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Supplement of

Trichodesmium and nitrogen fixation in the Kuroshio

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SUPPORTING METHODS

Nitrate, nitrite, and ammonium contamination in $^{15}N_2$ gas

$^{15}N_2$ gas supplied by SI Science Co., Ltd was used during all of the cruises. We performed the following experiments to examine possible contamination of nitrate, nitrite, and ammonium in the $^{15}N_2$ gas (Dabundo et al., 2014).

Experiment 1: Ten 250 mL PC bottles were filled with aged subtropical surface water (ASSW), and were sealed with thermoplastic elastomer caps without headspaces. 2 mL 98+ at% $^{15}N_2$ gas (batch #11059) was added to five of these bottles and mixed well. All of the bottles were kept at room temperature for at least 12 h, and the water was subsampled to a 30 mL polypropylene tube.

Experiment 2: Degassed seawater was mixed from the ASSW using a Sterapore membrane unit (20M1500A: Mitsubishi Rayon Co., Ltd., Tokyo, Japan) (Shiozaki et al., 2015), and stored in three 1 L Tedlar bags without headspaces. A different batch number of $^{15}N_2$ gas (#11059 and #11143) was added to two of these bags. The $^{15}N_2$ gas was injected at a ratio of 10 mL per 1 L seawater, and dissolved completely using a ruler (Großkopf et al., 2012). The $^{15}N_2$-enriched and non-$^{15}N_2$-enriched seawater samples were subsampled to five 30 mL polypropylene tubes from each Tedlar bags.

We determined the concentrations of nitrate, nitrite, and ammonium at the nanomolar levels using supersensitive colorimetric systems (Hashihama et al., 2009, in press). The detection limits of nitrate, nitrite, and ammonium were 3, 2, and 4, respectively.

Statistical analysis of environmental variables

We used non-metric multi-dimensional scaling (nMDS) to investigate the spatial differences in the environmental variables that could influence *Trichodesmium* growth and bulk water nitrogen fixation; temperature, mixed layer depth, nitrate, dissolved iron, and phosphate. The environmental variables were transformed by $\log_{10}(x + 1)$ prior to analysis. A dissimilarity/similarity matrix between stations was constructed using the Bray-Curtis index. The nMDS was used to visualize similarities in the environmental variables among the stations. An Analysis of Similarity (ANOSIM) was used to test the differences in the environmental variables among the stations.

SUPPORTING RESULTS AND DISCUSSIONS

Nitrate, nitrite, and ammonium contamination in $^{15}N_2$ gas

In both experiments, there were no significant differences between the control and $^{15}N_2$-added samples or among different batch numbers of $^{15}N_2$ gas, with one exception;

the nitrite concentration in the $^{15}N_2$ (#11143)-added samples was significantly lower than that of the control in Experiment 2 (Fig. S1a and b). Because we did not determine the $^{15}N/^{14}N$ ratios of nitrate, nitrite, and ammonium, the contamination at isotope level could not be evaluated. However, our results demonstrated negligible contamination of these nutrients in the $^{15}N_2$ gas.

Dabundo et al. (2014) found contamination of $^{15}N_2$ gas supplied from Sigma-Aldrich and from Cambridge Isotopes and suggested that the contamination could be caused by the production method of the $^{15}N_2$ gas. The $^{15}N_2$ gas of the two companies is produced by the catalytic oxidation of $^{15}NH_3$ gas with cupric oxide (Dabundo et al., 2014), whereas the SI Science $^{15}N_2$ gas is produced by oxidation of ^{15}N-labeled ammonium sulfate with potassium hypobromite (Nakane, 1963). Surplus potassium hypobromite is added to avoid ammonia and NO_x gas generation, and the $^{15}N_2$ gas is purified using a molecular sieve and liquid nitrogen. This different production process from that used by Sigma-Aldrich and Cambridge Isotopes probably explains why there is no contamination of nitrate, nitrite, and ammonium in the $^{15}N_2$ gas produced by SI Science.

Differences in environmental variables among the stations

The nMDS showed that the environmental variables at all of the stations were similar at the >80% similarity level, and except for station T0904, were similar at the >90% level (Fig. S4). The ANOSIM indicated no significant differences among the stations ($p > 0.05$).

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Table S1 Locations and obtained data at each station

Station	Area	Longitude	Latitude	Date (LST)	*Trichodesmium** [filaments l^{-1}]	N_2 fixation [μmolN L^{-1} d^{-1}]	Temperature* [ºC]	MLD [m]	NO_3^-+NO_2^{-*} [nM]	PO_4^{3-*} [nM]	DFe* [nM]
T0601	East China Sea	129.1	30.9	Sep 10, 2006	6	n.a.	29.3	32	3	9	0.89
T0602	East China Sea	129.35	30.71	Sep 10, 2006	6	305	29.4	39	10	32	n.a.
T0606	Kuroshio	131.17	28.92	Sep 11, 2006	4	377	30.5	21	<3	19	0.60
T0622	East China Sea	127.31	30.39	Sep 13, 2006	0	306	29	40	21	12	0.83
T0625	East China Sea	127.76	30.39	Sep 14, 2006	0	77.2	28.7	40	19	11	n.a.
T0702	East China Sea	128.38	30.82	Sep 6, 2007	4	38.4	28.7	13	9	25	0.55
T0706	Kuroshio	130.97	28.72	Sep 7, 2007	68	88.1	29.4	27	9	15	0.36
T0711	Philippine Sea	133.17	26.25	Sep 8, 2007	16	67.3	29.5	26	10	<3	0.39
T0715	Philippine Sea	133.16	23.81	Sep 9, 2007	0	77.7	29.3	21	5	<3	0.80
T0718	Philippine Sea	130.22	24.44	Sep 10, 2007	8	29.9	29.5	23	10	36	0.35
T0723	Kuroshio	129.85	29.05	Sep 12, 2007	26	251	29.1	39	3	4	0.39
GW-1	East China Sea	128.61	33.84	Jul 23, 2007	0	29.5	25.1	17	42	17	n.a.
GN-3	East China Sea	128.57	33.38	Jul 23, 2007	12	n.a.	27.6	14	16	22	n.a.
NW-8	East China Sea	129.25	32.24	Jul 25, 2007	0	113	28.6	12	17	5	n.a.
B	East China Sea	127.85	32.25	Jul 25, 2007	0	171	28.6	17	32	5	n.a.
CK-10	East China Sea	127.5	31.75	Jul 27, 2007	194	418	29.4	13	17	16	n.a.
T0902	Miyako Islands	125.67	25.13	Sep 10, 2009	2	62.7	29.2	51	15	<3	0.19
T0903	Miyako Islands	125.43	25.12	Sep 10, 2009	6	n.a.	29.2	24	15	<3	0.35
T0904	Miyako Islands	125.20	25.07	Sep 10, 2009	2	58.9	29.0	48	374	23	0.39
T0905	Miyako Islands	125.42	24.93	Sep 10, 2009	102	n.a.	29.7	31	6	4	0.20
T0906	Miyako Islands	125.73	25.19	Sep 10, 2009	>20000	753	29.6	60	3	14	0.25

T0907	Miyako Islands	125.57	24.67	Sep 11, 2009	2	49.1	29.2	27	53	9	0.89
T0908	Miyako Islands	125.61	24.92	Sep 11, 2009	10	105	29.4	40	4	5	0.40
T1001	East China Sea	129.28	30.66	Sep 4, 2010	6	70.0	29.1	28	21	9	n.a.
T1004	Kuroshio	126.90	28.40	Sep 9, 2010	72	79.0	28.6	22	19	20	n.a.
T1007	Miyako Islands	125.75	24.60	Sep 11, 2010	24	179	29.2	40	15	<3	n.a.

*values in the surface water, n.a. = no data available

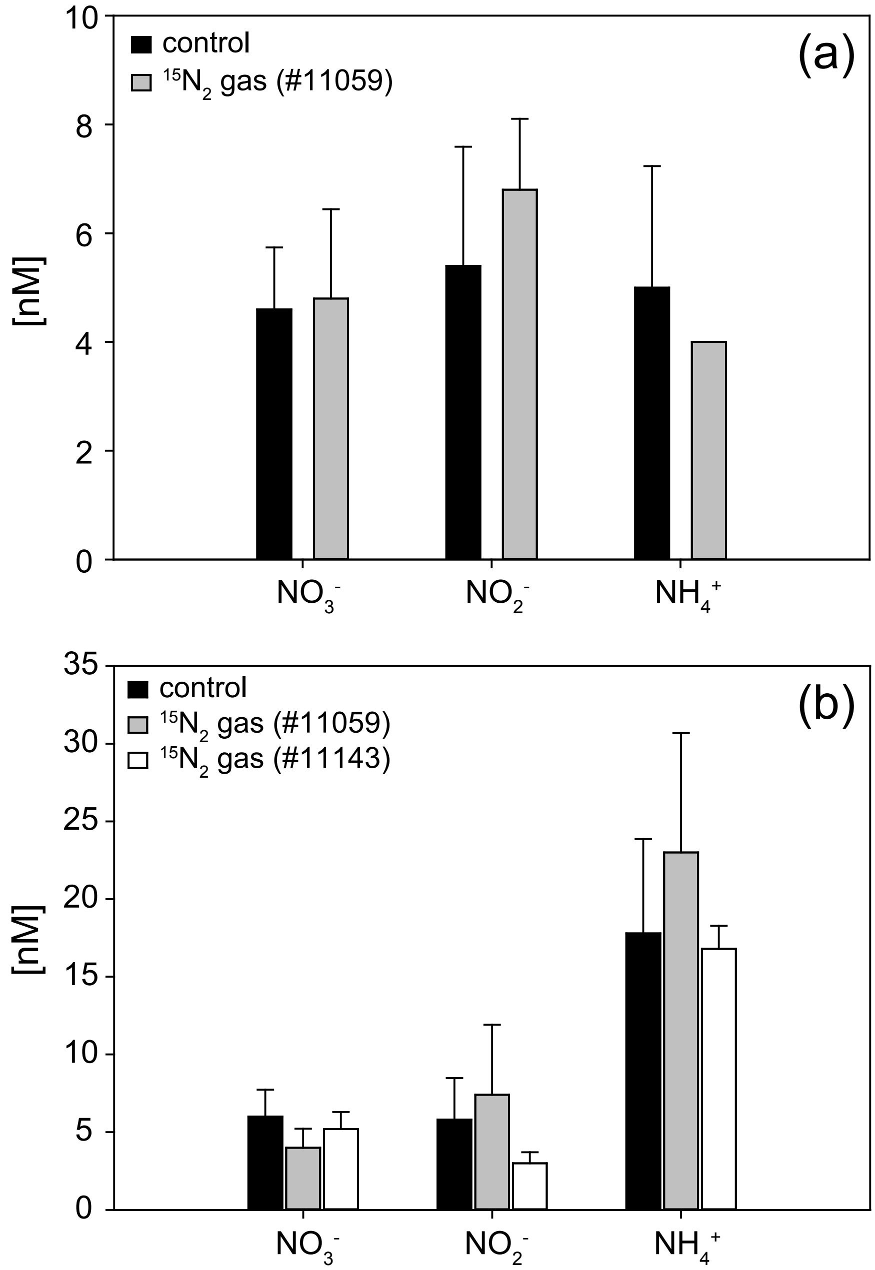

Fig. S1 Nitrate, nitrite, and ammonium concentration in control and $^{15}N_2$-gas added waters in experiment (a) 1 and (b) 2

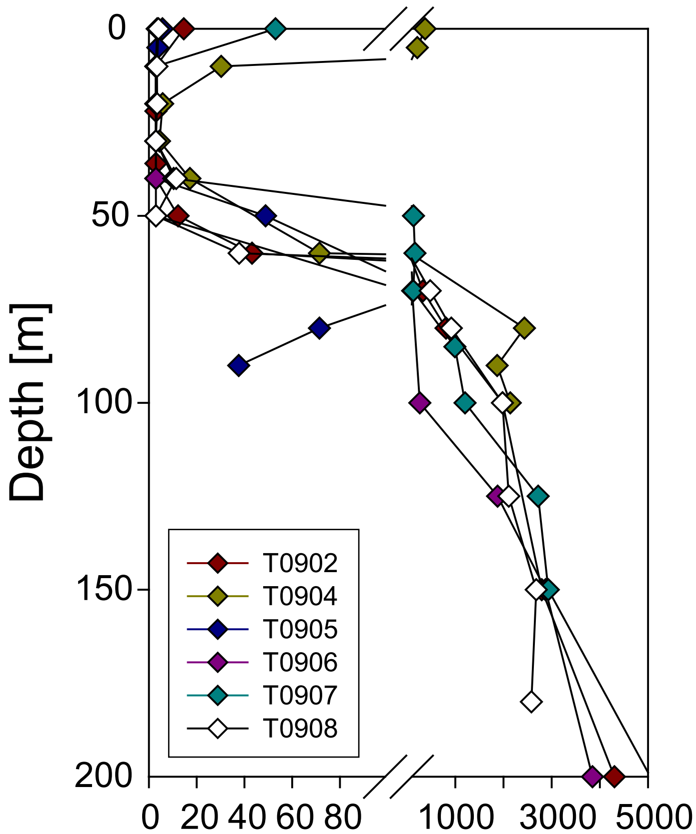

Fig. S2 Vertical profiles of nitrate during the KT-09-17 cruise

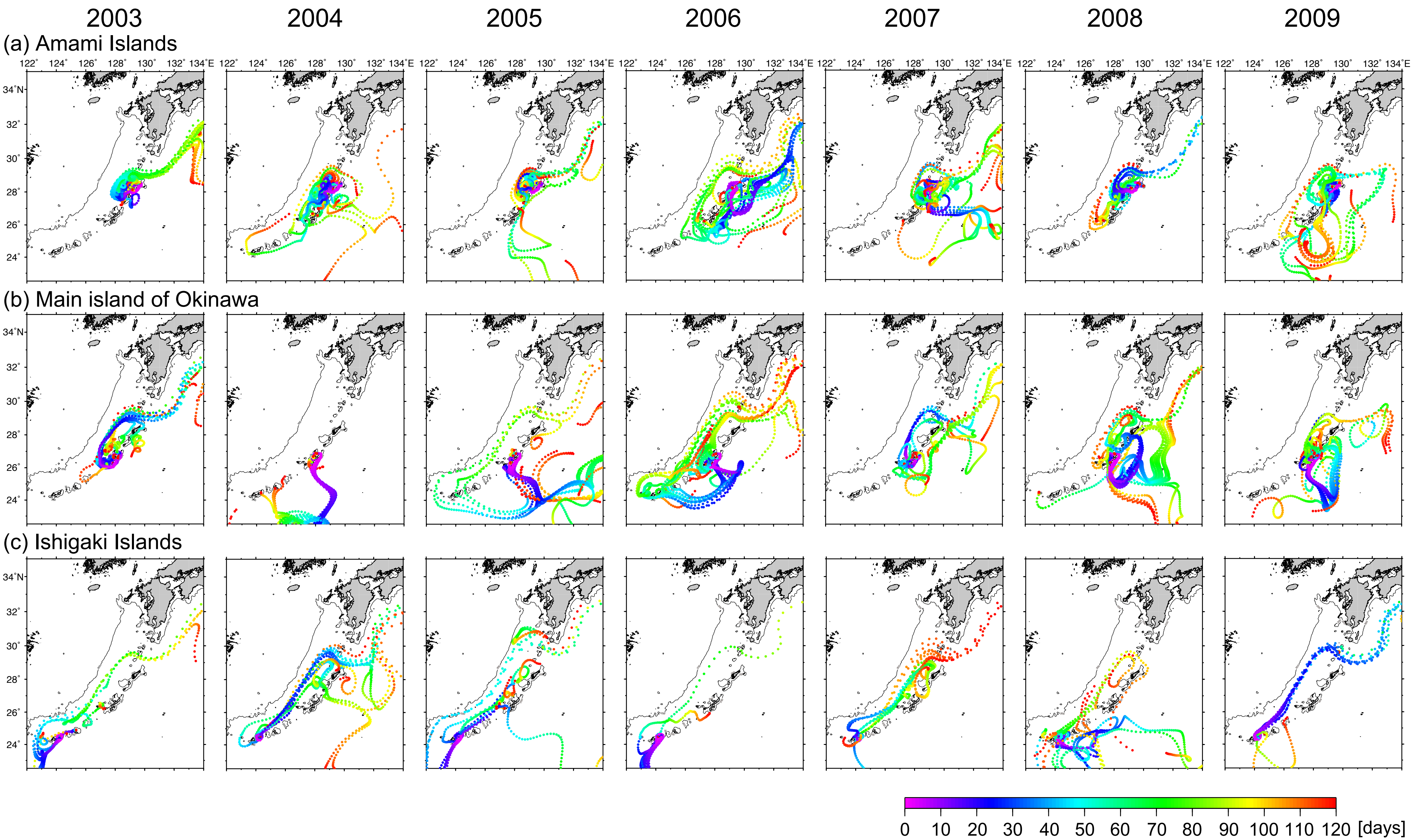

Fig. S3 Particle trajectories from the release points around (a) Amami Islands, (b) Main island of Okinawa, and (c) Ishigaki Islands. The released points of particles were selected at model grid points around the coastal waters of each island

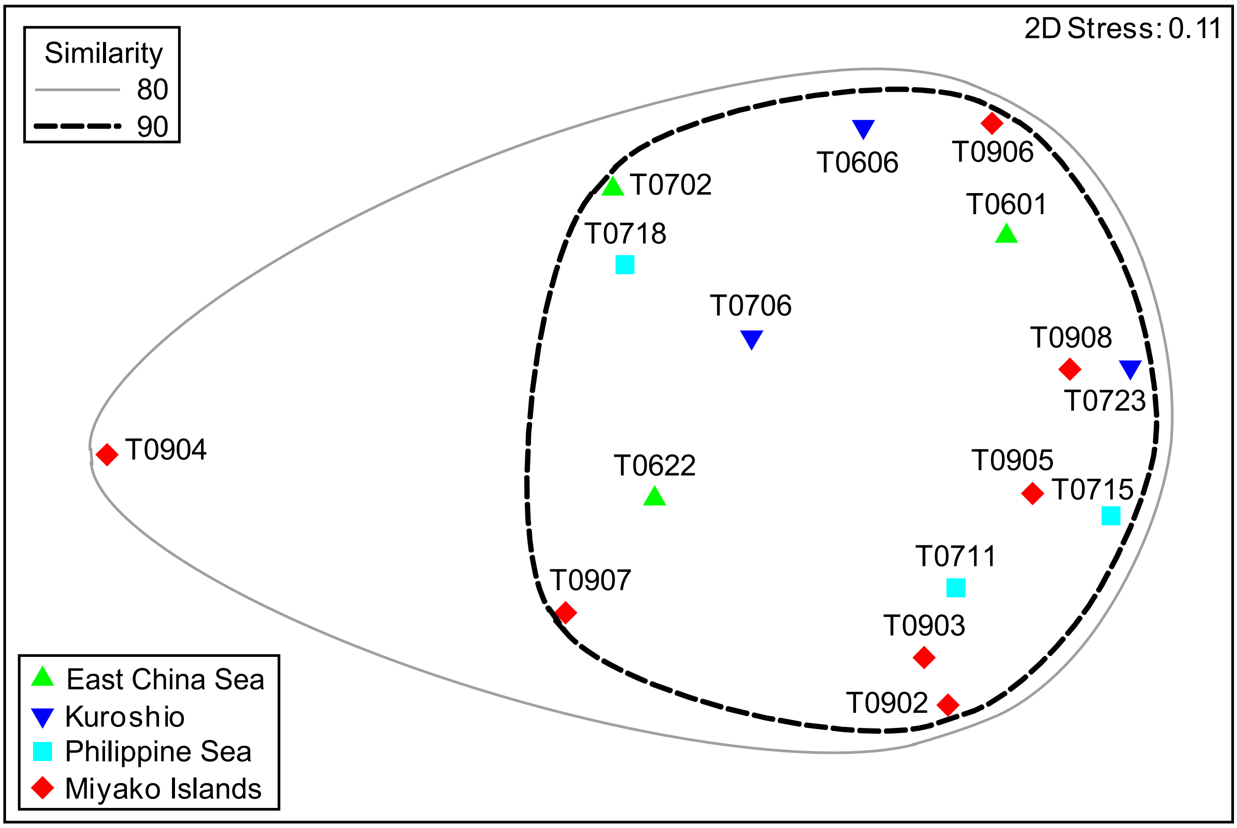

Fig. S4 nMDS ordination of sampling stations with environmental variables