compared with less than 30% from ballast water. In New Zealand close to 60% of non-indigenous marine species are thought to have been introduced through fouling, compared with less than 25% through ballast water. The percentage in San Francisco Bay, California, USA is 55% (Davidson & Simkanin. 2012). It is reasonable to assume that 55% of non-indigenous marine species detected around the world could have been introduced by biofouling.

In the Port of Manila, marine invasive aquatic species have been detected using intensive ecological sampling for 10 years (Vallejo et al 2017; Vallejo et al 2019, Trinidad et al 2019). Intensive sampling has been able to detect incipient biological invasion of the Tropical American mytlid mussel species *Mytella striata* (2014) and *Mytilopsis sallei* (2011). *M. striata* is now a documented IAS with serious economic and ecological effects on estuarine environments.

The International Maritime Organization (IMO) has recognized the biological invasion threat posed by invasive aquatic species (IAS) through ballast water release and ship biofouling. The IMO has presented two conventions, the Ballast Water Management of 2004 and the Anti Fouling Systems of 2001 that mandate signatory maritime states to implement appropriate measures to prevent the introduction of and manage the adverse environmental impacts of IAS. The Ballast Water Convention mandates two methods of ballast watermanagement. The D1 standard requires ships to exchange ballast water in open waters 200 km from port. The D2 standard requires ships to have ballast water treatment on-board after which ballast water can be discharged with minimal risk for the introduction of invasive species.

In countries where some ships have not been configured to D2 standards, ballast water can be treated in on-shore facilities for compliance. The IMO requires that all ships follow the D2 standard by 2024. Table 1 lists the IMO parameters for safe ballast water discharge. Most treatment methods employ mechanical filtration and the use of UV sterilizers as a biocide solely or with IMO approved biocides. Other methods include ozonation and electrochlorination.



Fig 1. Design of a ballast water treatment facility for Philippine ports

The University of the Philippines and the University of Cebu have fabricated a prototype ballast water treatment system that is capable of treating ballast water on shore. A conceptual schematic is shown in Fig 1. Gollasch & David (2010) and (2015) describe standard techniques for sampling the biota of ballast water. Rapid identification and taxonomic assessments of IAS in ballast and port water are time consuming and requires manual counting and microscopy. Most of the organisms that will be observed are planktonic and for the estimation of density, flow cytometry (Veldhius & Kraay, 2000) has been used. eDNA has been used in a few instances.

Empty Cell	Empty Cell	<b>Discharge limitation</b> * <i>colony</i> <i>forming unit (cfu)</i>
Indicator microbes	Toxicogenic Vibrio cholerae	<1 cfu per 100 mL
	Escherichia coli (E. coli)	<250 cfu per 100 mL
	Intestinal Enterococci	<100 cfu per 100 mL
Size of microorganisms	≥50 μm	<10 viable organisms per m <sup>3</sup>
	${\geq}10~\mu m$ and ${<}50~\mu m$	<10 viable organisms per mL

Table 1. Microorganisms discharge limitations based on IMO D-2 Standard. (IMO, 2004)

There is a need for a more rapid technique for assessment for target MNIS. In this light, eDNA (environmental DNA) approaches will be employed as well. Environmental DNA has become prevalent in conservation and has become rapidly developed for aquatic invasive species surveillance. In recent times, potentials of methods which detect DNA traces within

any environment have been looked into. This non-invasive method promotes a reliable and cost-effective method for monitoring various organisms in an aquatic environment, particularly when the populations are low in abundance .This has become really powerful and cost-effective because the DNA could be sourced from mucus, feces, sloughedoff cells, and decomposing organisms can introduce eDNA in a system and this can be identified through the use of the Polymerase Chain Reaction (PCR) (Gingera, et al, 2017). Therefore, environmental samples can be obtained anywhere, anytime, and the presence of target specimens from trace DNA can be detected. As an alternative or complement to some traditional ecological methods, eDNA technologies provide a cost effective and sensitive detection tool to monitor species' distribution over large geographic areas quickly and accurately (Gingera, et al, 2017). It was suggested that for early detection of exotic species, novel tools such as the environmental DNA should be used. The techniques are becoming less costly, are non-invasive, highly sensitive, independent of weather conditions for sampling, and may help to control the target species (Clusa et al, 2017).

Environmental DNA can be done using two approaches – a species specific approach and the approach that deals with a large number of species. In species specific approach, the sample is analyzed using Polymerase Chain Reaction or quantitative PCR and when the DNA of the target species is in the sample, the PCR will amplify the DNA of the sample and the primers would be created to target a short DNA fragment and it would be tested *in silico, in vitro* and *in situ*. For the detection of large number of species, the multi-species approach which uses several species specific primers and the universal approach via eDNA metabarcoding which uses primers that amplify a whole group of species, can be done (Geiger et al., 2014).

Many studies support the potential of the eDNA approach for the early detection of the invasive species. In Iberian Peninsula, three rivers near the most populated inland cities were monitored for the presence of the invasive molluscs and by designing new primers and using BLAST methodology and *in vitro* experiment, *Corbicula fluminea* was in Ebro River (near Zaragoza); *Mytilopsis leucophaeata* in Guadalquivir River (near Sevilla). *Melanoides tuberculata* and *Sinanodonta woodiana* were found from enclosed areas (lake and reservoir respectively) upstream, respectively, Zaragoza and Madrid (Clusa et al, 2017). In the North Sea, environmental sequences through a multigene approach detected 114 species belonging to 12 phyla (Gunther et al., 2018). Geiger et al. (2014) also presented some species in natural freshwater and their detection probability using qPCR.

In addition, BWMC enumerated indicator microbes and under regulation D-2 of the BWMC, these microbes must be treated to the following standards: (1) *Vibrio cholerae* (serogroups O1 and O139) b1 CFU/100 mL or b1 CFU/g of zooplankton samples (wet weight); (2) *Escherichia coli* b 250 CFU/100 mL; and (3) Intestinal *Enterococcus* b 100 CFU/100 mL.

Evidence links cargo ships as transport vectors of cholera and *Vibrio*-related disease (McCarthy and Khambaty, 1994; Mimura et al., 2007). There is growing evidence that the combined effect of extreme weather events, rising sea surface temperatures, and increasing

anthropogenic discharge into the marine environments not only exacerbates the spread of *Vibrio*-related diseases in the Americas and Asia, but also facilitates the displacement of marine organisms from distant areas (Martinez-Urtaza et al., 2016).

These approaches, which typically involve DNA sequencing of the bacterial 16S ribosomal RNA gene (16S rRNA) and subsequent matching of DNA sequences to known bacterial groups, provide a vastly more detailed view of the microbial community in ballast water. However, metagenomics studies on the bacterial ballast water community are still rare.

## 2. Methodology

#### 2.1 Water sampling

Water samples with a volume of 2.5 liters each were sampled every quarter in 2022 at international ports of Manila, Cebu and Matnog. These are 3 international ports out of six in the Philippines where eDNA assessment were performed (Fig 2). These were sent to the DNA laboratory of the Institute of Biology of the University of the Philippines in Diliman, Quezon City for DNA extraction and amplification within six hours of sampling. Manila and Cebu are the busiest ports in the country with 1,481 and 648 port calls respectively in 2022. Matnog is assumed to be less impacted by human activities as it has the least number of port calls with 12 in 2022.



**Fig. 2** International ports in the Philippines where port ecological studies for IAS were conducted. Manila, Cebu and Matnog are where eDNA assessments were conducted.

#### 2.2 Primer and Probe Design

Conventional Polymerase Chain Reaction (PCR) methods were used for analyzing eDNA. In qPCR, primers were used to amplify a portion of DNA that target organism-specific and a probe was used to provide additional specificity and quantitative information.

#### 2.2.1 Laboratory Optimization

The primer and probe design was tested to make sure that the qPCR reaction would always yield to positive detection in the presence of the DNA of the target organism. The DNA was screened before the environmental samples were analyzed to ensure test sensitivity. DNA can be extracted from preserved samples using commercially available kits.

The species-specific primers and probes for qPCR were designed by creating an inclusive consensus sequence that are included within all the species variability for a species in a well-known DNA region (Meusnier et al 2008). Mitochondrial DNA was used since it is more abundant than the nuclear DNA. Sequences in GenBank would be used for the tissue samples of the target species. For the selected probe chemistry, setting an appropriate qPCR primer is important to design short, unique sequences for use as forward- and reverse primers and probes. The universal primers and probe amplify and detect the target sequence (90—120 base-pair length is recommended). The result was compared with the sequences in GenBank to determine if the sequence will cross-amplify with other species.

#### **2.2 DNA Extraction and Amplification**

DNA extracted from the water sample was purified and amplified each PCR reaction according to protocols of Hinlo et al (2017).

For the extraction of DNA from water samples, the water was first filtered through glass fiber filter paper. Thus, extraction makes use of a modified kit extraction process that had to be first optimized to obtain consistently high DNA concentration and purity.

Before processing of samples, parameters involved in DNA kit extraction (using Thermo-Fischer GeneJet Extraction Kit) are: 1) filter paper cutting, 2) digestion and incubation time, 3) amount of reagents to be added, 4) addition of RNase A, 5) pooling in purification columns, and 6) amount of elution buffer. Moreover, multiple characterization steps were checked which include the use of Nanodrop machine for checking of concentration and purity, a Qubit fluorometer to determine accurate double stranded DNA concentration, and agarose gel electrophoresis to determine possible presence of DNA degradation.

Following DNA extraction, qPCR analysis was used to quantify DNA yield. This gives information about the detection of the target species' DNA. Although the amount of the target DNA present in the field samples may be quantified, the study focuses primarily on presence or absence of target taxa.

#### Design Molecular Assay

Variation within species and among species was accounted for to determine the utility

of the markers selected in distinguishing taxa. These will be based on the actual sequences that will be obtained as well as the sequences available in GenBank and BoLD. We used Folmer primers.

#### 2.2.1 Multiple Species eDNA Approach

The water sample was sent to a third-party laboratory for analysis and identification of species

#### 2.2.2 Phylogenetic Analysis

Sequences identified were manually extracted from the next generation sequencing (NGS) output files identified in the operational taxonomic unit (OUT) list and aligned using the BioEdit Software. Haplotypes will then be identified via a different software. Phylogenetic analysis were conducted using MEGA v.6. Phylogenetic tree containing the reference and the BW sequences was tested with maximum likelihood. Robustness of the tree topology was assessed using 1,000 bootstrap replicates.



**Fig. 3**. Process of species identification for the DNA barcoding (species-specific approach) and DNA metabarcoding (multispecific approach).

## 3. Results and Discussion

The DNA extracted was determined enough for sequencing. The average DNA yield from samples is 1.41 ng/ul. The samples were then amplified, Sanger sequenced and metabarcoded. Species identification was done by BLAST search. The metabarcoding results are shown in Figure 3 and 4.

A phylogenetic analysis (Figure 6) of the sequences may assist in the identification of previously unknown or cryptic species of *Nereis* 



Fig 4. Metabarcording results from samples taken in Cebu International Port Using the sequences amplified, it was possible to tentatively identify to the genus level polychaete ship biofoulers, such as *Nereis* in a shorter time. Morphological identification would have taken 2 weeks at least.



Fig. 5 Initial metabarcoding results for Nereis from the Port of Manila.



**Fig. 6** Phylogenetic tree of *Nereis* sequences with possible new or cryptic species circled in red.



Fig. 7 eDNA barcode and identification of Mytilopsis sallei

#### **DNA sequence:**

TACTTTGTATCTTATCTCTGGTGTGTGGGGCTGGGTTAATAG GAAGAAGGTTGAGATTGATTATCCGTATTCAACTTTCCCAC CCTGGAGGAAATTTTCTAAAAAACGAAAGCTTGTATAATGT AGTGGTAACTACTCATGCTTTAGTGATAATTTTTTTGCTG TTATACCACTTTTAATCGGTGCATTTGGGAATTGACTTCTT CCTTTATGTATCGGTGGTTGTGATTTGATTTTTCCTCGTCT TAATAATTTAAGATTTTGGTTGGCTCCTAATGCTTTATATTT ATTAATCTTATCTTTCATAACTGAAAAGGGAGCAGGGACA GGATGAACAATTTATCCTCCTTTGTCTTCTAGATTGTACCA TACTGGTCCTGCAGTAGATATCTTAATTACTTCTTTCATTT GATCGGTTTAAGTTCTTTATTAGGTTCGATTAATTTTGTAA GCACTAATAAGGATATACCTACAACTAAAATAAAAGGGGA GAAGTCAGAGTTATACTTATGAAGAATTACTGTGACTGGT GTTCTGTTAATTATTTCGGTTCCTGTTCTTGCTGGCGGAAT CACTATGCTTTTGTTCGATCGAAATTTTAACACTAGGTTTTT CGATCCAATTGGTGGGGGGGGGGGTGATCCTGTACTATTTCAACAT GTTTTT



Cunanan Wharf 4th Retrieval

#### Fig 8. eDNA barcode and identification of Mytella striata

#### **DNA** sequence:

TACCTTATATATTTTATTTGGCATATGATCAGGA CTAGTTGGTACAGCTCTTAGACTGTTAATTCGA GCAGAACTAGGTCAGCCCGGAGCTCTCTTAGG AGATGATCAACTATATAACGTAATTGTAACCGCC CATGCTTTTGTTATAATTTTCTTCCTAGTAATACC TATGATAATTGGAGGATTCGGAAACTGGCTGGT TCCCTTAATATTAGGAGCCCCAGACATAGCATT CCCTCGTTTAAATAATAATAAGTTTCTGACTCCTC CCTCCTGCCCTGCTACTGCTCCTTTCTTCAGCC GCGGTTGAAAGCGGAGTCGGAACCGGATGGAC CGTCTACCCTCCTTTAGCTGGAAACCTTGCCCA CGCTGGTGGATCTGTAGATCTTGCCATCTTCTC CCTTCACTTAGCAGGTGTTTCGTCAATTTTAGGT GCTGTAAACTTTATTACAACTATTATTAATATAC GGTGACGAGGTATACAGTTTGAACGACTTCCTT TGTTTGTATGATCCGTAAAAATCACCGCCATTCT CCTTCTTCTCTCTCTACCAGTTTTGGCCGGGGC CATTACAATGCTGCTGACAGATCGAAACTTTAAT ACTGCTTTCTTCGACCCGGCCGGAGGTGGAGA TCCTATCTTATATCAACACTTGTTC



Matnog Port 3rd Retrieval

Percent Identity: 99.85%

Fig 9. eDNA barcode and identification of Monetaria moneta.

eDNA is also able to rapidly identify known invasive IAS in ports such as tropical American *Mytilopsis sallei* (Figure 7) or the very invasive *Mytella strigata* (Figure 8) as well as indigenous species such as *Monetaria moneta* (Figure 9).

eDNA and metabarcoding promises to be an efficient technique in rapid assessment of the presence of IAS in ports and harbors as well as in ballast water discharge (Rey at al 2019).

However, it has limitations of the data available in existing sequence databases. There is a need to expand the catalogue of available markers in the reference databases which are taxonomically verified (Zaiko et al 2015) . These limitations make eDNA not applicable for management and enforcement purposes under the BWMC without standard ecological methods of identifying and assessing IAS. Metabarcoding also may allow for discrimination of harmful and invasive algae if this was taken as ballast water from certain ports with documented algal blooms (Shaw et al 2019). There is evidence that ship ballast water release is related to the spread of harmful algal blooms worldwide. These phenomena together with the movement of waters through shipping activities may result in serious implications for the emergence, spread of phytoplankton especially in a highly interconnected international shipping network (Trinidade de Castro & Veldhius, 2019).

The methods describe in this study show that eDNA and metabarcoding of indigenous and non-indigenous and possibly IAS is possible. Ports such as Manila and Cebu have high species diversity as revealed by ecological studies (Vallejo et al 2019) and supported by the present eDNA assessment of port and ballast water discharge which are able to detect previously ecologically detected IAS, indigenous species and possible new or cryptic species. There is growing evidence that ports and harbors show high  $\beta$  diversity turnover as a result of ballast water discharge and biofouling as revealed by eDNA (Andres et al 2023). This implies that these environments are biotically homogenized and this is supported by ecological studies as well.

## 4. Conclusion

eDNA and species metabarcoding show great possibility for the rapid assessment of ballast water for IAS. However, limitations such as presented by insufficient sequence information in DNA databases and primer design presents challenges for its use for ballast water monitoring and standards enforcement. Also, the technical aspects of these techniques need to be communicated to maritime industry stakeholders, national flag state authorities and coast guards.

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# Artificial drivers of jellyfish blooms and transport of nonnative species

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Abstract: Recent studies have perceived that the jellyfish increase is a global trend, portrayed as a symptom of ocean degradation. Changes in marine environments due to human activities could enormously contribute to the jellyfish abundance; overfishing, marine construction, eutrophication, global warming, etc., may benefit jellyfish populations over finfish. However, others suggested that this perception is based primarily on a few case studies and anecdotal evidence. Thus, the perception of a worldwide trend toward increased jellyfish abundance may still be unsupported because of many exaggerated information and lack of confidence in existing evidence. We have examined the causation and major factors contributing to jellyfish increase, non-indigenous species and the impact on the endemic zooplankton communities, and unknown behavior of a box jellyfish species that may have extended its distributional latitude. For the purpose of study, we have dealt with *Aurelia coerulea*, *Blackfordia* spp., and *Carybdea brevipedalia*, the ecologically interesting but often problematic jellyfish species.

**Keywords:** polyps on artificial structures, non-indigenous species, feeding and swimming behaviour, distributional extension

## Highlights

- Increase in coastal development and construction has been the primary cause of the rise of *Aurelia coerulea*.
- *Blackfordia virginica*, a non-indigenous hydromedusa, blooms every year in Shihwa Lake, exhausting zooplankton preys. *Blackfordia virginica* and *B. polytentaculata* were newly found in Seomjin River mouth located near the international trading port.
- *Carybdea brevipedalia* is venomous, an agile swimmer, and a voracious nocturnal feeder. Even though high predatory pressure on nocturnal preys such as mysids, decapods and polychaetes was observed, the jellyfish is fishing rather than hunting.

Light pollution affecting the jellyfish's productivity has not been clearly elucidated. New reports of sting by the species and population genetics showed possibly the recent northward shift onto the eastern coast of Korea.

### **1. Introduction**

Increasing of jellyfish populations has been the general trends in large marine ecosystem (Brotz et al. 2011) and may be a consequence of climate change (Frolova & Miglietta 2020). It is suggested that many changes that have occurred in coastal waters due to human activities could benefit jellyfish populations over those of fish. For example, finfish has a similar ecological niche with jellyfish, and overfishing by humans can lead to jellyfishes' predominance. According to the 'jellyfish spiral theory', if jellyfish once predominate an ecosystem, the ecosystem would be caught in a vicious circle that the ambient environment benefits jellyfish more and more, then fish would be eradicated. Multiple explanations have been suggested for possible drivers of the apparent increase of jellyfish blooms in many coastal waters around the globe, including the depletion of predators and competitors of jellyfish by overfishing, accidental translocations, eutrophication of coastal waters, changes in freshwater flows, human modification of coastal geomorphology, and climate change (Uye & Ueta 2004, Purcell et al. 2007, Fujii et al. 2011, Purcell 2012).

However, there are somewhat different perceptions of jellyfish trends in which a global trend toward increased jellyfish blooms is unsubstantiated. Condon et al. (2013) suggested that recurrent jellyfish blooms are a consequence of global oscillations. Duarte et al. (2015) insisted that jellyfish increasing trend is still unsupported because of many exaggerated, and sometimes even distorted information by citation mistakes and lack of confidence in existing evidence. Furthermore, we need a paradigm shift that jellyfish is an important trophic element in marine ecosystem instead of simply harmful organisms (Hays et al. 2018). In recent years, a range of studies, using a variety of techniques have shown that a broad range of marine predators including fish, birds, green turtles, and various invertebrates frequently feed on jellyfish.

A recent study using long-term data of biogeographic changes in the jellyfish risk showed that jelly risk has steadily spread over the last six decades, 1960 s - 2010 s (Lee et al. 2023). And East Asian marginal seas, i.e., the East China Sea, the Yellow Sea and East Sea/Sea of Japan, have faced the highest level of cumulative jellyfish hazards. In Korea, about 150 – 300 US dollars were estimated as the economic loss per year caused by harm from jellyfish such as impingement into cooling water system of NPP, damage of fishery products and fishing gears, and stings in beach and fisheries. However, little has been explained for the causation, changing trend and the consequence because of lack of quantitative long-term data on the jellyfish abundance as well as the ecology related to change in marine environment. We have examined the causation of jellyfish blooms, the impacts of newly found non-indigenous species on the endemic zooplankton communities, artificial effects on jellyfish feeding and distribution for *Aurelia coerulea*, *Blackfordia* spp., and *Carybdea brevipedalia*, the scyphozoan, hydrozoan and cubozoan species, respectively.

## 2. Methodology

#### 2.1. Polyps of Aurelia coerulea, the most abundant species

Because the population size of polyps may be a crucial factor in determining the intensity of medusa blooms in the next seasons, we have located polyp populations of *Aurelia coerulea* and determined their abundance around the Korean coast using underwater photographs collected by SCUBA diving. Diving observation has been made in ca 2,600 sites (Fig. 1). According to the substrates where polyps were found, we have earned underwater photographs of the polyps with various methods to calculate the amount of them; continuous attaching photographs through transects or joining the pieces of the photographs together. When needed, we collected representative samples and compare the polyp numbers between underwater photographs and the photographs taken from the various angles in the lab to compensate errors from 2-dimensional images of underwater photographs into real polyp numbers of 3-dimensional distribution. We also occasionally confirm the species identification with species specific DNA probes.



**Figure 4**. Polyp searching sites in Korea coast from 2013 to 2022. Numbers indicate that the numbers of examining sites in each spatial area and numbers in parentheses indicate the no. of sites where significant numbers of polyps have been found.

#### 2.2. Blackfordia virginica and B, polytentaculata, hydrozoan invaders

Since 2013 when *Blackfordia virginica* was first found in Shihwa Lake, the jellyfish had been continuously examined every year until 2020 except for 2019. Samples were collected from upper region (brackish waters) of Shihwa Lake from late spring to summer. Stable isotope ratio was measured to determine the major jellyfish's food items using samples collected from May to August in 2018. After preliminary finding of *Blackfordia* jellyfish in the Seomjin River, the jellyfish samples were collected quantitatively from seven stations where fresh- and seawater are mixed due to saltwater intrusion in Aug 2021. Possible dispersion or new invading of *Blackfordia* species had been separately monitored in a spot of each nine river mouths from April to June in 2019 to 2020.

#### 2.3. Carybdea brevipedalia, the venomous box jellyfish

Swimming and feeding behavior of Carybdea brevipedalia were numerically observed

on the base of 24 h rhythm; Depth distribution, feeding rhythm and gut contents, tentacle lengths, digestion time and pulsation. In situ feeding rate and abundance of the jellyfish and prey zooplankters on the carbon base were also measured. Data under natural dark and artificial light condition were compared. Distributional change was examined through collecting the samples of the species from various sites of Jeju-do, Namhae-do, Sacheon, and eastern coast of Korea. Then, genetic variation of populations of Namhae-do and eastern coast were also compared.

## 3. Results and Discussion

#### 3.1. Polyps of Aurelia coerulea

We have found substantial (significantly large) polyp populations of *Aurelia coerulea* from more than 800 sites. Downward-facing surfaces of various underwater artificial structures were the most frequently observed habitats of the jellyfish polyps (Fig. 2). We found polyp populations in only one natural habitat. These results provide compelling evidence that the increase in coastal development and construction is the primary cause of the rise of *Aurelia coerulea*, the most frequent and large-scaled blooming species.



**Figure 5.** Polyp distribution and abundance of A. coerulea along the Korean coast and schematic drawings of typical existing mode of the polyps under the artificial structures.

#### 3.2. Blackfordia virginica and B, polytentaculata

We first found *Blackfordia virginica*, the world-wide brackish invading hydromedusa from the Shihwa Lake in 2013. Nine years ago, in 2004, unidentified polyps were photographed from the abandoned fish-net in the same area, and their morphology was found to be identical to those of the species. Medusae of *B. virginica* bloomed every year from 2012 to 2020 except for 2017, showing extremely high density generally in Jun and Jul of which the maximum mean-density was 679 ind m<sup>-3</sup> in Jun 2015. Stable isotope ratio showed that copepods and barnacle larvae are the major prey items for the jellyfish. Every year, copepods were rapidly decreased after blooming of *B. virginica* (Fig. 3), and the bell diameter of the jellyfish shrunk after exhausting of its preys.

We had not found the individuals of *Blackfordia virginica* in other 9 brackish waters in 2016, 2019, and 2020, however, *B. polytentaculata* as well as *B, virginica* were found in Seomjin River mouth in Aug 2021 with high densities of which the maximum was above 2 ind m<sup>-3</sup> and

 $2 \times 10^2$  ind m<sup>-3</sup>, respectively. Thus, the Seomjin River mouth is the second after the type location of *B. polytentaculata*, Jiulong River, Fujian, China.



**Figure 6**. Population dynamics of Blackfordia virginica and copepods in 2013 – 2020 (no data in 2019).

#### 3.3. Carybdea brevipedalia

Larger zooplankters such as decapod and fish larvae, mysids, and swimming polychaetes (>  $0.2 \text{ Cmg}^{-1} \text{ ind}^{-1}$ ) were the major prey items of *Carybdea brevipedalia*, a cubozoan medusae, while copepods (<  $0.05 \text{ Cmg}^{-1} \text{ ind}^{-1}$ ), the most predominant in the study area were little found in the gut. While feeding was minimal during the day, more than 80 % of individuals observed at 20:30-03:00 involved 1 - 6 prey in their digestion pouch (Fig. 4).



**Figure 7.** Diel change of gut fullness. Ratio of individuals with/without prey in the gastric pouch (A), and the no. of preys in each species per jellyfish (B). White and black bars indicate the jellyfish with and without prey organisms, respectively. Shaded area indicates night.

It was a strong vertical migrator, conspicuously ascending right before sunset (Fig. 5). They distinctly elongated their tentacles under dark conditions. PIV analysis shows that it swims a long distance at night, the feeding time. Even though the species is a fast swimmer and voracious predator, having a complex visual structure including lens eyes, it is a passive hunter, fishing rather than hunting. Tentacle elongation and agile swimming are the most important

means to succeed in feeding on the faster swimming prey. Ascending to the surface water at sunset may lead its position to more frequently encounter nocturnal prey species. We assumed the impacts of artificial light on their feeding rate because of the solid positive phototaxis to the collimated light, both predators and prey; however, the light pollution affecting their productivity has not been elucidated.



**Figure 8.** Diel change of distributional depths of *Carybdea brevipedalia*. Blue-shaded area indicates observations at night (left). Diel change of tentacle length. Gray and white bars indicate days and nights under light source. Black bar indicate nights in continuous dark condition (right).

*Carybdea brevipedalia* is a small to medium sized cubozoan only known to be distributed in Japan and southern coast of Korea, Jeju-do, Namhaedo and Sacheon. We recently found that it is also distributed in the eastern coast of Korea in 2021. Mitochondrion gene analysis suggested the possibly recent northward shift of this species onto the eastern coast of Korea (Fig. 6).



**Figure 9.** COI TCS haplotype network of *Carybdea brevipedalia* distributed in Namhae-do and eastern coast of Korea.

## 4. Conclusion

The artificial effects on jellyfish abundance, population sizes, frequency or scales of the blooms, and changes in distribution, may vary from species to species; Coastal construction and facilities have provided broad and optimal habitat for polyps of *Aurelia coerulea*. Recent

increase of marine transport may introduce many non-indigenous jellyfish such as *Blackfordia virginica*, leading to disturbance of endemic zooplankton communities. We investigated feeding and swimming behavior of the box jellyfish, *Carybdea brevipedalia*, and found that they are agile swimmers, but fishing rather than hunting. Light pollution which may affect their productivity was not clearly elucidated. They showed recent northward shift in distribution onto the eastern coast of Korea, which may be the north-limit of the species' distribution.

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# Key Indicator Species and Habitats for Marine Biodiversity Change in East Asia

