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Guidelines for Authors

Introduction

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Seascape ecology is an emerging research field that applies the conceptual and methodological frameworks of landscape ecology to the marine environment. It aims to gain a deeper understanding of the complex and dynamic ecological patterns found over space and time across the oceans through the advancement of ocean observation systems, geospatial data and approaches. This research field is expected to contribute to quantifying biodiversity and cultural conservation efforts, assessing ecosystem services, and helping decision-making on complex social issues related to the blue economy and sustainable development. Furthermore, in terms of the phylogeography and conservation of marine organisms, visualization of the seafloor topography and the dynamics of physical and chemical components in the ocean, which cannot be observed from the land, makes it possible to explain the degree of connectivity among marine communities and contributes to the detection of so-called “soft” isolation barriers. This symposium gathers researchers who integrate seascape ecological methods into the study of marine communities, species and genetic diversity, and discusses the role of seascape ecology in understanding the driving mechanisms of marine biodiversity to formulate optimal management and/or conservation strategies for the marine environment.



Symposium mini review



Seafloor Heterogeneity: Offshore Oil and Gas Platforms and Marine Ecosystem Dynamics in the North Sea

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Keywords

artificial reefs, offshore man-made structure, decommissioning, anthropogenic influences, multiple stressors, ecological restoration

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Abstract

The North Sea has long been a vital ground for the exploitation of natural resources, supporting one of the world's most active fisheries as well as extensive hydrocarbon exploration, the latter of which has led to the installation of over 500 offshore oil and gas platforms since the 1960s across the region. Initially, offshore oil and gas installations were considered as one of the major stressors which have negative impacts on the seafloor. However, recent studies have shown that the physical presence of such installations may in fact have beneficial effects on the surrounding ecosystems because they provide extensive surface area of hard substrata for fouling organisms, including species of conservation importance (e.g., cold-water coral *Lophelia pertusa*), and thereby serve as artificial reefs that provide habitat for diverse marine life including a variety of commercially important fish species. Majority of these structures have been in place for more than decades and they may therefore have functioned as mature reef systems potentially acting as a network of *de facto* marine protected areas (MPAs). However, many offshore petroleum fields are currently approaching the end of their commercial lives, and the focus is now shifting towards the fate of these obsolete oil and gas facilities since different decommissioning options may make significant differences in both environmental effects and economic consequences. Here I present the current understanding of marine ecosystem dynamics in relation to various environmental parameters with particular reference to the physical presence of offshore oil and gas platforms in order to better understand the potential role of offshore sub-sea artificial infrastructure in the ecology of fish populations in the North Sea.

Introduction

Marine ecosystems have played a vital role in supporting human well-being since ancient times by providing a wealth of resources and services (Katsanevakis, et al., 2011; Thurber, et al., 2014; Bennett, et al., 2015). However, contemporary marine ecosystems have been placed under ever increasing anthropogenic pressure resulting from: e.g., climate change, fisheries, aquaculture, shipping, navigation, tourism, mining, ocean pollution, coastal development and installation of offshore energy infrastructure (Benn, et al., 2010; Hoegh-Guldberg and Bruno, 2010; Foden, et al., 2011; Katsanevakis, et al., 2011; McCauley, et al., 2015). To visualise the influences of various anthropogenic pressures on marine

ecosystems, Halpern et al. (2008) developed a pioneering methodology to quantify and map the cumulative impacts of multiple anthropogenic stressors and applied it to marine ecosystems globally (Halpern, et al., 2008; Halpern, et al., 2019). Such cumulative impact maps may indeed provide insight into how intensely different human influences are overlapped at certain geographical regions on a global scale. However, spatial data used are typically at coarse resolution, and individual stressor impacts are assumed to be "additive" and of "equal importance" without accounting for the potential for interaction ("synergisms", "antagonisms" or "ecological thresholds") (Halpern and Fujita, 2013). Further, the cumulative impact scores are neither ground-truthed nor compared to some measurements of actual change in

ecosystem condition (Halpern and Fujita, 2013). As a result, the utility of such simple mapping approach may be limited or even misleading if this is to guide and inform smaller-scale decisions about the costs and benefits of a specific management action.

In the North Sea, the impacts of offshore oil and gas production on the surrounding environments have long been the major policy issues because of the potential for them to generate negative effects either by increasing local pollution levels resulting from hydrocarbon extraction or by making some fishing areas inaccessible for fishing vessels. Although the installation of subsea man-made infrastructure is generally considered to have negative impacts on surrounding marine ecosystems (e.g., Benn, et al. 2010; Foden, et al. 2011; McCauley, et al. 2015), there are still major knowledge gaps in our understanding of the diversity of human activities operating in the marine environment, the nature of their cumulative impacts on marine ecosystems and the responses of marine populations, communities and ecosystems (Fujii et al., 2020; Fujii et al., 2023). Here I present the current understanding of marine ecosystem dynamics in relation to various environmental parameters with particular reference to the physical presence of offshore oil and gas platforms in order to better understand the potential role of offshore sub-sea artificial infrastructure in the ecology of fish populations in the North Sea.

Seafloor heterogeneity and marine ecosystems in connection with offshore oil and gas infrastructure

The North Sea once harboured extensive biogenic reefs which supported rich and diverse marine communities (Birkeland, 2010). However, many of these were destroyed by bottom trawl fisheries in the last couple of centuries (Gilbert, A.J. et al., 2015; Duarte et al., 2020). Hundreds of offshore man-made structures have since been installed for the oil and gas industry across the region (Martins et al., 2023). In view of measuring human pressures on marine seafloor habitats, offshore oil and gas installations are typically considered as one of the major stressors which have negative impacts on the seafloor (e.g., Halpern, et al., 2008; Benn, et al., 2010; Foden, et al., 2011; McCauley, et al., 2015; Halpern, et al., 2019). However, recent studies show that the physical presence of such installations may in fact have beneficial effects on the surrounding ecosystem (Steimle and Zetlin, 2000; Loke, et al., 2015). This is because they provide extensive surface area of hard substrata for fouling organisms, including species of conservation importance (e.g., cold-water coral *Lophelia pertusa*) (Gass and Roberts, 2006; Bergmark and Jørgensen, 2014), and thereby serve as artificial reefs that provide habitat for diverse marine life (Claisse, et al., 2014; Fujii, 2016; Meyer-Gutbrod et al., 2019), including commercially important fish, such as saithe *Pollachius virens*, haddock *Melanogrammus aeglefinus* and cod *Gadus morhua* (Fujii, 2015). Majority of these structures have been in place for decades and they may therefore have functioned as mature reef systems potentially acting as a network of *de facto* marine protected areas (MPAs), which may have enhanced habitat diversity, biological connectivity and productivity over wider geographical scales (Claisse, et al., 2014; Lawrence et al., 2024).

It follows that all human induced drivers do not necessarily have a uniform negative influence on ecosystem condition, and certain types of drivers could in fact have positive effects on certain ecological metrics, which is in contrast to the views predicted by the current cumulative impact mapping approach. Furthermore, stressors may act interactively, as opposed to additively, in their effects on marine organisms (McCauley, et al. 2015; Gunderson, et al. 2016; Liess, et al. 2016). The assessment of multiple anthropogenic stressors therefore needs to formulate better approaches showing the nature of human influences (i.e., negative or positive), where they affect, and how they interact in order to better describe changes in the state of the marine ecosystems as a function of human uses, climate change and environmental variation. In the North Sea, many offshore petroleum fields are currently approaching the end of their commercial lives, and the focus is now shifting towards the fate of these obsolete oil and gas facilities since different decommissioning options may make significant differences in both environmental effects and economic consequences.

Decommissioning the aging offshore oil and gas production infrastructure

The majority of oil and gas production in Europe comes from mature offshore facilities that are closing on the end of their lifespan (Fowler et al., 2014; Burdon et al., 2018), and many producing regions have regulatory frameworks requiring the entire infrastructure to be removed (e.g. Stagg, 1998; Jørgensen, 2012). These regulations were put in place principally in the 1980s and 1990s to ensure that the environment used for oil and gas production would be restored to its pristine conditions once this activity ceased. Our understanding of ecosystem dynamics has drastically changed since those operations started more than half a century ago and a wealth of studies have shown since then that ecosystem restoration is complex and that removing the obsolete production facilities might not necessarily result in a return to the ecosystem's original state (Fortune and Paterson, 2020). The scientific community is uncertain on meeting the policy objective of a restored ecosystem by following these regulations and, more importantly, the demolition work associated with decommissioning may itself impact ecosystem functioning and current species-at-risk while also incurring substantial environmental costs including carbon emissions (Fortune and Paterson, 2020; Fowler et al., 2020). It may be possible to meet some regulations with new engineering solutions, but many questions remain open about, for example, the re-suspension of contaminants and lengthy episodes of extreme noise. The North Sea has sustained oil and gas extraction for over five decades and, at the same time, the climate and fisheries pressures have changed with a variety of marine species have utilised subsea offshore structures as substrate and as locations for safety from fisheries operation. There is at the moment no way to predict what will be the ecological consequences of subsea structure removals in a system that may now benefit from hundreds of *de facto* MPAs across the North Sea (Burdon et al., 2018).

Emerging opinions and future directions

The North Sea presents the complexities in which managing the restoration of ecosystem functions and services is linked to a complex social, economic, and environmental setting (Nicolette et al., 2023). For example, decommissioning confronts both economic challenges and opportunities for nations and the private sector, and it will also have positive and negative impacts on coastal communities through employment diversification and the opportunity for skills and training (Macreadie et al., 2011; Fowler et al., 2020; Nicolette et al., 2023). Emerging low-carbon policy, renewable energy infrastructure and carbon sequestration initiatives will also be critical considerations when charting decommissioning processes (Burdon et al., 2018). In view of this, the scientific foundations across multiple areas of study need to be integrated synergistically to appraise the net costs and benefits of alternative options for decommissioning. The original motivation of decommissioning regulations was to minimise the environmental effects of offshore oil and gas production. However, a number of studies now show the importance of subsea structures for productivity and ecosystem functions, potentially enhancing regional productivity, indicating current approaches to decommissioning may pose the danger of having a counter-effect of the offshore operation (Van Elden et al., 2019; Fortune and Paterson, 2020; Fowler et al., 2020). While some removal may well be essential, there needs to be a more holistic approach that integrates the environmental costs and benefits at all spatio-temporal scales to yield beneficial outcomes for protecting ecosystem functions as well as for navigating towards more sustainable coastal socio-ecological systems.

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Symposium mini review



Multidisciplinary Seascape Ecology Approaches for Sustainable Fisheries Management and Biodiversity Conservation in the Skagerrak Region

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Keywords

connectivity, population structure, genetics, genomics, oceanography, biophysical modelling, fjords

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Abstract

Understanding patterns and mechanisms driving biodiversity structure and functional connectivity in the oceans is fundamental for management, conservation and restoration purposes. Integration of complementary data obtained using different types of approaches and methods can help to elucidate patterns, causes and mechanisms shaping marine connectivity. Focusing on the Skagerrak region, this manuscript revises earlier work integrating molecular and biophysical modelling within a seascape framework. It also discusses how comprehensive integration of 'omics approaches, oceanographic modelling with different types of geospatial, ecological, environmental and behavioral data under natural and experimental settings will provide crucial information into functional ecological connectivity across complex seascapes for sustainable fisheries management and biodiversity conservation in the Skagerrak region.

Introduction

Understanding patterns and mechanisms driving biodiversity structure and functional connectivity in the oceans is fundamental for management, conservation and restoration purposes. The adoption of integrative approaches incorporating ecological, oceanographic, environmental and genetic data has added a new dimension towards a comprehensive understanding of seascape ecology (Selkoe et al. 2016; Pittman et al. 2021). Focusing on the Norwegian Skagerrak coast, this manuscript highlights how combining multidisciplinary approaches can provide novel insights into functional ecological connectivity across a mosaic of seascapes.

The Skagerrak Sea and the complex Norwegian fjord system: a mosaic of seascapes

The Skagerrak Sea is located in the southern part of the Scandinavian Peninsula, in transition between the brackish Baltic Sea waters flowing near the surface along the Swedish coast and the nutrient-rich saline Atlantic and North Sea waters traveling deeper eastwards (Sætre, 2007). As these water masses of contrasting temperature and salinity profiles meet off the Norway-Sweden border, they turn counterclockwise

and flow westwards as part of the Norwegian Coastal Current. On its way out of the Skagerrak up north to the Arctic, the Norwegian Coastal Current encounters many coastal fjords and rivers draining large amount of freshwater and nutrient run-off from land. Riverine discharges have increased over the last decades and the Skagerrak coast has experienced a significant coastal darkening with an increase of suspended particle materials, a reduction of surface salinity and a structural shift in the community composition of hard-bottom benthic communities (Frigstad et al. 2023). Although southern Skagerrak fjords are usually shorter and shallower than those found in the western and northern regions, they often present a sill or threshold of relatively shallow depth near the fjord mouth. Fjord sills can restrict water exchange and oxygenation between the deeper fjord basin and the outer coastal waters, and foster vertical stratification and the establishment of strong environmental gradients crucial for ecosystem functioning. The presence of islets and skerries in the outlet adds complexity to the hydrodynamics and the degree of connectivity in coastal fjords. Thus, the Skagerrak coast represents a mosaic of distinct seascapes with unique attributes that promotes local adaptation and spatial patterns of genetic differentiation and population structure.

Genetic population structure and barriers to seascapes connectivity in the Skagerrak

The complex seascapes of the Skagerrak are evidenced by the large number of species displaying spatiotemporal patterns of genetic population structure both among coastal populations and between coastal and offshore populations despite high connectivity (Gustafsson et al., 2024; Henriksson et al. 2024; Robert et al. 2025). In their extensive literature review on more than 170 studies including 48 different taxa displaying a wide range of life history patterns, from large marine mammals and fish to macrophytes and small protists, Henriksson et al. (2024) concluded that the presence of population structure in the Skagerrak is the rule, and not the exception for a few

species. While patterns of population structure have attracted much attention, efforts to unveil the underlying mechanisms promoting population differentiation and the putative presence of barriers to connectivity are less common (Gustafsson et al., 2024; Henriksson et al. 2024; Robert et al. 2025). These barriers to dispersal may be related to gradients in environmental selection variables (i.e. temperature, salinity); oceanographic and metamorphological features (i.e. ocean currents including eddies and bathymetry); as well as historical reproductive isolation with reduced gene-flow due to either prezygotic barriers preventing fertilization (i.e. assortative mating) and/or postzygotic barriers acting against hybrid zygotes (i.e. hybrid sterility and hybrid inviability).

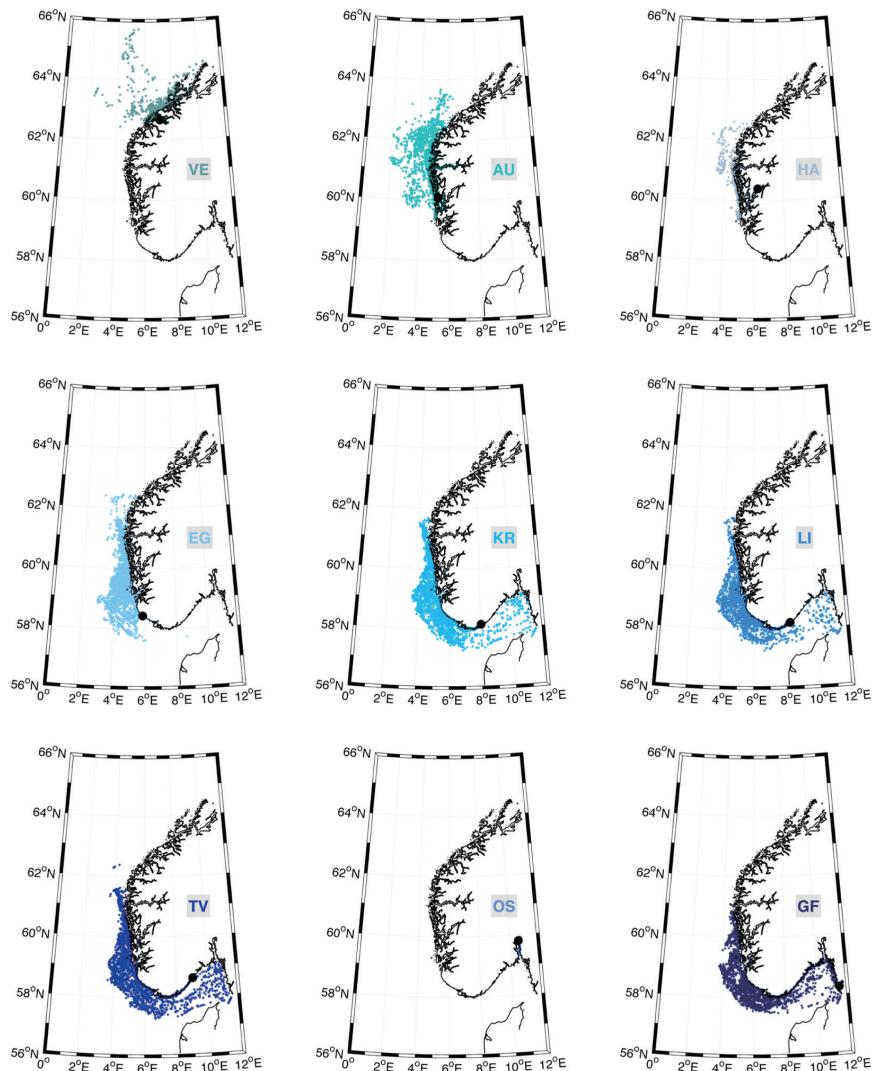


Fig. 1: Modeled oceanographic drift of particles released at several coastal fjords along the western (VE, AU, HA, EG) and southern Scandinavian coastline (KR, LI, TV, OS, GF) conducted as described in (Knutson et al. 2013). The black filled circles denote the location where 1800 virtual particles were released during summer from 12 subsequent years (2000–2011), and the colored clouds of dots denote where the particles ended up after 25 days, the maximum pelagic larval duration for corkwing wrasse. Note that all particles are released offshore due to coarse resolution in the ocean current model. Locations for particular releases are VE: Vestnes; AU: Austevoll; HA: Hardanger; EG: Egersund; KR: Kristiansand; LI: Lillesand; TV: Tvedrestrand; OS: Oslo; and GF: Gulmarfjord.

Coupling genetics with biophysical modelling to infer barriers to connectivity in the complex Skagerrak fjord seascape

Biophysical modelling coupling oceanographic and particle-tracking models can be integrated with genetic data to locate oceanographic barriers to dispersal and disentangle to what extent patterns of genetic differentiation can be explained by the dynamics (Jahnke and Jonsson, 2022). In the Skagerrak, a few studies have successfully coupled genetic data and biophysical modelling to understand the role of oceanographic connectivity and locate barriers to dispersal to the adjacent North Sea and Baltic Sea in fishes (Blanco Gonzalez et al. 2016; Seljestad et al. 2020; Knutsen et al. 2022), a crab (Moksnes et al. 2014) and an eelgrass species (Jahnke et al. 2018). Surprisingly, despite the strong environmental stratification and complexity of the fjords, seascapes are predicted to limit organismal dispersal and impose strong selective pressures, only two recent studies have reported barriers to gene flow within the Skagerrak fjords system; one conducted on eelgrass *Zostera marina* (Jahnke et al. 2020) and the other on blue mussel *Mytilus edulis* (Gustafsson et al., 2024). While biophysical modelling has proven very useful to investigate oceanographic barriers to connectivity and to understand source-sink dynamics in coastal and offshore systems (e.g. Huserbråten et al. 2018; Myklevoll et al. 2018), the implementation of ocean current models in complex fjords systems affected by many physical factors presents major limitations due to coarse resolution of the coastline and fine-scale bathymetry as fjords sills and skerries (Jahnke and Jonsson, 2022; Gustafsson et al., 2024). Fig. 1 illustrates the challenges of a biophysical model implemented on corkwing wrasse (*Syphodus melops*) along the Scandinavian Peninsula. The model shows predominant drifting along the Norwegian coastline following the direction of the Norwegian Coastal Current, except in Oslo (OS) where virtually all larvae are retained inside the fjord. It is important to note, however, that the trajectories presented in Fig. 1 are based on particles released offshore. Typically, the small fjords with sills separating the inshore and on-shore waters usually found along the Skagerrak coast have a higher degree of retention than the more open fjord systems along the west coast of Norway. Meanwhile, the fact that the trajectories in the central part of the Skagerrak drift a bit far off the coast responds to the apparent weakness of the model to deal with shallow waters along stretches of skerries and islands (Gustafsson et al., 2024).

Future perspective of seascape ecology to assist sustainable fisheries management and biodiversity conservation in the Skagerrak

An increasing number of genomic studies are contributing to gain new insights into demographic and adaptive responses of non-model organism to environmental clines in complex seascape such as the Skagerrak fjords seascape (Mattingdal et al. 2020; Knutsen et al. 2022; Gustafsson et al., 2024). Computational and analytical developments will help to incorporate more complete information on species' biology

and hydrodynamic processes to improve the resolution of the biophysical models to be coupled with 'omics data (Jahnke and Jonsson 2022). Genetic information has been also successfully coupled with several types of markers and tags in behavioural seascape studies in the Skagerrak region (Kristensen et al., 2021; Henriksson et al. 2023) and the field is expected to expand further integrating new knowledge generated from 'omics approaches. Also, oceanographic models are continuously being improved and it is becoming more common to use them with higher spatial resolution, which then makes it possible to reproduce egg/larval drift with a higher degree of realism. Future holistic studies integrating 'omics approaches, oceanographic modelling with different types of geospatial, ecological, environmental and behavioral data under natural and experimental settings will continue providing crucial information into functional ecological connectivity across complex seascapes for sustainable fisheries management and biodiversity conservation in the Skagerrak region.

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Symposium mini review



The Model of Marine Speciation: Evolution of Abalone Species due to Vertical and Horizontal Heterogeneity in Coastal Environments

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Keywords

Japanese Archipelago, ecological speciation, habitat depth, environmental heterogeneity, population genomics, speciation with gene flow, fertilization gene

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Abstract

The Japanese large abalone species, Ezo, Kuro, Madaka, and Megai abalones, have recently evolved following the expansion of their North American ancestor species to the Japanese Archipelago. Although these abalones are genetically similar, they exhibit distinct ecological characteristics. Kuro, Madaka, and Megai, whose distribution areas overlap along the southern coast, occupy different habitat depths. In contrast, Ezo and Kuro are believed to represent cold- and warm-water adapted subspecies, with distributions in the north and south, respectively. The evolution of abalone species, which are related to vertical and horizontal heterogeneity in coastal environments, serves as a crucial model not only for understanding the “beginning” of the speciation process in marine invertebrates but is also highly significant from a fisheries perspective. In this mini-review paper, I would like to introduce key findings from several population genomic researches on the Japanese large abalones. Conclusively, these findings highlight that speciation of abalones is a continuous process with gene flow driven by unstable ecological barriers, emphasizing the need for conservation strategies that account for such continuity.

Introduction

How have marine invertebrate species speciated in the ocean? The ocean, which covers approximately 70% of the Earth’s surface, is home to numerous species, and it is believed that gene flow between populations occurs actively in the ocean, hindering the accumulation of variation and subsequent speciation. Thus, the question of how speciation is initiated remains an important topic in marine biology (Palumbi, 1992). In particular, speciation is thought to be even more unlikely to occur in marine invertebrates because of the extensive gene flow that occurs as eggs and sperm are released into the ocean.

One of the few examples that explains the speciation process in marine invertebrates is the mutation of fertilization genes (*Lysin* on the sperm and *VERL* on the egg) in abalones (Clark et al., 2009). In abalone species from the west coast of the North America, it has been suggested that these mutations prevent fertilization between different species, resulting in reproductive isolation. Therefore, such coevolution of fertilization genes may have driven speciation (Van Doorn et al., 2001) (Fig. 1A). However, the divergence of these abalone

species is old, and it is unclear whether their speciation was truly initiated by a mutation in the fertilization gene. For example, it has been shown that the genetic basis for ecological isolation, such as habitat differences, may drive speciation in marine invertebrates (Palumbi, 2009), but no evidence has been found to suggest this. In fact, the North American abalone species are known to exhibit differences in habitat depth and geographic distribution among species (Lindberg et al., 1992). Given this fact, it is possible that ecological factors such as those described by Nosil (2012) may have driven speciation (Fig. 1B), but it is impossible to test this retrospectively.

The abalone species of the Japanese Archipelago, Ezo (*Haliotis discus hawaii*), Kuro (*Haliotis discus discus*), Madaka (*H. madaka*), and Megai (*H. gigantea*) abalones (Fig. 2A) have recently evolved following the expansion of their North American ancestors to the Japanese Archipelago (Hirase et al., 2021). Although these abalones are genetically similar, they exhibit distinct ecological characteristics (Fig. 2). Kuro, Madaka, and Megai, whose distribution areas overlap along the southern coast, occupy different habitat depths (Ino, 1952). Ezo and Kuro are believed to represent cold- and warm-water

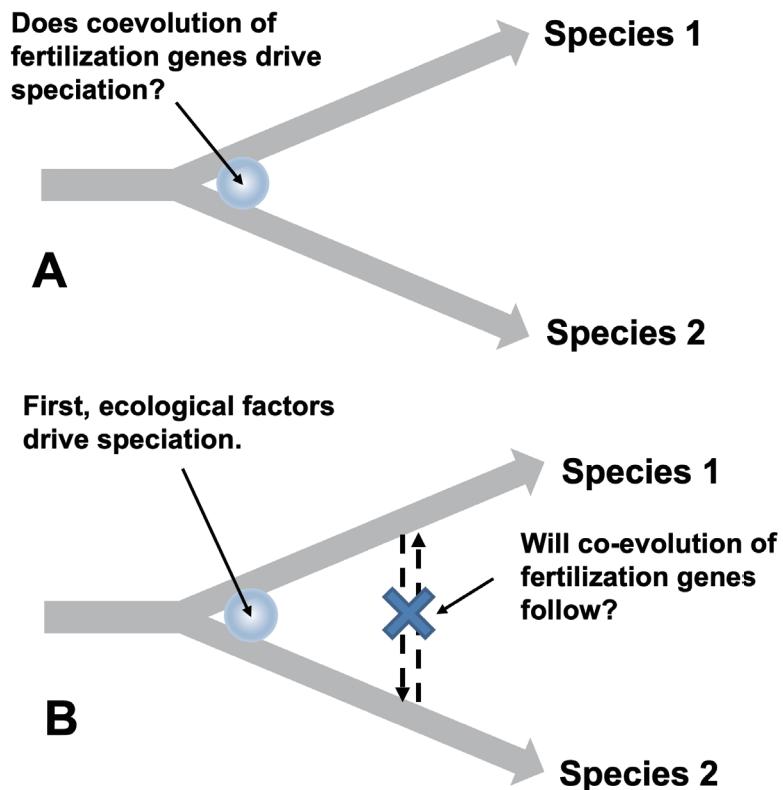


Fig. 1: Two possible scenarios in the speciation process of abalone species.

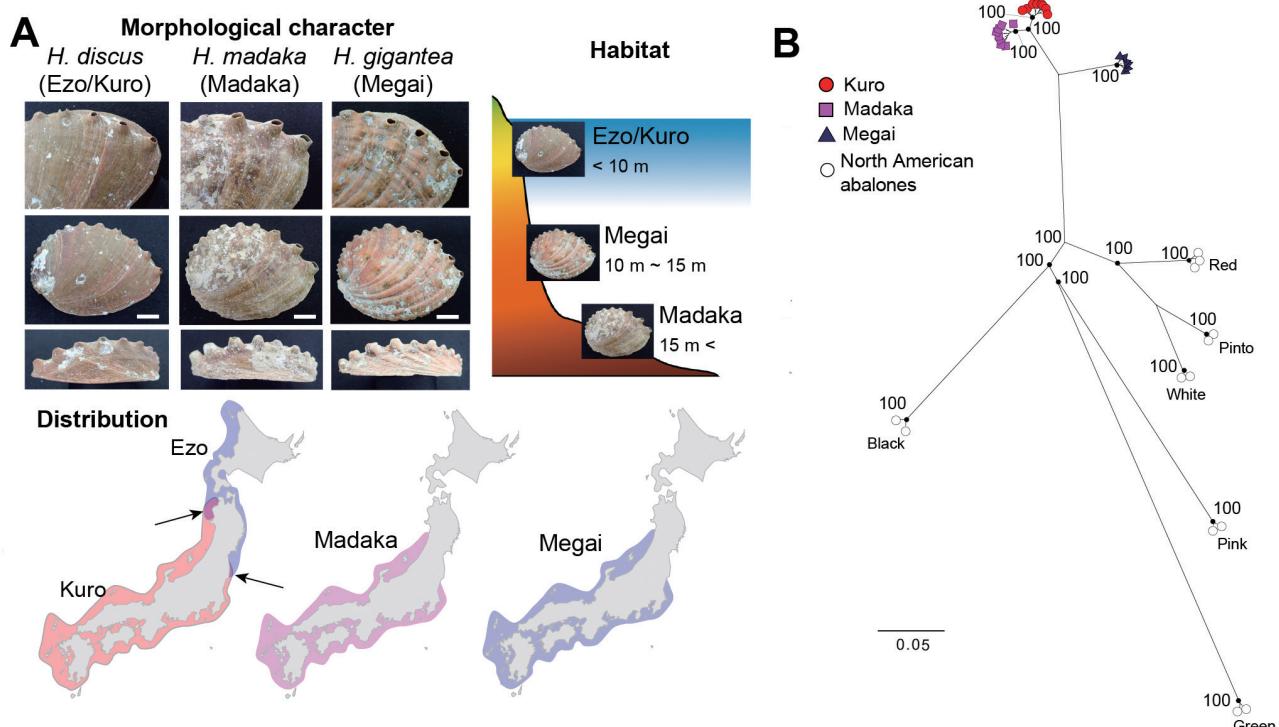


Fig. 2: **A.** Morphological and ecological features, as well as geographical distributions of the large abalones in Japan. The scale bars in photos correspond to 2 cm. Arrows in the distribution map of Ezo/Kuro show boundary zones between the two subspecies **B.** Maximum-likelihood tree of the Japanese and North American abalones (Red: *H. rufescens*, Pinto: *H. kamtschatkana*, White: *H. sorenseni*, Black: *H. cracherodii*, Pink: *H. corrugata*, Green: *H. fulgens*) based on whole-genome sequencing data (46,366 SNP loci). Adapted from Hirase et al. (2021).

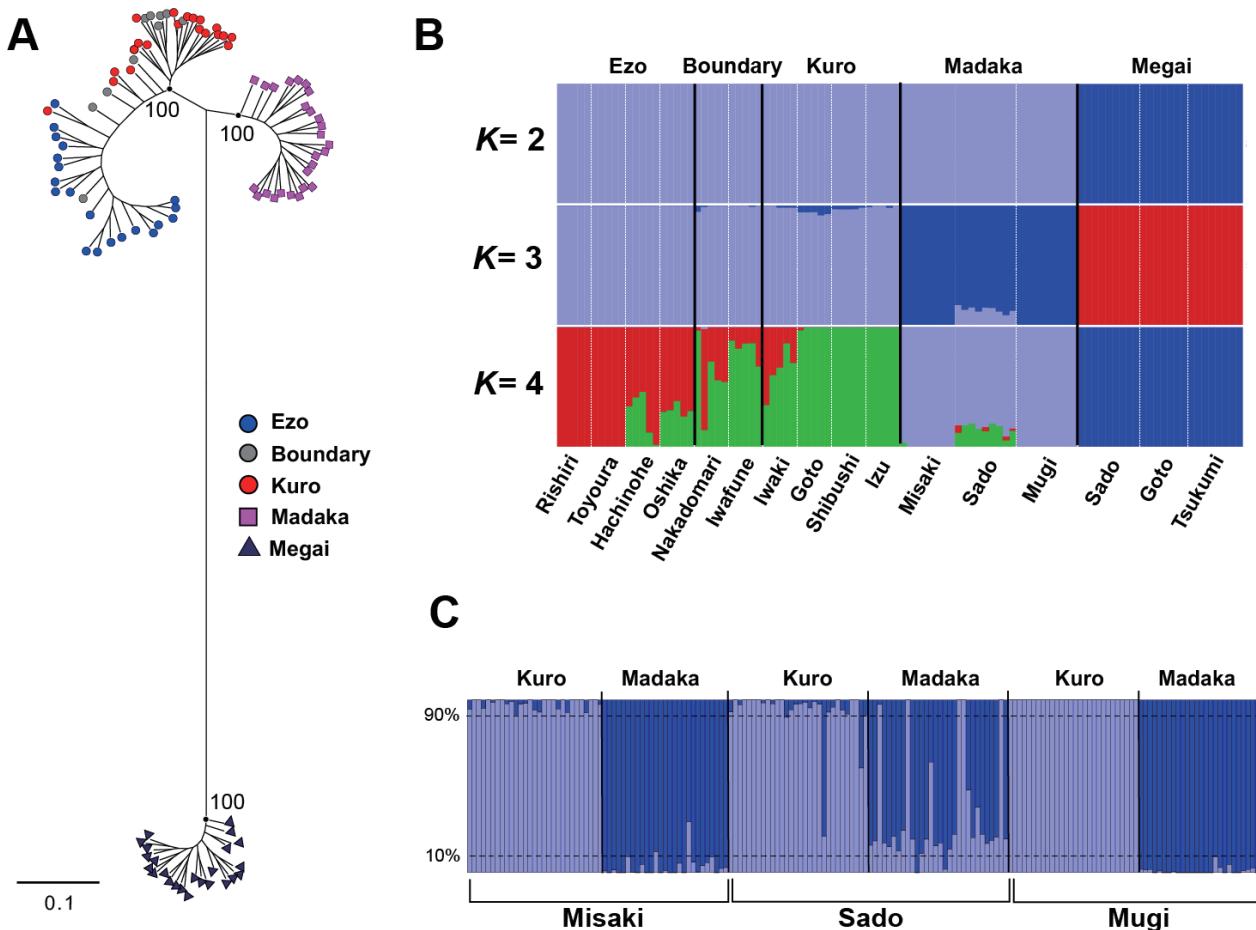


Fig. 3: A. Maximum-likelihood tree of the Japanese large abalones based on GRAS-Di data (18,109 SNP loci). **B.** Individual admixture proportions (q -values) among the large abalones estimated by ADMIXTURE. **C.** Individual admixture proportions (q -values) using sympatric populations of *H. discus discus* and *H. madaka* in three regions by ADMIXTURE. A and B were adapted from Hirase et al. (2021).

adapted subspecies, with distributions in the north and south, respectively (Hara and Sekino, 2005). On the other hand, their crossbreeding produces fertile offspring under laboratory conditions. These facts suggest that their evolution, which is related to vertical and horizontal heterogeneity in coastal environments, is maintained by ecological isolation rather than gametophytic isolation and serves as a crucial model for understanding mechanisms driving marine biodiversity. At the same time, they indicate that reproductive isolation between species is unstable and that there is a risk of species uniqueness disappearing due to environmental changes in the field, called as speciation reversal (Frei et al., 2022). Therefore, the process of the speciation of abalone in the Japanese Archipelago is not only important in terms of elucidating the initiation process of speciation in marine invertebrates, but also provides important information for conserving genetic resources of abalone, whose catches continue to decline recently. In this article, I would like to introduce population genomics studies of these large abalone species that I have been conducting.

Ecological speciation of three large abalones

In the past, evolutionary relationship among the three large abalone species (Ezo/Kuro, Madaka, Megai) was studied using molecular markers such as allozyme (Hara and Fujio, 1992), mitochondrial (mt) DNA (An et al., 2005), and microsatellite

(ms) DNA (Sekino and Hara, 2007). These previous studies showed that the Megai were differentiated from the other two species. However, the degree of genetic differentiation between Ezo/Kuro and Madaka was very small, it was not clear whether they were distinct species. To investigate whether the three large abalone species are reproductively isolated in nature, Hirase et al. (2021) conducted a population genomics analysis using 18,109 single nucleotide polymorphism (SNP) loci. A phylogenetic tree was constructed by concatenating sequences of the SNP loci, and which showed three clades corresponding to each species (Fig. 3A). Thus, it was confirmed that the three species are reproductively isolated in nature. A clustering analysis also showed that the three species are assigned to their own genetic clusters. On the other hand, this analysis showed that genomic introgression from Kuro to Madaka in Sado island, Niigata prefecture (Fig. 3B). This finding suggests that the intensity of reproductive isolation between Ezo/Kuro and Madaka abalones varied by region, indicating that speciation is ongoing with gene flow.

However, Hirase et al. (2021) used only 10 specimens of both Kuro and Madaka abalones in their analysis, and these species were collected from different locations within the same prefecture. Then, Hirase et al. (unpublished data) investigated the reality of hybridization between sympatric Kuro and Madaka abalone populations by amplicon sequencing for divergent SNP loci where allele frequencies between the two

species are different (Hirase et al. 2024). Clustering analysis based on the divergent SNP loci showed that all Kuro and Madaka individuals from Mugi, Tokushima Prefecture have admixture proportion (q) of more than 90%. Following the approach of Vähä and Primmer (2006) for assessing the level of genomic introgression (they set q values between 0.1 and 0.9 as indicators of genomic introgression), this suggests no genomic introgression (Fig. 3C). In contrast, some Kuro and Madaka individuals from Misaki, Kanagawa prefecture and from Sado Island, Niigata prefecture have less than 90% admixture proportion (q), suggesting genomic introgression. In particular, all Madaka individuals from Sado Island have undergone genomic introgression, supporting the previous studies (Fig. 3C). In addition, since only a few Kuro individuals from Sado Island showed genomic introgression, the degree of genomic introgression from Kuro to Madaka abalone was considered to be higher than the reverse pattern. Such directional gene flow was also supported by a demographic modeling analysis, which estimates past population dynamics from allele frequency patterns (allele frequency spectrum), in Hirase et al. (2021), and this analysis suggested that Ezo/Kuro, Madaka, and Megai abalones have speciated with gene flow due to hybridization, which is called as “speciation with gene-flow” model (Hirase et al., 2021).

Genetic differentiation between Ezo/Kuro and Madaka abalones is very low (genome-wide $F_{ST} = 0.007$) due to ongoing gene flow. At this stage of speciation, while the degree of genetic differentiation between species is low across genomic regions, genetic differentiation in genomic regions related to reproductive isolation is considered to be high, and such genomic regions are called “differentiation islands” (Burri 2017). Hirase et al. (2021) found 27 differentiation islands where genetic differentiation between Ezo/Kuro and Madaka

has accumulated. Therefore, the genetic basis for reproductive isolation between the two species is thought to exist around these differentiation islands.

Ecogeographical evolution of subspecies

Ino (1952) compared the external morphology of the shells of Ezo and Kuro, and found that the shell width ratio (shell width to shell length) of Kuro was greater than that of Ezo (Fig. 4A). Subsequent studies also showed that the shell width ratio was greater in the south (Kuro) than in the north (Ezo) on both sides of the Pacific Ocean and Sea of Japan (Kobayashi et al., 1992). On the other hand, there are individuals showing intermediate traits around the distribution boundary of these subspecies, suggesting that shell traits show geographic continuity (Ino 1952). There have also been reports of Ezo individual becoming Kuro shells in transplantation and release experiments into Kuro distribution areas, as well as reports of Kuro individual becoming Ezo types when reared in low-temperature environments (Ino, 1952; Hara, 1992). Therefore, the differences in shell morphology mentioned above may be attributed to variations in the external environment. Although population genetic analysis using microsatellite DNA markers indicated distinct clusters corresponding to Ezo and Kuro, the geographic distribution pattern of these clusters has not been fully explored. Consequently, it remains unclear whether these differences stem from genetic subspecies or phenotypic plasticity.

Hirase et al. (in revision) performed population genomic analyses based on 38,090 SNP loci and revealed the existence of genetic clusters corresponding to Ezo and Kuro and their admixture in the boundary zones (Fig. 4B). On the other hand, the population structure throughout the distribution

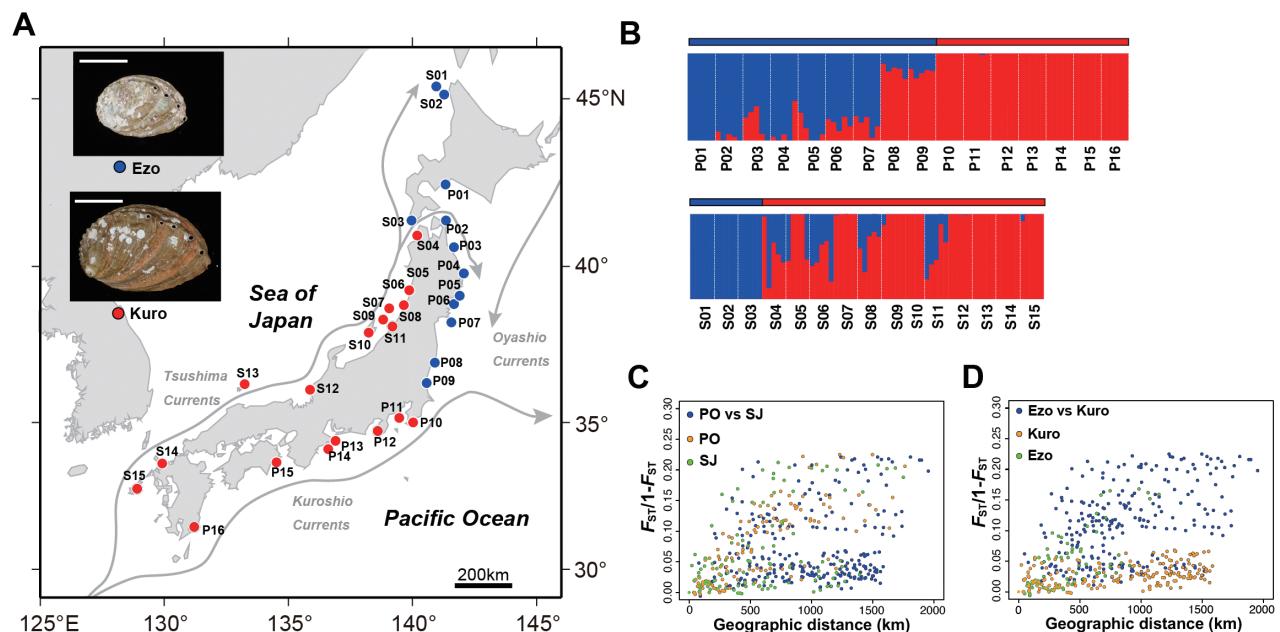


Fig. 4: **A** Sampling locations of Ezo and Kuro abalones along the coastline of the Japanese Archipelago. The blue and red circles indicate populations of Ezo and Kuro, respectively. The scale bar in the photo of each subspecies represents 5 cm. **B**, Individual admixture proportions (q -values) among populations using SNP data are also shown ($K = 2$). The top bars on the plots show the subspecies classification (blue: Ezo; red: Kuro). **C, D**, The relationship between genetic ($F_{ST}/1-F_{ST}$) and geographic distances across populations were calculated using SNP data. The color pattern of the plot is varied between C (Pacific Ocean: PO vs Sea of Japan: SJ) and D (Ezo vs Kuro).

area showed a pattern of isolation by distance (IBD) (Fig. 4C), which is commonly observed as intraspecific genetic structure in marine organisms. This pattern suggests that these population structures can be explained without assuming two subspecies. However, it was shown that geographic distance alone cannot explain the pattern of genetic differentiation (genetic differentiation within subspecies was small regardless of geographic distance), supporting the existence of genetic groups corresponding to the two subspecies (Fig. 4D).

The Japanese Archipelago stretches approximately 3,000 km from north to south, along which flow large ocean currents, including warm currents from the south and cold currents from the north. This geographical situation results in large environmental heterogeneity along a latitudinal gradient may be important factor for the geographic distribution of genetic groups corresponding to Ezo and Kuro. Therefore, Hirase et al. (in revision) summarized the environmental differences between the sampling sites for each subspecies by principal component analysis (PCA) based on 33 environmental variables and calculated the environmental distance between the sites by the first principal component (PC1). The results of Mantel and partial Mantel tests indicated that environmental distances between populations based on PC1 scores were related to genetic distances (Fig. 5A), suggesting that the external environment may be involved in shaping the geographic distribution patterns of Ezo and Kuro. Furthermore, outlier SNP loci showing significantly greater degree of genetic differentiation between Ezo and Kuro were extracted by multiple genome scan methods, and the relationship between genotype and environment was further examined, suggesting that factors such as mean sea water temperature in autumn are strongly associated with genotype (Fig. 5B).

Perspectives

Genomic data suggested that species and subspecies of Japanese large abalones are ecologically isolated due to differences in habitat depths and environmental differences between geographical regions. In other words, their genetic distinctness is maintained by seascape structure. On the other hand, genomic data also indicated that their speciation events have been accompanied by continuous gene flow. Genomic introgression from Kuro to Madaka occurs in certain geographic regions, suggesting that speciation is ongoing with incomplete ecological isolation probably due to differences in the habitat depth. Ezo and Kuro formed two distinct genetic clusters but exhibited genomic admixture along boundary coastal regions. Considering that the same ecological differences as above have occurred in North American abalones and that fertilization gene mutations has occurred among these abalones, it is expected that Japanese large abalone species are at an earlier stage of speciation than North American abalone species (Fig. 1B). These findings highlight new issues, such as whether Kuro, Ezo, and Madaka, where gene flow is occurring, can be managed as separate units of fisheries resources, and how to handle hybrid individuals among them.

As mentioned above, we have identified genomic regions that may be involved in the evolution of abalone species, which are related to adaptation to vertical and horizontal heterogeneity in coastal environments. Hirase et al. (2021) proposed the hypothesis that same genomic regions may have been used repeatedly in the speciation of abalone species. Population genomics analysis using chromosomal-wide genomic information is the next approach in identifying such genomic regions and associated genes. However, the reference genome used in these studies was not at the chromosome level, so the genome-wide distribution pattern

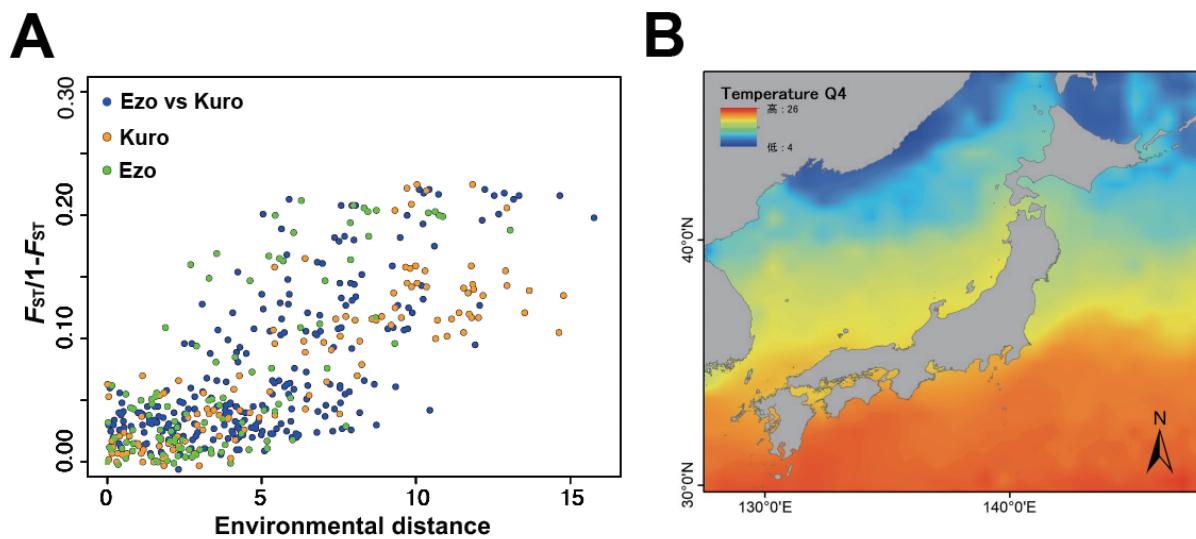


Fig. 5: **A.** The relationship between genetic ($F_{ST}/1-F_{ST}$) and environmental distances across populations. Environmental distances were estimated based on the first principal component scores that calculated from the principal component analysis of 31 populations using environmental variables. **B.** Mean seawater temperature in autumn (Q4) along the coast of the Japanese Archipelago.

of such genetic differentiation islands was not clear. Recently, we have constructed chromosome-level genome assembly of Megai (Hirase et al. 2025). In the future, it is expected that the genetic basis of speciation will be identified by improving the accuracy of the reference genome of large abalone species.

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Symposium mini review



How Geotopology and Ecology Affect Genetic Structure: Insights from Seascapes Genetics of Marine Snails

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Keywords

Phylogeography, Specialist-generalist variation hypothesis, Genetic boundary, Sanriku coast, ddRAD-seq, Marine snail

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Abstract

In general, marine ecosystems are characterized by a higher level of gene flow among local populations compared to terrestrial ecosystems. However, accumulating molecular studies have demonstrated that various marine species can be genetically differentiated. Naturally, coastlines are not just straight stretches, but also consist of complex structures such as bays, capes, and various shores. For instance, exposed shores are constantly subjected to the effects of strong waves, while sheltered areas, such as inner bays, are not. These differences in geotopology (the structure of the coastline) provide various types of coastal habitats for marine species. In this context, the principal goal of seascape genetics is to understand how coastlines, fringed by various environments, affect genetic population structures. Here, I focus on the relationships between geotopology and the genetic structure of marine species. Firstly, I demonstrate a comparative phylogeography of co-distributed intertidal snails with different habitat usage patterns concerning complex coastlines from sheltered bays to exposed shores. Based on the results, I discuss whether the specialist-generalist variation hypothesis is applicable in the marine environments. Specifically, habitat specialists that prefer certain coastal environments tend to be genetically differentiated and have low genetic diversity. Next, I introduce our recent work in seascape genetics along the Sanriku coast in the Tohoku District of Japan. The Sanriku coast presents an interesting seascape transition between a relatively smooth coastline and a complex rias coast. To examine the influence of coastline complexity on genetic structure, a high-resolution genome-wide SNPs dataset was obtained by ddRAD-seq from two direct-developing snail species. The results demonstrate that a genetic boundary coincides with the seascape transition. Finally, I emphasize the importance of seascape genomics focused on various taxonomic groups within the same seascape to better understand the relationships between coastal geotopology and species-specific ecological traits on genetic structure.

Seascape genetics and ecological traits

Seascape genetics is conceptually linked to landscape genetics, but there are significant differences between terrestrial and marine ecosystems. Landscape genetics is a field of research that combines population genetics and landscape ecology, quantifying the effects of spatio-temporal factors on microevolutionary processes such as gene flow, drift, and selection (Balkenhol et al., 2015). From a landscape perspective, the level of gene flows among populations is determined by “Isolation by Barrier” model and “Isolation

by Resistance” model, rather than a simple “Isolation by Distance” model (Ohnishi, 2021). Specifically, gene flows among populations tend to be restricted in terrestrial environments, because environmental heterogeneity, habitat patchiness, and mosaic landscapes give rise to various types of clear barriers or resistances to dispersal. In contrast, seascapes generally lack such clear barriers due to interconnected environments via seawater. Therefore, genetic population structures in marine ecosystems are often characterized by a higher level of gene flow among local populations compared to terrestrial ecosystems.

In recent, however, accumulating molecular studies have demonstrated that various marine species can exhibit genetic differentiation. Indeed, some comparative genetic studies revealed that the level of population genetic differentiation often varies even in co-distributed marine species within the same seascape (Kelly & Palumbi, 2010). In this context, the principal goal of seascape genetics is to understand the relationships between spatial environmental factors and the genetic patterns of marine species (Riginos & Nachman, 2001, Selkoe et al., 2016). Genetic patterns are influenced by the interaction of both physical and biological factors. Although the ocean seems to be open and interconnected, gene flow among populations is restricted by various physical factors such as island structure (Meyer et al., 2005, Hirase et al., 2012), oceanic current (White et al., 2010), and salinity (Teacher et al., 2013). Besides, biological factors, such as ecological traits concerning the level of dispersal ability, can influence the level of genetic differentiation (Cowen & Sponaugle, 2009). Recent molecular studies demonstrated that habitat usage patterns influence the level of genetic connectivity among populations (Robuchon et al., 2014, Wort et al., 2019). In the field of seascape genetics, it is important to understand how coastlines, fringed by various type of habitat environments, affect genetic population structures.

In the present review, I focus on the relationships between coastal geotopological structure and population genetic connectivity of marine species in shallow water. Naturally, coastlines are not just straight stretches, but also consist of complex structures such as bays, capes, and various shores. For instance, exposed shores are constantly subjected to the effects of strong waves. In contrast, sheltered shores like inner bays are not. These differences in coastal structure provide various habitat types for marine species. Many species have species-specific habitat usage patterns related to coastal exposure (e.g. Tam & Scrosati, 2014). Hence, complexity of coastlines is important influential factor for the inhabitation of marine species and biological community varies by position of coastlines (Burrows, 2012). To advance seascape ecological study, we need a better understanding of the relationships between habitat usage patterns linked to coastline and genetic structure.

Here, I first demonstrate comparative seascape genetics of co-distributed intertidal snails with different habitat usage patterns in the Japanese and Ryukyu archipelagoes. In the first chapter, I discuss whether specialist-generalist variation hypothesis (SGVH; Li et al., 2014) is applicable for habitat usage patterns in coastal geotopological structure. Next, I introduce our recent work of seascape genetics conducted in the Sanriku coast, Tohoku District, Japan, which includes famous rias coastline. Interestingly, the Sanriku coast has seascape transition between a relatively smooth coastline and complex rias coast. In the second chapter, I examine the influence of the complexity of the continuous coastline on genetic structure of snail species with direct development type. Lastly, I discuss the need for comparative seascape genetics to better understand the relationships between geotopology, ecology, and genetic structure. In the last chapter, I emphasize future perspective of “seascape genomics” which is expected to reveal the locus-by-environment associations and its contribution to address various modern issues.

Relationships between coastal geotopology and genetic structure

One important research question in seascape genetics is why co-distributed species exhibit different levels of genetic differentiation among populations, despite being affected by the same physical conditions. According to SGVH, populations of ecological specialists with limited or narrow range of habitats tend to be subdivided than those of generalists. Besides, the genetic diversity of a habitat specialist is lower than that of a generalist (Lavm & Nevo, 1981, Noy et al., 1987). Species with different habitat usage patterns in coastline are thus expected to differ genetic structure. For instance, populations of specialist species that are restricted to inner bays or exposed shores are predicted to be genetically more differentiated than habitat generalist species which can inhabit various coastal environments. However, evaluating SGVH in marine system is generally difficult because of chaotic genetic patterns and/or the general lack of population structure (Selkoe et al., 2010). Consequently, empirical tests of the SGVH have been predominantly conducted in terrestrial environments (e.g. Li et al., 2014, Mathee et al., 2018, Pasinelli, 2022). Although habitat specialization in marine environments has been suggested to promote genetic differentiation among populations (e.g. Selkoe et al., 2014, Wort et al., 2019), the general applicability of the SGVH remains unclear. In particular, no studies have quantitatively assessed coastal exposure—an important ecological factor—when testing the SGVH. That is, the question is as follows: do habitat usage patterns related to coastal exposure influence the levels of genetic diversity and population genetic structure?

To answer the above issue, Yamazaki et al. (2021) tried to evaluate the role of ecological traits in the genetic variation by comparative phylogeography using congeneric closely related species. We compared ecological traits concerning habitat usage patterns and genetic variation in three species of the intertidal snail genus *Monodonta* (*M. labio*, *M. perplexa* and *M. confusa*). These species co-occur in the Japanese and Ryukyu archipelagoes and show different habitat usage patterns. Specifically, GIS based statistical analysis has revealed two of three species are habitat specialists, but the other is a generalist. *M. labio*, a specialist in environments with low wave action, is dominant in sheltered habitats such as the inner side of bays. In contrast, *M. perplexa*, a specialist in strong wave action, is dominant in wave-exposed environments facing the open ocean. However, *M. confusa* showed no such habitat specificity and can inhabit various coastal environments, demonstrating that it is a generalist species.

Based on the above results of habitat utilization of *Monodonta* species for coastal structures, SGVH is evaluated by molecular analyses using the mitochondrial cytochrome c oxidase subunit I gene (COI) and a partial 28S ribosomal DNA. Concisely, the genetic results match well with SGVH. Specifically, two habitat specialists (*M. labio* and *M. perplexa*) showed greater genetic differentiation among populations and lower genetic diversity than generalist species (*M. confusa*). These findings of seascape genetics demonstrated the existence of the SGVH pattern concerning habitat utilization in connected coastal environments. Ecological factors related

to coastal complexity are thus suggested to affect genetic connectivity among populations.

Interestingly, some congeneric habitat specialists also show the different levels of genetic differentiation. Sister species of genus *Tegula* (*T. xanthostigma* and *T. kusairo*) show contrasting habitat usage patterns in coastline. *T. xanthostigma* often lives on exposed shores, while *T. kusairo* lives in sheltered environments, such as inside bays (Yamazaki et al., 2019, 2020). Comparing the genetic structure of these two species, *T. xanthostigma* has a lower level of genetic population differentiation and higher genetic diversity than *T. kusairo*. This indicates that a habitat specialist in weak wave environments has a higher level of population genetic differentiation than that in strong wave exposed environments (Yamazaki & Chiba, 2022). From the above, coastal structure appears to be an important factor influencing the genetic structure of marine species.

Detection of common genetic boundary in the Sanriku seascape

In general, one of the important ecological factors affecting the level of population genetic differentiation is dispersal ability, which is linked to planktonic larval duration (PLD) (Cowen & Sponaugle, 2009). Many marine invertebrate species have a planktonic larval stage in their life cycle and can disperse depending on their PLD. In contrast, the dispersal ability of direct-developing species, which lack a PLD, is very low. This promotes the accumulation of local genetic variation and genetic differentiation among populations (Mae et al., 2013, Azuma et al., 2017). These patterns indicate that the history of a given seascape can be imprinted in the genomes of low-dispersal species.

The Japanese archipelago has very complex coastal structure. The rias of Sanriku coastal area, the Pacific Ocean side of the northeastern region of Honshu in the Japanese Archipelago, is particularly well known. Interestingly, the northern part of the Sanriku coast is a relatively straight coastline, but the southern one is characterized by rias coast. The transition point is around the middle of the Sanriku area, Omo Peninsula (Miyako city, Iwate prefecture). In seascape genetics, it is important to understand the relationship between these differences in coastal geotopology and genetic structure. Yamazaki and Ikeda (2025) conducted comparative genetic study using the COI marker on six snail species with different PLD in the Sanriku coast. The results provided evidence for existence of genetic population differentiation of direct-developing snail species (*Nucella heyseana*) inhabiting along the Sanriku coast. However, it was difficult to examine a clear pattern of population genetic structure corresponding to the coastal structure.

To resolve the above issues, the resolution of genetic marker is very important. Marine species particularly have higher genetic connectivity than terrestrial species, often making it difficult to determine the degree of genetic differentiation through genetic analysis based on partial sequence information. In recent progress of molecular technique, we can elucidate genetic structure by using a high-resolution genome-wide SNPs datasets such as restriction site-associated DNA sequencing (RAD-seq), double-digest RAD

sequencing (ddRAD-seq), multiplexed ISSR genotyping by sequencing (MIG-seq), and genotyping by random amplicon sequencing (GRAS-Di). These methods have enabled us to understand the genetic divergence of marine species at a much higher resolution than limited partial sequence like Sanger methods (e.g. Yamazaki et al., 2022, Kagawa et al., 2024).

Here, I briefly introduce our recent results of seascape genetics in the Sanriku coast. To understand the relationship between coastline and genetic structure, our research system adopted two direct-developing snail species: *N. heyseana* and *Littorina sitkana*. Besides, we used high-resolution SNPs datasets obtained by ddRAD-seq for genetic analysis. Cluster analysis revealed that the two species have clear genetic structures and share a common genetic boundary within the Sanriku coast. Specifically, this boundary coincides with a transition in seascape—from a smooth coastline to the complex rias coast—around the Omo Peninsula. Furthermore, network analysis of *N. heyseana* demonstrated the level of genetic population differentiation differs between northern straight coast and southern rias coast. The southern populations showed a higher level of divergence than the northern ones. It indicates complex geotopology like rias promotes genetic differentiation even in interconnected marine environments. Besides, the level of genetic differentiation of *L. sitkana* is higher than that of *N. heyseana*. This phenomenon seems to be explained by the different depth of habitat ranges of the two species (e.g. Robuchon et al., 2014). While the mechanism by which coastal geotopology promotes genetic differentiation is not yet fully understood, the present study indicated seascape transition can drive genetic divergence.

Perspectives for seascape genomics

In this review, I highlighted the effects of coastal geotopology on the genetic structure of marine species in Japanese and Ryukyu Archipelagos fringed by complex coastal environments. Conventionally, it has been difficult to examine genetic structure in marine environments due to large population sizes and high dispersal potential among populations (Crandall et al., 2008). However, seascape genetics, through the use of multiple species, provides important insights into the evolutionary history of marine organisms (Selkoe et al., 2016). To better delineate the spatial arrangements of genetic variation along coastal geotopology, it is necessary to conduct not only comparative analyses within a certain taxonomic group, but also comparisons focused on cross-taxa distributed in same seascape. Under those circumstances, high-resolution genetic markers are powerful tools. Furthermore, energetic seascape geneticists hope that future advances in molecular techniques can elucidate the effects of coastal environments that lead to various types of natural selection on the genomes of marine species. In this emerging research field of “seascape genomics”, the principal theme is to identify the locus-by-environment associations by quantifying nuanced habitat heterogeneity that can point to genomic signatures of selection (Riginos et al., 2016). In the modern era—often referred to as the Anthropocene—seascape genomics is expected to detect genomic responses of marine species to rapid environmental shifts, making it a vital approach for understanding the impacts of ongoing climate

change and/or increasing human activities (Poloczanska et al., 2013, Hirase et al., 2025). Thus, seascape genomics can provide essential knowledge not only for understanding spatio-temporal microevolutionary process in marine life but also for addressing key issues such as biodiversity conservation, sustainable fishery strategy, and marine ecosystem-based management.

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Symposium mini review



Monitoring of Seaweeds and Seagrasses Using Remote Sensing Data in Coastal Areas

- Integration with Ground Surveys -

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Keywords

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Abstract

We have conducted an integrated survey to assess the distribution of seaweeds and seagrasses beds (marine vegetation) in coastal areas using ship-based surveys, diving surveys, UAV (aerial drone), and satellite remote data analysis. The seaweeds and seagrasses beds are significant sources of carbon dioxide absorption, with the carbon captured by marine organisms referred to as “blue carbon.” Remote sensing from above is a powerful tool for investigating the areas of seaweeds and seagrasses beds distributed in shallow waters. Satellite remote sensing allows for broad area observation, while UAV remote sensing enables the acquisition of high-resolution images at any time. Ship-based surveys using marine drones and cameras, along with diving surveys, are suitable for obtaining detailed information about seaweeds and seagrasses species. The study area is Hirota Bay, located in Rikuzentakata City, Iwate Prefecture. Hirota Bay was severely damaged by the tsunami caused by the Great East Japan Earthquake in 2011. We have been conducting ground surveys since 2022. The Pleiades NEO satellite images obtained in 2023 were analyzed. The seaweeds and seagrasses beds extracted through classification processing of satellite images, confirmed by UAV images as ground truth, closely matched the results from underwater and UAV surveys. Changes from *Zostera* beds to patches of foliose red algae were observed during underwater surveys in July 2024 and January 2025, and we assume this change corresponds to seasonal variations. By comparing with previous studies based on satellite images taken between 2014 and 2018, our survey suggests an increase in marine vegetation since then. Hanging cameras from vessels are a low-cost and effective method. Surveys conducted by local communities will be beneficial for coastal management and the preservation of marine vegetation.

Introduction

The seaweeds and seagrasses beds (marine vegetations) distributed in coastal areas serve as a significant carbon dioxide absorption source. They also play an essential role in water quality purification and biodiversity maintenance (Fujita et al., 2023). It is pointed out that over half of the biological carbon in the world is captured by marine organisms, and they

are collectively referred to as “blue carbon” (Nellemann et al., 2009). These blue carbon ecosystems, including seagrass meadows, salt marshes, and mangroves, sequester carbon at rates up to 50 times greater than terrestrial forests per unit area, making them critical natural solutions for climate change mitigation (Mcleod et al., 2011). Furthermore, blue carbon ecosystems provide multiple ecosystem services beyond carbon sequestration, such as coastal protection, nursery

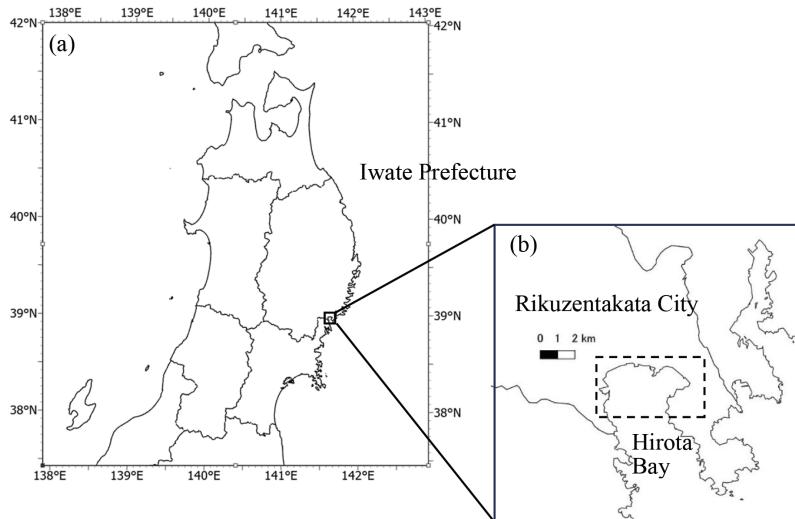


Fig. 1: Study Area. The dashed box in (b) shows the area presented in Figs. 3 and 6.

habitats for commercially important fish species, and nutrient cycling (Barbier et al., 2011).

Recent studies have demonstrated that seagrass meadows can store carbon in their sediments for millennia, with global carbon burial rates estimated at 48–112 Tg C yr⁻¹ (McLeod et al., 2011). Macroalgal beds, while traditionally not included in blue carbon assessments due to their limited direct sediment carbon storage capacity, have recently gained recognition for their significant contribution to carbon sequestration when their biomass is exported to the deep sea or adjacent ecosystems (Krause-Jensen and Duarte, 2016).

Japan, an island nation surrounded by the sea, possesses extensive coastal ecosystems with considerable potential for blue carbon projects. Several blue carbon offset credit projects for seagrass meadows, macroalgal beds, and macroalgae farming have been implemented by national and local governments (Kuwae et al., 2022, Sugimura et al., 2023). The ‘J-Blue Credit’ system, which began in fiscal year 2020, provides a framework for quantifying and crediting carbon sequestration by coastal vegetation, promoting market-based conservation approaches (Kuwae, 2024). To monetize carbon dioxide absorption, it is necessary to estimate the area of seaweeds and seagrasses beds. Remote sensing is a method for investigating the area of seaweeds and seagrasses beds distributed at depths less than 10 m (Fisheries Agency, 2024). Major platforms for remote sensing from above include satellites, aircraft, and Unmanned Aerial Vehicles (UAVs). UAVs are also called aerial drones. Satellite remote sensing allows for observing wide areas at once, making it possible to understand the distribution of seaweed beds across broad regions. Remote sensing sensors for observing the Earth’s surface are divided into two categories: optical sensors that use sunlight as a light source, and Synthetic Aperture Radar (SAR) that emits microwaves and receives their reflections. For observing seaweeds and seagrasses beds, optical sensors are used. Optical sensor images record the differences in reflectance from target objects for each observation wavelength band over an area. This

allows for the extraction of seaweeds and seagrasses beds through image classification processing. When extracting the marine vegetations from satellite images, the reliability of image classification can be improved by using ground truth data that confirms actual locations of marine vegetations on site. On the other hand, UAV remote sensing allows for acquiring high-resolution images whenever the researchers decides, although the observable area in a single capture is limited. In UAV observations, by creating orthomosaic images through Structure from Motion (SfM) from continuously captured images with overlap, high-resolution images of wide areas can be obtained. However, to create good orthomosaic images for extracting marine vegetations distribution areas, observations must be conducted during low tide or under low turbidity conditions (Murata et al., 2021). Meanwhile, ship-based surveys and diving surveys are appropriate for obtaining detailed information about seaweeds and seagrasses species (Fisheries Agency, 2024).

We report on integrated surveys conducted to assess the distribution of marine vegetation in Hirota Bay, located in Rikuzentakata City, Iwate Prefecture. This study combines satellite image analysis, UAV remote sensing, and ship-based surveys (Yonezawa et al., 2024). We have been continuing observations using UAVs and satellite image analysis in Hirota Bay since 2022. Hirota Bay suffered catastrophic damage from the tsunami caused by the Great East Japan Earthquake that occurred on March 11, 2011. Based on field survey results, we attempted to extract marine vegetations from high-resolution satellite images.

Materials and Methods

The target area is shown in Fig. 1. In the inner of Hirota bay, oysters and scallops are cultivated. After the damage caused by the tsunami, it has been confirmed that more than 1,000 aquaculture facilities were established by 2015 (Murata et al., 2018).

Surveys from the fishing boat were conducted on October

18, 2023, July 5, 2024, and January 25, 2025. The survey on October 18, 2023, aimed to validate the classification results of satellite images through field surveys, which included diving surveys conducted by divers and observations using underwater drones. The survey in July 2024 involved diving, underwater drone surveys, and video recordings from a camera suspended from the vessel (Fig. 2). Survey points were determined based on the analysis results of the satellite images. A total of 30 locations were surveyed using the suspended camera, 3 locations with the underwater drone, and 3 locations by diving. In the January 2025 survey, no diving was conducted, and the same locations as in July 2024 were surveyed using the underwater drone and the suspended camera. Surveys by UAV synchronized with the underwater survey conducted on October 17 and 18, 2023, July 4 and 5, 2024, and January 24 and 25, 2025. Broad-area images were created from continuously captured images by ortho-mosaicing using SfM software.

A satellite image observed by Pleiades NEO on August 2, 2023, was analyzed. Pleiades NEO has six multispectral bands and a spatial resolution of 1.2 m. Based on the broad-area images from UAV observations, *Zostera* (eelgrass) beds, macroalgal beds, water areas, and land areas were visually selected to create training data, and supervised classification was applied.



Fig. 2: A camera hanging from a fishing boat for underwater surveys.

Results

The extraction results of the marine vegetations from the Pleiades Neo image are shown in Fig. 3. The *Zostera* beds and macroalgal beds extracted through the supervised classification were correspond with the results from the underwater surveys. However, some areas classified as *Zostera* beds were coexisting with macroalgal beds.

The changes in the *Zostera* beds were confirmed from the underwater drone surveys conducted in July 2024 and January 2025. Fig. 4 shows images captured by the underwater drone on the same point observed in July 2024 and January 2025. It was observed that foliose red algae were growing in the

Zostera area identified in July, indicating that the *Zostera* that inhabited the area during the summer had died. Images observed from the UAV at the same location are shown in Fig. 5. Comparing July (a) and January (b), it can be discerned that the black shadows visible in the water decreased in January, corresponding to a reduction in the *Zostera* beds.

Discussion and conclusions

Marine vegetations appear as dark shadows beneath the water surface in satellite images. They may be misidentified as reefs, necessitating verification through ground truth. The seaweeds and seagrasses beds extracted via classification processing from satellite images, confirmed by UAV images as ground truth, closely matched the results from underwater and drone surveys. This suggests that using UAV images as ground truth data for more extensive observations in satellite image classification is effective (Murata et al., 2024). UAV can capture images with sub-centimeter resolution, making them a valuable tool for on-site surveys. By continuously observing along flight paths covering the target area and performing orthomosaic synthesis, it is possible to understand the spatial distribution of marine vegetations in a defined area. Furthermore, applying UAV survey results as training data for satellite image analysis enables scaling up the observation range.

Fig. 6 shows the distribution of seaweeds and seagrasses beds based on high-resolution satellite images taken between 2014 and 2018 (Biodiversity Center, Nature, Ministry of the Environment., 2021). The analysis results from the 2023 Pleiades NEO image shown in Fig. 3 extracted several areas of the *Zostera* that were not reported in the 2014-2018 images. The underwater surveys in July 2024 confirmed the presence of the *Zostera* and *Zostera marina* in these locations. It has been confirmed that the seagrass beds in Hirota Bay decreased due to the tsunami from the Great East Japan Earthquake (Sanyo Techno Marine Co., Ltd., 2022), and subsequent increases (Biodiversity Center, Ministry of the Environment., 2023). Our survey was conducted over five years after the previous investigation, suggesting further recovery of marine vegetation.

It is estimated that there are seasonal changes in the marine vegetation. The observed change from *Zostera* beds to patches of foliose red algae is presumed to be seasonal. For this verification, it is necessary to conduct further surveys in the summer. Additionally, for estimating the area to qualify the seaweeds and seagrasses beds as carbon credits, seasonal changes should also be taken into account.

Weather conditions are crucial for on-site investigations using UAV, underwater drones, and diving. It is difficult to conduct surveys immediately on days with favorable weather conditions for the researchers who operate drones and serve as divers who reside in Sendai. Sendai is more than two hours away by car from Hirota Bay. Citizen science—surveys conducted by local community members—is expected to play a significant role. If local governments, national authorities, and researchers utilize the survey results, coastal management will become more efficient.

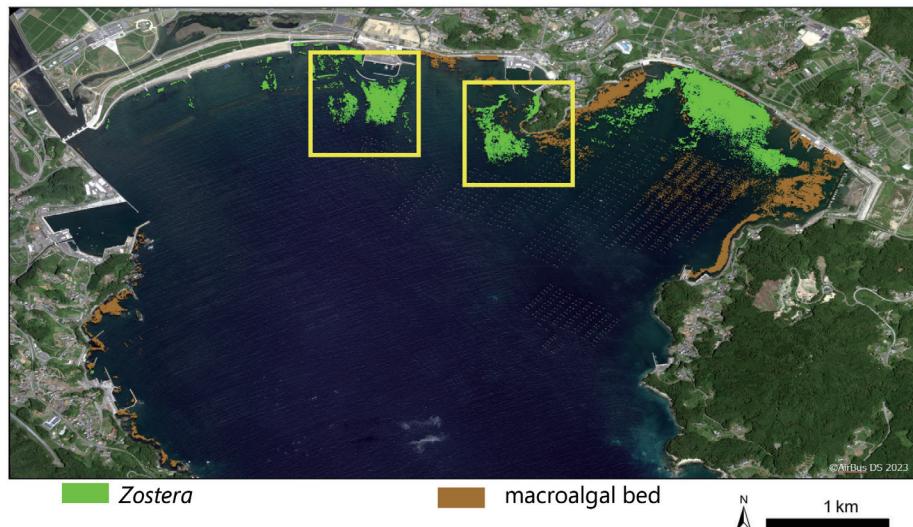


Fig. 3: Extracted areas of seaweeds and seagrass from the Pleiades NEO image obtained on August 2, 2023. Yellow box areas correspond to the area where the marine vegetation was not found on previous survey (shown in Fig. 6).

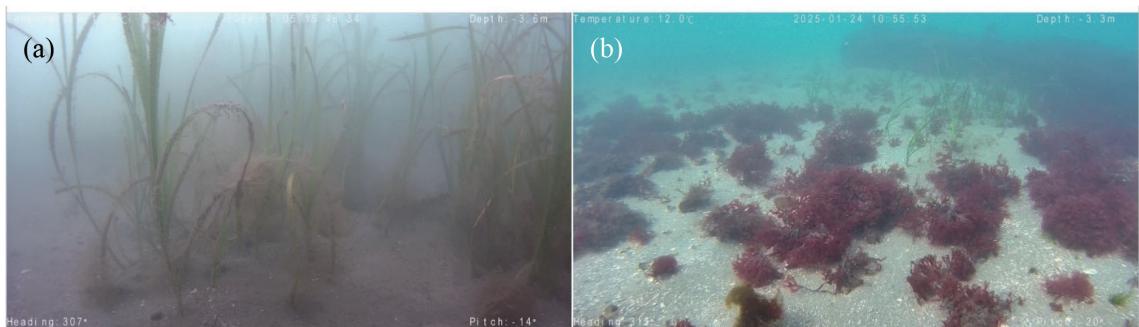


Fig. 4: Observed images taken by an underwater drone on (a) July 5, 2024, and (b) January 25, 2025.

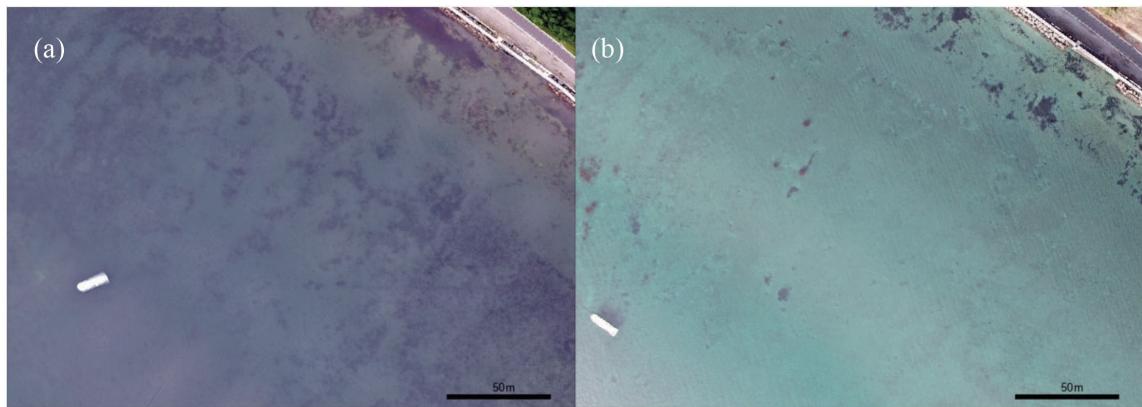


Fig. 5: UAV images including the underwater observation points shown in Fig. 4, captured on (a) July 5, 2024, and (b) January 25, 2025.

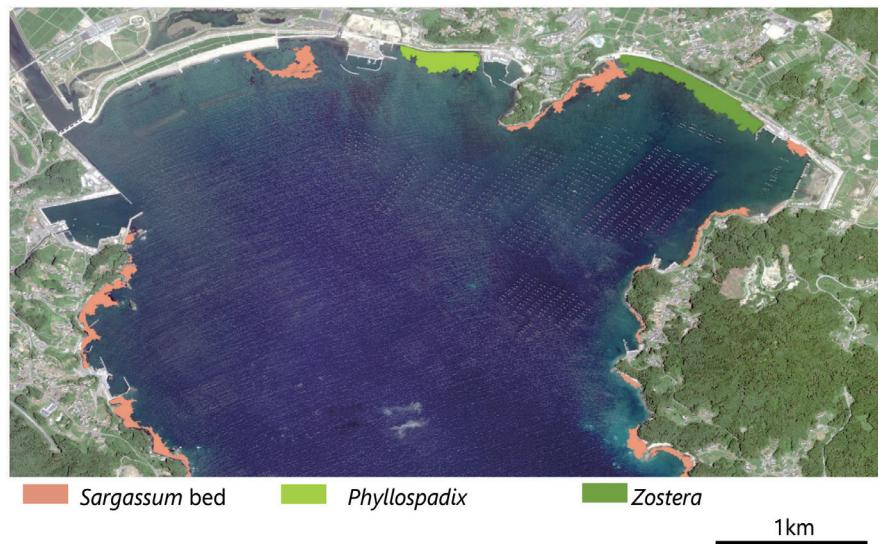


Fig. 6: Distribution of marine vegetation based on high-resolution satellite images taken between 2014 and 2018 (Biodiversity Center, Nature, Ministry of the Environment, 2021). The background image is a Pleiades NEO image obtained on August 2, 2023.

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Symposium mini review



Addressing Knowledge Shortfalls for Marine Biogeography with eDNA and Species Distribution Models

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Abstract

Shortfalls in our knowledge of biogeography for the marine realm limit our ability to understand biodiversity patterns and inform management and conservation. In this mini-review, I summarize how environmental DNA (“eDNA”) can be used to detect rare and cryptic marine species, thus providing occurrence data for species distribution models to make predictions of potential geographic distributions. I also describe how we can address some uncertainties in sampling eDNA and using it for modeling analyses, as well as how new distribution models can take advantage of the high-frequency community data that eDNA makes available to improve current and future predictions.

Introduction

Geographic distributional data for species provide fundamental information for empirical and applied fields. The sizes, shapes, locations, and dynamics of species distributions dictate biodiversity patterns and inform studies in ecology and evolution. Species distributions are also heavily relied on as base data for conservation assessments and environmental management plans. However, there remain broad distributional knowledge gaps for many taxonomic groups—this is referred to as the Wallacean shortfall (Hortal et al. 2015), after the pioneer of biogeography Alfred Russel Wallace. This shortfall is particularly severe in the marine realm, where much biodiversity remains undescribed due to difficulties regarding survey accessibility and costs, detectability of cryptic species, among others (Appeltans et al. 2012). Without more comprehensive geographic distributional data for marine species, we are unable to assess conservation priorities or make predictions about the effects of future climate change for many taxonomic groups in coastal and ocean environments. In this mini-review, I will highlight the benefits of collecting environmental DNA for marine biodiversity surveys and the applications of using these data with species distribution models to predict current and future ranges for understudied marine species.

Environmental DNA can provide occurrence data for cryptic marine species

Extensive biodiversity databases like GBIF (gbif.org) and OBIS (obis.org) have revolutionized the open accessibility of georeferenced species’ occurrence records, but for understudied taxa these data remain either scarce or nonexistent. Traditional survey methods for marine organisms such as underwater visual surveys, trawls, or nets can often be ineffective for rare or cryptic species (Westgaard et al. 2024, Polanco et al. 2024), and detectability can vary by taxon, life stage, ocean stratum, and behavior, among others (Pastor Rollan et al. 2024). All organisms shed their DNA into the environment, called “eDNA”, and sampling with simple kits provides a relatively low-cost and low-effort method to monitor species that are difficult to detect (e.g., Ames et al. 2021). Compared to traditional survey approaches, eDNA surveys recover similar community data with much less effort and they are more likely to detect cryptic species (Polanco et al. 2022), making it a promising technology to address the Wallacean shortfall for marine biodiversity. The relatively low cost of eDNA surveys also enables more frequent sampling over time, which allows for better tracking of range dynamics (Closek et al. 2019). Moreover, eDNA collection is noninvasive and can be preferred for monitoring endangered or sensitive species (Qiao et al. 2024).

As eDNA is subject to the complex physical and ecological dynamics of the marine environment, many challenges remain

to use these data for analysis, particularly for cryptic species that typically have smaller populations and are less active. For example, species with low detection rates tend to have inflated false positives (Darling et al. 2021), and there is often considerable spatial and temporal uncertainty regarding the origin of eDNA samples (Hansen et al. 2018). Techniques are being developed to address this uncertainty. For example, biophysical models can estimate eDNA dispersion extents to help optimize sampling and reduce false positives, and they can also predict geographic origins of samples by tracking simulated particles from point sources (Pastor Rollan et al. 2024).

New efforts to make marine eDNA open to the research community are gaining traction. One example is the ANEMONE database (<https://db.anemone.bio/>), which provides open access to eDNA samples from across Japan since 2019, mainly in estuaries and along coastlines but also increasingly in upland freshwater areas and the open ocean. A new effort called ANEMONE Global is collating eDNA data from numerous countries in Asia and beyond using a standardized sampling regime, which should prove revolutionary in providing global data for poorly known marine biodiversity.

Predicting current and future distributions of cryptic marine species with eDNA and species distribution models

Species distribution models (SDMs) make predictions of species' potential geographic distributions based on records of their occurrences and environmental variables (Guisan et al. 2017). Potential distributions refer to areas that have suitable environmental conditions for the species yet may not be currently occupied due to extirpations, dispersal limitations, or species interactions (Peterson et al. 2011). Over the past 20 years or so, these models have seen many methodological developments, including bias correction (Phillips et al. 2019), model complexity tuning (Kass et al. 2021), and community-level joint SDMs (Warton et al. 2015), many of them easily accessible via open packages in the R programming language (Kass et al. 2025).

Species distribution models typically use opportunistic data found in large occurrence databases that originate from a combination of museum collections, field studies, and citizen science. As a result, SDMs for marine species often suffer from data deficiencies arising from taxonomic sampling biases (Robinson et al. 2017), and they often use environmental variables averaged over decadal timescales to account for the poor temporal resolution of occurrence records (e.g., Chen et al. 2023). However, SDMs that alternatively use georeferenced records from structured eDNA sampling regimes with precise timestamps will suffer from fewer biases and additionally allow occurrences to be matched to environmental values representing seasonal conditions or climate anomalies.

Although eDNA data is associated with various methodological uncertainties, techniques have been proposed to address them; an example is adapting hierarchical SDMs that account for imperfect detection (i.e., occupancy models) for eDNA workflows (e.g., Fukaya et al. 2022). Moreover, eDNA datasets likely have fewer samples and smaller extents

per species than opportunistic occurrence datasets, but SDM techniques tailored for small datasets can still make useful predictions for low-data species (Shcheglovitova & Anderson 2013, Breiner et al. 2015). Low-data species can also be addressed by leveraging the community data in eDNA samples with a joint SDM approach, which can "borrow strength" from species with more data to improve predictions for low-data species and use latent variables to explain residual variance in the model (Warton et al. 2015). Joint SDMs can also infer potential interactions between species based on the residuals of co-occurrence patterns after explaining shared environmental preferences, though there are considerable caveats (Zurell et al. 2018), and thus eDNA data can be used to generate new hypotheses for poorly understood communities.

A common application for SDMs is to project range shifts under different climate change scenarios, and this can help guide marine conservation policy to prioritize protected area locations (Wilson et al. 2020). Future projections have been made for some marine taxa (Robinson et al. 2011), but as there are still considerable gaps in marine distributional data (Menegotto & Rangel 2018), many species remain overlooked. However, SDM estimates based on eDNA data can expand projections to entire marine communities that include cryptic, low-data species. Additionally, eDNA can help improve the monitoring of range dynamics in response to climate change. Frequent sampling with traditional survey methods can be prohibitive due to high costs, and opportunistic occurrence data often lacks structured samples over time. But eDNA sampling with high frequency over time can produce the data needed to accurately model how ranges of marine species dynamically shift in response to ongoing climate change.

Conclusions

The use of eDNA data in SDMs is relatively new, but along with the adoption of other new data sources such as acoustic and visual monitoring, there is great potential for expanding our knowledge of the biogeography and community ecology of cryptic species (Hartig et al. 2024). Methodological uncertainties remain surrounding the collection, processing, and application of eDNA data to model species distributions and biodiversity patterns, but as technology advances, we should see more applications of biophysical models to address spatial uncertainty (Pastor Rollan et al. 2024) and hierarchical models to address detection uncertainty (Fukaya et al. 2022). Lastly, SDM frameworks that integrate different models can leverage the benefits of individual data types and scales (Isaac et al. 2020), and as these can be effective for modeling geographic distributions of rare and cryptic species (e.g., Neto et al. 2020), we need more applications using eDNA data in the marine realm.

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Graduate School of Agricultural Science, Tohoku University
The 22nd International Symposium on Integrated Field Science

Seascape Ecology: Towards Understanding the Mechanism of Biodiversity Structure and Better Management Strategy in the Oceans

Date: March 14 (Fri), 2025

**Venue: Lecture Room No. 1 in Aobayama Commons, Tohoku
University**



PROGRAM

10:00–10:30 Registration

10:30–10:35 Opening Remarks
Mizuhiko Nishida (Tohoku University, Japan)

10:35–10:50 Introduction
Minoru Ikeda (Tohoku University, Japan)

10:50–11:20 *Seafloor Heterogeneity: Offshore Oil and Gas Platforms and Marine Ecosystem Dynamics in the North Sea*
Toyonobu Fujii (Tohoku University, Japan)

11:20–11:50 *Multiscale Seascape Ecology Approaches Unravels Barriers to Marine Connectivity along Southern Norwegian Fjords*
Enrique Blanco Gonzalez (University of Agder, Norway)

-Lunch Break-

13:00–13:30 *Evolution of Abalone Species due to Vertical and Horizontal Heterogeneity in Coastal Environments*
Shotaro Hirase (The University of Tokyo, Japan)

13:30–14:00 How Geotopology and Ecology Affect Genetic Structure: Insights from Seascape Genetics of Marine Snails
Daishi Yamazaki (Toho University, Japan)

14:00–14:30 Monitoring of Seaweeds and Seagrasses Using Remote Sensing Data in Coastal Areas: Integration with Ground Surveys
Chinatsu Yonezawa (Tohoku University, Japan)

14:30–15:00 Data and Techniques for Biodiversity Modeling in the Marine Realm
Jamie M. Kass (Tohoku University, Japan)

15:00–15:30 General Discussion

15:30–16:00 Lightning Talk Session

*16:00–17:30 Poster Session

* The start time may be moved up if there are not sufficient applicants for the lightning talks.

Seafloor Heterogeneity: Offshore Oil and Gas Platforms and Marine Ecosystem Dynamics in the North Sea

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The North Sea has long been a vital ground for the exploitation of natural resources, supporting one of the world's most active fisheries as well as extensive hydrocarbon exploration, the latter of which has led to the installation of over 500 offshore oil and gas platforms since the 1960s across the region. Initially, offshore oil and gas installations were considered as one of the major stressors which have negative impacts on the seafloor. However, recent studies have shown that the physical presence of such installations may in fact have beneficial effects on the surrounding ecosystems because they provide extensive surface area of hard substrata for fouling organisms, including species of conservation importance (e.g., cold-water coral *Lophelia pertusa*), and thereby serve as artificial reefs that provide habitat for diverse marine life including a variety of commercially important fish species. Majority of these structures have been in place for more than decades and they may therefore have functioned as mature reef systems potentially acting as a network of *de facto* marine protected areas (MPAs). However, many offshore petroleum fields are currently approaching the end of their commercial lives, and the focus is now shifting towards the fate of these obsolete oil and gas facilities since different decommissioning options may make significant differences in both environmental effects and economic consequences. Here I present the current understanding of marine ecosystem dynamics in relation to various environmental parameters with particular reference to the physical presence of offshore oil and gas platforms in order to better understand the potential role of offshore sub-sea artificial infrastructure in the ecology of fish populations in the North Sea.

Multiscale Seascape Ecology Approaches Unravels Barriers to Marine Connectivity along Southern Norwegian Fjords

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Ocean currents and environmental heterogeneity can generate inconspicuous boundaries to marine connectivity, preventing gene flow and shaping the genetic architecture of the species. In Norway, coastal species are highly influenced by the complex dynamics of the fjords systems. Organisms are exposed to strong environmental clines driven by the mixing of freshwater and saltwater, while retention mechanisms are also common during the planktonic stages. As a result, populations of many coastal species display genetic patterns of population structure and signatures of local adaptation. Understanding population connectivity and the mechanisms driving the spatiotemporal patterns of genetic structure results essential for fisheries management and conservation. In this study, I will present recent experiences implementing multiscale seascape ecology approaches in coastal species along the southern Norwegian fjords. Integrating genetics and ‘omics approaches, oceanographic modelling and environmental data has proven to be a very powerful tool for unravelling subtle patterns of genetic population structure and elucidating the mechanisms driving reproductive isolation in the absence of physical barriers to gene flow.

Evolution of Abalone Species due to Vertical and Horizontal Heterogeneity in Coastal Environments

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The Japanese large abalone species, Ezo, Kuro, Madaka, and Megai abalones, have recently evolved following the expansion of their North American ancestor species to the Japanese Archipelago. Although these abalones are genetically similar, they exhibit distinct ecological characteristics. Kuro, Madaka, and Megai, whose distribution areas overlap along the southern coast, occupy different habitat depths. In contrast, Ezo and Kuro are believed to represent cold- and warm-water adapted subspecies, with distributions in the north and south, respectively. The evolution of abalone species, which are related to vertical and horizontal heterogeneity in coastal environments, serves as a crucial model for understanding mechanisms driving marine biodiversity. Population genomics analysis of Kuro, Madaka, and Megai revealed three distinct clades corresponding to each species, suggesting that they are reproductively isolated in natural environments, despite the fact that their crossbreeding produces fertile offspring under laboratory conditions. We also found that their speciation events have been accompanied by continuous gene flow and that genomic introgression from Kuro to Madaka occurs in certain geographic regions, suggesting that speciation is ongoing with incomplete ecological isolation probably due to differences in the habitat depth. Ezo and Kuro formed two distinct genetic clusters but exhibited genomic admixture along boundary coastal regions. Furthermore, we found a significant relationship between genetic structure of the two subspecies and coastal environmental factors, such as mean seawater temperature in autumn. These findings highlight that speciation of abalones is a continuous process driven by unstable ecological barriers, emphasizing the need for conservation strategies that account for such continuity.

How Geotopology and Ecology Affect Genetic Structure: Insights from Seascapes Genetics of Marine Snails

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In general, marine ecosystems are characterized by a higher level of gene flow among local populations compared to terrestrial systems. However, accumulated molecular studies have demonstrated that various marine species can be genetically differentiated. Naturally, coastlines are not just straight stretches, but also consist of complex structures such as bays, capes, and various shores. For instance, exposed shores are constantly subjected to the effects of strong waves, while sheltered areas, such as inner bays, are not. These differences in geotopology (the structure of the coastline) provide various types of coastal habitats for marine species.

In this context, the principal goal of seascape genetics is to understand how coastlines, fringed by various environments, affect genetic population connectivity. Here, I focus on the relationships between geotopology and the genetic structure of marine species. Firstly, I demonstrate a comparative phylogeography of co-distributed intertidal snails with different habitat usage patterns concerning complex coastlines such as sheltered bays and exposed shores. Based on the results, I discuss whether the specialist-generalist variation hypothesis is valid in the marine environment. Specifically, habitat specialists that prefer certain coastal environments tend to be genetically differentiated and have low genetic diversity. Next, I introduce our recent work in seascape genetics along the Sanriku coast in the Tohoku District of Japan. The Sanriku coast presents an interesting seascape transition between a relatively smooth coastline and a complex rias coast. To examine the influence of coastline complexity on genetic structure, a high-resolution genome-wide SNPs dataset was obtained by ddRAD-seq from two direct-developing snail species. The results demonstrate that a common genetic boundary of the two species coincides with the seascape transition. Finally, I emphasize the importance of comparing various taxonomic groups within the same seascape to better understand the relationships between coastal geotopology and species-specific ecological traits on genetic structure.

Monitoring of Seaweeds and Seagrasses Using Remote Sensing Data in Coastal Areas: Integration with Ground Surveys

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In coastal area, seagrass and seaweed beds play an important role as carbon sinks. They also contribute to water purification and biodiversity conservation. Remote sensing images obtained from drones, satellites, and aerial photography are useful for assessing the seaweed and seagrass beds in coastal shallow waters. Ground surveys, including scuba diving, are appropriate for obtaining detailed information about their species.

We assessed seaweed and seagrass beds using underwater drones with cameras, diver-based surveys, aerial drone observations conducted on the same day in October 2023, July 2024 and January 2025. Additionally, we analyzed high resolution satellite images acquired by Pleiades NEO in August 2023. The study area was the coastal region of Hirota Bay in Rikuzentakata City, Iwate Prefecture, which was damaged by the tsunami during the 2011 Great East Japan Earthquake.

Zostera (eelgrass) beds and macroalgal beds were extracted through satellite image classification. We identified *Zostera* beds that had not been recorded in previous surveys conducted between 2014 and 2018 based on high resolution satellite images from this study. These seagrass and seaweed areas correspond well with the results of the underwater surveys. However, some areas classified as *Zostera* beds were actually mixed with macroalgal beds. Images acquired by drones can be used as the ground truth data for classification of satellite images that observe a wider area. Additionally, it was confirmed that deploying a submerged camera is a low-cost and effective method for identifying the presence and types of seaweeds and seagrasses.

Data and Techniques for Biodiversity Modeling in the Marine Realm

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Most of Earth's life remains poorly understood or completely unknown to us, particularly in the oceans where gaping knowledge shortfalls remain for many species. Biodiversity models can make use of the limited data we have on species and predict their individual ranges and the broader biogeographical patterns of diversity, so they hold much promise for addressing these shortfalls. Occurrence data for marine species is now available in large volumes thanks to data sharing platforms, citizen science, and new monitoring techniques. Progress in remote sensing and marine data collection has led to extensive environmental variable datasets to model marine biodiversity. Biodiversity models can use these data to generate maps that indicate range limits and biodiversity hotspots, but also to make predictions of how these patterns will change in the future. This talk will focus on marine data sources, biodiversity modeling frameworks, and their applications for the marine realm.

Population Genetics of Three Economically Important Rockfishes (*Sebastes* spp.) along the Coastal Waters of Japan

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Ignoring genetic diversity information when designing harvest plans can lead to loss of diversity, especially in small populations. Significant levels of gene flow are required to restore small populations, as relatively few migrants may be insignificant for long-term conservation or sustainable harvest. In Japan, three closely related rockfishes (*Sebastes cheni*, *S. inermis* and *S. ventricosus*) are harvested by local communities in coastal waters. However, information on the genetic diversity and level of gene flow among populations of these species is still lacking. We analysed genetic data from ten microsatellite loci amplified in four to five populations per species. All populations showed high levels of genetic diversity. The three species showed different patterns of gene flow despite being collected from the same sampling sites. There was genetic homogeneity between populations of *S. cheni* separated by more than 1500 km. In contrast, populations of *S. inermis* and *S. ventricosus*, which are geographically much closer than those of *S. cheni*, had significantly different genetic distances. We hypothesised that these contrasting patterns may result from the interplay between the lifestyle of each species and oceanographic conditions, ruling out a significant influence of isolation by distance between populations. We thus presented preliminary results on the genetic make-up of these economically important rockfishes.

Jellyfish Blooms and Climate Change: Understanding Reproduction and Environmental Drivers

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Reports of jellyfish blooms are increasing worldwide, and the consensus is that their proliferation is linked to multiple anthropogenic factors, such as overfishing and nutrient enrichment. However, climate change and rising sea temperatures may also play a significant role. These blooms have profound ecological and economic impacts, particularly on fisheries and tourism. Jellyfish blooms are intimately connected to the survival and reproduction of these marine organisms. Therefore, understanding the life cycles and environmental factors inducing or inhibiting their transitions among the separate developmental stages is essential for forecasting future bloom events and mitigating associated problems. To better understand jellyfish sexual reproduction in the mature pelagic stage (medusa) and asexual reproduction in the sessile stage (polyp), we will use a combined lab and field experimental design. The study will focus on cultivating the asexual polyp phase of the life cycle and tracking its transition through the juvenile ephyra stages. By analyzing the specific conditions driving their proliferation, we can determine how different species respond to environmental stressors which can be validated with in situ analysis to clarify transcriptional responses to abiotic stressors. We expect to apply this knowledge to characterize underlying mechanisms of jellyfish proliferations in Japan and neighboring coastal waters. This integrative approach will enhance our ability to forecast and manage jellyfish blooms in a changing ocean.

Molecular and Morphological Study of Rhizostome Jellyfishes (Cnidaria, Scyphozoa, Rhizostomeae): Symbiodiniaceae Associations and Cassiosome Production in Japanese Species

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Jellyfish of the order Rhizostomeae (Cnidaria: Scyphozoa) play important ecological and economic roles, yet their taxonomy and symbiosis remain underexplored, particularly in Japan. Many rhizostome species host endosymbiotic dinoflagellates of the family Symbiodiniaceae, which contribute to their development and physiology, but the extent of species-specificity and their possible patterns of coevolution is not well understood. This study integrates molecular phylogenetics, symbiont profiling, and microscopy-based observations to characterize some Rhizostomeae jellyfishes of Japan. Using mitochondrial (16S rRNA, COI) and nuclear (28S) gene regions, we analyzed 18 individuals from five genera—*Mastigias*, *Rhopilema*, *Netrostoma*, *Cephea*, and *Cassiopea*—including publicly available sequences. Phylogenetic reconstruction confirmed the identity of *M. albipunctata* in Japan and suggested a novel *Cassiopea* species in Kagoshima. Symbiodiniaceae analysis using specific ITS2 gene region identified three dominant genera (*Symbiodinium*, *Cladocopium*, and *Durusdinium*), with variability among species, indicating host-specific and environmental influences on symbiont composition. We also provide the first microscopy confirmation of cassiosome production in *Netrostoma setouchianum*, and confirmation in additional *Cassiopea* taxa, while also noting variance in cassiosomes production during different developmental stages in *Mastigias*. These findings strengthen knowledge of phylogenetics, Symbiodiniaceae associations, and cassiosome production in Rhizostomeae jellyfishes.

Full details are available in a separate manuscript *Tan et al. (in prep)*

***Physalia mikazuki* sp. nov. (phylum Cnidaria; class Hydrozoa) from Tohoku (Northeast) Japan: Highlighting Taxonomic Complexity of a Previously Monospecific Genus**

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The discovery of *Physalia mikazuki* sp. nov. from the temperate waters of Sendai Bay (Miyagi Prefecture) in the Tohoku (northeast) region of Japan, represents a significant addition to the taxonomic and ecological understanding of this genus. Detailed morphological analyses reveal distinct traits, including a pneumatophore size of up to 70 mm, blue float and red-green crest coloration, and six cormidia in the oral zone, distinguishing *P. mikazuki* sp. nov. from its congener, *Physalia physalis*. Phylogenetic analyses of the 16S rRNA gene and COI (cytochrome c oxidase subunit 1) regions further confirm its classification as a distinct species, forming a well-supported monophyletic clade separate from other *Physalia* lineages. Oceanographic data and particle trajectory simulations suggest that this species may have been carried from southern regions to Sendai Bay via meandering currents and localized oceanographic processes, potentially facilitated by shifting environmental conditions. However, the limited molecular data available in the public domain complicates the taxonomic context, as *P. mikazuki* sp. nov. matches an unpublished sequence from Pakistan, raising questions about its geographical origins and connectivity. The introduction of *P. mikazuki* sp. nov. into the Tohoku coastal ecosystem raises ecological and public health concerns, including potential disruptions to local food webs and risks of envenomation to humans. These findings underscore the dynamic nature of marine ecosystems in response to climate change and emphasize the need for integrative research to explore the ecological and public health implications of expanding marine species ranges.

Full details are available in a separate manuscript (Yongstar et al., *in review*).

A Comparison of *Olindias formosus* and *Olindias deigo* (Hydrozoa: Limnomedusae) through Literature Review and Molecular Analysis

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The genus *Olindias* includes seven jellyfish species worldwide, with *Olindias formosus* and *Olindias deigo* found in Japan. *O. formosus* has been observed for over 100 years, while *O. deigo* was newly described in 2019, with its ecology and genetics still under studied. This study examines the phylogenetic relationship and distribution of *O. formosus* and *O. deigo* through literature review, molecular analysis, and morphological comparison.

Sequences of *O. formosus* from the Sea of Japan are unavailable while those from the Pacific Ocean exist in public databases. Phylogenetic analysis of 16S sequences confirmed no significant genetic differences between *O. formosus* populations from the Sea of Japan and the Pacific. *O. deigo* sequences showed slight branching within Okinawa samples. COI (Cytochrome oxidase subunit I) analysis requires further validation. Morphological comparisons suggest tentacle number as a diagnostic feature, and microscopy implied the presence of gonadal nematocysts in *Olindias* from Japan for the first time.

Census of Octopus Fisheries in Japan from Molecular and Legacy Data

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Octopuses hold deep cultural and culinary significance in Japan, with human consumption dating back to prehistory. Today, Japan imports over 40% of its octopus from China, Mauritania, and Vietnam, yet these goods are labelled with their localized Japanese names, masking their true origins and potentially their species. This lack of transparency reflects broader challenges in fisheries management, where species identification and geographic distribution data remain underutilized. Marine regime shifts due to climate change further complicate ecosystem assessments and impede fisheries. For a general background of octopuses, including commercial species, we accessed five databases, including four accessed for research globally and a collection from Japan's National Museum of Nature and Science. Over 80,000 unique specimen records spanning 147 years (1875–2022) were analyzed and mapped with a custom pipeline developed with the freeware RStudio. Within the Tohoku region, three species of octopus –*Enteroctopus dofleini* (A. d'Orbigny 1841), *Octopus sinensis* A. d'Orbigny, 1834, and *Octopus conispadiceus* (Sasaki, 1917)– are highly commercially relevant and known by Japanese common names, but caught individuals are typically labelled based on morphological traits or capture season alone. Tissue samples were collected from octopuses caught in Tohoku area by fisheries during seasons where each of the three species had been reportedly caught. 16S and COI mitochondrial regions of the DNA for these species were obtained and aligned with the program MAFFT for the construction of phylogenetic tree based on publicly available molecular specimen data from the online repository GenBank.

Full details are available in separate manuscripts: *Torgovitsky & Ames (In review)* and *Ames et al. (In prep)*.

PlanDyO: Meta-Epigenomic Monitoring of Marine Plankton Dynamics in Onagawa and Mutsu Bay

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Marine ecosystems, characterized by complex physicochemical properties and diverse biomes, present significant challenges for ecological research. Coastal areas, in particular, exhibit heightened complexity as they are influenced by both local and open ocean environments, as well as human activities such as fisheries, aquaculture, and coastal development. The Sanriku Coast, located in northeast Japan, is a dynamic region where the Kuroshio and Oyashio currents converge, creating a highly productive yet sensitive marine ecosystem that is particularly vulnerable to global environmental changes, including climate change and ocean acidification.

In Onagawa Bay, the southernmost part of the Sanriku Coast, we are conducting monthly sampling to perform physicochemical measurements and genomic analyses. These efforts aim to provide a comprehensive understanding of ecosystem dynamics by quantifying the abundance, condition, and function of all individual planktonic species in the bay. This approach allows us to monitor seasonal and interannual variations in biodiversity, nutrient cycling, and ecosystem health.

To address these challenges, we propose a novel marine metagenomic platform leveraging Nanopore sequencing technology to analyze the epigenomics of marine plankton. This platform focuses on two key objectives: (A) generating reference genomes by reconstructing genome sequences from metagenomic data (MAGs), particularly for eukaryotic species with larger genomes, and (B) characterizing genes and genomes through epigenetic profiles, abundance profiles, and Single Nucleotide Polymorphism (SNP) profiles. By integrating metagenomic and epigenomic data, we aim to uncover the molecular mechanisms driving ecosystem responses to environmental changes. We present the current status of this platform's development, which is expected to be publicly available in 2025, providing valuable resources for marine ecological research and conservation efforts.

Spatio-Temporal Variability in Chlorophyll-a Concentrations Estimated by GCOM-C/SGLI in Comparison with *in situ* Vertical Observations Conducted by Fluorometer in Onagawa Bay, Japan

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Sea surface chlorophyll-a (Chl-a) concentrations can be used as a proxy for phytoplankton abundance in the marine environment. Distribution of Chl-a concentration is associated with local primary production, carbon and nutrient cycling, marine trophic interactions and marine ecosystem dynamics, and hence serves as an important indicator for the production of fisheries resources. For example, along the Sanriku Coast, changes in Chl-a concentrations are an important factor for shellfish aquaculture operations (e.g., oysters, scallops) as the cultured species feed entirely on naturally occurring phytoplankton for food. Global Change Observation Mission - Climate (GCOM-C) satellite was launched on December 27, 2017, equipped with an optical sensor, Second-Generation Global Imager (SGLI), which allows for multi-channel observation at wavelengths from near-UV to the thermal infrared. In this study, we compared changes in Chl-a concentrations estimated by GCOM-C/SGLI with those observed by a fluorometer along both vertical (depth) and horizontal gradients over the duration between January 2018 and December 2023 in Onagawa Bay. The Satellite observations showed that Chl-a concentrations were higher in spring and autumn at all monitoring stations during the study period. Although the fluorometer observations showed similar seasonal patterns at the surface, Chl-a concentrations were markedly higher at deeper depths in spring whereas higher at shallower depths in autumn. Fluorometer observations also indicated that Chl-a concentrations were high near the surface at the nearshore stations during the summer. We discuss how the spatio-temporal patterns of Chl-a concentrations measured by the fluorometry translate to the variations found in the GCOM-C observations.

Extraction of Bamboo in Narrow Areas from High-Resolution Satellite Imagery Combined with a Digital Canopy Model

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Insufficient bamboo management may lead to their expansion into surrounding forests, contributing to biodiversity loss and soil degradation. Most previous studies on bamboo extraction using satellite imagery have been relied on images with a spatial resolution of 10–20 meters. The aim of this study is to develop an automated method for extracting bamboo in narrow areas by analyzing images with high resolution satellite imagery. The study area is located in Osaki City, Miyagi Prefecture, Japan. The analysis utilized multispectral data from the WorldView-3 satellite collected in 2015 and Digital Canopy Model (DCM) from 2011. The DCM was generated from LiDAR data obtained by the Geospatial Information Authority of Japan. Five-band GeoTIFF images were created by combining multispectral data and the DCM. The U-Net deep learning model was employed for bamboo extraction, and four data combinations were compared to evaluate accuracy. The results show that the inclusion of DCM improved extraction accuracy, whereas Short Wave Infrared (SWIR) bands had little effect. The limited effectiveness of SWIR bands may be attributed to their spatial resolution of 3.7 meters, which is insufficient for extracting bamboo in narrow areas. In contrast, the DCM, derived from LiDAR data with a point spacing of approximately 1 meter, closely matches the 1.24-meter nadir resolution of the WorldView-3 imagery. Additionally, because bamboo grows in dense clusters and maintains a relatively uniform height, it forms a distinct pattern in the DCM. This characteristic makes it easier to distinguish bamboo from other vegetation and structures, thereby improving extraction accuracy. The data generated by our method is provided as GeoTIFF images and can be applied to GIS-based time-series analyses and bamboo management planning. Our approach enables the early detection of bamboo encroachment around residential areas and other tree species.

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Estimation of Bamboo Dynamics around the Hirota Bay Using High-Resolution Satellite Imagery

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The lack of management of bamboo may lead to their expansion and encroachment into other land cover types. Bamboo expansion affects local ecosystems and forest biomass. The tsunami disaster caused by the 2011 Tohoku earthquake may have influenced bamboo dynamics in damaged coastal areas. We estimated the bamboo dynamics in the tsunami-affected area using high-resolution satellite image analysis. We analyzed a WorldView-2 image acquired on April 6, 2011, and a WorldView-3 image acquired on April 12, 2021. The images cover Hirota Bay area in Rikuzentakata City, Iwate Prefecture and part of Kesennuma City, Miyagi Prefecture, Japan. Land cover classification was performed to estimate bamboo area. From the classification results, we identified areas of bamboo expansion and reduction between 2011 and 2021. We extracted 19 bamboo expansion areas and 10 reduction areas. The land cover in 2011 before the bamboo expansion mainly consisted of “grasslands” and “others”. Several of these areas were damaged by the tsunami. Immediately after the tsunami damage, many grasslands and bare lands emerged. The disaster caused land abandonment, which may have contributed to bamboo expansion. The land cover in areas where bamboo had been reduced was mainly classified as “others” in the 2021 image. Many of these reductions were due to artificial development, such as housing, parking lots, and farmland. A significant reduction in bamboo was found in Kesennuma area, where it seems to have been extensively cut down. There may be regional differences in bamboo management between Rikuzentakata and Kesennuma areas, which should be examined. An assessment of bamboo dynamics affected by the tsunami disaster in another region will be the focus of future work.

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Estimation of Nitrogen Uptake of Dry Direct-Seeded Rice Using Vegetation Indices by a Drone-Mounted Multispectral Camera

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In Japan, the population involved in agriculture is expected to decline sharply over the next 20 years, while the cultivating land per farmer is gradually increasing. Dry direct-seeded rice (DDSR) cultivation is becoming an increasingly important alternative for rice farmers in Japan, as it provides a labor-saving advantage over traditional transplanted rice cultivation. However, its low N use efficiency has been an obstacle to reducing cost. We estimated the nitrogen uptake of DDSR based on vegetation indices, Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI), and Visible Atmospherically Resistant Index (VARI), calculated from multispectral images captured by a drone-mounted sensor. Approximate equations were derived from the relationship between vegetation indices and measured nitrogen uptake. As growth progresses, the values of the vegetation indices tended to saturate at high densities of vegetation cover, and the accuracy of nitrogen uptake estimation declined. However, before saturation occurs, the vegetation indices performed well for nitrogen uptake estimation. In early July, the correlation coefficients (R^2) between vegetation indices and nitrogen uptake were 0.88-0.94 and the root mean square error values used to measure the model accuracy were 0.40-0.80g/m². Result of this study demonstrates the potential for using NDVI, GNDVI and VARI for nitrogen diagnosis for DDSR.

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Drone Observation and Sample Plot Survey of Thinned Test Plots in the Forest Area at Kawatabi Field Science Center

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A large part of Kawatabi Field Science Center, Tohoku University in northern Japan is covered by forest. In the conifer plantation forest, there are nine adjacent small patches of which area of ca.0.5 ha, which were set up for a thinning experiment in 2003. Three replicates of three treatments with different thinning intensities: unthinned, weakly thinned, and intensively thinned were set for these stands. Previous studies showed that the thinning intensities, in number and volume, were 33.4% and 28.3% in weakly thinned plot, and 66.5% and 60.5% in intensive, respectively. Frequent forest surveys and experiments have been conducted in these thinning test plots.

We conducted drone observation and sample plot surveys in November 2024 for these thinning test plots. We observed the thinning test plots with drone-mounted camera from height of 120.5 m. An ortho-mosaic image with a 0.043 m resolution was generated using Structure from Motion. In the sample plot survey, a circular plot of 400 m² was established around the reference point, and the height, diameter at breast height (DBH), and crown bottom height of 10 representative coniferous trees were measured. Additionally, the tree species and DBH of all trees within the plot were recorded. Sample plot surveys were conducted for the six patches.

The tops of the coniferous trees were extracted successfully from the ortho-mosaic image using a local maximum filter. However, there are limitations in extracting the top of broadleaves. The DBH of the representative trees in each plot was 38.3 cm and 46.0 cm in the unthinned plots, 55.3 cm and 52.2 cm in the weakly thinned plots, and 55.5 cm and 65.5 cm in the intensively thinned plots. The next step will involve comparing these results with previous survey data and examining the temporal changes in biomass.

Distribution of Cattle Manure Compost Nitrogen to Plants and Soils in a Paddy Field Crop Rotation System

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Introduction The application of cattle manure compost to farmland is crucial not only for supplying nutrient, including nitrogen (N), to crops but also for establishing nitrogen cycles in agro-ecosystems. However, studies on its N dynamics in paddy field crop rotation systems, which are commonly practiced in Japan, remain limited. This study aimed to elucidate the multi-year dynamics of N derived from cattle manure compost in a rice-soybean rotation system using a ¹⁵N tracer technique.

Materials and Methods Rice and soybean were cultivated in microplots installed in experimental fields. In the first year, ¹⁵N-labeled cattle manure compost was applied into the microplots, and its derived N (N_{dfc}) in crops and soils were traced over six years by measuring ¹⁵N abundance. The rotation was conducted from 2019 to 2024, alternating annually between rice and soybean, starting with rice. A continuous rice treatment (c-rice) was established for comparison. Additionally, two soil types, gleiyic fluvisol (fluvisol) and aluandic andosol (andosol), were used to evaluate soil effects on the N dynamics.

Results and Discussions N_{dfc} was taken up by crops in all six years in all treatments. Rice plants in the rotation system exhibited higher N_{dfc} uptake than c-rice, except for andosol in 2021. This trend may be attributed to the upland management in the rotation, which kept soil Eh high, thereby enhancing rice growth in subsequent years. The effect of the rotation on rice growth was less pronounced in andosol than in fluvisol. In contrast, soybean exhibited significantly lower N_{dfc} uptake than rice across all years and soil types. Residual N_{dfc} in the soils remained above 50% across all treatments after six years. While no significant differences in the residual N_{dfc} were observed among the treatments during the study period, long-term investigation may reveal further trends.

Dynamics of Weeds-Derived Nitrogen Returned to Paddy Soils in Organic Farming

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In organic paddy fields, weeds thrive compared to conventional farming. Nitrogen (N) is crucial for crop cultivation, and understanding its cycle—including the role of weeds—is essential. While weeds compete with rice for N, they may also serve as a N source once returned to the soil. Therefore, this study examined N dynamics from weeds returned to the soil at weeding and after harvest.

To trace N from weeds, two experiments were conducted using ¹⁵N-labeled *Monochoria vaginalis* (MV) and *Echinochloa oryzicola* (EO) in single-plant cultivation frames (17 cm × 30 cm). The first experiment simulated the return of weeds at weeding (maximum tillering stage). In July 2023, ¹⁵N-labeled weeds were returned to the soil, and ¹⁵N distribution was evaluated in rice and soil in 2023 and 2024. In the first year, 31% of N from MV and 10% from EO were absorbed by rice, with higher aboveground dry weight of the rice in MV plots, suggesting rapid mineralization after weeding. In 2024, the N uptake from weeds was lower than in 2023, but EO-derived N (3.2%) was absorbed more than MV-derived N (2.1%). The second experiment simulated the return of weeds after harvest. In October 2023, ¹⁵N-labeled weeds were mixed into the soil, and N distribution was evaluated in 2024. Regardless of weed species, about 6% of N was absorbed by rice, with no significant growth differences.

These findings indicate that N from weeds returned at weeding is rapidly absorbed. In contrast, N from weeds returned after harvest could be lost or converted into organic forms, limiting availability. Differences in N uptake between MV and EO at weeding may be due to mineralization characteristics linked to their C/N ratios (MV ~20, EO ~80). Overall, the contribution of N from weeds depends on the species and timing of their return.

Effect of the Degradation Product from Polycarbonates on Komatsuna (*Brassica rapa* var. *perviridis*) Growth

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Recently, a novel concept of “Plastic to fertilizer” wherein urea derived from polycarbonate (PC) serves as a fertilizer source has been presented (Abe et al., 2021). This concept was raised based on the ammonolysis process of PC degrading into urea and the derivative monomers. The aim of this study is to evaluate the effectiveness of the urea derived from petroleum-based PC and bio-based PC (PIC, poly isosorbide carbonate) on crop growth in pot cultivation. For the growth test we used komatsuna (*Brassica. Rapa* var. *perviridis*) and determined whether the polycarbonate-derived urea is available on the agricultural fertilizer or not. Growth test was conducted in 1/5000a pot applied with the five treatments as follows: (1) urea derived from petroleum-based PC (U-PC); (2) PIC degradation product (a mixture of urea and starch-based monomer of isosorbide, U+ISB-PIC); (3) commercially available urea (U); (4) commercially available urea and isosorbide (U+ISB); (5) no urea application (−N). Each urea application rate equivalent to 0.5 g N pot⁻¹ was applied. Fifteen komatsuna seeds were sowed into each pot and finally seedlings were thinned to 3 plants for the harvesting period. The values of the dry weight, plant height and nitrogen uptake and nitrogen content in komatsuna in the U-PC treatment showed no significant difference compared with these values in the U and DP-PIC treatments. No significant difference between the values in the DP-PIC and U+ISB treatments was also detected. The results indicated that urea derived from petroleum- and bio-based polycarbonates are as effective for komatsuna growth as commercially available urea.

Reference: Abe et al. 2021. *Green Chem.*, (23), 9030–9037.

Dynamics and Interactions of Trace Elements in Soil Solution and Rice under Long-Term Application of Inorganic and Organic Amendments in Paddy Fields

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The necessity of soil fertility management in paddy fields has been re-evaluated in recent years due to soil degradation and increasing fertilizer costs. As a result, the utilization of various inorganic amendments and organic fertilizers, including sewage sludge, has gained attention. However, limited studies have examined the dynamics and interactions of trace elements in soil solution and rice under long-term amendment application.

This study focused on two long-term amendment plots at the Yamagata Agricultural Research Institute: (1) a sewage sludge-applied field, where elemental fluctuations during midseason drainage were analyzed, and (2) a field treated with organic matter and silica amendments, where a controlled pot experiment under continuously flooded conditions was conducted to assess the arsenic (As) reduction effect of silica amendments. Soil solution and rice samples were collected and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) and mass spectrometry (ICP-MS).

In the sewage sludge-applied field, midseason drainage-induced fluctuations in element concentrations showed similar trends within two groups: Mn, Co, P, Fe, (G-A), and Cu, and S (G-B). Elements in G-A decreased, while those in G-B increased during drainage, suggesting redox-driven processes, including Co adsorption/release on Mn oxides and P adsorption onto Fe hydroxides.

In the pot experiment, arsenic concentrations in brown rice decreased by 14.7%, 11.3%, and 27.3%, respectively, across all treatments with Si amendments. These findings confirm the effectiveness of long-term silica amendment in reducing arsenic accumulation in rice, highlighting its potential for sustainable soil management.

The Effects of Rice Husk Biochar Application in Paddy Fields on Methane Emissions and Rice Growth

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The application of biochar to paddy fields is expected to mitigate CH_4 emissions due to the oxidative condition of the soil. This study focuses on rice husk biochar (RHB) and investigated the optimal RHB application rate to simultaneously achieve CH_4 mitigation and maintain both rice yield and quality.

In a paddy field at the Furukawa station, test plots were established with RHB application rates of 0, 10, 30, and 60 Mg ha^{-1} , which were applied in April 2024. Following this, rice (cultivar Hitomebore) was transplanted in May, and cultivation was carried out according to the standard practices of the station. Rice growth and CH_4 emissions were subsequently measured.

Regarding rice growth, there was a tendency for the number of panicles to increase with higher RHB application rates. However, the grain yield and quality were nearly the same across all plots. Examining the inorganic component contents in the rice plants, a slight decrease in N content was observed with higher RHB application rates, while no clear differences were found in Si levels. In the post-harvest soil, C content, C/N, and exchangeable K content clearly increased with higher RHB application rates. A slight increase was also observed in available Si content. When observing the changes in soil Eh, the plots with 30 Mg and 60 Mg showed higher values until early July, just after mid-season drainage, but the difference became smaller after mid-July. In terms of CH_4 flux, no significant differences were observed across the test plots until late July. However, from August, when the rice began to heading, a slight decrease in CH_4 emissions was observed in the 60 Mg plots. The total CH_4 emissions during the measurement period were 8.60, 8.48, 8.73, and 5.68 for the 0 Mg , 10 Mg , 30 Mg , and 60 Mg plots, respectively.

Plasticity of the Host Range and Infectivity of Jumbophages Isolated through *Ralstonia* spp. and *Burkholderia* spp.

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Phages with more than 200 kbp genomes are specifically called “jumbophages,” and are known to infect a broader range of bacterial hosts than ordinary phages. We have previously isolated multiple jumbophages that lyse plant-pathogenic bacteria belonging to *Ralstonia* spp. and *Burkholderia* spp. from leaf litter compost. Of these, jumbophages FLC4-4c and FLC6 isolated using *R. pseudosolanaceae* and *B. glumae* as the host, respectively, shared high nucleotide sequence similarity (97.3% identity in 97.2% coverage); however, they showed different infectivity to host bacteria. FLC4-4c showed high infectivity (10^3 ~ 1 pfu/genome) to *R. pseudosolanaceae* MAFF106603 strain (hereafter, Rp), while showing low infectivity (10^5 ~ 4 pfu/genome or no plaque formation) to *B. glumae* MAFF301169 strain (Bg) and *B. plantarii* MAFF302475 strain (Bp); conversely, FLC6 showed high infectivity to Bg but low infectivity to Rp and Bp. To test if such a difference is a result of jumbophage adaptation to each host, we carried out experimental evolution of the phages, by repeatedly inoculating FLC4-4c or FLC6 to liquid cultures of Bg, Bp, or Rp, for 10 generations in three independent trials. As a result, progeny phages obtained through each bacterial host gained higher infectivity to the new host bacterium in most combinations. Genome sequence analysis showed that the progeny phages evolved in each host bacterium obtained mutations in the same gene in the three independent trials, suggesting that mutations in these genes caused adaptation to the host. These results suggest that the host range and infectivity of jumbophages are highly plastic, and such plasticity may help jumbophages to hitchhike different hosts in the soil for their survival.

Exploration of Cardinal Lipases and Their Producing Bacteria from Anaerobic Digester Sludge through Functional Metaproteomics

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Anaerobic digestion offers fundamental benefits by converting various organic wastes into biogas. Lipids are commonly found in wastewater from sources such as food processing plants, slaughterhouses, and edible oil production facilities and are considered fundamental energy resources. However, the microorganisms responsible for lipid hydrolysis remain largely unknown. In this study, we aimed to identify the key lipase and its secreting microorganisms in anaerobic digestion sludge through functional metaproteomic approach. We performed 2D-gel zymography followed by two-dimensional polyacrylamide gel electrophoresis, which led to the identification of four protein spots exhibiting lipase activity. These spots were excised from the gel and analyzed using nanoLC-MS/MS to determine the partial amino acid sequences of the lipases. To facilitate protein identification, we constructed a custom metagenomic database through metagenomic analysis using a PacBio Sequel IIe and DNBSEQ sequencer. Using this database, we successfully identified six hypothetical proteins as potential lipase candidates. To assess their lipase activity, we cloned the corresponding genes into *Escherichia coli* for recombinant protein expression. One of these hypothetical proteins was successfully expressed, and its lipase activity was confirmed. The DNA sequence of this lipase was found within a metagenome-assembled genome classified as belonging to the Oscillospiraceae family, implying that unfamiliar bacterium involved in lipolysis in anaerobic digestion process. We hope the presented approach will facilitate the understanding of lipid hydrolysis in anaerobic digestion, contributing to the optimization of this bioprocess.

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