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Note

Spatio-temporal distribution of environmental DNA derived from Japanese sea nettle jellyfish *Chrysaora pacifica* in Omura Bay, Kyushu, Japan

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Abstract: We surveyed the spatial and temporal distribution of Japanese sea nettle jellyfish *Chrysaora pacifica* in Omura Bay, Japan, using an environmental DNA (eDNA) method. In 2018, the *C. pacifica* eDNA concentration increased from March–May at all depths. The seasonal pattern of *C. pacifica* eDNA was consistent with previous reports based on visual observations along the Japanese coast. Thus, the eDNA method might have advantages to follow the seasonal pattern of *C. pacifica* while being less time-consuming and less laborious compared with traditional methods. The eDNA concentrations tended to reach a maximum near and/or below the pycnocline throughout this study. Therefore, the vertical distribution of *C. pacifica* medusae may have been restricted by strong pycnocline formation in July and August 2018. However, even with a weak pycnocline, which *C. pacifica* should be able to swim across, the apparent distribution of *C. pacifica* eDNA seems to be restricted by the pycnocline. Therefore, the eDNA method cannot, currently, accurately assess the absolute vertical distribution pattern of *C. pacifica*, especially when a pycnocline is formed.

Key words: *Chrysaora pacifica*, environmental DNA, Jellyfish, Omura Bay

Reports of jellyfish blooms have increased in recent years (Purcell et al. 2007), and anthropogenic disturbances such as climate change, eutrophication, the construction of coastal artifical structures, and overfishing are considered to be potential causes (Purcell et al. 2007). Jellyfish blooms may damage fisheries, injure humans, and cause problems with cooling water intakes at coastal power plants (Purcell et al. 2007). The number of Japanese sea nettle jellyfish *Chrysaora pacifica* (Goette, 1886) might have increased in recent years in the Inland Sea of Japan (Uye & Ueta 2004, Yoshioka & Kamizono 2005). Accordingly, there are reports of severe injuries to humans caused by *C. pacifica* stings (Ikawa et al. 2016, Yoshioka & Kamizono 2005). To reduce the risk of human injuries caused by *C. pacifica*, a full understanding of the mechanism controlling *C. pacifica* blooms and the effects of environmental conditions on *C. pacifica* abundance is indispensable. To achieve this goal, there is a fundamental need for information regarding the distribution of *C. pacifica*. Unfortunately, few quantitative evaluations of the distribution of *Chrysaora* species and their dynamics have been conducted (Kinoshita et al. 2006, Toyokawa 1995) due to the methodological difficulties in observing jellyfish. Traditionally, visual observation from the ocean surface and net-sampling have been conducted to determine jellyfish distribution (Purcell 2009). However, jellyfish distribution is hard to determine by visual observations, especially in deep layers and very turbid water, and net-sampling is difficult to apply for multidepth and multipoint surveys due to it being time-consuming and laborious. In addition, net cloging by abundant jellyfish leads to the underestimation of jellyfish abundance (Brierley et al. 2001).

Environmental DNA (eDNA) is extracellular DNA that originates from organisms present in the environment; it has been used by researchers to detect the presence of macroorganisms in the environment. Because the eDNA concentration may reflect the abundance or biomass of target organisms (Yamamoto et al. 2016), the distribution of eDNA concentrations provides important information about the distribution of organisms. For example, the distribution of a fish species (Japanese jack mackerel *Trachurus japonicus* Temminck & Schlegel, 1844) was estimated using eDNA, and the eDNA concentrations were positively correlated with fish abundance, as estimated using the echo sounder method (Yamamoto et al. 2016). Minamoto et al. (2017) recently developed an eDNA quantification method for *C. pacifica*, which revealed that temporal fluctuations in the eDNA concentration of *C. pacifica* were positively correlated with the number of the visually observed medusae. These technical advances pro-

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vide an opportunity to infer the spatio-temporal distribution of *C. pacifica* more easily and efficiently, especially in deep layers where visual observations are difficult.

In this study, we surveyed the distribution of *C. pacifica* eDNA as a proxy for the distribution of *C. pacifica* abundance and biomass in Omura Bay, Japan. These fundamental data will help clarify the mechanism and dynamics of *C. pacifica* blooms.

Omura Bay is located in Nagasaki prefecture, western Kyushu, Japan. The bay is connected to the open ocean by narrow channels, and water exchange between the bay and the open ocean is limited. Due to the low transparency of surface water (<5.0 m, Hamabe 2004), it is difficult to visually observe jellyfish.

We performed a monthly water sampling at station 1 (approximately 20 m depth) in the central part of the bay from March–September 2018 (Fig. 1). In addition to station 1, water samples were collected at station 0 (approximately 20 m depth) in the northern part of the bay, and stations 4 (approximately 14 m depth) and 5 (approximately 11 m depth) in the southern part of the bay in May 2019 (Fig. 1). All sampling was conducted from 8:00–10:00 am. Seawater samples were collected using a 5 L Niskin bottle (Niskin-X; General Oceanics, Miami, FL, USA) or a 3 L Van Dorn water sampler (RIGO, Tokyo, Japan) from depths of 0, 5, 10, 15, and 19 m at stations 0 and 1; from depths of 0, 5, and 13 m at station 4; and from depths of 0, 5, and 10 m at station 5. Water temperature and salinity at each sampling depth was measured with an EC meter (EC300A; YSI, OH, USA) from March–September 2018. The density of the seawater (σ_t) was calculated from the water temperature and salinity based on Fofonoff & Millard Jr (1983). In May 2019, water temperature, salinity, and σ_t was measured at 0.5–1 m depth intervals from the surface to the bottom with a multiparameter water quality meter (WQC-24; DKK-TOA, Tokyo, Japan). As an index of the strength of stratification, we utilized maximum σ_t ($\Delta\sigma_{t\ max}$) at each site, where $\Delta\sigma_t$ was calculated as the difference between adjacent depths in σ_t. In this study, the pycnocline was defined as the depth of maximum $\Delta\sigma_t$. Samples for dissolved oxygen (DO) analysis were poured into 100-mL BOD bottles. Samples for eDNA analysis were poured into 1000-mL polycarbonate bottles that had been washed with a commercial bleach solution.

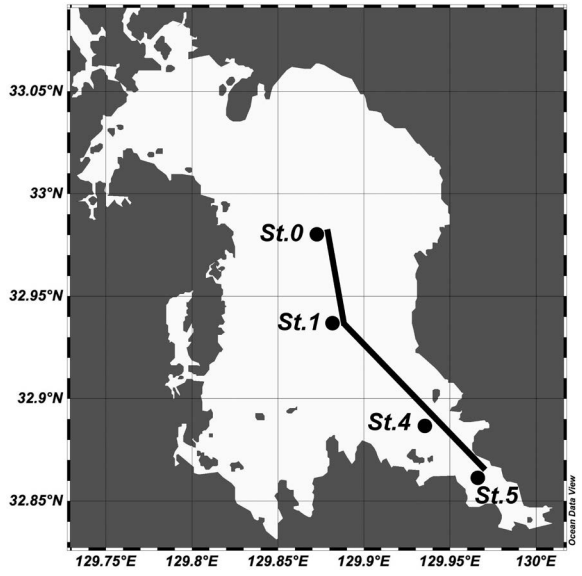

Fig. 1. The study area and sampling locations. The line indicates the axial section in Fig. 3.

The number of jellyfish medusae, including *C. pacifica*, was visually monitored by one of the authors during the water sampling (approximately 30 minutes) at each station. For each observation, jellyfish were counted within a distance of 2–3 m from the ship.

The DO concentrations were measured by the Winkler titration method (Dickson 1994) using an automated titration system (AT-710; Kyoto Electronics, Kyoto, Japan).

To determine the *C. pacifica* eDNA concentrations, 0.5–1 L water samples were filtered through 0.7-μm GF/F filters (General Electric Company, Coventry, UK). The filter funnels and measuring cylinders used for filtration were washed by a commercial bleach solution after every filtration. Additionally, 0.5 L of ultrapure water (MilliQ, Direct-Q UV3, Merck Millipore, MA, USA) was filtered through a 0.7-μm GF/F filter for use as an extraction blank.

A DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was used to extract DNA from material trapped on the filters according to Minamoto et al. (2017). Briefly, a sample filter was placed into a Salivette tube (Sarstedt, Nümbrecht, Germany). After 20 μL Proteinase K and 400 μL buffer AL (Qiagen, Hilden, Germany) were added, the tube was incubated for 30 minutes at 56℃. The liquid held in the filter was collected by centrifugation. Next, 200 μL of tris-ethylenediaminetetraacetic (TE) buffer was placed on the filter, and the liquid was again collected by centrifugation. Buffer AL (200 μL) and absolute ethanol (600 μL) were added to the collected liquid and the mixture was transferred to a DNeasy spin column. The manufacturer's instructions were followed for subsequent steps.

The eDNA concentration was quantified by a quantitative polymerase chain reaction (qPCR) using a MyGo mini Real-Time PCR system (IT-IS Life Science, Cork, Republic of Ireland) according to Minamoto et al. (2017). Briefly, each reaction contained 900 nM primer and 125 nM TaqMan Probe in a 1× TaqMan gene expression master mix (Thermo Fisher Scientific, MA, USA) and 2 μL of DNA sample to reach a final volume of 13 μL. Cpa_COI_F and Cpa_COI_R primers and a Cpa_COI_P probe were used for the quantification of *C. pacifica* mitochondrial DNA.

Dilution series containing 4×10^1 to 4×10^4 copies per PCR tube were prepared and used as quantification standards. Cytochrome c oxidase subunit I (COI) DNA sequences were cloned into pUC57 plasmids and amplified to obtain a standard curve. The qPCR conditions were as follows: 2 minutes at 50℃, 10 minutes at 95℃, 45 cycles of 15 seconds at 95℃, and 60 seconds at 60℃. Two replicates were used for samples collected in 2019, and a negative control containing ultrapure water (UltraPure DNase/RNase-Free Distilled Water; Invitrogen,

Table 1. Maximum $\Delta\sigma_t$ ($\Delta\sigma_{t\,max}$) and depth of maximum $\Delta\sigma_t$.

Year	Month	Station	$\Delta\sigma_{t\,max}$ and depth (m)
2018	March	1	2.7 at 5
	April	1	0.6 at 5
	May	1	0.6 at 10
	June	1	0.8 at 16
	July	1	3.0 at 5
	August	1	1.4 at 10
	September	1	0.6 at 15
2019	May	0	0.3 at 9
		1	0.4 at 9.5
		4	0.4 at 1
		5	0.7 at 3

CA, USA) was included in all PCRs instead of template DNA.

In 2018, the water temperature increased gradually at all water depths from March–August. The salinity was high in the bottom layers from May–September. As a result, an obvious pycnocline was observed in July ($\Delta\sigma_{t\,max}$=3.0) and August ($\Delta\sigma_{t\,max}$=1.4, Table 1, Fig. 2a). DO in the bottom layer was low (Fig. 2b) from July–September, and hypoxia (defined as DO levels lower than 2.0 mg O_2 L^{-1}) occurred in the bottom layer in July. In May 2019, a weak thermal and salinity stratification was observed at all stations ($\Delta\sigma_{t\,max}$=0.3–0.7, Table 1, Fig. 3a). DO in the bottom layer was still high at all the sampling stations (Fig. 3b).

Although two individuals of *Aurelia aurita* (Linnaeus 1758) s.l. medusae were observed at station 4 in 2019, *C. pacifica* medusae was not observed at every sampling station. Nevertheless, *C. pacifica* eDNA was detected in most of the surface water samples (Figs. 2c; 3c). In 2018, the *C. pacifica* eDNA concentration increased from March–May at all depths, and high eDNA concentrations were observed throughout the water column in May (Fig. 3c). Although *C. pacifica* medusae were not visually observed throughout this study, the seasonal pattern of *C. pacifica* eDNA was consistent with previous reports from visual observations, which have indicated that the medusae of *C. pacifica* are most abundant in April–June in the Buzen Sea (Yoshioka & Kamizono 2005), Osaka Bay (Yamada et al. 2010), and Tokyo Bay (Toyokawa 1995) in Japan. Additionally, a previous study reported that 44% of *C. pacifica* eDNA degraded within 24 h (Minamoto et al. 2017). Thus, medusae of *C. pacifica* might be most abundant from April–June in Omura Bay, as also reported in the Buzen Sea (Yoshioka & Kamizono 2005), Osaka Bay (Yamada et al. 2010), and Tokyo Bay (Toyokawa 1995).

From June–September 2018 and in May 2019, eDNA concentrations reached the maximum near and/or below the pycnocline (Figs. 2a, c; 3a, c). The sharp pycnocline is one of the major factors that ristricts the vertical distribution of jellyfish, because they are unable to readily swim across it due to the strong buoyancy force (Suzuki et al. 2018). Assuming that a sharp pycnocline with a $\Delta\sigma_t$ maximum of >0.7 restricts the vertical distribution of *C. pacifica* medusae in a similar way as reported for *Aurelia* medusae (Suzuki et al. 2017), the vertical distribution of *C. pacifica* may also be restricted by strong pycnocline formation in July ($\Delta\sigma_{t\,max}$=3.0) and August ($\Delta\sigma_{t\,max}$=1.4, Fig. 2a). However, the pycnocline should not have been strong enough ($\Delta\sigma_{t\,max}$=0.3–0.7, Fig. 3a) to restrict

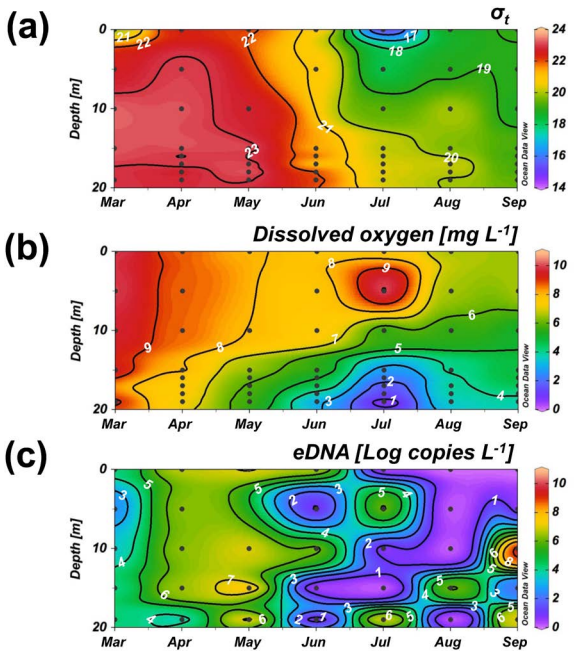

Fig. 2. Seasonal changes in σ_t (a), dissolved oxygen (b), and *Chrysaora pacifica* eDNA concentration (c) at St.1 from March to September 2018. The black dots in the graphs indicate the observation layers.

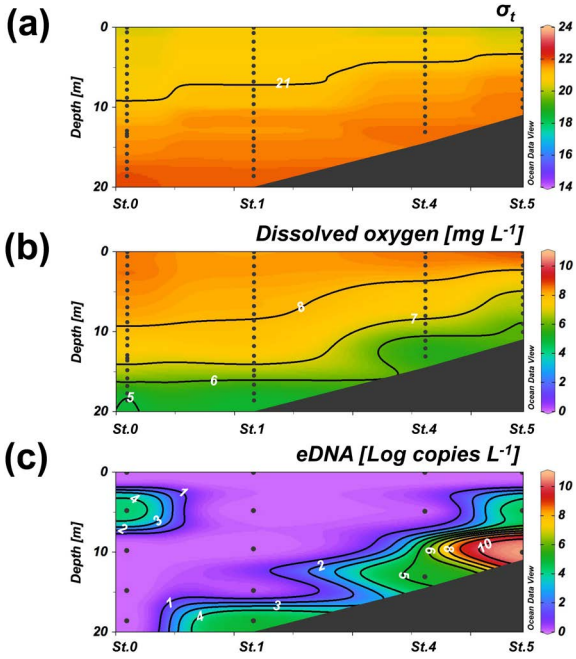

Fig. 3. Horizontal distribution of σ_t (a), dissolved oxygen (b), and *Chrysaora pacifica* eDNA concentration (c) in May 2019. The black dots in the graphs indicate the observation layers.

the vertical migration of *C. pacifica* in May 2019. This suggests that only the eDNA and not the medusae of *C. pacifica* were restricted by the pycnocline, though the possibility of pycnocline-restricted *C. pacifica* medusae distribution under these weak pycnocline conditions cannot be totally ruled out due to a lack of direct observations of *C. pacifica* medusae distribution. Further studies of the relationship between the strength of stratification and *C. pacifica* medusae distribution or *C. pacifica* eDNA dispersion and/or degradation is needed to achieve reliable estimation of *C. pacifica* medusae distribution using the eDNA method.

We found a relatively high concentration of *C. pacifica* in hypoxic conditions in July 2018 (Figs. 2b, c). This observation suggests that *C. pacifica* may survive in the bottom layer where the DO concentration is low, which might imply that *C. pacifica* has a high tolerance of low DO levels, like the medusae of *Chrysaora quinquecirrha* (Desor, 1848) (Grove & Breitburg 2005). However, *C. pacifica* may not migrate intentionally into an oxygen-depleted water mass. Condon et al. (2001) demonstrated that *C. quinquecirrha* polyps also have a high tolerance to low DO levels. Toyokawa (2011) found that *C. pacifica* polyps were attached to bivalve shells on the sea floor. Thus, the relatively high concentration of *C. pacifica* eDNA in the hypoxic conditions on July 2018 may have been derived from *C. pacifica* polyps, although data regarding eDNA release from *C. pacifica* polyps are not available.

This study was the first spatio-temporal survey of *in situ C. pacifica* eDNA. The seasonal pattern of *C. pacifica* eDNA was consistent with previous reports based on visual observations along the Japanese coast. Thus, the eDNA method might have advantages to follow the seasonal pattern of *C. pacifica* in a less time-consuming and less laborious way compared with traditional methods especially in deep layers and very turbid water. The relationship between the density of *C. pacifica* medusae and the amount of eDNA released into the seawater has been proven in a tank experiment (Minamoto et al. 2017). However, under field conditions there are many different factors that influence the distribution of eDNA. Even with a weak pycnocline, which *C. pacifica* should be able to swim across, the distribution of *C. pacifica* eDNA seems to be restricted by the pycnocline. The degradation and dilution of eDNA may also influence the estimation of the density of *C. pacifica* medusa using the eDNA method. Therefore, without visual observations, it is difficult to blindly believe the results of eDNA analysis at present. Further studies are needed to address the aforementioned points in order to achieve reliable estimation of *C. pacifica* medusae distribution using the eDNA method.

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