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Effects of causeway construction on environment and biota of subtropical tidal flats in Okinawa, Japan

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ABSTRACT

Okinawa, Japan is known for its high marine biodiversity, yet little work has been performed on examining impacts of numerous large-scale coastal development projects on its marine ecosystems. Here, we examine apparent impacts of the construction of the Kaichu-Doro causeway, which was built over 40 years ago. The causeway is a 4.75 km long embankment that divides a large tidal flat and has only two points of water exchange along its entire length. We employed quadrats, transects, sampling, visual surveys, and microbial community analyses combined with environmental, water quality data, and 1 m cores, at five stations of two paired sites each (one on each side of Kaichu-Doro) to investigate how the environment and biota have changed since the Kaichu-Doro was built. Results indicate reduction in water flow, and site S1 was particularly heavily impacted by poor water quality, with low diversity and disturbed biotic communities.

1. Introduction

The coral reefs, tidal flats, and other coastal ecosystems of southern Japan are not only known for their high biodiversity but also for the critical threat these ecosystems face from coastal development and overexploitation (Roberts et al., 2002). Some recent studies have suggested that when combining biodiversity levels and effects of human impact, efforts to protect and properly manage southern Japanese ecosystems are more urgently needed than anywhere else on the planet (Roberts et al., 2002). Despite their high diversity, many of the coastal areas of this region, primarily the southern half of the main island of Okinawa, have been degraded or destroyed by landfill, construction of shore protection and roads, dredging and harbors, and terrestrial outflow of soil (McCormack, 1999; Coral Reefs of Japan, 2004). Negative human impacts have been documented dating back to the 1930s and 1940s, when an undescribed endemic clam species in the genus *Meretrix* went extinct on the Sashiki tidal flats of Okinawa Island (Fig. 1a) (Yamakawa, 2014).

Over the past four decades, environmental rules and regulations for large-scale developments have become stronger in Japan. Despite legal strengthening, compared to many Western countries there are weak points with the Japanese assessment system. For example, EISs are performed by the project proponents and not by an independent agency (Environmental Impact Assessment Division, 2012). Additionally, assessment results from many past (pre-1999) projects have not been released to the public. Public hearing sessions before construction and formal post-construction assessments (=Impact Mitigation Reports) have only been required since 2012 and 2013, respectively (Environmental Impact Assessment Division, 2012).

The result is a lack of scientific literature and validation on the impacts of coastal development on neighboring marine ecosystems

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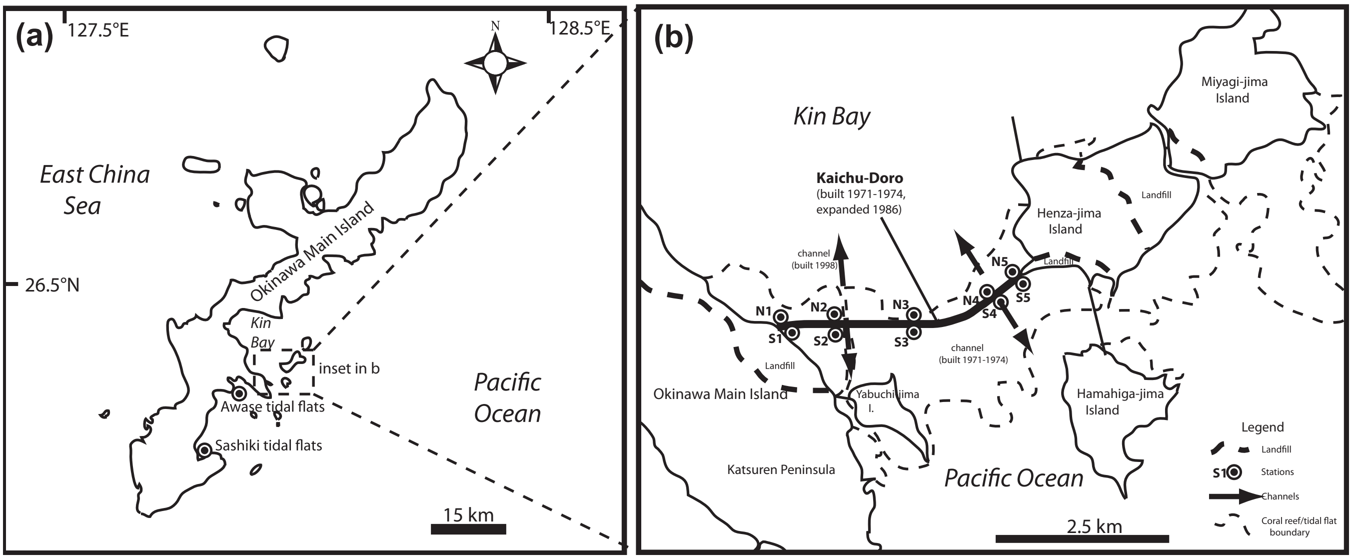

Fig. 1. Map of (a) Okinawa Main Island, and (b) the study location at Kaichu-Doro. Other tidal flats mentioned in text also shown in (a). Circles show sites investigated in this study with name (N1, S1, etc.). Arrows indicate channels connecting two sides of the Kaichu-Doro. Large dashed lines indicate areas of landfill, narrow dashed lines extent of tidal flats.

in Japan as a whole and in Okinawa in particular. Furthermore, there are very few examples in the literature discussing post-construction assessment of causeways on subtropical or tropical tidal flat ecosystems. Focused research from developed and developing countries in the Asian-Pacific are therefore urgently needed to provide information and data for the impact of planned and future coastal development projects, especially given the fast economic development of this region.

One example of large-scale coastal development/reclamation on the main island of Okinawa in southern Japan is the 4.75 kilometer-long "Kaichu-Doro" causeway in Uruma City (Fig. 1b) (literally "Road in the Sea", hereafter called Kaichu-Doro). Kaichu-Doro connects Katsuren Peninsula of Okinawa Main Island to the smaller Henza-jima Island across extensive subtropical tidal flats that stretch between the two islands (Fig. 1b). There are three other small, inhabited islands joined by bridges to Henza-jima Island (collectively called the Ikei Group), and Kaichu-Doro is the only link for all of these islands to Okinawa Main Island. Overall, greater than 35% (237/609 ha) of the tidal flats around Katsuren Peninsula and the Ikei Group have been lost to land reclamation (Uruma City Cultural Sea Museum, 2007).

Kaichu-Doro is a causeway built up from the tidal flats as a large embankment. Water can flow between the north and south sides through only two channels where there are bridges. The eastern channel is shallow and exposed at low tide, while the western channel is deep enough (approximately 5 m) for small boats to pass. Aside from these two channels, the north and south sides of the tidal flat have been effectively divided into two separate flats. The northern side faces Kin Bay, and the southern side is bound by Katsuren Peninsula of Okinawa Main Island, Yabuchi-jima Island and Hamahiga-jima Island (Fig. 1b).

Before construction of Kaichu-Doro, locals either moved goods to and from the Ikei Islands by boat, or crossed the tidal flats on foot during low tides. Early attempts at making a causeway were started in 1960 during the post-World War II American occupation of Okinawa, but these efforts were unsuccessful, with portions being swept away during large typhoons in 1961. Large-scale construction of the current Kaichu-Doro was conducted between 1971 and 1974 and paid for by the Gulf Oil Company (now merged with Chevron) to link its oil base on Henza-jima Island with the main island. Originally a two-lane road, it was upgraded to a four-lane road in 1999. No pre-construction environmental assessment data for this project are publically available.

Since construction of Kaichu-Doro, anecdotal evidence suggests the marine ecosystems on each side of the road have diverged from the other (e.g. McCormack, 1999 and references within). Areas near the Katsuren Peninsula, particularly on the south side of Kaichu-Doro, have been apparently negatively impacted from sewage/pollution runoff (Seki, 2001), a loss of water circulation (Saji and Seki, 2001), red tides (Saji and Seki, 2001), and landfill (in 1986; Fig. 1b). The western channel was constructed from 1986 to 1998, with the hope that it would rejuvenate the area by increasing water flow, but locals believe it has resulted in more mud in the area, and that this degraded southern area has less sand now than prior to construction (I. Maeda pers. comm.). Pre-Kaichu-Doro, the tidal flats were used by local people for small-scale fisheries, but local fishermen have not harvested from this area for approximately 20 years (I. Maeda pers. comm.). Fishermen have noted the decline and/or local extinction of the Japanese tiger prawn (*Marsupenaeus japonicus*), flounders (Paralichthyidae, Bothidae), gobies (Gobiidae), and other commercially important marine organisms, and there has been a decline in benthic animals in the area (Henza Jichikai, 1985), although no hard data are publically available. Locals also believe that other areas along the Kaichu-Doro further away from Katsuren Peninsula, such as the middle area (around sites N3 and S3; Fig. 1b), are in better health (I. Maeda pers. comm.). Seasonal, local harvesting of *Abdopus aculeatus* octopus occurs in this area during low tides (Uruma City Cultural Sea Museum, 2007).

In this study, we aimed to examine the impacts of the construction of Kaichu-Doro on the surrounding tidal flats by asking and investigating the following questions:

1. Can the environment and ecosystem of the pre-construction tidal flat ecosystem be estimated? Can impacts of construction on the environment and/or ecosystem be seen along either or both sides of Kaichu-Doro?
2. Do differences exist in the present environment and biota between the two sides of the Kaichu-Doro tidal flat?

To answer these questions, we utilized one meter cores and data acquired from them (sediment size and composition, sediment and hard coral dating with isotopes, hard coral and

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foraminifer diversity). Permanent transects and quadrats were utilized at five paired locations on both sides of the road to observe and record the diversity of a variety of taxa, including fish, echinoderms, amphipods, and macroalgae. We also examined the relative diversity of bacteria and eukaryotes from both seawater and sediments utilizing molecular methods. Amounts and description of shoreline garbage were also recorded. Finally, various environmental data including water temperature, water current, and seawater quality (including salinity, dissolved oxygen content, particulate organic matter, turbidity, conductivity, pH, phosphate, nitrite, nitrate and ammonium levels) were obtained from each site. In this study, we examine the results of our multi-disciplinary survey to draw conclusions on the possible impacts the construction of Kaichu-Doro has had on the biodiversity of the surrounding tidal flat.

2. Materials and methods

2.1. Station locations and experimental design

Paired sites at five stations (sites $n = 10$ total) were arranged along the Kaichu-Doro from west to east, numbered N1 (north 1) and S1 (south 1) to N5 and S5 (Fig. 1b). The paired sites were directly opposite one another on each side of the road, and were separated from each other by distances of 96–167 m (Table 1). Sites were placed at least 10 m away from the edge of Kaichu-Doro to ensure that sediment cores (see below) could be taken from areas undisturbed by the road's construction. In this study, 'station' refers to paired sites (e.g. station 1 = sites N1 + S1), while 'site' refers to a single or specific location.

At each site, a permanent 5 × 5 meter (=25 m^2) quadrat was placed. Environmental data and most taxa data were recorded from each quadrat. Cores were also collected from within 5 m of the edge of quadrat.

The various surveys (described in detail below) were conducted in October to November 2011 (autumn), and most environmental data were also obtained at this time, with water current data acquired in 2012.

2.2. Environmental data

2.2.1. Seawater conditions

Parameters for analyzing seawater conditions were nutrients (NO_2–N + NO_3–N, PO_4–P and NH_4–N), particulate organic matter (POM), dissolved oxygen (DO), pH, salinity, seawater temperature, turbidity, and flow intensity (current).

Methods for analyzing nutrients, POM, DO, pH, and salinity followed Yang et al. (2013). Turbidity was measured using the same method as pH, salinity and DO, with a multi-parameter water quality meter (model WQC-24, DKK-TOA, Tokyo). All samples from 10 sites were sampled on November 17–18, 2011.

Seawater temperature was logged every 30 mins at each site/station from November 17–30, 2011 using HOBO water temperature data loggers (model U22-001, Onset, Massachusetts, USA).

Differences in water motion among sites were compared by analysing differences in dissolution rate of two plaster balls (10.5 cm diameter) (Komatsu and Kawai, 1992) placed 10 cm above the substrate at each site for the period of November 15–23, 2012. As the sites were intertidal, we did not calculate water flow speed as in previous studies for subtidal research (e.g. Yang et al., 2013), but instead used the differences in dissolution weights as comparative data.

2.2.2. Garbage

The amount and diversity of garbage was assessed at each site by counting all garbage within 10 × 2 m transects set parallel to the coastline directly in front of each quadrat at the high tide line. All garbage items within each transect were within transects were counted, sorted into categories (e.g. plastics, metals, glass, others), dried at 40 °C for 24 h and dry weight was estimated in grams.

2.3. Biota sampling/observation

For echinoderm information see Supplementary Text 1.

2.3.1. Fish

Visual fish surveys were conducted on October 22, 2011 at all sites except for N2 and S2, due to boats using the channel at this station by 50 m transects placed perpendicular to Kaichu-Doro (e.g. heading directly out from the coastline). All transects started at the shoreline within 10 m of the permanent quadrats.

All fishes within 0.5 m of the transect line were counted and identified to lowest possible taxonomic level by two observers while snorkeling.

2.3.2. Amphipoda

Amphipods were collected from sediment and coral rubble samples at the 10 sites along Kaichu Doro on November 17, 2011. Following the methods of White (2013), a 12-liter bucket was filled one fourth full with sediment from just outside each permanent quadrat and filled halfway with water from the sample site. The lid was kept on the bucket to prevent loss of specimens during collection. Coral rubble was added to the bucket at each quadrat until the bucket was full.

Immediately after collection at the shore, approximately 10 ml of formalin was added to each bucket. After five minutes, the contents of the bucket were stirred and poured through a 500 μm mesh sieve. New seawater was added to the bucket and the contents were stirred and sieved again; this was repeated seven times per bucket.

The contents of the sieve were then transferred to a vial and preserved in 99.5% EtOH. The next day samples were

Table 1
Locations and general information on sites examined in this study. Site numbers match those in Fig. 1b.

Site number	Latitude	Longitude	Distance from paired station (m)	Substrate	Other notes
N1	26°19′54.90″N	127°54′25.30″E	140.2	Sand, small rubble	Human influence?*
S1	26°19′51.10″N	127°54′22.50″E		Mud/sand, some small rocks	Runoff outflow
N2	26°19′55.70″N	127°54′52.50″E	96.3	Rubble	
S2	26°19′52.70″N	127°54′51.50″E		Rubble	
N3	26°19′58.20″N	127°55′52.20″E	167.6	Seagrasses, sand	Human influence?*
S3	26°19′52.80″N	127°55′53.10″E		Mud, macroalgae	
N4	26°20′9.80″N	127°56′20.80″E	113.5	Macroalgae, rubble	
S4	26°20′7.10″N	127°56′23.50″E		Rubble	
N5	26°20′25.60″N	127°56′42.40″E	168.9	Sand, rubble	
S5	26°20′20.20″N	127°56′42.90″E		Sand/mud, rubble	

* Sites N1 and N3 are popular with beach-goers, and occasionally and irregularly cleaned of garbage, which may influence results of garbage surveys.

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rough sorted and all amphipods were separated from the collection, and identified to morphospecies at the family level and counted.

2.3.3. Macroalgae and seagrasses

All macroalgae (green algae, red algae, brown algae) and seagrasses appearing within designated locations within the 5 × 5 m quadrats for all ten sites were identified to lowest possible taxonomic level by two snorkeling observers. The designated locations were 1 × 1 m sub-quadrats located at each corner of the 5 × 5 m quadrat ($n = 4$) plus one centrally located sub-quadrat (total $n = 5$ sub-quadrats). Numbers or relative cover of macroalgae and seagrasses were not recorded. The survey was conducted on November 17, 2011.

2.3.4. Microbial community (Eukaryota and Bacteria)

Total DNA was isolated from sediments and seawater at each site. Sediment samples were collected on November 17, 2011 using sterilized 50 ml falcon tubes in two different locations within each quadrat and then placed in a cooler box with dry ice and transported to the laboratory (transportation time < 1 h). DNA was extracted from 1 g of sediment using a PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, California, USA) following the manufacturer's instructions. For seawater samples, 10 l of seawater was collected at each sampling point during high tide on November 17, 2011. 2 l of seawater was filtered using Supor 200 S-Pack Membrane Filters with a pore size of 0.2 μm (Pall, Port Washington, New York, USA) in the laboratory. DNA was extracted from the filter using a modified method of the WaterMaster™ DNA Purification Kit (Epicentre, Madison, Wisconsin, USA) protocol.

For analyses of community structures, we performed denaturing gradient gel electrophoresis (DGGE) and amplicon pyrosequencing of eukaryotes, and bacteria. To create amplicons, we used 10 ng/μl of template DNA and amplified the target region of 16S and 18S rDNA using Phusion DNA Polymerase (New England BioLabs, Ipswich, MA USA). Primer pairs used in PCR reactions are listed in Table S1. PCR amplification for DGGE was performed by touchdown PCR with a decrease in annealing temperature of 1 °C for every cycle down from an annealing temperature 72 °C to 62–68 °C (depending on domain, see Table S1). For tag sequencing, DNA was amplified following the manufacturer's protocol with annealing temperatures as listed in Table S1. After cleaning PCR products using FastGene Gel/PCR Extraction Kit (Nippon Genetics, Tokyo Japan), an appropriate amount of DNA was used for DGGE (Table S1). DGGE was carried out using DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA USA). We used 8% acrylamid gels containing denaturant gradient of 30–45% and 40–60% for eukaryotic and bacterial sequences, respectively. Gels were stained with CYBR® Gold Nucleic Acid Gel Stain (Invitrogen, Carlsbad, CA USA) for 30 minutes. Digitized DGGE gel images were analyzed on GelCompar II (Applied Maths, St-Martens-Latem, Belgium). We visually checked band patterns to remove false-positive bands after automated band calling. The analysis parameters including background subtraction and similarity coefficient were optimized for each gel. Amplicon sequencing was applied to seawater and sediment samples from station 1 and sediment samples from station 2. Sequencing was performed on a Roche Genome Sequencer FLX + System by Hokkaido System Science (Sapporo, Hokkaido, Japan). Quality-checked reads were analyzed using the QIIME 1.8.0 package (Caporaso et al., 2010).

Cluster analyses were performed with Dice similarity resulting in the forms of an unweighted pair group method using an arithmetic averages (UPGMA) dendrogram.

3. Cores

3.1. Collection

Sediment cores of approximately 60–80 cm length were obtained from each site at points of approximately 1 m water depth within 10 m of the 5 × 5 m quadrats (Table S2, Fig. 2a). Sampling of cores followed the methods of Adachi et al. (2010). The sediments of cores mostly consisted of sands with middle and coarse grain sizes. After obtaining cores, they were transported to the laboratory at the University of the Ryukyus and stored at −80 °C until further analyses. Cores were utilized for several different analyses detailed below.

3.1.1. Sediment size and composition

Sub-samples of the top (0–3 cm below surface) and bottom layers (Table S2, Fig. 2a) of each core were analysed for sediment mud content. After removing coarse sand and gravels using a 1-mm sieve, <1-mm fractions of the sediment samples were sifted through a 63-μm sieve. The dry weights of sediment were measured before and after the sifting. Sediment mud content was calculated as (<63-μm fraction sediment/<1-mm fraction sediment) × 100 (%, by weight).

3.1.2. Sediment dating

The lead-210 (^{210}Pb) method was attempted to estimate the sedimentation rate of the core sample collected from site S1. The method is based on the theory that introduced excess $^{210}Pb_{ex}$ to the sediment decreases with the time (Krishnaswami et al., 1971).

The frozen core was cut in half vertically using a diamond saw and 2 cm thick measurement samples were taken from five to six layers from top to bottom of the core. Samples were dried, disaggregated, passed through a 0.5 mm sieve and transferred to polyethylene containers. The radionuclides in the samples were determined using a low background germanium detector (Canberra GCW4023 well-type). The total ^{210}Pb in the samples were obtained using the 46.5 keV gamma ray from ^{210}Pb, and the ^{226}Ra concentrations were obtained using the 351.9 keV gamma ray from ^{214}Pb, a short-lived daughter of ^{226}Ra. Excess $^{210}Pb_{ex}$ in the samples were calculated by subtracting the ^{226}Ra-supported ^{210}Pb from the total ^{210}Pb concentrations.

3.1.3. Coral identification and geochemical analyses

52 samples of fossil coral gravel with a size of >1 cm³ were taken from the 10 cores for X-ray diffraction (XRD) and stable carbon and oxygen isotope analyses. The samples were cut using a diamond saw and inner fragments with relatively well-preserved skeleton were taken and then cleaned ultrasonically in ultrapure milli-Q water (18.2 MΩ). The fragments were dried in an oven at 60 °C for one day and ground to a powder. The powder samples were used for XRD and stable carbon and oxygen isotope analyses. Any visible evidence of sample contamination was removed. XRD analysis of the samples was performed by following the method of Asami et al. (2014) to determine calcite/aragonite component. The stable isotope analyses of calcium carbonate were performed using a continuous flow isotope ratio mass spectrometer (Delta V Advantage: Thermo Fisher Scientific Inc.) attached to a Gasbench II and a GC-PAL auto-sampler (Thermo Fisher Scientific Inc.) at the University of the Ryukyus. The analytical method followed Asami et al. (2014).

Isotopic ratios were reported in the conventional d notation relative to the Vienna Pee Dee Belemnite (VPDB) and were calibrated to the NBS-19 international standard. External precision of replicate measurements of GSJ/AIST-JCp-1 (aragonite, *Porites* spp.) throughout the analyses was ±0.05‰ and ±0.06‰ for carbon and oxygen isotope composition, respectively.

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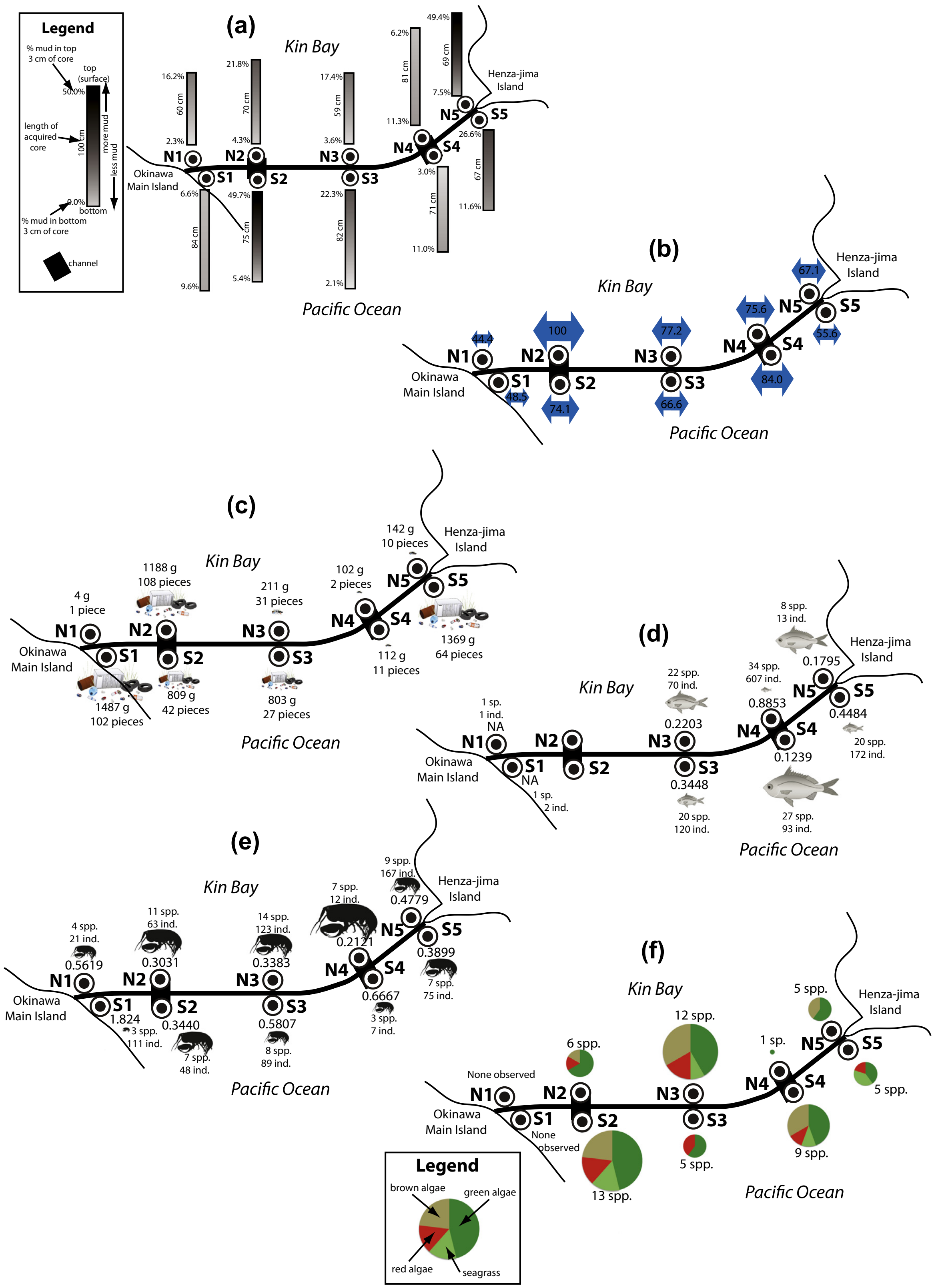

Fig. 2. Summary of core, environmental, and biotic data obtained on Kaichu-Doro. (a) information of cores acquired and examined in this study. Sizes of cores acquired in this study from each site along Kaichu-Doro, and percentage of mud content from tops and bottoms of each core; (b) relative water energy levels at each site along Kaichu-Doro. Numbers in arrows and sizes of arrows are water energy levels as a percent of the level at site N2 (=100%) expressed in percentages (see data in Table S5); (c) relative weight (g) and numbers (pieces) of garbage in transects adjacent to each site along Kaichu-Doro. Garbage images' sizes are relative to weight of garbage at each site (data in Table S6); (d) numbers of individual fishes (=ind.) and species (=spp.), as well as Simpson's Diversity Index weight (large number next to sites) for each site along Kaichu-Doro. Fish images' sizes are relative to Simpson's Diversity Index at each site (data in Table S8); (e) numbers of individual amphipods (=ind.) and morphospecies (=spp.), as well as Simpson's Diversity Index weight (large number next to sites) for each site along Kaichu-Doro. Amphipod images' sizes are relative to Simpson's Diversity Index at each site (data in Table S9); and (f) numbers of species (=spp.) of macroalgae and seagrasses for each site along Kaichu-Doro. Circle sizes are relative to total number of species at each site (data in Table 3).

3.1.4. Foraminifera

Three-cm-thick samples were collected from the top, middle and bottom (=for depths see Table S3) of sediment cores obtained from N1, S1, N3 and S3. A total of 12 samples were processed. In the laboratory, each sample was washed on a 63-μm sieve, and dried at 60 °C. Then, each sample was dry-sieved into a 2-mm and 0.5-mm mesh sieves to separate gravel and coarse-grained sand fractions. Foraminiferal shells in a coarse-grained (2-0.5-mm) size fraction were picked, identified to genus or species level, and counted. The FORAM (the Foraminifera in Reef Assessment and Monitoring) Index, which is a foraminiferal-based indicator for the water-quality suitability for reef communities, was calculated using the equation proposed by Hallock and Lidz (2003).

To find samples with similar taxonomic composition, Q-mode cluster analysis was performed on non-transformed, relative abundance data of foraminiferal taxa (>3%) using a group-average linking method. A Bray-Curtis (BC) dissimilarity coefficient was used to calculate a similarity matrix of taxonomic composition among samples. Q-mode cluster analysis and BC dissimilarity coefficients were computed using the software PRIMER ver. 5 (PRIMER-E Ltd., England).

3.2. Combined data analyses

Three different methods were utilized to examine "combined" datasets of more than one variable in order to examine the relationships between and among the 10 different sites. Simple hierarchial cluster analyses were used for both environmental data and biotic datasets to show the similarity among sites. Principle component analyses (PCA) were utilized for a combined environmental dataset to summarize main characteristics of the data. Finally, multi-dimensional scaling (MDS) was used for both environmental and biotic data sets for comparison.

The results of cluster analyses, PCA and MDS were analyzed using PRIMER v6 (Clarke and Gorley, 2006), and all the prerequisites for datasets followed the instructions of the PRIMER v6 user manual (PRIMER-E Ltd., England).

3.2.1. Combined environmental data

Principle Components Analysis (PCA) was used to illustrate the environmental variables' relationships among sites. All the environmental data (water temperature (average, high, and low temperatures during research period), water flow (average weight change per site), mud/sand change in content between top and bottom of cores, garbage (weight only), as well as pH, DO, salinity, turbidity data obtained from multi-parameter water quality meter, and nutrients and POM from seawater analyses) were first checked with draftsman plots to distinguish parameters that needed to be transformed. NO_2–N + NO_3–N, PO_4–P and NH_4–N were then log transformed and square root transformed, respectively. All parameters were normalized and then used in PCA analysis. Cluster analysis was used to distinguish the similarity of the environment among all sites. The resemblance of the dataset after normalization was calculated using D1 Euclidean distances before cluster analysis. A similarity profile test (SIMPROF) was used in cluster analysis to test the significant differences among the groups. Finally, the similarity among sites was plotted using Non-metric Multi-dimensional Scaling (MDS) with cluster analysis.

3.2.2. Combined biota data

Similar to the combined environmental data, the biota data were analysed using cluster analysis and multiple dimensional scaling (MDS). The combined biota dataset utilized in these analyses only included invertebrate (finest level to genus), fish (with sites N2 and S2 as zero counts), and algae data (species numbers of green, red, brown, seagrasses), as the other biotic datasets did not have data for all 10 sites (foraminifer and core-related data), or were generated by other software and could not be translated into the combined biota analyses (DGGE data). The datasets that were utilized were first standardized, overall fourth root transformed, and then resemblance was analyzed using Bray Curtis similarity. A SIMPROF test was then applied in cluster analysis. MDS with cluster analysis was plotted to illustrate the similarity among sites.

4. Results

4.1. Environmental data

4.1.1. Seawater conditions

A maximum of 37.04 °C was observed at site N5 on November 23, while a low of 14.98 °C was observed at site S1 on November 25. Generally, seawater temperatures varied on average 3.40 °C (site S2) to 6.69 °C (site S1) each day at each site between observed maximum and minimums for the period of November 17–30, 2011, and variation of up to 14.06 °C on extreme days (site S1 on November 23) was observed.

Despite such daily differences in temperatures, on average, differences between paired north and south sites at each station were generally small (Table S4), and no differences of >0.3 °C were observed.

From the total weights of dissolution of plaster balls, very clear significant differences in the water flow regime of different stations and sites were observed (ANOVA, $p < 0.001$). Water flow was clearly lowest at station 1 (S1 > N1), followed by station 5 (N5 > 5S), then station 3 (N3 > S3), and then the two stations by channels, station 4 (S4 > N4) and then station 2 (N2 > S2). Water flow at site N2 was over two times higher than at both sites at station 1 (Table S5, Fig. 2b).

Site S1 had the highest levels of nitrites and nitrates, ammonium, phosphate, and POM (Fig. 3, Table 2) of all sites. Site N5 also had high levels of nitrites and nitrates and ammonium. Generally, levels of all of these parameters were lower at all other stations, and lowest at stations 3 and 4 on both sides of the Kaichu-Doro. Particulate organic matter (POM) was over ten times higher at S1 and S2 than all other sites (9.5 mg/L for S1 and S2 opposed to less than 0.6 mg/L at all other sites), and turbidity showed a similar pattern, being much higher at 1S and 2S than all other sites. Dissolved oxygen (DO) was generally similar at all stations on both sides of the Kaichu-Doro, and lowest at site N5 (Table 2). pH ranged between 8.28 (S4) to 8.08 (S1) (Table 2).

4.1.2. Garbage

More garbage by weight was consistently seen on the south side of Kaichu-Doro compared to the north side, with all paired sites except for N2 and S2 showing this trend. At stations 1 and 5, this was very pronounced, with S1 and S5 having the most garbage of all stations, with 1487 g and 1369 g, respectively. Conversely, N1 and N5 had only 4 g and 142 g, respectively. Other low garbage weights were seen at N4 and S4, with 102 g and 112 g, respectively (Fig. 2c, Table S6).

The trends seen for pieces of garbage were more mixed, with the highest numbers seen at N2 (=108 pieces), followed by S1 (=102) and S5 (=64). Low numbers of garbage were seen at N1 (=1) and N4 (=2) (Fig. 2c, Table S6).

Overall, by weight plastics made up 61%, followed by glass at 16%, others at 15%, and metal at 8%. By numbers, plastics made up 85% of all garbage, with the other three categories accounting for 5% each (Table S6).

For pieces of garbage that we were able to determine country of origin, 31 pieces were from Japan, 3 from the Philippines, and 2 each from Korea, China, and Taiwan.

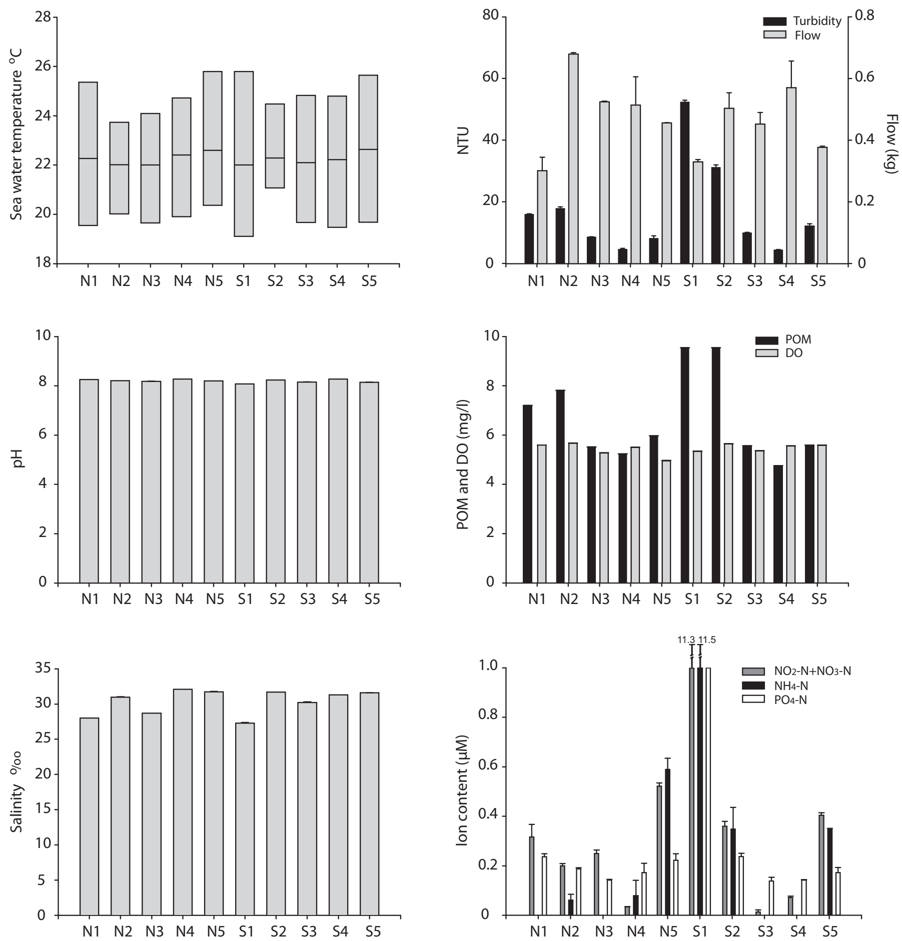

Fig. 3. Environmental information at each site along Kaichu-Doro. For details see Table 2.

Table 2
Water quality and environmental data from sites along the Kaichu-Doro in November 2011.

Site number	pH	DO (mg/l)	Conductivity (S/m)	Turbidity (NTU)	Salinity (‰)	POM (mg/l)	NO_2–N + NO_3–N (μM)	NH_4–N (μM)	PO_4–P (μM)
N1	8.26	5.59	4.34	15.83	28.0	7.20	0.317	<0.001	0.236
N2	8.21	5.67	4.75	17.70	31.0	7.81	0.199	0.061	0.187
N3	8.18	5.28	4.43	8.47	28.7	5.52	0.249	<0.001	0.142
N4	8.27	5.50	4.88	4.50	32.1	5.24	0.034	0.080	0.172
N5	8.20	4.96	4.84	8.03	31.7	5.97	0.522	0.589	0.222
S1	8.08	5.34	4.26	52.23	27.3	9.54	11.356	11.561	1.025
S2	8.24	5.64	4.85	31.07	31.7	9.54	0.359	0.349	0.237
S3	8.15	5.36	4.63	9.87	30.2	5.56	0.013	<0.001	0.139
S4	8.28	5.55	4.77	4.20	31.3	4.76	0.071	<0.001	0.143
S5	8.15	5.59	4.82	12.10	31.6	5.59	0.404	0.346	0.172

4.2. Biota sampling/observation

4.2.1. Fish

On the Kin Bay (=north) side of Kaichu-Doro, only one fish was observed at site N1, while at N3, N4, and N5 10 families, 17 genera, 19 species (70 total fish); 17 families, 20 genera, 28 species (607 fish); and 7 families, 8 genera, 8 species (13 fish) were observed, respectively. In total, 25 families, 37 genera, and 48 species, and 689 fish were observed on the north side of Kaichu-Doro (Fig. 2d, Table S7, Table S8).

On the south side of Kaichu-Doro, at sites S1, S3, S4, and S5, 1 species (2 fish); 10 families, 15 genera, 19 species (120 fish); 14 families, 19 genera, 26 species (93 fish); and 12 families, 15 genera, 16 species (172 fish) were observed, respectively. In total, 18 families, 31 genera, and 38 species, and 383 fish were observed on the south side of Kaichu-Doro (Table S7).

Overall, 26 families, 46 genera, 61 species, and 1078 individual fish were observed during the survey (Fig. 2d, Table S7).

4.2.2. Amphipoda

Thirty-five morphospecies in 20 families were collected from all stations along Kaichu-Doro (Table S9, Table S10). Site N3 was the most diverse with 14 morphospecies collected from 11 families. Site S4 was the least diverse with three morphospecies collected from two families. Sites N1 and S1 showed the next lowest diversity with four morphospecies collected from four families at each site.

Site N5 also showed high diversity, with the highest number of individual amphipods collected here (167). Site S1 appeared to be the most disturbed site, with a known opportunistic genus, *Corophium* sp., dominating the amphipod community and comprising 106 of the 111 total amphipods collected (Fig. 2e, Table S9).

Table 3
Numbers of species of green algae, seagrasses, red algae, and brown algae observed at each site along Kaichu-Doro in November 2011.

Site	Green algae	Seagrass	Red algae	Brown algae	Total species
N1	0	0	0	0	0
S1	0	0	0	0	0
N2	4	0	1	1	6
S2	6	2	2	3	13
N3	5	1	2	4	12
S3	3	0	2	0	5
N4	1	0	0	0	1
S4	4	1	1	3	9
N5	3	0	0	2	5
S5	2	2	1	0	5

The overall amphipod biodiversity was different on the north and south sides Kaichu-Doro and between the paired stations. The numbers of morphospecies common to both the north and south sides of each paired station were as follows: N1 + S1, one common morphospecies of seven observed; N2 + S2, two of 16; N3 + S3, four of 18; N4 + S4, two of eight; and N5 + S5, four of 13 (Fig. 2e, Table S9).

4.2.3. Macroalgae and seagrasses

From the November 2011 surveys, a total of 23 species (9 spp. of green algae, 5 spp. of red algae, 7 spp. of brown algae, 2 spp. of seagrasses) were observed from the stations around Kaichu-Doro (Fig. 8, Table 3). Of particular interest was the presence of the siphonous yellow–green alga *Pseudodichotomosiphon constricta* (Xanthophyceae) at site N3. This species is included on the Okinawa Red List of Endangered Species.

By site, the highest diversity was observed at sites S2 and N3, with 13 and 12 species, respectively. On the other hand, sites N1 and S1 did not have any macroalgae or seagrasses present. Site N4 was completely dominated by the green alga *Halicoryne wrightii*. This contributed to the largest difference observed between paired sites, with N4 having only one species, while S4 had nine species (Fig. 2f, Table 3).

4.2.4. Microbial community (Eukaryota and Bacteria)

For a comparative overview of the bacterial and eukaryotic communities of the whole sampling area, water and sediment samples from all sites were analyzed by DGGE. Cluster analyses of the band patterns results showed the eukaryotic communities in the seawater column at each site were different from those contained in the sediments (Fig. 4a). The eukaryotic community from the seawater showed stations 1, 2 and 5 had most similar compositions between north and south paired sites, but no such similarities were observed in the sediment results, with no observed pairing of north and south sites at any station.

Bacterial community results were somewhat similar to those of the eukaryotic seawater column (Fig. 4b), as the bacterial composition of the sediment at stations 3, 4, and 5 had most similar DGGE results between their north and south sites. For the bacterial composition in the seawater column all five stations were paired by the north and south sites.

Thus, overall, for seawater, the grouping patterns from both eukaryote and bacteria were similar (Fig. 4a and b). From both community results, N1 was most similar to site S1, N5 was most similar to S5, and stations 2, 3 and 4 had higher similarity to each other than to stations 1 and 5.

To get more detailed insights into community structures of selected sites, samples from station 1 (seawater and sediment)

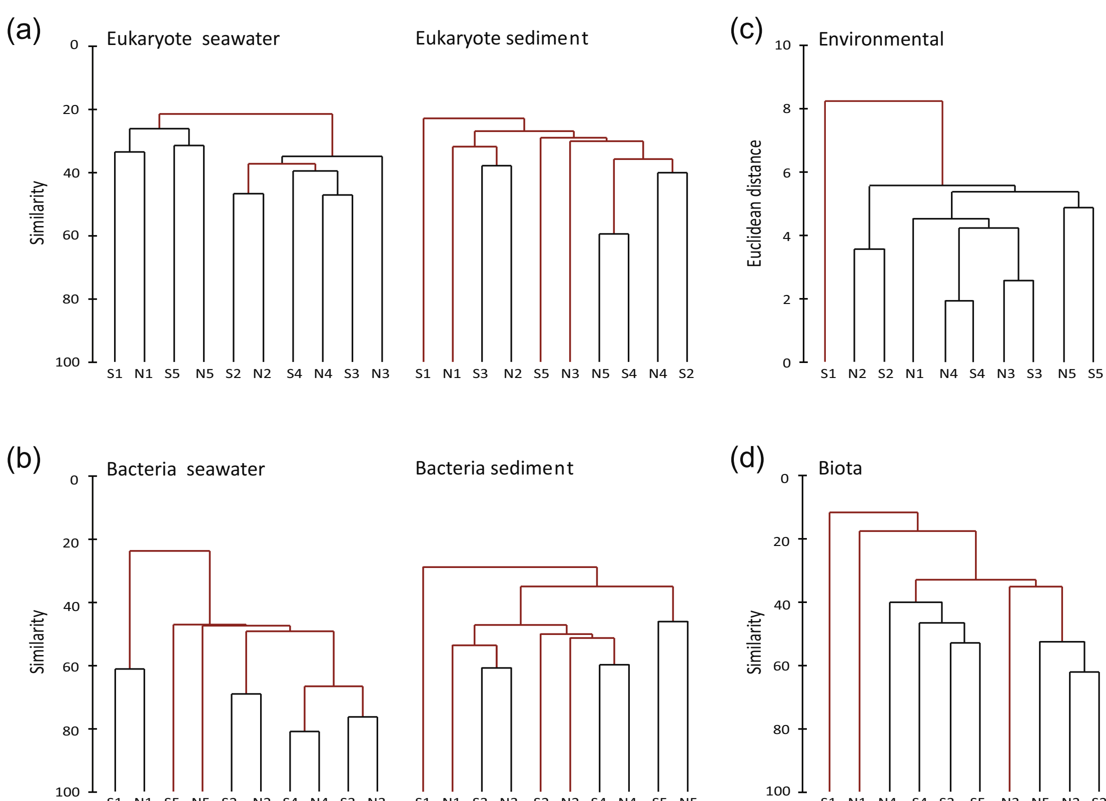

Fig. 4. Cluster analyses of similarity of (a) eukaryotic community in seawater and sediment; (b) bacterial community in seawater and sediment; (c) water environmental parameters among sites; and (d) biota diversity among sites. Significant differences were tested using the similarity profile test (SIMPROF). Red lines represent significant differences ($p < 0.05$).

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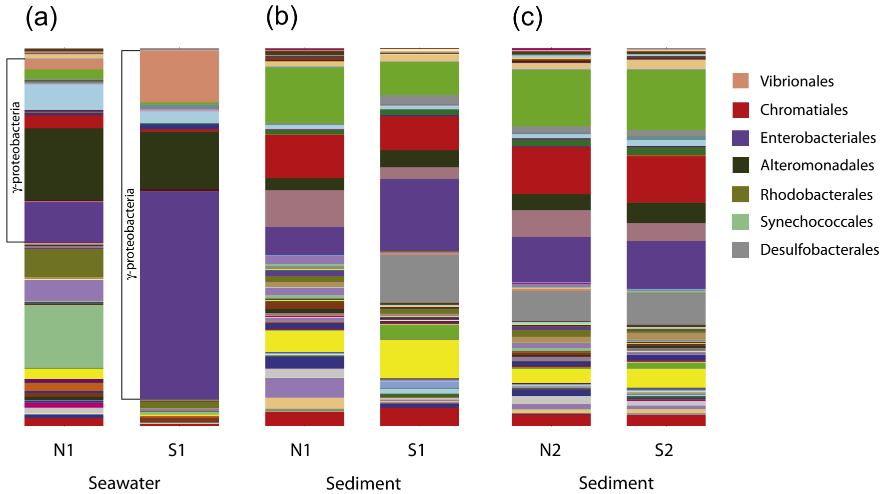

Fig. 5. Bacterial community composition in seawater and sediment samples. Affiliations to bacterial orders are shown for sequences retrieved by amplicon pyrosequencing from (a) seawater of station 1, (b) sediment of station 1 and (c) sediment of station 2. Order names are given for a selection of the most relevant groups.

and station 2 (sediment) were subjected to pyrosequencing of amplicons targeting the small ribosomal subunit of both bacteria and eukaryotes. Sites N1 and S1 showed profound differences in their bacterial seawater communities (Fig. 5a). At N1 cyanobacteria constituted 21.2% of the bacterial reads with the clear majority (80%) belonging to the order Synechococcales, whereas S1 contained only 2.1% cyanobacterial reads. Even more substantial was the variation of gamma-proteobacterial sequences. They constituted 49.6% of the reads at site N1 but 92.9% at S1. This extreme dominance owed mainly to Vibrionales (2.7% at N1 vs. 13.7% at S1) and Enterobacteriales (10.6% vs. 55.1%). The enterobacterial reads at S1 were heavily loaded with four very similar sequences, which formed 51% of the total reads for this site. The closest match (99% identity) revealed by a BLAST search was *Serratia marcescens*, an opportunistic human pathogen (Mahlen, 2011), which has been identified as the causative agent of white pox disease of corals (Patterson et al., 2002).

In the sediment samples of station 1 (Fig. 5b), clear differences could also be detected, though they were less substantial than for the seawater column. The larger proportion of Enterbacteriales at the south site was quite prominent with 19.1% compared to 7.1% at N1, a difference that possibly reflects the extremely different proportions found in the water columns of station 1. Moreover, a large population of Desulfobacterales was found in the sediment of S1 constituting 12.7% of the total reads, while at N1 only 0.7% of the reads belonged to this order. In contrast to station 1, the bacterial communities in the sediments of station 2 were virtually identical (Fig. 5c), differing only in some minor details.

The eukaryotic communities in the seawater columns of station 1 showed substantial variations. N1 was dominated by brown microalgae (Phaeophyceae) amounting to 28.4% of the reads, whereas S1 contained only 6.7%. The protist order of Jakobida also differed considerably with 19.0% at the north site but only 4.4% in the south. On the other hand, the water column at S1 was heavily loaded with copepod sequences (30.4%), while N1 contained only 11.3%. Dinoflagellate and ciliate communities did not differ significantly at N1 and S1.

Diatoms dominated the sediment samples. Site N1 contained 29.2% diatom reads, which was similar to the values found at sites N2 and S2 with 29.6% and 35.5%, respectively. At site S1, however, this dominance by diatoms was extreme having a proportion of 71.9% of total reads with a majority belonging to the benthic genus *Sellaphora*.

5. Cores

5.1. Collection

5.1.1. Sediment size and composition

The sediments of all 10 cores were mostly (light-) dark in color and showed no distinct variations in color. Obtained cores were 60–84 cm in length (Fig. 2a, Table S2). The sediment mud content was generally higher in the top layer than in the bottom layer at most (7/10) of the sampling sites (Fig. 2a, Table S2). Site S1 showed a slight decrease in mud content from top compared to bottom, and both sites at station 4 also showed some decrease. On the other hand, of the seven sites at which mud content increased in the top layer of cores, the level of increase was much greater than those of the decreases; the lowest increase (site S5) was still over 200% (Fig. 2a, Table S2).

5.1.2. Sediment dating

As water content in the samples from the core taken at site S1 was 25 ± 1%, it was assumed compaction effects were negligible and they were not considered.

Fig. 6 presents the total ^{210}Pb, excess $^{210}Pb_{ex}$, and ^{226}Ra concentrations in the samples. The total ^{210}Pb varied from 6.5 to

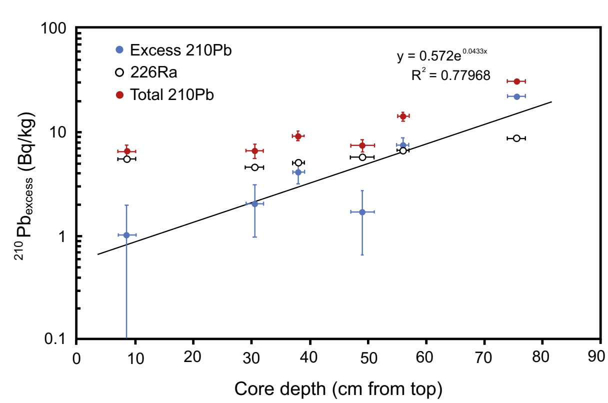

Fig. 6. Vertical profiles of total ^{210}Pb, excess ^{210}Pb, and ^{226}Ra in sediment from site S1 at Kaichu-Doro. Excess ^{210}Pb activities were determined by subtracting the activity of ^{214}Pb (considered as the ^{226}Ra in equilibrium with ^{210}Pb) from the total ^{210}Pb.

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30.8 Bq/kg and for ^{226}Ra ranged from 4.6 to 8.7 Bq/kg. These values are considerably low comparing with that in the sediment from Kin Bay (7–140 Bq/kg) (Taira et al., 1989). The core was formed mainly by sand and contained a small amount of mud (top 6.6%, bottom 9.6%), which included the ^{210}Pb scavenged by suspended particulate matter. The low ^{210}Pb values indicate that the fine particles of sand with low ^{210}Pb concentrations were involved in the measurement samples.

The total ^{210}Pb in the samples showed an increase with depth while ^{226}Ra concentrations were almost constant (Fig. 6). Excess ^{210}Pb was calculated by subtracting the ^{226}Ra from the total ^{210}Pb and therefore showed an increase with the depth.

Thus, for site S1, it was not possible to determine the sedimentation rate. This could be due to several factors. Atmospheric ^{210}Pb is normally assumed to have a constant flux at any given locality. If the steady-state sedimentation is not fulfilled, specific activities of ^{210}Pb will be varied. If ^{210}Pb was not redistributed after deposition and the regional ^{210}Pb flux was constant at Kaichu-Doro, the depth profile of $^{210}Pb_{ex}$ (Fig. 6) suggests that rapid deposition might have occurred in short period at site S1.

5.1.3. Coral distribution, identification, and geochemical composition

As mentioned above, the sediments of all 10 cores were mostly (light-) dark in color and showed no distinct variations in color. Fossil coral gravels with a size of >1 cm^3 were more frequently found in the south cores (58%) than in the north cores (42%). This is further supported by the fact that coral samples were rarely included in the cores N1, N3, S1, and S5 (Table S11). Identified coral included eight genera of corals; *Acropora* sp., *Cyphastrea* sp., *Leptastrea* sp., *Montipora* sp., *Pavona* sp., *Porites* sp., *Stylophora* sp., and *Turbinaria* sp. The south-side cores included all eight genera of corals. On the other hand, the north-side cores included only five genera and did not include *Cyphastrea*, *Leptastrea*, and *Porites*. Unlike the edges of Kaichu-Doro (stations 1 and 5), the south cores S2, S3, and S4 had much gravel consisting of several species of corals implying that there could be a higher diversity in coral assemblages on the southern area of the Kaichu-Doro relative to the northern area, and that there is a difference in transporting capacity and current direction between the middle and edges of the Kaichu-Doro.

XRD analysis showed that most of coral samples exclusively consisted of aragonite skeleton. Only six and one of all 52 samples had slight calcite components of <1% and <3%, respectively. These results indicate that inner portions of fossil coral gravel are well preserved. Interestingly, the cores from N3 and S3 had relatively large amounts of altered coral samples with calcite components to those from all other sites (Table S11). This is probably due to the two sites being very shallow and resultantly subject to meteoric diagenesis. Carbon and oxygen isotope composition of coral samples fall within a range of −2.5‰ to 1.3‰ and from −4.5‰ to −2.6‰, respectively (Fig. S1). The stable isotope data cover a period of one-to-several years (i.e., annual-to-interannual average data) due to the bulk sampling from coral gravel. Fig. S1 shows the scattered plots of isotope data; indicating no distinct change or tendency in isotope signals. This is caused by the small number of samples, the inclusion of various species corals, and by differences in ages of corals. In this study, therefore, time determination of sediment cores (i.e. determining the core depth at the boundary before/after the construction of Kaichu-Doro) could not be conducted based on the coral data. However, one characteristic of coral distribution from the cores indicates that there is a large decrease in coral gravels in the middle of the cores compared to the bottoms. One possibility is that the bottom parts of cores were deposited before the construction of Kaichu-Doro.

5.1.4. Foraminifera

A total of 32 taxa were identified from sediment core samples, of which 15 taxa have a symbiotic relationship with microalgae (i.e., mixotrophic) (Table S3). Foraminiferal assemblages were dominated by *Calcarina* spp, and associated with *Amphistegina* spp., Soritinae, *Peneroplis* spp., and smaller Miliolida. The above taxa comprise more than 90% of the assemblages in all the samples examined. The FORAM Index was approximately 9 for all samples examined. Q-mode cluster analysis clearly indicated that foraminiferal assemblages differed by sites and by core depths (Fig. 7). Foraminiferal assemblages at both N1 and S1 were clearly distinguished from N3 and S3. Within station 1, foraminiferal assemblages at site S1 were similar over time except for slight changes in the middle part, while those at site N1 changed over time with the assemblages in the top section becoming similar to those at site S1. At station 3, foraminiferal assemblages differed by core depth (i.e., top vs. bottom) rather than by sides of Kaichu-Doro (north vs. south), indicating that foraminiferal assemblages at station 3 changed over time. In particular, relative abundances of *Calcarina*

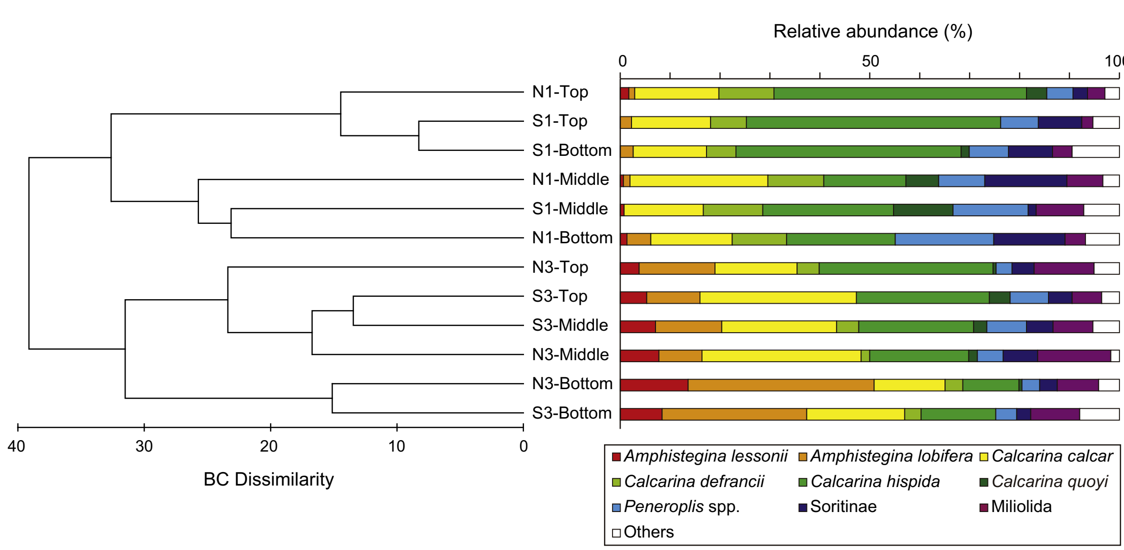

Fig. 7. A dendrogram of Q-mode cluster analysis and taxonomic composition of foraminiferal assemblages from the top, middle and bottom of sediment cores obtained from sites N1, S1, N3 and S3.

spp. increased and those of *Amphistegina* spp. (*A. lobifera* and *A. lessonii*) decreased at the top compared with the bottom.

5.2. Combined data analyses

5.2.1. Water environment

From the results of the PCA analyses (Fig. S2), turbidity was seen to be the strongest factor for PC1, along with PO_4–P, NO_2–N + NO_3–N, POM, and garbage weight. Site S1 was separated from other sites due to highly turbid water, high nutrients and garbage input, low pH and less water flow. S2 and N2 were less turbid, with lower amounts of nutrients and garbage than S1. On the other hand, S4 and N4 had clearer water, with less nutrients and garbage. Temperature was the strongest factor for PC2, along with salinity and mud content change in cores (top to bottom). This separated N5, S5 and S2 from the other sites, as these three sites had slightly higher seawater temperatures. Coupled with cluster analyses (Fig. 4c) on the PCA analyses, we observed four main groups of sites. Only site S1 was significantly distinct from all other sites. N1 was most similar with a group consisting of sites N3, S3, N4, and S4, which were less turbid, with lower nutrient levels and garbage. Besides station 1, sites from north and south sides had high similarity, even without the presence of a channel (stations 3, 5).

5.2.2. Biota

The MDS results of the biota data with cluster analyses (Fig. 8a, Fig. 4d) separated all the sites into four main groups. Site S1 and N1 each formed their own distinct group. Sites N5, N2, S2 and N3 formed another group, with N3 being less similar than the others. N2 and S2 had higher similarity to each other than to N5, however these three sites were not significantly different. Finally, sites S3, S4, S5 grouped with N4, and there were no significant differences in this group despite N4 being less similar than the other three sites.

5.3. Comparison of water environment and biota

By comparison of the MDS (Fig. 8) with clustering results (Fig. 4), environmental conditions appear to not totally explain the biotic results, except for sites S1, N2 and S2. Site S1 had the most different environmental conditions, biotic results, and sediment microbial compositions from other sites, and was the most significant outlier for all data sets. The microbial community composition in the seawater column may be explained by our observed environmental results, as asides from site N1, results were very similar (Fig. 4).

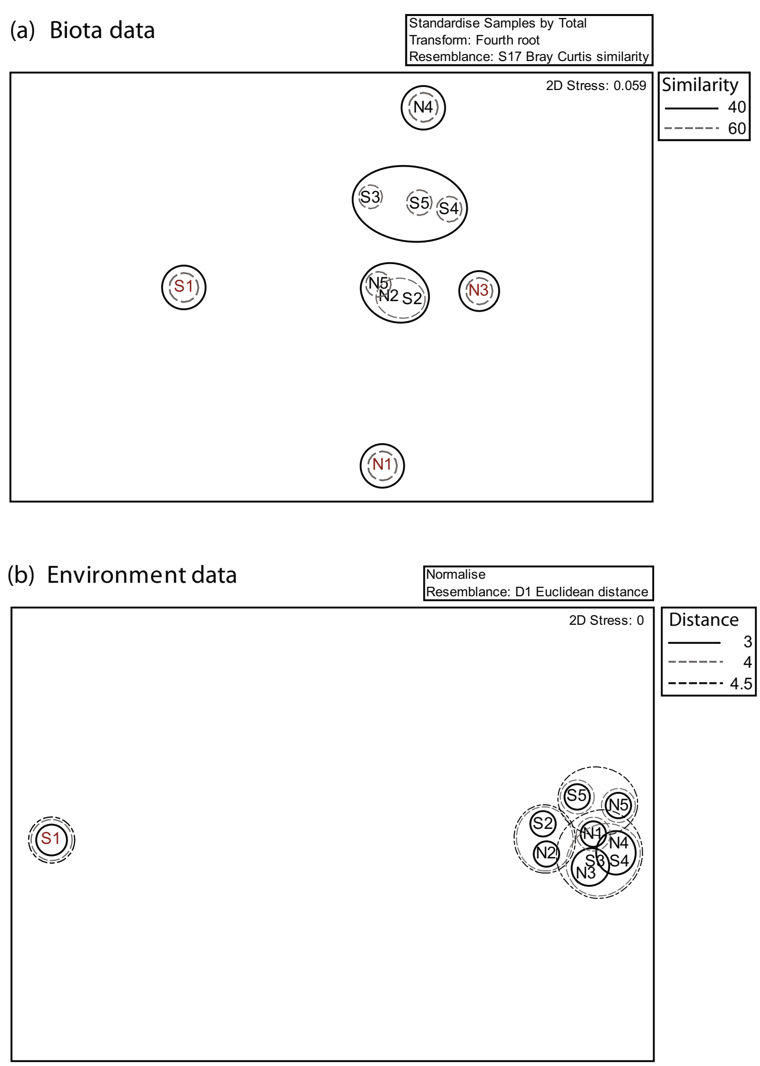

Fig. 8. (a) Non-metric multi-dimensional scaling (MDS) of biota, similarity of sites are grouped by cluster analysis (Fig. 4d) in similarities of 40% and 60%; and (b) MDS of water environmental parameters among sites. Distance among groups is clustered in distances 3, 4, and 4.5 (Fig. 4c). Sites that are marked in red represent significant differences (SIMPROF, p<0.05).

6. Discussion

Seven of the 10 sites at Kaichu-Doro showed evidence of large reductions of water flow from the analyses of the cores. In particular, site S1 had poor water quality and reduced biodiversity, and was an outlier in all analyses. At this site, enterobacterial *Serratia* and amphipod *Corophium* sp. dominated, indicating environmental degradation. Temperature and water quality did not appear to cause differences between or among sites, and a loss of water flow appears to be the main cause of this degradation. Below, we discuss the various impacts of Kaichu-Doro's construction in detail, compare these results to similar past studies, and then finally make recommendations for future coastal construction work and assessment in Okinawa.

6.1. Results from sediment cores - can we estimate the past environment?

An inherent problem with many environmental impact studies is the lack of pre-impact baseline data, and this study was no exception. We attempted to overcome the lack of pre-Kaichu-Doro construction data on the fauna of the area via sediment cores, and utilized the cores for analyses of past and present environmental and biotic data. Analyses of the sediment composition, foraminiferal assemblages, and coral rubble identification analyses provided data that had utility when considering past conditions in the tidal flats where Kaichu-Doro was built.

The fact that the top layer sediments of the majority of cores (7/10 sites; Table S2) were much higher (e.g. >200%) in mud content compared with the bottom layer sediments indicates that the depositional environment distinctly changed some time during formation of the sampled sediment layer. In particular, an increase of mud content to > 15% suggests a transition of sedimentation regime (Mitchener and Torfs, 1996), and all seven sites with increases in mud content had top layers with >15% mud content (Table S2). Sediment mud content is known to non-linearly increase and sediment shifts from non-cohesive sandy (generally mud content <∼5%) to cohesive muddy sediments (generally mud content > 15%) when the currents/waves of overlying water weaken to below a certain threshold (Sakamaki and Nishimura, 2007). The construction of Kaichu-Doro may have led to such a change of hydrodynamic conditions as well as sedimentation processes in the study area. Furthermore, the accumulation of mud fraction in the sediment has the potential to alter benthic biota (e.g. Thrush et al., 2003).

Indeed, benthic biota changes in the cores between top and bottom were observed in the Foraminifera data. Foraminiferal assemblages in sediment cores at stations 1 and 3 were dominated by algal symbiont-bearing mixotrophs. *Calcarina* spp. live commonly in back-reef environments, while *Amphitegina* spp. generally live in reef margins and fore-reef environments (Hohenegger, 1994; Hallock, 1999). Other mixotrophic taxa (Soritinae, *Peneroplis* spp.) and heterotrophic miliolids are also common in back-reef environments (Hohenegger, 1994; Ujiié and Hatta, 1995). A high FORAM Index, which was attributed to the dominance of mixotrophic taxa in foraminiferal assemblages, indicates good water quality suitable for reef growth. However, this was partly due to the coarser size-fraction examined in this study (2–0.5 mm). In addition, foraminiferal shells in sediments generally have a long residence time (up to 10^3 years; Martin, 1999), being derived from adjacent reefs, transported and accumulated for a long time. Thus the abundance of mixtrophic taxa may not always indicate the conditions of water quality at the time of deposition (Hallock, 2012).

Based on foraminiferal results, the benthic environments on both sides of station 1 have not changed much over time. The predominance of *Calcarina* spp. indicates shallow-intertidal environments through time. Temporal differences in the taxonomic composition at sites N1 indicated that the site became shallower and similar to site S1 with time. In contrast, relative increases of *Calcarina* spp. and decreases of *Amphistegina* spp. at both N3 and S3 suggest a decline in water energy, less water-exchange with open water, and/or shallower water depths with time. In summary, the foraminiferal results suggest that the present tidal flats along Kaichu-Doro have less water energy (i.e., more calm conditions) or have become shallower via sediment filling than past conditions (e.g. before the leeway construction; in particular at station 3).

As shown in previous research (e.g. Ruiz-Fernández et al., 2002; Chen et al., 2005; Domitsu and Oda, 2006), sediment cores can provide invaluable data that can help reconstruct past environmental and biotic conditions. However, our attempts to date when changes in the environment occurred via coral rubble analyses from the cores failed, and thus while we can state that the water energy appears to have decreased (e.g. from data from tops of cores) compared to the past (data from bottoms of cores), we cannot conclusively provide a time scale. Still, in this study, the sediment composition, coral rubble frequency, and foraminiferal data suggest that water currents around Kaichu-Doro have decreased compared to the past.

6.2. Differences in the present environment and biota between the two sides of Kaichu-Doro

Compared to reconstructing the past environment, we were able to obtain a wide variety of data that aided in analyzing the current environment and ecosystem around Kaichu-Doro. The majority of biotic data were used for combined analyses.

6.2.1. Present environment of Kaichu-Doro

Combined analyses of all environmental data showed the same general results regardless of the type of analyses. Both the cluster analyses (Fig. 4) and the principal component analyses (Fig. S2) of the combined environmental data showed that all paired stations grouped together with the exception of station 1, and also a slight separation between S5 and N5 (Fig. S2). The results showed that as far as the marine environment is concerned, each paired station was most similar to its partner on the opposite side of the Kaichu-Doro, despite the presence of the Kaichu-Doro. The exception to this was site S1, which had very divergent environmental results for most parameters examined.

Site S1 is located between Katsuren Peninsula and Kaichu-Doro, and based on our results suffers from relatively low water current, poor water quality (high turbidity, POM, phosphate, nitrite, nitrate, ammonium levels; low pH and salinity), and heavy garbage pollution. However, the sediment composition analyses showed a slight decrease in mud content from bottom to top of the core. There is a small drainage outflow from residential and agricultural land adjacent to the coast, and this can explain the poor water quality levels, similar to as seen in other locations in Okinawa (West and van Woesik, 2001; Ramos et al., 2004). Outflow from periodic heavy rain (typhoons) and subsequent heavy freshwater outflow could also cause the seemingly paradoxical results of less mud content with reduced water flow, and also contribute to the apparent rapid sediment deposition at the site that resulted in the failure to obtain proper sediment deposition data.

Overall, although it appears from the sediment core results that water current has been reduced from the past, the construction of Kaichu-Doro has not disproportionately affected the environment of one side of the road over the other. This does not mean the environment has not been impacted, but that impacts appear to relatively equal on both the north and south sides, with the exception of site S1.

6.2.2. Present biota of Kaichu-Doro

Overall, low numbers of fish species and individuals were observed at both sites at station 1, while at stations 3 and 4, higher numbers of both species and individuals were observed. One possible explanation of these general trends is that the marine environment near Katsuren Peninsula has been degraded from runoff and pollution. Additionally, low numbers of fishes were observed at site N5, and this may be due to landfill around this area. However, large numbers of fish (species, individuals) were observed at the paired site S5, perhaps due to its location near the channel Henza-jima Island and Hamahiga-jima Island (Fig. 1b).

When comparing the north sites on Kin Bay with the sites on the south side of Kaichu-Doro, despite some similarities, there are clear differences in the fish taxa that were observed. Thus, it appears that the construction of Kaichu-Doro and subsequent changes in the environment have had at least some influence on the fish communities surrounding the road.

We also observed differences in the macroalgae and seagrass compositions between sites and between the north and south sides of Kaichu-Doro. However, subtropical macroalgae communities have strong seasonal component influences (Lefèvre and Bellwood, 2010), and thus for these taxa it is difficult to discuss possible effects of the construction of Kaichu-Doro for these taxa without obtaining data for a full calendar year.

Clear differences in amphipod assemblages were evident between sample sites both along and across the Kaichu-Doro causeway. Overall, amphipod species diversity appears to be higher at the sites on the north side of the Kaichu-Doro. Amphipod species diversity appears highest at stations 2, 3, and 5, suggesting that there has been some kind of disturbance at stations 1 and 4. The low amphipod diversity at these sites indicates low ecosystem health (Bellan-Santini, 1980; Zakhama-Sraieb et al., 2006). Additionally, 11 of the 35 amphipod species were collected on both sides of the bridge, with stations 3 and 5 sharing the most similar species assemblages (four species each). However, stations 1, 2, and 4 only shared one, two, and two species respectively, suggesting a significant difference in species assemblages and a major impact on the ecosystem from the construction of the Kaichu Doro.

Interestingly, site S1, the most degraded area along the leeway, had the highest numbers of amphipods. However, 95 percent of the specimens belonged to one species in the genus *Corophium* Latreille, 1806, which is well known for colonizing disturbed intertidal ecosystems (van den Brink et al., 1993; Marsden et al., 2000; Rinderhagen et al., 2000; Buckley et al., 2004). Amphipod assemblage changes after river damming of the Danube in Romania have been clearly shown to reflect habitat and sediment changes (Popescu-Marinescu et al., 2001). Estuarine amphipods, such as those in the closely related genus *Paracorophium*, have been documented as particularly useful bioindicators based on their tolerance of changes in salinities and sediment types (Marsden et al., 2000). The dominance of corophiid amphipods at site S1 very likely reflects change(s) in habitat type as do the differences in amphipod assemblages at all sites along Kaichu-Doro.

6.3. Comparison with past results

Studies similar to the current one have been performed in temperate regions, and the results of our study appear to fit well with conclusions from past research. Perhaps the most well documented case is in the Bay of Fundy in Nova Scotia, Canada, where the Windsor Causeway was built across the Minas Basin in 1970. Post-construction studies found that the causeway reduced tidal flushing, increased siltation, and also noted changes in fauna and seagrasses, with increases in salt marsh area due to these coastal modifications (van Proosdij and Townsend, 2006). Another example is from Tasmania, where the Orielton Lagoon was created increased siltation from a causeway across the Pitt Water (Buttermore 1977). Based on the limited routes for water exchange at the Kaichu-Doro, our observed results of higher mud content in the tops of cores demonstrate reduced tidal flushing and increased siltation.

A smaller amount of research has been performed on causeway construction in subtropical or tropical ecosystems. One study in Micronesia examined the effects of an ancient causeway (500–700 years ago; now gone), and found higher mud content and lower diversity of seagrasses, macroalgae, and fauna shoreward, even in the absence of a barrier to water flow now (Coles et al., 2005). In the current study, the sides of the Kaichu-Doro near Katsuren Peninsula and Henza-jima Island can be thought of as 'shoreward', and also have higher mud content and lower diversity as seen in Coles et al. (2005). Results from Coles et al. (2005) demonstrate that the effects of causeways on the environment may be on the scale of hundreds of years, and continue even after the effective life of coastal modification, emphasizing the need for more rigorous pre- and post-construction assessment in Okinawa.

6.4. Conclusions: combined analyses, overall trends, future investigation, and recommendations

When examining the overall data as well as the combined analyses, it is clear that site S1 is an outlier compared to all other site. This site is dominated by an amphipod species known to prefer disturbed environments, has almost no macroalgae or seagrass, and has an unusual microbial community extremely dominated by the enterobacterial genus *Serratia*. Species belonging to this genus are known to act as opportunistic pathogens in humans with reported cases ranging from eye infections and pneumonia to fatal sepsis (Mahlen, 2011). Moreover, *Serratia* can also be pathogenic for fish (Baya et al., 1992; Loch et al., 2012) and was identified as the causative agent of the white pox disease of corals (Patterson et al., 2002). Therefore, the disturbed environment found at site S1 not only results in decreased biodiversity but also creates severe disease risks for humans and marine organisms including both invertebrates and vertebrates. From the environmental and biotic combined analyses, this site was consistently shown to be far different from all other investigated sites.

However, potential impacts were not only seen at site S1. Most sites showed indications of a changed environment from the past, likely the result of reduced water flow. Aside from site S1 discussed above, seven of nine sites had marked increase in mud content in recent sediment layers. This reduction in water flow was further supported by foraminiferal data. Other environmental factors such as temperature and water quality did not appear to be causes of differences between and among sites (aside from S1 with poor water quality), and all sites except for 1 showed the environments between north and south sides were still similar.

A similar trend was observed for the microbial communities in the seawater, with north and south sides of each site most related to each other. However, the sedimentary microbial data did not show such strong linkages, and other biotic data also often showed the closest links between sites were not the paired site on the other side of the Kaichu-Doro. We recommend the collection of many different kinds of data as in this study in other environmental impact studies, as it is clear that different data sets show different trends. In this study, data on microbes in the sediment and amphipods, as well as the fish and macroalgae data, clearly displayed the differences of site S1 that the poor water quality indicated may have existed.

However, given the paucity of past data available, the conclusions we can make based on this study are somewhat limited.

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Aside from the current data obtained in this study, other data would also allow a better understanding of the environment of Kaichu-Doro. Current and water flow data for the entire Kin-wan Bay would allow further analyses of the role Kaichu-Doro plays in this area. Other future research looking at parts of the tidal flats away from Kaichu-Doro to examine the effects of distance from the road are also needed. Seasonal surveys are also needed for many of the taxa investigated in this study that have seasonal movement and distribution patterns, such as fish and macroalgae, as this study represents a single point (November 2012) in time. Unfortunately, there are no 'pristine' tidal flats remaining in Okinawa to serve as a control. We attempted to overcome this problem with the utilization of cores and subsequent analyses as a way to "backcast" environmental (e.g. mud content), and by extension, biotic conditions (e.g. foraminifer content) at the study sites. Although not perfect, the combination of geological and biological surveys shows much promise, and we hope future researchers utilize such a combined methodology.

As of the writing of this paper (December 2014), additional dredging is ongoing at sites N4 and S4 in order to deepen and widen the channel running under Kaichu-Doro, and to increase water circulation in the area. Continued surveys analyzing future changes at these sites are needed to monitor how increased circulation impacts on the marine flora and fauna. As the tidal flat surrounding Kaichu-Doro is the largest in Okinawa, and with large landfills to the south on the large Awase tidal flats, more research is needed to better understand and protect the Kaichu-Doro tidal flats.

In the future, in order to better conserve marine natural resources and in particular tidal wetlands, Okinawa and Japan would be well-served by following past recommendations and guidelines made to help protect coral reefs and associated ecosystems (e.g. Maragos, 1993). Additional post-construction impact assessments from already completed projects should help contribute to avoiding further unwanted environmental degradation of Okinawa.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2015.02.037.

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