

## Abstracts of Poster Presentations

### 1: Possible effects of terrestrial managements on seagrass ecosystem functionings

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#### Abstract

Seagrass species function as typical foundation species which support high productivity and diversity because of their high rates of primary production and their ability to provide associated organisms with trophic support, refuge from predation, and spawning substrates. Seagrass associated species, especially herbivorous invertebrates, can regulate the ecosystem functioning of seagrass beds, suggesting that this interaction is an important key to clarify the relationship between biodiversity and ecosystem functioning in coastal ecosystems. Seagrass communities are characterized by wide occurrence in shallow nearshore areas, where there is a boundary between terrestrial and marine ecosystems. Therefore, seagrass communities are often influenced by various factors derived from terrestrial habitats, such as agrichemicals and nutrients through fresh water input. Although terrestrial nutrients are essential for productivity for nearshore marine plants including seagrass species, increased nutrients cause eutrophication and phytoplankton blooming resulting in the degradation of seagrass growth and distribution. Agrichemicals from river, adjacent agriculture fields and forests also have serious effects on seagrass communities; in particular insecticide has caused mass mortality of the seagrass-associated invertebrates, resulting in vulnerable ecosystem functioning of seagrass beds. The inflow of both agrichemical and nutrients into nearshore areas has increased with the change of the adjacent terrestrial management. In this study, we demonstrated

interactive effect of insecticide with eutrophication on ecosystem functioning of seagrass beds using manipulative experiments in Seto Inland Sea, Japan. In the manipulation, we used the insecticide carbaryl to exclude invertebrate herbivores and the fertilizer to add nutrients, which is a technique developed by the *Zostera* Experimental Network (ZEN), a global collaborative network of scientists studying the structure and functioning of ecosystems supported by eelgrass. The technique enables us especially to demonstrate the indirect effect of epiphyte grazing by the associated invertebrates on eelgrass growth (e.g. Whalen *et al.*, 2013). The carbaryl loading significantly decreased the density and diversity of herbivorous invertebrates and indirectly increased epiphyte biomass, resulting in a significant difference in seagrass growth, although carbaryl concentrations were within the safety standards even at the experiment site. However, the nutrient addition significantly changed the indirect effect of carbaryl loading on seagrass growth. These results suggest that seagrass-associated herbivores can regulate ecosystem functioning of seagrass beds, and indicate the possibility that both agrichemical and nutrient loading can easily change the seagrass ecosystem functionings even in lower concentration. Both agrichemical and nutrient loading from terrestrial habitat to nearshore areas would be easy to change and vary spatially with land use change, so that we should pay attention to the management for the adjacent terrestrial habitats.

#### Annotated Bibliography of Key Works

(1) Duffy J., Reynolds P., Boström C., Coyer J., Cusson M., Donadi S., and Fredriksen S., 2015: Biodiversity mediates top-down control in eelgrass ecosystems: a global comparative-experimental approach. *Ecol. Let.*, **18**(7), 696-705.

This paper investigates top-down and bottom-up effects of adding nutrient and deterrents to eelgrass (*Zostera marina*) beds at 15 sites across the range of this species. They found that removing grazers had a stronger average effect on epiphytic

biomass than local addition of nutrients, revealing stronger top-down control by grazers. Also, they found that the influences of biodiversity in their global analysis are very similar to results from other small-scale experiments. These include sites with more genotypically diverse eelgrass with higher crustacean biomass and lower algal biomass at sites with increased grazer species.

(2) Reynolds P., Stachowicz J., Hovel K., Boström C., Boyer K., Cusson M., and Duffy J., 2017: Biogeography of predation pressure in eelgrass across the Northern Hemisphere, Unpublished manuscript, Virginia Institute of Marine Science, Virginia USA.

Studies across broad geographic ranges exploring drivers of change in predator interactions are relatively rare. The authors surveyed predation on a common amphipod prey in eelgrass (*Zostera marina*) beds at 42 sites across the Northern Hemisphere. At all coasts, predation declined with latitude, but declined more in areas where temperature gradients are steeper.

(3) Whalen M., Duffy J., and Grace J., 2013: Temporal shifts in top-down vs. bottom-up control of epiphytic algae in a seagrass ecosystem. *Ecology*, **94**, 510-520.

The authors used a cage-free approach of manipulating mesograzers abundance and epiphytic loading over a temporal period, by using deterrent and nutrient treatments. They found that reduction of mesograzers abundance and increase in nutrients can allow increased epiphytic algae growth on eelgrass (*Zostera marina*). By performing the experiment in the fall and summer, they found that the dominant factors that controlled epiphytic algae abundance changed. In fall, there was a natural decrease in mesograzers abundance, and by adding nutrients, which caused bottom-up factors to dominate, increased epiphyte biomass. In summer, added deterrents, stimulating strong top-down factors, decreased mesograzers abundance and caused an increase in epiphyte biomass. Also, unexpectedly, they found that drift macroalgae indirectly reduced epiphytes by providing structure for mesograzers.

## 2: Genetic effects of the tiger puffer stock enhancement program on wild population in the sea around Japan

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### Abstract

Since 1977, millions of hatchery-reared tiger puffer, *Takifugu rubripes*, juveniles have been released for stock enhancement in the sea around Japan. The stock enhancement program has contributed to catches, however, no information about genetic effects of the program on wild population is available. The genetic effects were evaluated with highly polymorphic DNA marker sets (1962 bp mitochondrial DNA sequences and 11 microsatellite DNA loci) using 316 mature wild adults from nine identified or possible spawning sites in the sea around Japan, 276 wild juveniles in the Ariake Sea which is a possible spawning site and an identified nursery, 85 hatchery-reared juveniles, 150 mature hatchery-released adults in the Ariake Sea. Both types of markers indicated that hatchery-reared and hatchery-released populations had lower genetic variability than wild populations, however, hatchery-released population had closer genetic variability to wild populations. Microsatellite  $F_{ST}$  estimates indicated hatchery-reared and hatchery-released populations were significantly different from wild populations. On the other hand, significant differences were observed only between hatchery-reared population and wild populations with mitochondrial  $\Phi_{ST}$  estimates. Microsatellite  $F_{ST}$  estimates and Bayesian clustering analysis revealed population structure of the tiger puffer, however, the population structure was not associated with the history and scale of stock enhancement program. The genetic variability was almost equivalent among wild populations, no significant deviation from Hardy-Weinberg equilibrium and significant linkage disequilibrium were detected from wild populations. Moreover, putative wild juveniles

having a high possibility of being related to mature hatchery-released adults were detected based on relatedness among individuals in the Ariake Sea. These results suggested that there is a high possibility that hatchery-released fish breed in the wild, however, negative genetic effects of the stock enhancement program on wild populations were not likely in the tiger puffer. As one of the factors, it is considered that mature hatchery-released population is composed of multiple groups and year classes and have close genetic characteristics to wild populations.

#### Annotated Bibliography of Key Works

(1) Araki H., Cooper B., and Blouin M. S., 2007: Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, **318**, 100-103.

Captive breeding is used to supplement populations of many species that are declining in the wild. The suitability of and long-term species survival from such programs remain largely untested, however. We measured lifetime reproductive success of the first two generations of steelhead trout that were reared in captivity and bred in the wild after they were released. By reconstructing a three-generation pedigree with microsatellite markers, we show that genetic effects of domestication reduce subsequent reproductive capabilities by ~ 40 % per captive-reared generation when fish are moved to natural environments. These results suggest that even a few generations of domestication may have negative effects on natural reproduction in the wild and that the repeated use of captive-reared parents to supplement wild populations should be carefully reconsidered.

(2) Hamasaki K., Toriya S., Shishidou H., Sugaya T., and Kitada S., 2010: Genetic effects of hatchery fish on wild populations in red sea bream *Pagrus major* (Perciformes, Sparidae) inferred from a partial sequence of mitochondrial DNA. *J. Fish Biol.*, **99**, 2123-2136.

Variation in the mitochondrial DNA transcriptional control region sequence was investigated in wild and hatchery-released red sea bream *Pagrus major* from Kagoshima Bay, where an extensive hatchery-release programme has been conducted for > 30

years. The programme has successfully augmented commercial catches in the bay (released juveniles have been produced from the captive broodstock, repeatedly used over multiple generations). Samples were also obtained from outside the bay, where limited stocking has occurred. Genetic diversity indices measured as number of haplotypes, haplotype richness, haplotype diversity and nucleotide diversity were lower in hatchery-released fish than in wild fish. Genetic differences in wild fish from the bay, especially in the inner bay, compared with fish from outside the bay were detected in terms of decreased genetic diversity indices and changed haplotype frequencies. Unbiased population pairwise FST estimates based on an empirical Bayesian method, however, revealed low genetic differentiation between samples from the bay and its vicinity. Mixed stock identification analyses estimated the proportion of hatchery-released fish in wild populations in the inner and central bays at 39.0 and 8.7%, respectively, although the precision of the estimates was very low because of the small genetic differentiation between populations and relatively small sample sizes. Hence, the long-term extensive hatchery release programme has affected the genetic diversity of wild populations in the bay; however, the genetic effects were low and appeared to remain within the bay.

(3) Blanco Gonzalez E., Aritaki M., Sakurai S., and Taniguchi N., 2013: Inference of potential genetic risks associated with large-scale releases of red sea bream in Kanagawa prefecture, Japan based on nuclear and mitochondrial DNA analysis. *Mar. Biotechnol.*, **15**, 206-220.

Since 1978, millions of hatchery-reared red sea bream (*Pagrus major*) juveniles have been released in Sagami Bay and Tokyo Bay in Kanagawa Prefecture, Japan. The stock enhancement program has contributed to total catch; however, no information regarding the genetic interactions with wild counterparts is available. Here, we combined 15 microsatellite loci and mitochondrial D-loop sequencing to characterize the genetic resources of red sea bream in Sagami Bay and Tokyo Bay and to elucidate the potential harmful genetic effects associated with fish releases. Both types of markers evidenced higher levels of genetic diversity in wild

samples (SB and TB) compared with offspring before stocking (H07 and H08) as well as a hatchery-released sample recaptured in Sagami Bay (HR). Microsatellite  $F_{ST}$  estimates and Bayesian clustering analysis found significant genetic differences among samples ( $F_{ST}=0.013-0.054$ ), except for the two wild samples ( $F_{ST}=0.002$ ) and HR vs. H07 ( $F_{ST}=0.007$ ). On the other hand, mitochondrial-based  $\Phi_{ST}$  suggested haplotypic similarity between SB, H07, and HR. The low effective number of females contributing to the offspring over multiple generations may be responsible for the lack of haplotypic differentiation. Moreover, the putative hatchery origin to three fish (8 %) without deformity in the inter-nostril epidermis was inferred for the first time. Our results showed the usefulness of combining nuclear and mitochondrial markers to elucidate genetic interactions between hatchery-released and wild red sea bream and warned about potential harmful genetic effects should interbreeding takes place.

(4) Nakajima K., Kitada S., Habara Y., Sano S., Yokoyama E., Sugaya T., Iwamoto A., Kishino H., and Hamasaki K., 2014: Genetic effects of marine stock enhancement: a case study based on the highly piscivorous Japanese Spanish mackerel. *Can. J. Fish. Aquat. Sci.*, **71**, 301-314.

We used a before–after control–impact design to quantify the genetic effects of the large piscivorous Japanese Spanish mackerel (*Scomberomorus niphonius*) stock enhancement program on wild populations in the Seto Inland Sea. Samples of 1424 wild and 230 hatchery fish collected from 13 sites around Japan were genotyped using five microsatellite markers. A total of 758 wild and 103 hatchery fish were sequenced for the mitochondrial DNA D-loop region. The population structure of Japanese Spanish mackerel was panmictic around Japan. Hatchery fish had significantly lower genetic diversity indices than did wild fish. However, there was no significant change in any of the diversity indices in the Seto Inland Sea, despite the substantial genetic mixing proportion of hatchery-origin genes (7.8 % – 14.5 % from releases in 2001 and 2002), a conclusion supported by simulations. The estimated effective population size was surprisingly small (~430 – 970) but stable in the Seto Inland Sea compared

with the large census size. A Ryman–Laikre effect was not likely in the Japanese Spanish mackerel.

### 3: Assessment of the ichthyotoxicity of harmful marine microalgae *Karenia* spp. using cultured gill cells from red sea bream (*Pagrus major*)

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#### Abstract

The present study reports the development of a method to investigate ichthyotoxicity of harmful marine microalgae using cultured red sea bream (*Pagrus major*) gill cells. The cultured gill cells formed adherent 1–2 layers on the bottom of the culture plate, and could tolerate seawater exposure for 4 h without significant alteration in cell survival. The microalgae *Karenia mikimotoi*, *Karenia papilionacea*, *K. papilionacea phylotype-I*, *Karenia digitate* and *Heterosigma akashiwo* were cultured, then directly exposed to gill cells. Live gill cell coverage after *K. mikimotoi*, *K. papilionacea phylotype-I*, and *K. digitate* exposure were significantly lower than in the cells exposed to a seawater-based medium SWM-3 and IMK (control cells;  $P < 0.05$ ). Toxicity of *K. mikimotoi* cells was weakened when the cells were ruptured, and was almost inexistent when the algal cells were removed from the culture by filtration. Significant cytotoxicity was detected in the concentrated ruptured cells, although cytotoxicity was weakened in the concentrated of ruptured cells after freezing and thawing; whereas, cytotoxicity almost disappeared after heat treatment. In addition, examination of the distribution of toxic substances from the ruptured cells showed that cytotoxicity mainly occurred in the fraction with the resuspended

pellet after centrifugation at  $3000 \times g$ .

**4: Asari clam predation by intertidal fishes: feeding habits of immature black porgy, *Acanthopagrus schlegelii* in Yamaguchi Bay, western Seto Inland Sea, Japan**

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**Abstract**

Black porgy, *Acanthopagrus schlegelii* (Family: *Sparidae*), is a commercially important fish in Japan. In 2015, commercial catches of the porgy in Japan and the Seto Inland Sea of western Japan were 3,181 metric tons and 1,508 metric tons, respectively. This species is known as a major predator of Asari clam, *Ruditapes philippinarum* in tidal flats. On the other hand, Asari clam is a commercially important species, too. Recently, the clam catches have decreased in Japan. Especially in the Seto Inland Sea, the catches have remarkably dropped to 137 metric tons in 2015 from 45,023 metric tons in 1985 (peak level). Several reports concerning protecting clam beds with netting suggest that predation is one of the most important factors for the survival of the clam. In order to restore Asari clam resource, it is necessary to clarify the habits of the porgy. In this study, we investigated the seasonal occurrence and feeding habits of immature-sized porgy (less than about 25 cm total length) in tidal flats from 2005 to 2017 in Yamaguchi Bay, western Seto Inland Sea. 102 individuals of the porgy (10.4-27.7 cm TL) were collected by rod-and-line fishing there. The monthly catch per unit effort (CPUE: number of fish caught/3 hours/person) of the porgy ranged 0 - 2.9. The CPUE rapidly increased in

June. In July, it reached 2.9, the highest annual value, after which the CPUE maintained high values from August to November. From December values were low (0 - 0.4) again. These results indicate that immature *A. schlegelii* occurs seasonally in the tidal flats from June to November. Bases on the stomach contents of the porgy, bivalves such as Asian mussel, *Arcuatula senhousia*, Japanese razor clam, *Solen strictus* were the most important prey items in wet weight. Next, Japanese mud shrimp, *Upogebia* major was the second important one. These benthic animals were in common and are filter feeders on phytoplankton, benthic microalgae, and detritus. Immature-sized porgy preyed on juvenile Asari clams ranging from 2.8 to 18.0 mm shell length. The values of stable isotopes of  $\delta^{13}C$  and  $\delta^{15}N$  on black porgy showed -17.6 ~ -16.0 ‰ and 16.2 ~ 17.7 ‰, respectively. In the Seto Inland Sea of Japan, resources of benthotrophic fish species, such as flatfishes and pufferfishes, which use the estuary in their early life history, have decreased remarkably. For recovery and regeneration of these critical resources, it is necessary to clarify the relationships between organisms and the estuarine ecosystem.

**Annotated Bibliography of Key Works**

- (1) Shigeta T. and Usuki H., 2012: Predation on the short-neck clam *Ruditapes philippinarum* by intertidal fishes: a list of fish predators. *J. Fish. Technol.*, **5**(1), 1-19. (in Japanese with English abstract)
- Recently, commercial catches of the short-neck clam *Ruditapes philippinarum* have decreased in Japan. Especially in the Seto Inland Sea, western Japan, the clam catches have remarkably dropped. Several reports suggest that predation by fishes is one of the most important factors for the survival of the clam. In this review, we made a list of fish species that forage on the short-neck clam in the field. Twenty-three fishes ranging from *Myliobatidae* to *Tetraodontidae* (12 families) are listed in the world. Among them, 21 intertidal fishes (12 families) occur in Japan. It was clarified that five fishes, the Longheaded eagle ray *Aetobatus flagellum*, the Black porgy *Acanthopagrus schlegelii*, the Yellowfin seabream *A. latus*, the Kyuusen wrasse *Parajulis poecilepterus* and the Grass puffer *Takifugu niphobles*, foraged

on whole adult-sized clam (> 20 mm shell length). At least, the siphon was cropped by eight fishes including the Stone flounder *Kareius bicoloratus*, the Marbled sole *Pseudopleuronectes yokohamae*, and the Japanese sillago *Sillago japonica*. Meanwhile, the Black porgy and the Kyuusen wrasse preyed on all parts except the foot of the clam. We are continuing to analyze details of the interaction between these intertidal fishes and the short-neck clam.

### 5: Evaluation of inexpensive Raspberry Pi-based time-lapse camera system for tidal flat ecosystem observation

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#### Abstract

In recent years, changes in the structure of coastal ecosystems, leading to a collapse of conventional fisheries, have been widely reported. Maintaining clam fisheries or aquaculture production in general has become increasingly difficult without adequate predator prevention measure, especially in the tidal flats of western Japan, indicating certain changes in the structure of the ecosystem. It is suggested that migratory fish species influence the benthic community structure through predation in the tidal flat ecosystem. However, inadequate information exists on the abundance or frequency of the appearance of migratory fish species in tidal flats; hence, its impacts on the benthic community are unclear. Observation using an underwater time-lapse camera system can be an effective approach to understanding the temporal change in the abundance of migratory fish species in tidal flats and their influence on the ecosystem. We have, therefore, developed and tested an inexpensive

Raspberry Pi-based underwater time-lapse camera system to observe the tidal flat ecosystem processes. The Raspberry Pi is an inexpensive single-board computer that can be connected to a dedicated camera module. Our system consists of Raspberry Pi with a camera module, USB battery, time-lapse power control unit, and optional light emitting diode (LED) module and temperature sensor. The camera system uses the command line interface for operation setting, i.e., setting the initial wakeup time, repeat times, and the interval. It takes still and/or video images after wakeup, and then shuts down until the next scheduled wake-up time. We placed the entire system, excluding the LED module and temperature sensor, in an inexpensive underwater housing structure made with polyvinyl chloride (PVC) water pipe sockets and an acrylic window. The camera system successfully captured underwater time-lapse images during observations, except under low visibility conditions, or when biofouling occurs. However, certain improvements are required, which include improving the image quality under low-light conditions by increasing the brightness of the light module, widening the field of view of the image, and adding an anti-biofouling mechanism.

### 6: Development of Free-Ocean Real-Time Experimental System (FORTES) for in-situ CO<sub>2</sub> manipulation in eelgrass beds

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#### Abstract

Ocean acidification (OA) causes various effect on

coastal resources. For eelgrass beds, positive and negative effects are expected on different functional groups of biological communities. In addition, the acidification level in coastal ecosystems vary greatly due to the CO<sub>2</sub> consumption and production by marine plants and input of freshwater. To understand the actual influences of OA under such complicated conditions, in-situ field experiments for manipulating CO<sub>2</sub> are promising. FOCEs (Free ocean carbon experiments, Gattuso *et al.*, 2014) have already been established in several marine habitats such as coral reefs and *Posidonia* seagrass beds. However, these systems have several problems such as changing in-site hydrodynamic conditions, the needs to electrical power to operate the system, and high installation cost. To overcome such difficulties, we have developed a newly CO<sub>2</sub> manipulating system on the site, named FORTES (Free-Ocean Real-Time Experimental System). The system allows control of the acidification level in the area of square meters of eelgrass beds by supplying a high concentration of CO<sub>2</sub>-dissolved water under mostly opened condition. No electrical power is required because this system could be operated by the pressure and buoyancy of injected CO<sub>2</sub>. The CO<sub>2</sub>-dissolved water is always emitted to the experimental area from the upstream direction even when the current direction changes with tide and other factors. In the test trials of FORTES in eelgrass beds in Oki Island and Akkeshi lagoon in Japan, the acidification level expected in 2100 was reproduced successfully. Furthermore, this system enables the supply of water-soluble nutrients and the deterrent of invertebrate grazers along with the CO<sub>2</sub> for simultaneous control of nutrient levels and grazer densities. This orthogonal design allows the experiments to examine interacting effects of ocean acidification and other factors in the eelgrass bed community.

#### Annotated Bibliography of Key Works

(1) Gattuso J. P., Kirkwood W., Barry J. P., Cox E., Gazeau F., Hansson L., Hendriks I., Kline D. I., Mahacek P., Martin S., McElhany P., Peltzer E. T., Reeve J., Roberts D., Saderne V., Tait K., Widdicombe S., and Brewer P. G., 2014: Free-ocean CO<sub>2</sub> enrichment (FOCE) systems: present status and future

developments. *Biogeosciences*, **11**, 4057-4075.

Free-ocean CO<sub>2</sub> enrichment (FOCE) systems are designed to assess the impact of ocean acidification on biological communities in situ for extended periods of time (weeks to months). They overcome some of the drawbacks of laboratory experiments and field observations by enabling (1) precise control of CO<sub>2</sub> enrichment by monitoring pH as an offset of ambient pH, (2) consideration of indirect effects such as those mediated through interspecific relationships and food webs, and (3) relatively long experiments with intact communities. Bringing perturbation experiments from the laboratory to the field is, however, extremely challenging. The main goal of this paper is to provide guidelines on the general design, engineering, and sensor options required to conduct FOCE experiments. Another goal is to introduce xFOCE, a community-led initiative to promote awareness, provide resources for in situ perturbation experiments, and build a user community. Present and existing FOCE systems are briefly described and examples of data collected presented. Future developments are also addressed as it is anticipated that the next generation of FOCE systems will include, in addition to pH, options for oxygen and/or temperature control. FOCE systems should become an important experimental approach for projecting the future response of marine ecosystems to environmental change.

#### 7: A comparison of environmental and biological parameters at asari, *Ruditapes philippinarum*, fishing grounds in Japan for understanding the cause of recent catastrophic decrease of asari clam

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### Abstract

The catastrophic decrease of catch of the asari clam, *Ruditapes philippinarum*, has been observed for the last three decades in Japan's coastal waters. Many factors including overfishing, disease, habitat loss, competition with invasive species, global warming, and altered trophic cascade are suggested to be involved. However, major factor is still not

well characterized to explain the widespread and long-term decrease of the catch. We conducted comprehensive comparison of environmental and biological characteristics among asari fishing grounds in Japan. Significant relationship was observed between water nutrient level (*i.e.*, total nitrogen and chlorophyll *a*) and the asari catch. Stable carbon isotope ratio of asari was found to be a useful indicator representing not only the nutrient level but also the asari catch per unit area. Significant relationship was also observed between the benthos biomass (macrobenthos, meiobentos, and nematodes) and the asari catch. All these observations suggest that recent catastrophic biomass decrease is occurring not only for asari but also for wide range of benthic organisms in Japan's coastal waters.

This study was conducted by a research fund entitled a feasibility study on biodiversity assessment methods in fishing ground environment from Fisheries Agency.