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Feasibility Study for Seagrass Beds Monitoring Using Environmental DNA

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Environmental DNA (eDNA) is DNA collected directly from environmental samples (soil, sediment, water, air, etc.) rather than from individual organisms. When organisms interact with the environment, DNA is released and accumulates in the environment. Hence, eDNA analysis facilitates efficient detection and monitoring of species in aquatic ecosystems. A monitoring method for seagrass beds using eDNA has been examined. Our study considered three requirements for monitoring seagrass bed using eDNA from a water sampling point. 1) An observation point recording eDNA originating exclusively from a specific area of seagrass bed. To get information about a specific area of the seagrass bed, eDNA from that area should not to be mixed with eDNA originating from other areas. 2) Long periods of continuous eDNA appearance at an observation point to enable water sampling. 3) Large amount of eDNA observed at a water sampling point. This study investigated the feasibility of the three requirements for monitoring seagrass beds using eDNA for 10 simplified bay models. The study used numerical simulation and particle tracking to study the transport and distribution of eDNA.

Keywords: *environmental DNA, eDNA monitoring, numerical simulations, particle tracking, tidal forcing*

1. INTRODUCTION

Environmental DNA (eDNA) analysis is an emerging approach for species detection using the genetic material – the DNA – extracted directly from environmental samples (soil, sediment, water, air, etc.) without isolating the target organisms. All organisms interact with the environment, and they release DNA which accumulates in their surroundings. Organisms' excrements (epidermis, feces, mucus, hair, gametes, etc.) in environmental samples contain residual DNA which can be traced using eDNA analysis. Hence, eDNA sourced from environmental samples offers a new avenue to efficiently detect, investigate and monitor species in marine ecosystems [1)–3)].

A network of water monitoring points makes it possible to monitor water quality and aquatic species in marine waters. However, for marine waters (complex reservoirs, bays, etc.), the question of sampling location and number of samples requires more than an intuitive choice of sampling design to obtain representative results for the entire water body. Nevertheless, the task of determining the optimal number of samples to take and the most appropriate locations in a water body in order to characterize its water quality and aquatic species in a meaningful and economical way can be quite challenging. Strategic selection of monitoring point locations can improve eDNA monitoring system. The water sampling locations can be designed with respect to the hydrodynamics, and eDNA transport characteristics in marine waters. An efficient eDNA monitoring design can enable researchers to predict water sampling locations and sampling depth, and eDNA sources given prevailing tidal and meteorological conditions.

Based on the literature review, it can be said that the standard objectives for determining the water monitoring network design are the detection and understanding of the spatial and temporal variations of eDNA; and the identification of the sources of eDNA [4)–6)]. In this scenario, monitoring and evaluating the eDNA is of fundamental importance for the sustainable management of aquatic species, as it allows the understanding of the current situation of marine waters and the main changes that have occurred over time. This makes it possible to identify trends and to develop diagnostics that can help in hydro-environmental restoration and management.

Based on the above considerations, the objective of this study is to propose a method to determine optimum eDNA monitoring point using numerical simulations and particle tracking, and applicable to coastal waters. Numerical simulations and particle tracking make it possible to understand transport and distribution characteristics of eDNA in marine ecosystems [7),8)]. The proposed methodology was applied to simplified bay models through the analysis of tidal transport processes of eDNA.

2. MATERIALS AND METHODS

(1) Simple bay models

Ten idealized bay models are studied (**Fig. 1**). The bay models are categorized based on the bay shape, and bay mouth location and size. "The Report of the Marine Biotic Environment Survey in the 4th National Survey on the Natural Environment. Vol.2 Algal and Sea-Grass Beds" indicated that there are 55 typical sea areas (bays and coastal lakes) with Zostera marina beds [9)]. These sea areas can be classified into

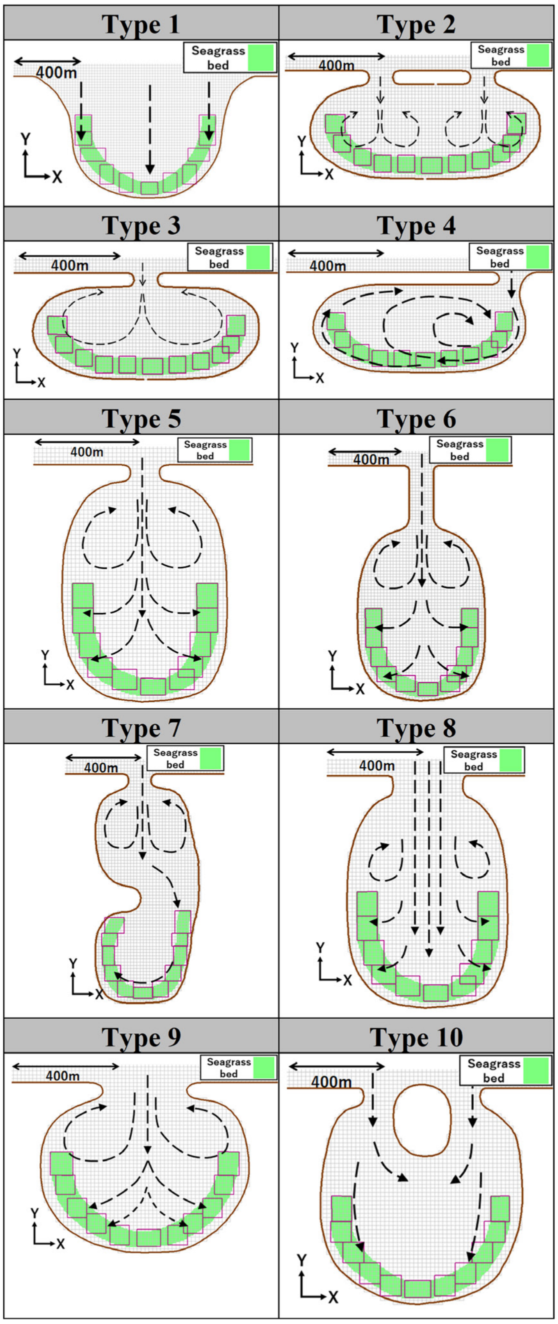


Fig. 1 Bay types and particle position at the start of the simulation

10 basic types [10)]. **Fig. 1** shows the ten simplified bay models. **Fig. 1** also show the flow patterns during flood tide. The bathymetry of the bays is parabolic with maximum depth 8 m at the center and minimum depth 0.5m along the circumference of the bays. **Table 1** shows numerical calculation conditions.

Table 1 Conditions for flow and particle tracking calculation

Average computation domain dimensions	East-West	3 km
	North-West	2 km
Plane coordinates	Cartesian coordinate system	
	Grid width	15m
Vertical coordinate	Layer	5
Analysis period	6 days (ramp-up period: 1 day)	
Time step	3 s	
Boundary condition	Tidal	M2
Particle input	Average total amount	2300
	Time interval	10 minutes

(2) Flow calculation conditions

The computational domain has a horizontal resolution of 15m × 15m and vertical resolution of 5 layers. Delft3D FLOW module, a three-dimensional hydrodynamic (and transport) simulation program is used to calculate hydrodynamics of the bays [11)]. Only one hydrodynamics condition is considered in this study, which is tidal forcing. The main assumption is that advection due to tidal forcing is dominant in inner bays. The tidal amplitude was set with reference to the spring tide in the inner bay.

(3) eDNA transport modeling conditions

A previous study states that eDNA transport dynamics seems to be comparable to those of fine particulate organic matter [12]. Hence, for the idealized cases, eDNA were considered as simple particles in this study. The study also assumes that eDNA degradation and gravity-induced eDNA deposition are negligible.

A particle tracking model is used to determine the movement of eDNA particles from the point of release. The flow simulation results from Delft3D FLOW module are used by the particle tracking model to simulate the transport of eDNA particles. The particles on each sampling point and depth layer are then counted to determine temporal and spatial variation of eDNA particles. The path taken by each eDNA particle can be traced from the release moment to the end of the simulation.

The study targets eDNA values obtained from filtration residuals of seawater. It has been confirmed that seagrass releases DNA of a sufficiently small particle size (6-0.7μm) [13]. The study considered these particle conditions: 1) fine particulate organic matter, 2) degrade in 5 days, 3) eDNA considered passive to flow due to its particle size. The main assumption is that the particles are carried along by the flow and correlation between positive advection diffusion and eDNA concentration [7].

In the simulations, at least 2300 eDNA particles were input every 10 minutes, i.e. released from a seagrass bed located in each bay. The particles passing through each observation point are counted. The particle count can be done for particles from a specific area. In this study particles originating from eleven specific locations are counted at each observation point for each bay.

The bays are divided into equispaced grid points, and each grid point is the center of an observation point. All the observation grid cells are of the same area, 2m × 2m. The depth of observation is from 1m to 3m below surface level, hence each observation point is 2m × 2m × 2m.

3. FEASIBILITY OF MONITORING USING eDNA

Three conditions are of interest in this study. 1) An observation point recording eDNA originating exclusively from a specific area of seagrass bed. To get information about a specific area of the seagrass bed, eDNA from that area should not to be mixed with eDNA originating from other areas. 2) Long periods of continuous eDNA appearance at an observation point to enable water sampling. 3) Large amount of eDNA detected at an observation point.

The ideal eDNA monitoring scenario will be the satisfaction of the conditions at a single observation point for multiple seagrass beds by changing water sampling time (**Fig. 2**). The other ideal scenario will be a sampling point far from the area of interest that meets requirements, making remote monitoring of the seagrass beds possible. On the other hand, if eDNA starting from different seagrass beds are

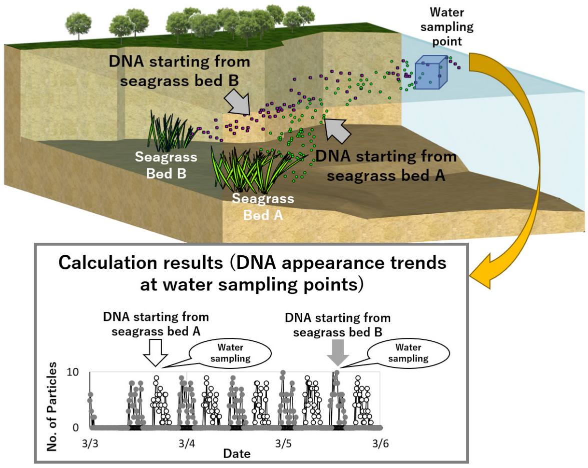
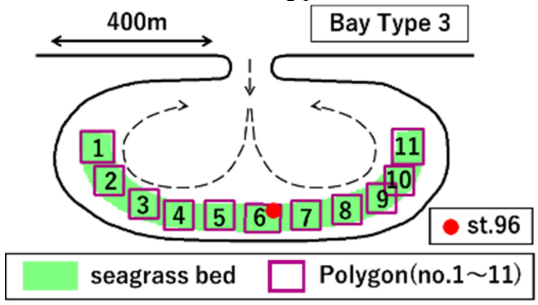
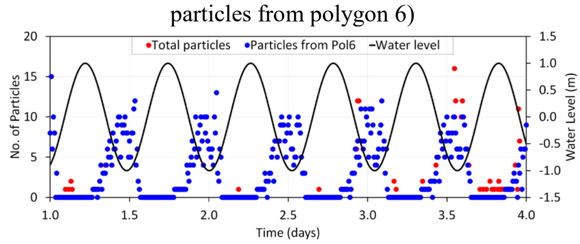


Fig. 2 eDNA originating from two different regions monitored at a single observation point.

(a) The general current circulation, seagrass bed, and monitoring point



(b) Particles at observation point 96 (red – total particles, blue – particles from polygon 6)



(c) Instances with all particles at observation point 96 are coming from polygon 6 only

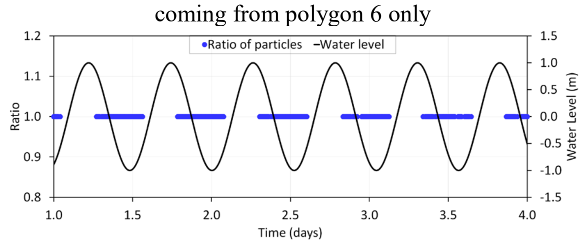


Fig. 3 Bay type 3

observed at the same point and at the same time, the information for the entire bay can be obtained from a single sampling point.

Fig. 2 shows a hypothetical situation whereby particles from two seagrass beds is monitored by sampling water at a single point. In **Fig. 2**, the particles from seagrass beds A and B appear at the sampling point at clearly separate and different times. However, predicting when particles from a certain area will appear at a sampling point is a challenge. Predictable appearance of particles at a sampling point simplifies monitoring of seagrass beds.

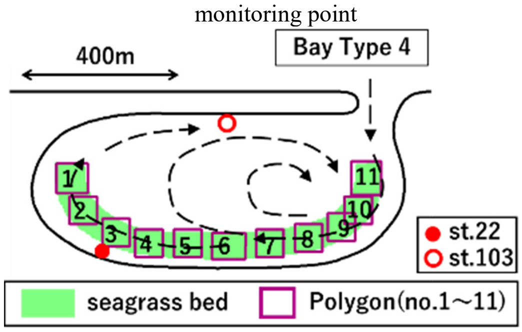
4. RESULTS AND DISCUSSION

The study monitored the transport and distribution of particles released from specific areas of the seagrass bed. **Fig. 3**, **4**, and **5** shows: (a) the general current circulation, seagrass bed, and monitoring point (b) temporal variation of number of particles, and (c) the duration of the appearance of particles. The water level in parts b and c of **Fig. 3**, **4**, and **5** is the input water level at the open boundary.

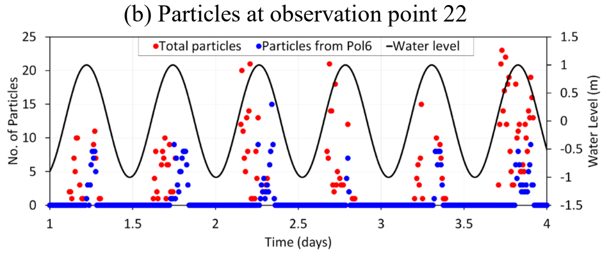
Fig. 3b, **Fig. 4**b, and **Fig. 5**b shows the time series of particle count at a particular observation point. The blue particles were released from a specific area of interest, and the red particles from the rest of the seagrass bed. Particles starting from polygon 6 in Bay Type 3 (**Fig. 3**), continue to be observed for a half of tidal cycle at observation point. 96. However, only observation points within or near the area of particles origin recorded high number of particles and/or long continuous particle appearance duration. This can be attributed to weak current flow at areas far from the bay mouth, the particles tend to remain close to the release point.

At observation point 22 in Bay Type 4 (**Fig. 4**), particles originating from polygon 6 (blue, **Fig. 4**b) alternate with the particles starting from the rest of the seagrass bed (red). The flood tide carries particles through observation point 22, including the particles from polygon 6 (**Fig. 4**b, c). Some of the particles pass through observation point 22 again during the ebb tide. The same phenomenon is observed at observation point 76 in Bay Type 8 (**Fig. 5**) with particles released from polygon 8. The particles from polygon 8 are transported through observation point 76 during the last quarter of the ebb tide and the first quarter of the flood tide (**Fig. 5**b, c). **Fig. 4**, and **5** indicate that the same sampling point can be used to monitor multiple regions of the seagrass bed. This implies that at a single sampling point, it is possible to distinguish particles released from a specific region of the seagrass bed from the particles released from other regions of the seagrass bed.

(a) The general current circulation, seagrass bed, and monitoring point



(b) Particles at observation point 22



(c) Instances with particles at observation point 22 all coming from polygon 6

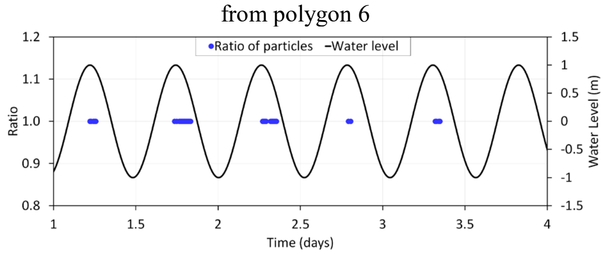
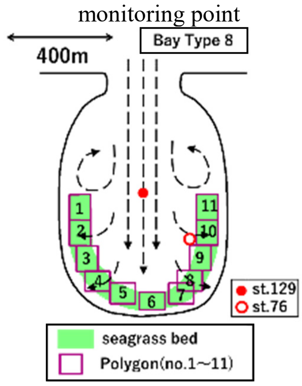
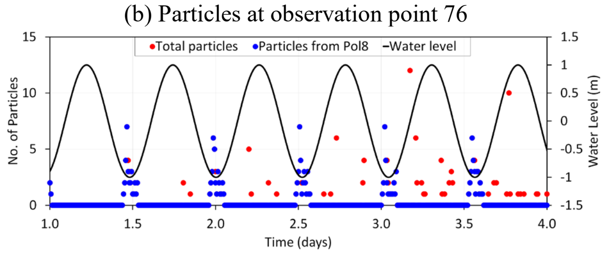


Fig. 4 Bay type 4

(a) The general current circulation, seagrass bed, and monitoring point



(b) Particles at observation point 76



(c) Instances with particles at observation point 76 all coming from polygon 8

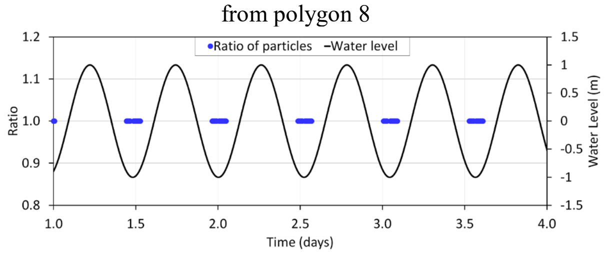


Fig. 5 Bay type 8

The particles passing through an observation point continuously for extended period makes it possible to monitor a specific region(s) of the seagrass bed. In this study, duration is the time the sampling work can be performed, and duration of at least one hour during one tide was deemed conducive. **Fig. 3**c, **4**c, and **5**c shows examples where duration of at least one hour, which in our opinion is long enough for sampling work. For Bay Types 3 and 8 (**Fig. 3**c, and **5**c) the duration of continuous particle appearance at observation points 96 and 76, respectively, is consistent and so does the timing of the appearance. The water sampling for the purposes of catching particles from polygon 6 (Bay Type 3) and polygon 8 (Bay Type 8) can be done with easy since the appearance of particles from these regions is predictable.

Fig. 6 shows the temporal variation of the number of particles released from two different areas of the seagrass bed at a sampling point. In **Fig. 6** (Bay Type 8), particles starting only from polygon 1 and 10 appeared at observation point 129 at clearly separate and different times, without mixing. This indicates that information on multiple regions of the seagrass beds can be obtained by changing the sampling time at the same sampling point.

In **Fig. 7** (Bay Type 4, St. 103), particles released from multiple polygons appeared at the same point and at same time. This shows the case where information on many regions of seagrass beds or multiple seagrass beds can be obtained at the same sampling point and at the same time. Hence, the whole bay can be monitored by sampling at one point. From the above, depending on the sampling point, the bay shape, and the location of seagrass beds, there are points that can be used for remote monitoring or to obtain the seagrass bed information of the entire bay.

In **Fig. 8**, for 10 bay types, the satisfaction of requirements for monitoring specific seagrass beds is categorized into three ranks. The requirements are the number of particles passing through a point, the duration of continuous observation of particles, and the number of seagrass beds or regions that could be monitored at a single point. Each requirement was divided into three classes; good (○), fair (△), poor (×) as shown in **Table 2**. Rank A, all conditions are met, that is, (1) duration of continuous particle appearance at observation point is greater than 2 hours, (2) the number of particles passing through the point at the time of sampling is greater than 4, and (3)

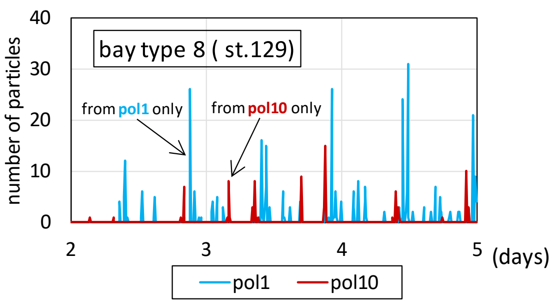


Fig. 6 Exclusive appearance of particles starting from polygons 1 or 10 at observation point 129, Bay Type 8

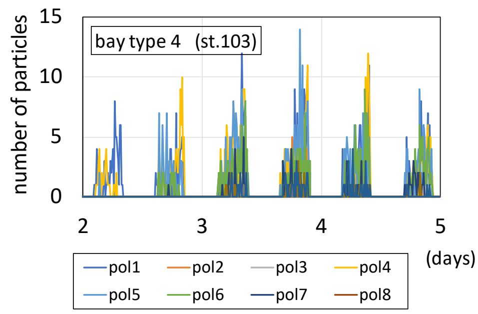


Fig. 7 Particles from multiple regions observed at point 103, Bay type 4

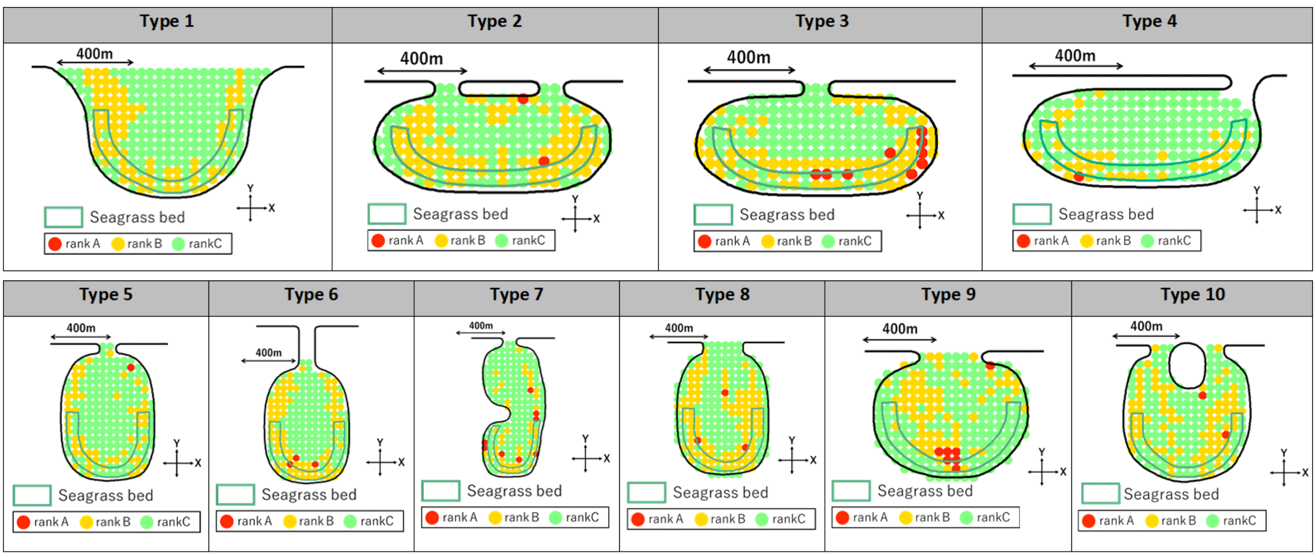


Fig. 8 Mapping of monitoring feasibility for 10 bay types

Table 2 Conditions for monitoring and ranking

Item	Time duration	No. of particles	No. of areas
Rank A	All are○		
Rank B	○ or △	○ or △	○ or △
Rank C	At least one is ×		
○	>120 min	>4	>4
△	60 – 120 min	2 – 4	1 – 3
×	< 60 min	< 2	1

the number of seagrass beds or regions that can be monitored is greater than 4. For Rank B, duration is between 1 hour and 2 hours, number of particles is between 2 and 4, and less than 4 seagrass beds or regions can be monitored. Rank C is the least of the ranks.

According to **Fig. 8**, although almost all the bays have Rank A, they have very few observation points with Rank A. Rank B is distributed in all bay types. Sampling points that meet the requirements for the monitoring a specific seagrass beds or regions of the seagrass bed can be obtained regardless of the bay shape. Therefore, the feasibility of the monitoring method was shown.

5. CONCLUSIONS

The study simulated hydrodynamics and eDNA particle tracking for ten simplified bay models. The study monitored the transport and distribution of eDNA particles released from eleven specific areas of the seagrass bed. The particle tracking simulation obtained information on many regions of seagrass bed from a single sampling point and during the same period. The particle tracking simulation can reveal the best water sampling point(s) that can provide information on the whole bay all at once or at different times. Most importantly, it will help with the determination of the best period to conduct water sampling for different goals. The simulation can also predict the source of particles and the time they are expected to pass through a specific sampling point, and whether particles from different sources will arrive together or separately. The results show the fluctuation of the number of particle with time. This is useful in the long-term monitoring of seagrass beds. A fixed observation station can be used to monitor the increase or decrease of seagrass in a specific area. Therefore, the monitoring method was shown to be feasible.

This project was designed with a goal of continuous improvement. The simulations were conducted for a few days with neither eDNA settling nor eDNA decay. It will be of interest to study the eDNA particle transport and distribution over extended periods of time considering eDNA settling and decay, and realistic bay and forcing conditions.

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