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Tracing Dynamics of Organic Material Flow in Coastal Marine Ecosystems: Results from Manila Bay (Philippines) and Kyucho Intrusion (Japan)

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Abstract—Organic material transfer in coastal marine ecosystems is an important physical process which often drives base production and higher trophic level interactions. However the differentiation of the source of this material can be difficult to establish. In two separate studies we utilized carbon and nitrogen stable isotope analysis to trace and understand the importance of organic matter flow and material contribution between adjacent marine pelagic ecosystems. In Manila Bay (Philippines), from February and July 2010 (representing dry and wet seasons, respectively) we performed an extensive survey throughout the bay (31 stations) and collected and analyzed particulate organic matter (POM) for stable isotope analysis. Results indicated strong correlations of carbon stable isotopes to levels of chl-*a* and nutrient loading. Nitrogen stable isotopes were less clear due to the multiple sources of N from different river systems with varying nutrient inputs. In a separate study we applied stable isotopes to examine the relationship between coastal and open-ocean organic matter during the occurrence of a mild Kyucho Current intrusion along the coast of Japan. The results from this study are presented and discussed.

Keywords: Kyucho, marine ecosystem, stable isotope analysis

INTRODUCTION

Stable isotopes of carbon and nitrogen have been shown to be effective tracers of the source of organic matter flow (Peterson and Fry, 1987), particularly within coastal ecosystems or where there are point sources of pollution which can provide a relatively high signal to noise ratio (Miller *et al.*, 2010). However the utilization of stable isotopes alone is often inadequate in tracing the ecosystem processes at play. For example physical and biological processes which occur in coastal environments can have terrestrial and marine end-members with distinctive isotope signatures, however increased δ^{13}C in diatom blooms (Miller *et al.*, 2010) or lower $\delta^{15}N$ values from N_2-fixed new nitrogen (Montoya *et al.*, 2002) can mask the origin of organic matter, thus making additional measures of environmental parameters essential in understanding the biological processes at play.

Stable isotopes are now commonly used in ecosystem studies where carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopes are measured and the stable isotope value is calculated (δ) as a ratio of the heavy to light isotope relative to a standard. Specifically, the following calculation is used: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$ where X is ^{15}N and R is the ratio of the heavy to the light isotope (Peterson and Fry, 1987). For nitrogen, at the base of the food web the source of N can have a particular $\delta^{15}N$ isotope signature with offshore ecosystems typically having a lower $\delta^{15}N$ value due to a greater contribution of N by N-fixation, whereas nearshore or littoral systems display higher $\delta^{15}N$ values due to a greater contribution of $\delta^{15}N$-enriched upwelled nitrogen (Michener and Schell, 1994). For carbon the pattern is similar in that $\delta^{13}C$ is less negative nearshore relative to offshore waters (Miller *et al.*, 2008). However the input by coastal rivers can also alter the signature with elevated $\delta^{15}N$ values associated with organic loading by sewage and subsequent denitrification (Miller *et al.*, 2010). Terrestrial-based sources of carbon are very similar to the offshore marine isotope signatures, with very low (more negative) $\delta^{13}C$ values, therefore requiring additional measures of the system (e.g., temperature, salinity and chl-*a*) to distinguish between marine and freshwater source of the organic matter.

Manila Bay of the Philippines is a semi-enclosed system with a surface area approximately 1,800 km^2 and a mean depth of 17 m (Jacinto *et al.*, 2006). Heavily relied upon for economic growth it is a center of industry and trade for the region and country as a whole, yet it is also heavily relied upon for commercial and subsistence fisheries and aquaculture. The mouth of the bay faces the South China Sea to the southwest, with Corrigedor Island in the center, with the eastern side of Manila Bay consisting of Metro-Manila (MM), a large city consisting of about 11 million people. The MM area is sandwiched between Manila Bay and a large freshwater lake (Laguna Lake) which is connected by the Pasig River. The Pasig R. is heavily polluted and contributes to a large amount of the freshwater input to Manila Bay. To the north and northwest of MB is the Pampanga River, which drains a large portion of central Luzon that largely consists of agriculture. Therefore the system consists of several major sources of organic matter flow that may contribute to production in the bay, but also may be detrimental by over-eutrophication. To examine these inputs, stable isotope analysis can, with additional environmental parameters (salinity, temperature, chl-*a*), help elucidate the relative inputs of urban, agricultural and marine-derived organic matter to the system. This can theoretically be done by considering Manila Bay as a large "mixing bowl" of marine and terrestrial-derived sources with their associated isotope values, and using the isotope values of the sources to come up with some estimation of relative contributions to the mixer.

Kyucho is known as a sudden and swift current of higher temperature water from the Pacific Ocean (Kuroshio Current) which is intruded into the Bung Channel (Takeoka and Yoshimura, 1988; Takeoka *et al.*, 1992), a major entrance between the Pacific Ocean and the Seto Inland Sea. Kyucho almost entirely occurs in summer months, and is mostly associated with the neap tide (Takeoka *et al.*, 1992). Kyucho events have been known by fishers and it has become of particular interest because of it's inherent ties to the Kuroshio Current and potentially climate change. Kyucho events are therefore perturbations in the system that are more like "pulse events" which can dramatically alter the characteristics of the plankton community through material transfer. The mass transfer of material from an external source such as what may occur through Kyucho may be a form of donor control where the local system is the receiving end of outside sources of organic material. The application of stable isotopes to these pulse events may be an effective method to trace the flow of organic matter between systems (offshore to inshore), because the end-member sources would presumably have very different δ^{15}N and δ^{13}C signatures. Determining whether organic matter is effectively transferred during Kyucho events is an essential component for accurate flow models and further hypothesis testing.

In this study we apply stable isotope analysis to two separate ecosystem-scale studies to examine the importance of inter-ecosystem transport of organic material and zooplankton through coastal advective processes. The first examination is the use of δ^{13}C and δ^{15}N to examine sources of organic matter within Manila Bay, Philippines. The second study is the tracing of organic matter during Kyucho intrusion into the Bungo Channel, Japan.

METHODS

Field collection

Manila Bay

Between 12 and 15 July 2010 a 31-station survey of Manila Bay was performed to examine the distribution of nutrients and environmental parameters that may be associated with DO conditions (Fig. 1). All sampling of the bay occurred from a Bureau of Fisheries and Aquatic Resources (BFAR) vessel in collaboration with University of the Philippines Diliman Marine Science Institute (MSI). Each station was measured for environmental parameters of DO, temperature, salinity, and nutrients. For stable isotope analysis of particulate organic matter (POM), surface water (upper 1 m) was collected and filtered onto a 0.2 um mesh glass fiber filter (GFF) until clogged. The filter was maintained under cool conditions (up to 48 hrs) and eventually dried at approximately 50°C for 24 hrs. A total of stations were sampled for stable isotopes within Manila Bay. River samples were also collected from 3 river sites representing levels of nutrient sources such as predominantly natural (near Ternate), urban (Pasig R.) and agricultural (Pampanga R.). The same methods for collection of Manila Bay samples were applied to rivers.

Kyucho

Between 17 June and 7 July 2010 we performed four sampling surveys (June 17, 19 and 29, and July 7) from pre-determined stations within the Bungo Channel (Fig. 2), which covered a period just prior, during and after occurrence of a

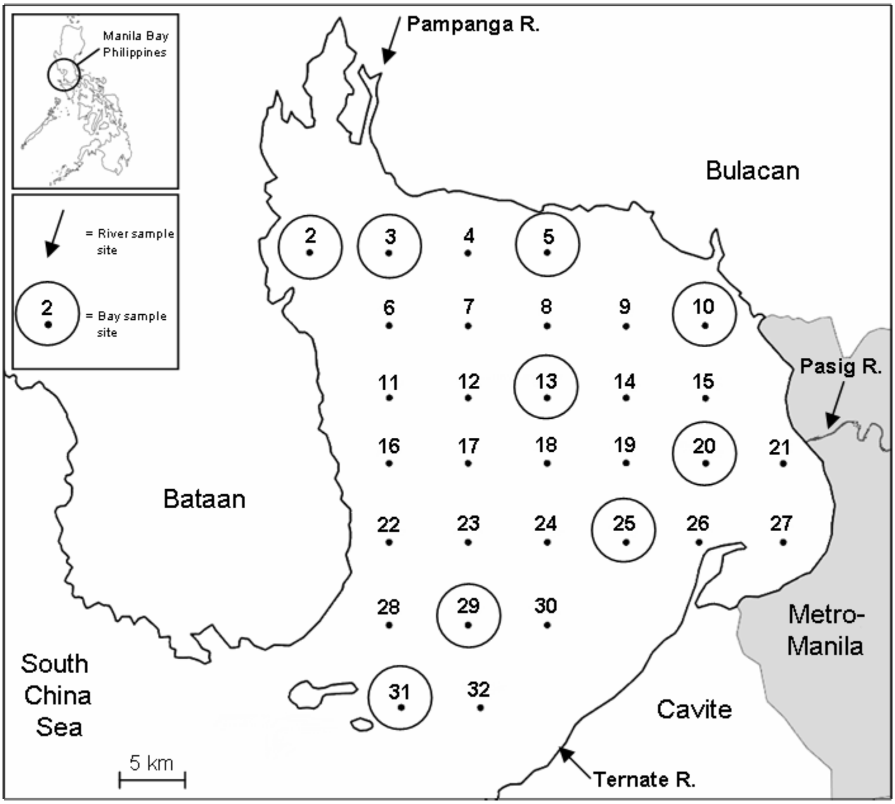

Fig. 1. Map of Manila Bay (Philippines) showing all stations (all numbered sites) and sample sites for stable isotope analysis of river (arrows) and bay (circled numbers) sites.

Kyucho event. The basic principal of this sampling plan was to capture progression of a Kyucho event and measure the corresponding environmental and organic matter isotopic shifts in the water mass. We sampled POM for stable isotope analysis using the same method described for Manila Bay. We also collected copepods for stable isotope analysis from the same stations and periods however results are not presented here.

Laboratory analyses

Stable isotope and data analysis

Stable isotopes were measured for δ^{13}C and δ^{15}N at the Center for Marine Environmental Studies, Ehime University (Japan) using a Carlo Erba Elemental Analyzer 2500 coupled to a Finnigan MAT Delta Plus stable isotope ratio mass spectrometer via a ConFlo-III continuous flow interface (measurement error for δ^{15}N and δ^{13}C ± 0.3‰). Calculation of the ratios relative to the standard for

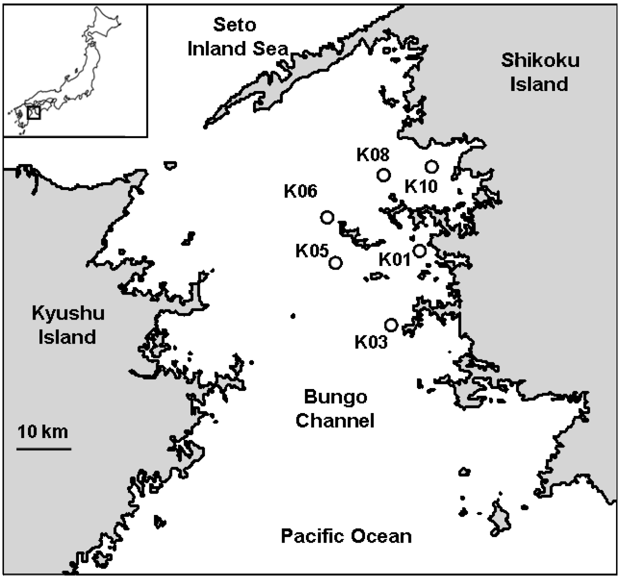

Fig. 2. Map of the Bungo Channel showing main sampling sites (circles, K01, 03, 05, 06, 08, 10) for the Kyucho event.

nitrogen (N_2) was done by the standard equation $\delta X = \{(R_{sample}/R_{standard}) - 1\} \times 1000$, where R is the ratio of the heavy to light isotope for the sample (R_{sample}) and standard ($R_{standard}$) in units of parts per mil (‰).

For the Manila Bay data we used principal components analysis (PCA) using variables $\delta^{13}C$, $\delta^{15}N$, temperature, salinity, and chl-*a*. We also applied linear regression methods to examine trends between the isotope and environmental data. For the Kyucho data we similarly applied linear regression analysis to examine stable isotopes with environmental parameters associated with Kyucho shift.

RESULTS AND DISCUSSION

Manila Bay

From the 9 Manila Bay stations sampled for stable isotope analysis, the range of surface salinity was from 28.4–33.3 ppt, indicating low freshwater/terrestrial input at the time of this study. Stable isotope values of $\delta^{13}C$ and $\delta^{15}N$ of POM varied considerably throughout the bay with no discernable trend between environmental variables and stable isotopes. The PCA was only able to explain ~20% of the total variability. The absence of a correlation may be due to two major reasons: the lack of a strong terrestrial input which is confirmed by high salinities, and the fact that high circulation in the bay combined with possible benthic-pelagic coupling in the shallow waters may allow for quick and thorough

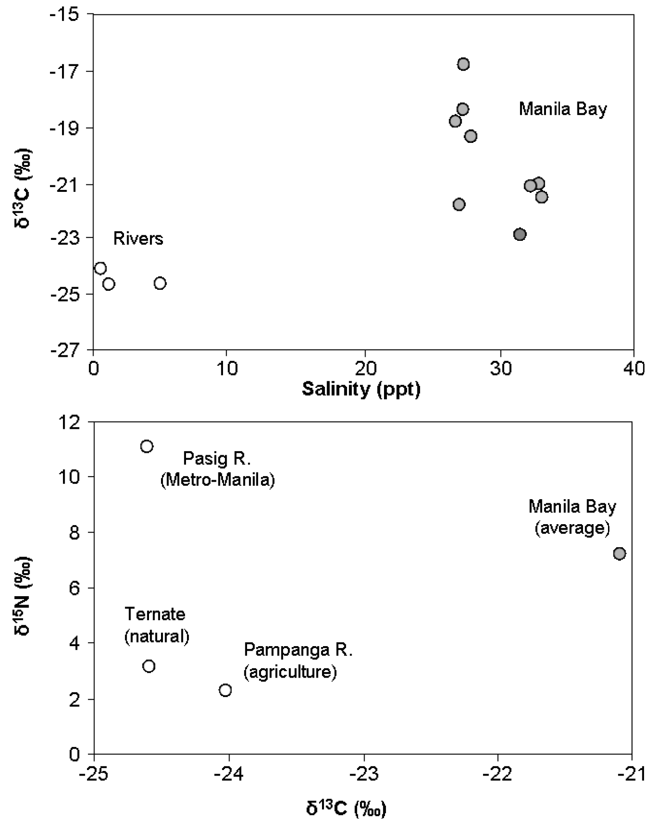

Fig. 3. Mean carbon and nitrogen stable isotope values from Manila Bay (all stations combined) and three rivers flowing into the bay.

mixing of organic matter throughout much of the bay.

Results from sampling river systems did show major differences in δ^{13}C and δ^{15}N with the river "type" and between rivers and Manila Bay (Fig. 3). Specifically, the Pasig R. which is most urban displayed very high δ^{15}N values (approximately 11‰) indicative of sewage and denitrified N, and the Pampanga River was very low in δ^{15}N indicative of more agriculture sources. Considering the high salinities of Manila Bay during the time of this study the system was largely marine. However, a mean δ^{15}N of ~7.2‰ of POM from Manila Bay suggests significant input from urban/industrial sewage because mean δ^{15}N values of Manila Bay were intermediate to values from the Pasig River (δ^{15}N ~11‰) and other more agricultural river sources (<4.0‰), as well as from marine waters (~5–6‰, Miller *et al.*, 2010).

During the time of this study the Philippines was experiencing drought conditions and therefore we would expect high salinities and relatively lower terrestrial inputs to Manila Bay. The absence of strong haline stratification within the bay likely allowed for greater benthic-pelagic exchange and with tidal and wind mixing, created fairly well-mixed conditions both vertically and horizontally. During the study we were able to collect water samples for $\delta^{18}O$ analysis from the same river and Manila Bay sites. Future research will involve analysis of $\delta^{18}O$ samples, which may help discern the relative contribution of water from different river systems (natural, urban and agricultural) even when inputs are relatively low. Future research is needed to compare more between wet and dry periods to examine seasonal effects. During wet periods inputs would be stronger and would undoubtedly be expressed in the stable isotope values of POM.

Kyucho

Results from monitoring Kyucho intrusion into the Bungo Channel showed a very clear shift in increasing temperature and decreasing salinity between 17 June and 7 July (Fig. 4). POM samples also showed a corresponding shift that was linearly correlated to a major shift in higher (less negative) $\delta^{13}C$ and lower $\delta^{15}N$ (Fig. 5). The increase in salinity and increase in $\delta^{13}C$ were unexpected, and this was mainly because we expected higher salinities with offshore intrusion, and lower $\delta^{13}C$ values associated with more offshore production. Further review of a satellite-derived chl-*a* data map (provided by Professor A. Isobe, CMES) indicated intrusion by Kyucho at the time of this study likely entrained a river plume from Kyushu Is. This would explain the sudden decrease in salinity and particularly the higher $\delta^{13}C$ values in POM that are often associated with new diatom production in the terrestrial-derived nutrients which mixed with offshore water intruding into the Bungo Channel. The decrease in $\delta^{15}N$ would explain the offshore source of N, however it is apparent that the low $\delta^{15}N$ and high $\delta^{13}C$ is indicative of significant mixing.

Additional study of Kyucho events is warranted and the use of stable isotopes may be beneficial in discerning dynamics of organic matter transfer in this process. Future work will involve analyzing copepods that were collected from the same sampling events to see if similar trends occur to the POM. Because pulse events such as Kyucho are strong currents that can, as shown here, entrain and transfer organic matter at great distances, it is possible this would have an impact on the larval and juvenile stages of fishes. Early life stages of fishes which consume POM (diatoms) and copepods may benefit from the added production through Kyucho entrainment of the river plume from Kyushu. This may be visible in $\delta^{15}N$ and $\delta^{13}C$ values of larval fishes because of their very high growth rates which are the primary source of isotope expression in poikilotherms (Miller, 2006). Furthermore, tracing the potential intrusion of offshore fishes may be done by examination of $\delta^{15}N$ and $\delta^{13}C$ of the larval fish community using the same sampling methods discussed here. We did not do this due to logistical constraints of ship time and the number of sites. Finally the use of oxygen isotopes ($\delta^{18}O$) would be an additional benefit in determining the extent of mixing between the offshore surface, deep, nearshore surface and deep, as well as the proportion from river sources.

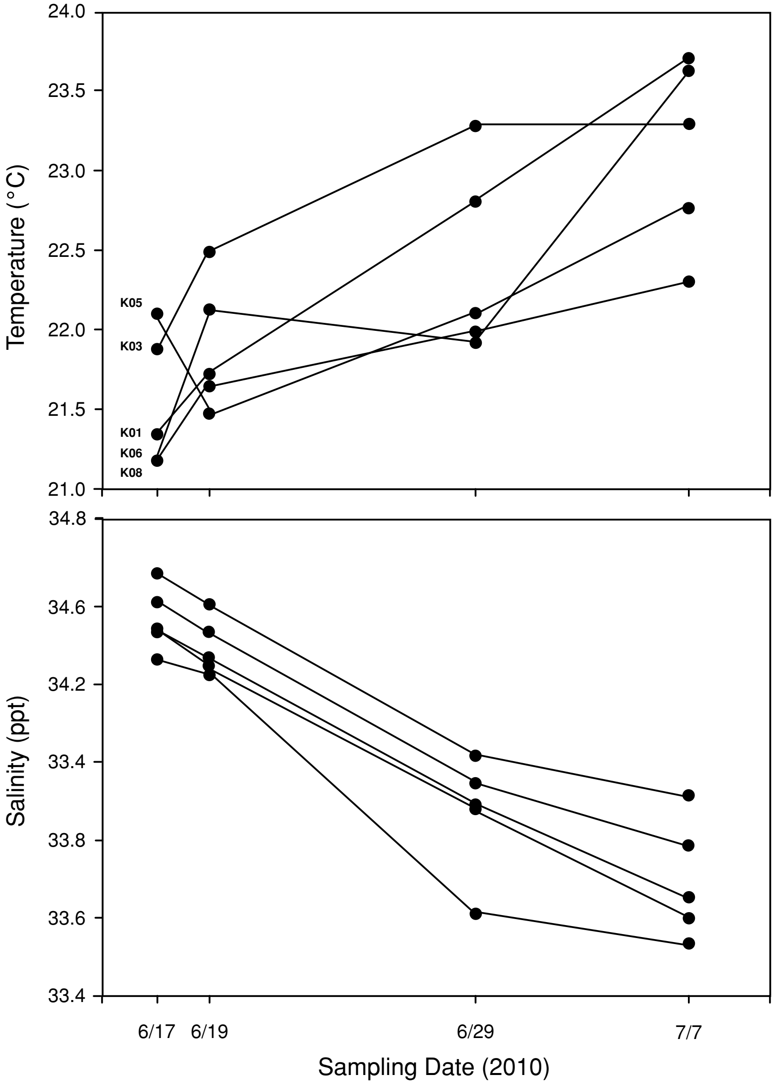

Fig. 4. Sea surface (3 m) temperature and salinity of sampling stations during temporal shift in a Kyucho event.

In summary we examined the use of stable isotopes in tracing organic matter flow in two coastal systems representing the river-estuary of Manila Bay, and the more coastal-oceanic system of the Bungo Channel, Japan. Results from Manila Bay showed rivers were all very low in δ^{13}C relative to the bay, but that the rivers differed markedly in their δ^{15}N values which were largely associated with their individual anthropogenic loads. The Pasig R. expressed the highest d^{15}N values,

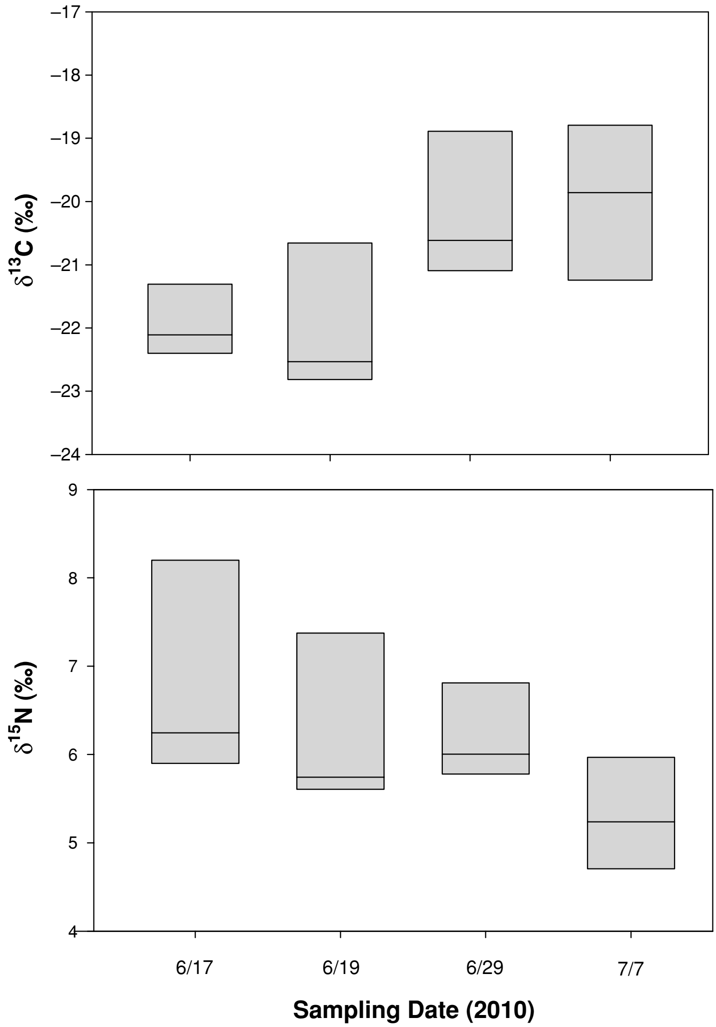

Fig. 5. Box plot of stable isotope (δ^{15}N) values from POM of 5 stations. Boxes denote the upper and lower 25% with midline as the mean.

with more agriculturally based Pampanga R. having very low values. Within Manila Bay, despite the absence of large salinity gradients, much of the bay's nitrogen is from terrestrial sources, specifically the Pasig R. and other similar rivers with high anthropogenic nutrient loading. Results from off the Pacific coast of Japan with the progression of a Kyucho event showed a distinct shift in increasing δ^{13}C and decreasing δ^{15}N. The unexpected shift in δ^{13}C with offshore intrusion was unexpected however after examination of chl-*a* and salinity data we were able to conclude that Kyucho intrusion retained and mixed with water from another coastal system that contained riverine water. This result underlies the importance of including multiple data sets to interpret stable isotope results.

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