Species richness and community structure of benthic macrofauna and megafauna in the deep-sea chemosynthetic ecosystems around the Japanese archipelago: an attempt to identify priority areas for conservation

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ABSTRACT

Aim To characterize the species richness, endemism and community structure of benthic macro-/megafauna in the deep-sea chemosynthetic ecosystems around the Japanese archipelago and determine the conservation priority for deep-sea chemosynthetic habitats.

Location Twenty-seven methane seeps from the Kuril–Japan Trench, Nankai Trough, Ryukyu Trench and Sagami Bay as well as 15 hydrothermal vents from the Izu–Bonin Arc and Okinawa Trough around the Japanese archipelago.

Methods Species richness and the number of endemic species from 42 vents and seeps were examined using the distributional data of 155 mollusc, annelid and arthropod species. Multivariate analyses were performed to identify groups of sites with similar assemblage composition. Complementarity analysis was performed to determine the minimum number of sites required to conserve at least one population of all species for all 42 sites and for groups of sites detected by the multivariate analyses.

Results The species richness of the deep-sea chemosynthetic sites around the Japanese archipelago was higher in the Okinawa Trough and Sagami Bay where higher methane concentrations were observed. Eight distinct biogeographic regions were detected at the community level, and the community structure was classified according to the geological variables of depth and latitude. Complementarity analysis for all 42 sites indicated 21 sites for conservation, while that for each distinct biogeographic region indicated 30 sites for conservation.

Main conclusions As unique communities exist in each geographically isolated region around the Japanese archipelago, priority areas should be identified for each type of bioregion for effective conservation. At least 30 (17 seeps and 13 vents) of 42 sites should be conserved to avoid the extinction of the local populations of deep-sea chemosynthetic macro-/megabenthos.

Keywords Benthic fauna, chemosynthesis, complementarity, deep-sea, Japanese waters.
INTRODUCTION

Deep-sea chemosynthetic ecosystems are fuelled by microbial primary production, in which inorganic carbon is fixed into organic carbon via reduced chemical compounds, usually hydrogen sulphide or methane (Jannasch & Wirsen, 1979; Cavanaugh, 1985; Jannasch & Mottl, 1985; Childress & Fisher, 1992). The biomass of benthic organisms in vent and seep habitats is often remarkably high – 500 to 1000 times greater than that on the surrounding sea floor (e.g. Tunnicliffe, 1991, 1992). The net primary productivity of deep-sea chemosynthesis at hydrothermal vents is comparable to that of tropical rain forests or coral reefs (Sarrazin & Juniper, 1999; Nyholm et al., 2008). Another remarkable trait of these habitats is the numerous rare and endemic species discovered from vent and seep communities geographically isolated in deep-sea areas (McArthur & Tunnicliffe, 1998; Van Dover et al., 2002; Little & Vrijenhoek, 2003). Despite the scientific importance of understanding ocean biodiversity and ecological connectivity, deep-sea chemosynthetic habitats remain mostly unexplored because of their inaccessibility (McClain & Hardy, 2010). The amount of data regarding species diversity of habitats in deep-sea chemosynthetic ecosystems is insufficient, and thus species diversity must be estimated from fragmented evidence.

In the last few decades, deep-sea vent and seep habitats have come under increasing threat from various human activities either underway or planned (Van Dover et al., 2011, 2012). Recent technological developments have overcome the barrier of water depth and distance from shore, allowing the exploitation of previously inaccessible areas. This has spurred the continuous expansion of human-related activities in these ecosystems, including destructive bottom trawling and the exploitation of energy and mineral resources. As a consequence, deep-sea ecosystems are experiencing serious damage and biodiversity degradation (Ramirez-Llodra et al., 2011; Van Dover et al., 2011, 2012). Setting up protected areas is a critical tool for maintaining habitat integrity and species diversity (e.g. Geldmann et al., 2013). To date, the majority of deep-sea chemosynthetic ecosystems are unprotected, and only a few chemosynthetic ecosystem sites have been partially or fully protected from human activities (Van Dover et al., 2011). To establish protected areas effectively, it is important to identify bioregions that contain geographically distinct assemblages of natural communities and identify the most important areas within these bioregions that will conserve targeted species, ecosystems and ecological processes (Green et al., 2009). As biodiversity is distributed heterogeneously, precise descriptions of biogeographic patterns and processes are vital to advance conservation efforts and set up protected areas (McClain & Hardy, 2010).

The sea around the Japanese archipelago is a suitable area for conducting studies on the deep-sea biodiversity of chemosynthetic ecosystems because of its geological setting on the complex plate boundary region involving four tectonic plates (i.e. the Eurasian and North American continental plates, and the Pacific and Philippine Sea oceanic plates), which develops many hydrothermal vents and methane seeps (Fujikura et al., 2008). More than 50 deep-sea chemosynthetic communities have been discovered around the Japanese archipelago. However, there are currently no protected areas or reserve systems in this region. Previous studies on the genetic connectivity of vesicomyid clam populations around the Japanese archipelago suggest dispersal barriers such as islands, subduction zones and interposing plates result in distinct populations in different geographic regions (Kojima et al., 2001, 2005). Therefore, it is conceivable that distinct biogeographic regions (i.e. bioregions) exist at the community level. This study examined the geographic distributions of the deep-sea chemosynthetic community structures of benthic macro-/megafauna at 42 seeps and vents around the Japanese archipelago and community composition data spanning 155 species belonging to three phyla: molluscs, annelids and arthropods. The objectives were as follows: (1) to characterize the species richness, endemism and structure of chemosynthetic communities; (2) to clarify the factors controlling species richness and community structure; and (3) to identify conservation priority areas for deep-sea chemosynthetic habitats on the basis of the above information.

METHODS

Data collection

Hydrothermal vent systems develop in association with the volcanic-arc basin of the Izu–Bonin Arc and back-arc basin of the Okinawa Trough in the eastern and western Philippine plate. Meanwhile, the methane-seep sites are distributed around the plate convergence regions including the Japan Trench, Nankai Trough, Ryukyu Trench, Sagami Bay, Suruga Trough and Japan Sea (Kojima, 2002; Fujikura et al., 2008). In this study, 42 sites from six regions located below 200 m water depth were selected: 27 methane seeps from the Kuril–Japan Trench, Nankai Trough, Ryukyu Trench, Sagami Bay, Suruga Trough and Japan Sea (Kojima, 2002; Fujikura et al., 2008). The benthic animal datasets were collected from the published literature (Fujikura et al., 1999, 2008; Watanabe & Kojima, in press), JAMSTEC databases (Biological Information System for Marine Life (BISMaL), http://www.godac.jamstec.go.jp/bismal/e; and Marine Biological Sample Database, http://www.godac.jamstec.go.jp/bio-sample/index_e), and unpublished data from a cruise (cruise number: NT11-20, JAMSTEC, Ogura et al., unpublished data). The datasets were compiled with respect to geographic distribution using the presence/absence data of 155 species including 91, 41 and 23 species of molluscs, annelids and arthropods, respectively (see Table S1 in Supporting information). As these
three phyla have been almost completely identified to species level by taxonomic experts across all 42 sites, only these have been included in the analysis, while other less comprehensively studied benthic taxa present at the sites, for example poriferans and echinoderms, have been excluded.

Data analyses

The species richness (i.e. the number of species) and endemic species at each chemosynthetic site were examined from the compiled species distribution data. The correlations between species richness and abiotic variables were calculated by Spearman rank-order correlation. The water temperature, depth, longitude and latitude of each habitat were the only environmental parameters available for all sites. The water temperatures were average values measured during animal collections by submersibles. The concentrations of methane and hydrogen sulphide at some the sites were collected from the published literatures (Sakai et al., 1990, 1994; Gamo et al., 1991; Masuzawa et al., 1992, 1995; Chiba et al., 1993; Tsunogai et al., 1994, 1996, 2002; Ishibashi et al., 1995; Kishida et al., 2004; Toki et al., 2004, 2007; Konno et al., 2006), and their correlations with species richness were examined.

Hierarchical cluster analysis using the group average method was performed on the species presence/absence data using the Sørensen’s index (Sørensen, 1948) to identify groups of sites with similar assemblage compositions. A similarity profile (SIMPROF) test was used as a prerequisite for defining the species composition zones (Clarke et al., 2008). Ordinations in reduced spaces were subsequently generated by non-metric multidimensional scaling (MDS) based on Bray–Curtis similarity (Bray & Curtis, 1957). One-way analysis of similarities (ANOSIM) was subsequently used to calculate the probability of acquiring a given level of clustering determined by the cluster analysis. The relationships between multivariate community structure and abiotic variables (i.e. water temperature, depth, latitude and longitude) were examined using the BIO-ENV procedure (Clarke & Ainsworth, 1993) to define suites of variables that best explain the biotic structure. The analyses were conducted using PRIMER, version 6 (Clarke & Gorley, 2006).

The most important areas for conservation are usually identified on the basis of high species richness and high concentrations of endemic, rare or endangered species (Tognelli et al., 2005). However, conserving sites that contain few but endemic species is also important from the perspective of biodiversity conservation. In this study, complementarity analysis was performed to determine the minimum number of sites required to conserve at least one population of all species in order to identify significant chemosynthetic sites that should be preferentially conserved. MARXAN software was used for complementarity analysis (Ball & Possingham, 2000). The analysis was repeated 1000 times for all 42 sites without grouping as well as for groups of sites detected by the multivariate analyses (i.e. cluster and MDS). A site that was selected 1000 times was considered irreplaceable (i.e. must be
| No. | Site                              | Latitude  | Longitude  | Depth   | Species richness | Endemic species richness | Marxan score (All sites) | Marxan score (by groups of sites) |
|-----|-----------------------------------|-----------|------------|---------|------------------|--------------------------|--------------------------|-----------------------------------|
|     |                                   | (N)       | (E)        | (m)     |                  |                          |                          |                                   |
| 1   | West Kuril Trench                 | 41°19'    | 144°48'    | 5131–5785 | 4                | 0                        | 0                        | 0                                 |
| 2   | Sanriku Escarpment                | 40°06'-08' | 144°10'-14' | 5343–6809 | 11               | 5                        | 1000                     | 1000                              |
| 3   | Near axis of Japan Trench         | 40°03'-04' | 144°16'-17' | 7326–7434 | 5                | 3                        | 1000                     | 1000                              |
| 4   | Sagami Knoll                      | 35°06'    | 139°20'    | 1400–1500 | 8                | 0                        | 0                        | 0                                 |
| 5   | Off Hatsushima Island             | 35°00'    | 139°14'    | 800–1300  | 29               | 5                        | 1000                     | 1000                              |
| 6   | Okinoyama                         | 34°59'    | 139°31'    | 750–1300  | 19               | 0                        | 0                        | 0                                 |
| 7   | Nankai Trough                     | 34°17'-18' | 138°15'    | 270–300   | 8                | 3                        | 1000                     | 1000                              |
| 8   | Omazesaki                         | 34°15'    | 138°02'    | 1100–1200 | 4                | 0                        | 0                        | 0                                 |
| 9   | Ryuyo                             | 34°12'    | 137°46'    | 1000–1100 | 8                | 1                        | 1000                     | 1000                              |
| 10  | Tokai                             | 34°06-07' | 138°07'-08' | 2120–2360 | 2                | 0                        | 0                        | 0                                 |
| 11  | Tenryu II                         | 33°40'    | 137°48'    | 500–900   | 3                | 0                        | 0                        | 0                                 |
| 12  | Yuki                              | 33°53'    | 137°54'-55' | 1940–2180 | 5                | 1                        | 1000                     | 1000                              |
| 13  | Tenryu III                        | 33°39'    | 137°55'    | 3700–3800 | 6                | 0                        | 502                      | 514                               |
| 14  | Atsumi II                         | 33°51'    | 137°23'    | 1042–1100 | 3                | 0                        | 0                        | 1000                              |
| 15  | Tenryu                            | 33°36 '-37' | 137°32'    | 3654–3855 | 7                | 2                        | 1000                     | 1000                              |
| 16  | Zenisu                            | 33°32'-7'  | 138°26'-3' | 3300     | 2                | 0                        | 0                        | 0                                 |
| 17  | Kumano IV                         | 33°59'    | 136°38'    | 2000     | 2                | 0                        | 318                      | 485                               |
| 18  | Kumano VI                         | 33°41'    | 136°34'    | 2000     | 2                | 0                        | 334                      | 515                               |
| 19  | Off Kumano 2100 m site             | 33°16'    | 136°43'    | 2100     | 3                | 0                        | 0                        | 1000                              |
| 20  | Off Kumano 2500–2900 m site        | 33°07-08' | 136°28-29' | 2500–2900 | 5                | 0                        | 348                      | 1000                              |
| 21  | Off Kumano 3250 m site             | 33°08'    | 136°34'    | 3250     | 3                | 0                        | 0                        | 0                                 |
| 22  | Off Kumano 3700 m site             | 33°02'    | 136°34'    | 3660     | 2                | 0                        | 0                        | 0                                 |
| 23  | Off Muroto 3200–3700 m site        | 32°32'-35' | 134°41-43' | 3260–3706 | 5                | 0                        | 498                      | 486                               |
| 24  | Off Muroto 4800 m site             | 32°21'    | 134°56'    | 4800     | 2                | 0                        | 0                        | 1000                              |
| 25  | Minami-Muroto I                   | 32°21'    | 134°32'    | 3540–3620 | 9                | 2                        | 1000                     | 1000                              |
| 26  | Off Kikaijima                     | 28°27'    | 130°19'    | 1400–1500 | 6                | 0                        | 0                        | 1000                              |
| 27  | Kuroshima                         | 24°07'-08' | 124°11'-12' | 636–812  | 14               | 5                        | 1000                     | 1000                              |
|     |                                   |           |            |          |                  |                          |                          |                                   |
|     | Hydrothermal vent                 |           |            |          |                  |                          |                          |                                   |
|     | Izu-Bonin Arc                     |           |            |          |                  |                          |                          |                                   |
| 28  | Myojin                            | 32°06'    | 139°53'    | 1200–1397 | 9                | 0                        | 0                        | 1000                              |
| 29  | Sumsu                            | 31°29'    | 140°02'    | 920      | 7                | 2                        | 1000                     | 1000                              |
| 30  | Suiyo                             | 28°35'    | 140°39'    | 1300–1400 | 4                | 0                        | 0                        | 1000                              |
| 31  | Mokuyo                            | 28°19'    | 140°34'    | 1200–1300 | 3                | 0                        | 0                        | 0                                 |
| 32  | Kaikata                           | 26°42'    | 141°05'    | 300–500   | 10               | 2                        | 1000                     | 1000                              |
| 33  | Nikko                             | 23°05'    | 142°20'    | 400–500   | 8                | 0                        | 0                        | 1000                              |
| 34  | Kasuga II                         | 21°36'    | 143°39'    | 300–400   | 7                | 1                        | 1000                     | 1000                              |
| 35  | Eifuku                            | 21°29'    | 144°02'    | -1580     | 6                | 1                        | 1000                     | 1000                              |
| 36  | Daikoku                           | 21°25     | 144°10'    | 350–450   | 6                | 0                        | 0                        | 0                                 |
|     | Okinawa Trough                    |           |            |          |                  |                          |                          |                                   |
| 37  | Minami-Ensei                      | 28°24'    | 127°39'    | 600–800   | 38               | 18                       | 1000                     | 1000                              |
| 38  | Iheya North                       | 27°48'    | 126°54'    | 900–1000  | 33               | 4                        | 1000                     | 1000                              |
| 39  | Iheya                             | 27°33'    | 126°58'    | 1300–1500 | 35               | 3                        | 1000                     | 1000                              |
| 40  | Izena                             | 27°13'-16' | 127°03'-06' | 1300–1600 | 26               | 2                        | 1000                     | 1000                              |
| 41  | Hatoma                            | 24°52'    | 123°51'    | 1400–1530 | 24               | 2                        | 1000                     | 1000                              |
| 42  | Yonaguni IV                       | 24°51'    | 122°42'    | 1320–1387 | 17               | 2                        | 1000                     | 1000                              |

Site numbers correspond to those in the map in Fig. 1.
protected to conserve targeted species), while other sites that were selected in some but not all replicates were considered flexible. Thus, excluding one or some flexible sites would still allow the conservation of all targeted species in combination with irreplaceable sites (Ball & Possingham, 2000).

RESULTS

Species richness and endemism

The species richness (i.e. number of species) of chemosynthetic-based communities was highly variable amongst the 42 research sites, ranging from two to 38 species per site (Fig. 2). The vent fields at Minami-Ensei (38 species), Iheya (33 species), Iheya North (35 species), Izena (26 species) and Hatoma (24 species) in the Okinawa Trough and the methane seep at Off Hatsushima Island (hereafter OHI, 29 species) in Sagami Bay contained double or more numbers of species compared with the other sites. Molluscs were the most species-rich phylum at all sites, comprising 68.5 ± 18.0% of the total species on average, followed by annelids (17.0 ± 18.0%) and arthropods (14.8 ± 21.5%). Bivalves were the most species-rich class, constituting 65.9 ± 34.7% of the molluscs, followed by gastropods (29.2 ± 31.5%) and polyplacophores (4.1 ± 10.6%). Annelids consisted of single class of polychaetes. Arthropods consisted of Malacostraca (81.5 ± 15.9%) and Maxillopods (18.5 ± 15.9%). Arthropods were common in the hydrothermal vents, but their distribution in seeps was restricted to Sagami Knoll, OHI and Okinoyama in Sagami Bay. The distribution of arthropods was greater in the vents of the Izu–Bonin Arc (except Sumisu), accounting for 33.0–66.7% (overall = 47.5 ± 20.5%) of the observed species. Regarding the accumulative number of species by region, the Okinawa Trough (82 species) had the most, followed by the Nankai Trough (35 species), Sagami Bay (29 species), Izu–Bonin Arc (27 species), Ryukyu Trench (18 species) and Kuril–Japan Trench (14 species).

The occurrences of endemic species were high. In total, 19 of the 42 chemosynthetic sites contained endemic species that were only recorded at a single site (Table 1); many endemic species were found at these 19 sites including the methane-seep sites at Sanriku Escarpment in the Kuril–Japan Trench (5 species), OHI in Sagami Bay (5 species) and Kuroshima in the Ryukyu Trench (5 species) as well as the vent sites at Minami-Ensei (18 species) and Iheya North (4 species) in the Okinawa Trough. Regarding the accumulated
number of endemic species by region, 56 endemic species exclusively comprised 68.3% of the total number of species in the Okinawa Trough, followed by the Nankai Trough (20 species, 57.1%), Sagami Bay (13 species, 44.8%), Izu–Bonin Arc (15 species, 44.8%), Kuril–Japan Trench (13 species, 92.9%) and Ryukyu Trench (5 species, 27.8%).

Although different regions had different numbers of sampling sites, the results regarding the numbers of species and endemic species standardized by the number of sites indicated the Okinawa Trough still had most species (13.7 species and 9.3 endemic species) followed by Sagami Bay (9.7 species and 4.3 endemic species), the Ryukyu Trench (9.0 species and 2.5 endemic species), the Kuril–Japan Trench (4.7 species and 4.3 endemic species), the Izu–Bonin Arc (3.0 species and 1.7 endemic species) and the Nankai Trough (1.8 species and 1.1 endemic species).

There were significant correlations between species richness and depth (Spearman rank-correlation coefficient \( n = 42, r_s = -0.39, P = 0.011 \)) or water temperature \( (r_s = 0.48, P = 0.0022) \), but no significant correlations were found between species richness and latitude \( (r_s = -0.28, P = 0.068) \) or longitude \( (r_s = -0.17, P = 0.25) \). The species richness of the faunal communities tended to be lower at the sites > 2000 m deep, while the species richness were highly variable amongst the sites < 2000 m deep (see Figure S1).

It is difficult to directly compare the chemical concentrations (i.e. methane and hydrogen sulphide) obtained in the seeps and vents because of differences in sample collection methods. For example, the values in seeps are from sediment pore water, while those in vents are from vent fluid. Regardless, if comparisons are to be made, methane concentrations were higher at the sites in the Okinawa Trough and Sagami Bay, while hydrogen sulphide concentrations were higher at Izena in the Okinawa Trough and Ryuyo in the Nankai Trough (Fig. 3a,b). Although the number of studies was limited (~11 sites), the species richness was significantly correlated with methane concentration (Spearman rank-correlation coefficient \( n = 11; r_s = 0.781, \) significant level of \( r_s = 0.618 \) (Fig. 3a). Conversely, species richness was not significantly correlated with hydrogen sulphide concentration \( n = 8, r_s = 0.0714, \) significant level of \( r_s = 0.738 \) (Fig. 3b).

**Classification of faunal community**

The assemblage compositions of the chemosynthetic-based communities were clustered into eight groups and two outliers by the SIMPROF test \( (P < 0.05, \) Fig. 4a). These groups were basically congruent with geographically different regions as follows: Group 1, all sites in the Kuril–Japan Trench; Group 2, nine sites in the Nankai Trough; Group 3, five sites in the Nankai Trough and one site from the Ryukyu Trench; Group 4, five sites in the Nankai Trough, one site from the Ryukyu Trench and one site from the Izu–Bonin Arc; Group 5, all sites (except Sagami Knoll) in Sagami Bay; Group 6, all sites (except Minami-Ensei) in the Okinawa Trough; and Groups 7 and 8, each four site in the Izu–Bonin Arc.

**Figure 3** Correlation between species richness and fluid chemical factors: (a) methane and (b) hydrogen sulphide. Sites in the Izu–Ogasawara Trench and Okinawa Trough are hydrothermal vents (closed symbols), while those in the Kuril–Japan Trench, Nankai Trough, Ryukyu Trench and Sagami Bay are methane seeps (open symbols). Linear fitting conducted by straight-line function \( (y = ax + b, \) where \( x \) is chemical concentration and \( y \) is species richness) was performed using the KALEIDAGRAPH© SYNERGY Software package. Chemical data are from pore water at seeps and from fluid at vents: Off Hatsushima Is. (Masuzawa et al., 1992, 1993; Tsunogai et al., 1996), Ryuyo (Tsunogai et al., 2002), Tenryu III (Tsunogai et al., 2002), Atsumi II (Toki et al., 2007), Off Kumano 2500–2900 m site (Toki et al., 2004), Suiyo (Sakai et al., 1994; Tsunogai et al., 1994; Kishida et al., 2004), Minami-Ensei (Chiba et al., 1993; Sakai et al., 1994), Ihey North (Gamo et al., 1991; Ishibashi et al., 1995), Ihey (Gamo et al., 1991, 1994; Ishibashi et al., 1995), Ihey North (Sakai et al., 1990), Yonaguni IV (Konno et al., 2006).
The two-dimensional MDS ordination of the assemblage compositions of the 42 chemosynthetic sites showed a stress value of 0.05 (Fig. 4b), indicating the ordination was acceptable with an excellent representation (Clarke & Warwick, 1994). The MDS ordination plots were consistent with the cluster analysis. All eight groups detected by the multivariate analyses were also consistent with geographically different regions and significantly different (ANOSIM test, Global \( R = 0.834, P < 0.001 \)). The Nankai Trough was divided into three groups (Groups 2, 3 and 4) basically with respect to depth and horizontal location. Group 2 comprised relatively deeper sites with depths from 2500 to 4800 m, while Group 3 comprised sites with depths from 1400 to 2360 m (1940–2360 m for sites from Nankai Trough). Group 4 comprised the north-eastern sites of the Nankai Trough with depths from 270 to 1200 m.

The BIO-ENV procedure (Table 2) showed that depth was the only abiotic variable that best grouped sites in a manner consistent with the faunal patterns (\( \rho = 0.551, P < 0.01 \)). Furthermore, the best 2-variable combinations were depth and latitude (\( \rho = 0.546 \)), and depth and water temperature (\( \rho = 0.463 \)).

**Complementarity analysis**

The complementarity analysis selected 24 sites to conserve all 155 species inhabiting the 42 sites in this study (Marxan score, 318–1000; Table 1). Of the 24 selected sites, 19 were determined to be irreplaceable sites (i.e. they were selected 1000/1000 times) and the remaining five were flexible sites. The 19 irreplaceable sites corresponded to the 19 sites where endemic species were only found at a single site. Thus, the sites selected on the basis of the principle of complementarity are strongly affected by the presence of endemic species (Table 1). Excluding three of the five flexible sites would still allow for the conservation of all 155 species populations (Table 3). Therefore, the complementarity analysis for all 42 sites concludes at least 21 sites (11 seeps and 10 vents, 50.0\% of all sites) including 19 irreplaceable sites, and two flexible sites are required to conserve all 155 species.

The results of the complementarity analysis targeted for each geographically distinct region detected by the multivariate analysis selected 32 sites for conservation (Marxan score, 485–1000; Table 1). The 32 sites selected contained an additional nine irreplaceable sites compared with the previous sites (i.e. the Tokai, Tenryu II, Atsumi II, Zenisu, Off Kumanono 2100 m site and Off Kumano 2500–2900 m site in the Nankai Trough, Off Kikaijima in the Ryukyu Trench and Suiyo and Nikko in the Izu–Bonin Arc), giving 28 irreplaceable sites and four flexible sites. Of the four flexible sites, two sites were in Group 2 (the Tenryu III and Off Muroto 3200–3700 m site in the Nankai Trough) while the other two sites were in Group 3 (Kumano IV and Kumano VI in the Nankai Trough), and one site from each group must be protected to conserve all species. Therefore, the complementarity analysis by geographically distinct groups indicated 30 sites (28 irreplaceable + two flexible sites, 71.4\% of all sites), comprising 17 seeps and 13 vents, must be protected to conserve at least one population of all 155 species. Although we conducted complementarity analysis for each geographically groups clustered by SIMPROF test, Off Kikaijima and Kuroshima (Ryukyu Trench) in Groups 3 and 4, respectively (Fig. 4a,b), where Nanaki Trough sites dominated, were placed in the Ryukyu Trench for the analysis because these two sites are far from those in the Nankai Trough (784–1768 km). Similarly, Sumisu in Group 4 was included in Izu-Bonin Arc (Group 7). Sagami Knoll, an outlier by the SIMPROF test (Fig. 4a), was also included and analysed in Sagami Bay because the site contained no endemic species compared with the other sites in Sagami Bay (i.e. OHI and Okinoyama, Table 1).

**DISCUSSION**

**Species richness**

This study describes the biogeographic information of the deep-sea chemosynthetic communities around the Japanese archipelago to identify priority areas for conservation. In the present study, 19 of 42 sites contained species only recorded at one site – and these species were therefore described as ‘endemic’ to those sites. However, it should be noted that as both cold seeps and hydrothermal vents are fundamentally ephemeral environments at the scale of individual vent field or seep site (Van Dover et al., 2002), it is almost certain that there are either additional as-yet-unknown sites in the area also hosting populations of those ‘endemic’ species, or the ‘endemic’ species may be present (but not yet sampled) at other known sites.

The species richness of the chemosynthetic-based communities in the area surrounding the Japanese archipelago was highly variable amongst the sites. Basically, vent fields in the Okinawa Trough and seeps in Sagami Bay (especially OHI) contained more species. Okinawa Trough and Sagami Bay are relatively well-studied fields (i.e. 30–92 dives in the Okinawa Trough and 10–200 dives in Sagami Bay; JAMSTEC Data Search Portal, http://www.godac.jamstec.go.jp/dataportal/index_eng.html), and the species richness could be influenced by sampling efforts. However, there are many other sites that have higher sampling efforts but lower species richness; for example, Suiyo in the Izu–Bonin Arc have 90 dives, but only four species were recorded there so far. Similarly, Myojin and Kaikata (Izu–Bonin Arc) have 72 and 33 dives but only nine and 10 species, Kurosima (Ryukyu Trench) have 53 dives but 14 species, and Off Muroto 4800 m site have 21 dives but only two species (JAMSTEC Data Search Portal). Therefore, it is highly likely that the high species richness in the Okinawa Trough vents and Sagami Bay seeps is real.

In this study, depth (along with decreasing temperature) was significantly correlated with species richness at the site level. Similarly, Sibuet and Olu (1998) report a general trend of decreasing species richness with depth in several deep-sea
Figure 4 The similarity of macro- and megafaunal community structure in different geological regions: (a) dendrogram of the group average cluster analysis and (b) non-metric MDS plots. Solid branches of the dendrogram indicate significant faunal groups in which the SIMPROF test \( (P < 0.05) \) suggested the structure is not random. The results of clusters detected by SIMPROF test are imposed as solid lines in the MDS plots.
Chemosynthetic ecosystems. The small number of species in deeper waters could be construed as limited larval dispersal there (Sibuet & Olu, 1998). However, although the species richness of the faunal communities tend to be lower at the sites > 2000 m deep in the present study, the species richness were highly variable amongst the sites < 2000 m deep and may be explained not only by the depth but also by habitat heterogeneity (Cordes et al., 2005, 2010). The major factor affecting species richness may include a variety of habitat conditions such as seabed substrata as a benthic habitat, composition and concentrations of fluid chemicals from vents and seeps (e.g. Levin et al., 2001; Bergquist et al., 2003; Cordes et al., 2005, 2010). For example, the seabed materials in the vent fields in the Izu-Bonin Arc are rather rocky with no sediment, while the Okinawa Trough vent fields are characterized by thick soft sediments (Narita et al., 1990; Hsu et al., 2004). These offer suitable habitats for endobenthic invertebrates even around hydrothermal vent site, allowing an inhabiting of both vent and seep communities in the area (Tunnicliffe et al., 1998; Watanabe et al., 2010). Indeed, representative species of endobenthic deep-sea clam Calyptogena are found in sediment-hosted seafloor in most of the seeps of Japan as well as the Okinawa Trough vent fields, but none of these clams have been reported in the Izu-Bonin vent fields (Fujikura et al., 2008).

Hydrogen sulphide and methane are common energy sources for chemosynthetic prokaryotes and are strong drivers that affect the habitability of prokaryote species including symbiotic species of benthos dwelling vent and seep areas (reviewed by Childress & Fisher, 1992; Nelson & Fisher, 1995). In this study, species richness tended to increase linearly with increasing methane concentration. Higher methane concentrations simply support the primary production of prokaryotes and secondary growth of invertebrates. The amount of energy available for chemosynthesis readily affects the biomass of invertebrates associated with bacterial symbionts such as Calyptogena clams, Bathymodiolus mussels and vestimentiferan tubeworms (e.g. Cordes et al., 2010). These deep-sea chemosynthetic-bearing animals favour high methane environments and may in turn act as ‘ecosystem engineers’ (Cordes et al., 2005, 2009, 2010), supporting a greater number of species in the structure of their beds. A preliminary observation at the vent sites in the Okinawa Trough where Bathymodiolus mussel beds and motile galatheid crabs (Shinkaiia crosnieri) colonies exist alongside each other showed the number of invertebrate species tend to be higher in the mussel beds than galatheid crab colonies, implying mussel beds can harbour more species (Watanabe et al., unpublished data). The multiple occurrences of the higher concentration of methane as well as diverse seabed materials may provide a highly diverse suite of potential habitats for chemosynthetic faunal community (Cordes et al., 2005) and may explain the higher number of species in the vent sites in the Okinawa Trough and seeps in Sagami Bay where higher methane concentrations were observed.

### Community structure

The deep-sea chemosynthetic sites of the Japanese archipelago were clustered into several groups with respect to faunal similarity; these groups were congruent with geographically different regions. These results suggest unique communities exist in each geographically isolated region. Watanabe et al. (2010) previously report that the vent and seep communities around the Japanese archipelago share 20% of their species, and there are no apparently distinct groupings of chemosynthetic communities consisting of distinct geographic regions. This discrepancy may simply be attributable to the fewer species examined in that study (i.e. 55 species in Watanabe et al., 2010 vs. 155 species in the present study).

It should be noted that the similarity index used in this study is influenced by absence of species shared between sites, in addition to presence of shared species, and that any influence of variable sampling effort on perceived patterns of
species richness may influence the multivariate patterns produced. The number of sampling effort varied depending on sites; the sites with more than 15 sampling efforts showed large variation in species richness, while those with less than 15 efforts showed lower species richness (see Figure S2a). These results imply the sites with < 15 efforts may contain insufficient sampling sites, which may have influenced on the entire structures of cluster analysis in this study (i.e. Fig. 4a). However, the significant groups constructed by SIMPROF test for only < 15 efforts sites were identical to those for all sites (see Figure S2b, except Sagami Knoll), and thus the < 15 efforts sites did not influence the entire significant groupings for all 42 sites. We therefore consider that our analysis is still valid although additional research will be required to acquire more concrete figures (especially for < 15 efforts sites).

Variance events have probably influenced the species distributions of biogeographic regions (Tunnicliffe et al., 1998). Cordes et al. (2007) report that depth appears to be a more important factor affecting community structure than distance in the deep cold seeps of the Gulf of Mexico. Similarly, the present BIO-ENV test results suggest depth is the best single variable that explains the faunal community grouping. Furthermore, the combinations of depth and water temperature as well as depth and latitude are secondary important drivers of the zonation of faunal communities. These results suggest depth, temperature and latitude may play pivotal roles in the differentiation of faunal community distribution in Japanese deep-sea waters. Although the present study could not statistically consider the habitat heterogeneity caused by vents/seeps and/or methane and sulphide concentrations, which may primarily determine the distribution of specialized symbiont macro-/megafauna (MacDonald et al., 1989; Sibuet & Olu, 1998), the chemosynthetic-based community structure in Japan can be classified according to the geological variables of depth (along with decreasing temperature) and latitude.

**Potential priority areas for conservation**

From the perspective of species richness and endemism, species-rich sites, which have both relatively more species as well as endemic species, should be primarily protected for biological conservation. Accordingly, the chemosynthetic sites at Sanriku Escarpment (Kuril–Japan Trench), OHI and Okinoyama (Sagami Bay), Kuroshima (Ryukyu Trench), Kaikata (Izu–Bonin Arc), and all sites from the Okinawa Trough should be preferentially selected as protected areas. However, from a perspective of species conservation, sites with their own endemic species should also be protected, even if they have few species overall.

The results of the complementarity analysis for all sites combined indicate that if 21 sites (19 irreplaceable sites + two flexible sites) are set aside as marine reserves, at least one population of each 155 species will be conserved in theory. However, the results of the multivariate analyses (i.e. cluster and MDS analyses) indicate unique communities exist in each geographically isolated region. Therefore, the chemosynthetic community should be protected separately in each geographic region. Some species are present in several geographically isolated regions, although larval supply between these regions is unlikely (Watanabe et al., 2010). It would be incorrect to use the results of the complementarity analysis targeting all sites pooled from several distinct bioregions, because it may result in the selection of sites that have no larval supply for some populations from other sites, and complementarity analysis targeting each distinct bioregion must be considered. The complementarity analysis results targeting each bioregion indicate that the conservation of 30 sites (28 irreplaceable sites + two flexible sites; 17 seeps and 13 vents) listed in Table 1 will provide more efficient species conservation. Thus, these would be the potential priority areas for the conservation of the deep-sea chemosynthetic communities around the Japanese archipelago.

We used only presence/absence data to identify significant sites in the present study, because they were the only data available for all sites at this time. Complementary studies on phylogenetic connectivity and population density of vent and seep communities will help refine which areas are significant for conservation. Information about the patterns of genetic connectivity will help identify which sites are population sources and sinks (Cordes et al., 2010). Information about the population size of a given species is also important, because a high-density site will serve as a source of propagates dispersing into relatively small habitats (Cordes et al., 2010). Therefore, detailed and comprehensive investigations of phylogenetic connectivity and population density are warranted.

In conclusion, the species richness of the deep-sea chemosynthetic sites around the Japanese archipelago is higher in the Okinawa Trough and Sagami Bay where higher methane concentrations were observed. In addition, the results show distinct biogeographic regions exist at the community level as previously observed at the population level. The community structure can be classified according to the geological variables of depth and latitude. Finally, complementarity analysis should be conducted for each different bioregion to effectively identify priority areas for conservation. Thus, the results indicate at least 30 sites (17 seeps and 13 vents) should be conserved to avoid extinctions of the local populations of the deep-sea chemosynthetic macro-/megabenthos (annelids, arthropods and molluscs) around the Japanese archipelago.

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REFERENCES

Ball, I. & Possingham, H. (2000) MARXAN (Marine Reserve Design using Spatially Explicit Annealing). The University of Queensland, Australia.

Bergquist, D.C., Ward, T., Cordes, E.E., McNelis, T., Howlett, S., Kosoff, R., Hourdez, S., Carney, R. & Fisher, C.R. (2003) Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. Journal of Experimental Marine Biology and Ecology, 289, 197–222.

Bray, J.R. & Curtis, J.T. (1957) An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs, 27, 325–349.

Cavanaugh, C.M. (1985) Symbiosis of chemosynthetic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. Bulletin of the Biologocial Society of Washington, 6, 373–388.

Chiba, H., Nakashima, K., Gamo, T., Ishibashi, J., Tsunogai, U. & Sakai, H. (1993) Hydrothermal activity at the Minami-Ensei Knoll, Okinawa Trough: chemical characteristics of hydrothermal solutions. Proceedings of JAMSTEC Symposium on Seep-sea Research, 9, 271–282.

Childress, J.J. & Fisher, C.R. (1992) The biology of hydrothermal vent animals: physiology, biochemistry, and autotrophic symbioses. Oceanography and Marine Biology: An Annual Review, 30, 337–441.

Clarke, K.R. & Ainsworth, M. (1993) A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series, 92, 205–219.

Clarke, K.R. & Gorley, R.N. (2006) PRIMER-E V6: User Manual/Tutorial. PRIMER-E, Plymouth.

Clarke, K.R. & Warwick, R.M. (1994) Similarity-based testing for community pattern: the 2-way layout with no replication. Marine Biology, 118, 167–176.

Clarke, K.R., Somerfield, P.J. & Gorley, R.N. (2008) Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. Journal of Experimental Marine Biology and Ecology, 366, 56–69.

Cordes, E.E., Hourdez, S., Predmore, B.L., Redding, M.L. & Fisher, C.R. (2005) Succession of hydrocarbon seep communities associated with the long-lived foundation species Lamellibrachia luymesi. Marine Ecology Progress Series, 305, 17–29.

Cordes, E.E., Carney, S.L., Hourdez, S., Carney, R.S., Brooks, J.M. & Fisher, C.R. (2007) Cold seeps of the deep Gulf of Mexico: community structure and biogeographic comparisons to Atlantic equatorial belt seep communities. Deep-Sea Research I, 54, 637–653.

Cordes, E.E., Bergquist, D.C. & Fisher, C.R. (2009) Macrocology of Gulf of Mexico cold seeps. Annual Review in Marine Science, 1, 143–168.

Cordes, E.E., Canha, M.R., Galéron, J., Mora, C., Olu-Le Roy, K., Sibuet, M., Van Gaever, S., Vanreusel, A. & Levin, L.A. (2010) The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. Marine Ecology, 31, 51–65.

Fujikura, K., Kojima, S., Tamaki, K., Maki, Y., Hunt, J. & Okutani, T. (1999) The deepest chemosynthesis-based community yet discovered from the hadal zone, 7326 m deep, in the Japan Trench. Marine Ecology Progress Series, 190, 17–26.

Fujikura, K., Okutani, T. & Maruyama, T. (2008) Deep-Sea Life—Biological Observations Using Research Submersibles. Tokai University Press, Kanagawa, Japan. (in Japanese with English figure captions)

Gamo, T., Sakai, H., Kim, E.-S., Shitashima, K. & Ishibashi, J. (1991) High alkalinity due to sulfate reduction in the CLAM hydrothermal field, Okinawa Trough. Earth and Planetary Science Letters, 107, 328–338.

Geldmann, J., Barnes, M., Coad, L., Craigie, I., Hockings, M. & Burgess, N.D. (2013) Effectiveness of terrestrial protected areas in reducing habitat loss and population declines. Biological Conservation, 161, 230–238.

Green, A., Smith, S.E., Lipsett-Moore, G., Groves, C., Peterson, N., Sheppard, S., Lokani, P., Hamilton, R., Almany, J., Aitsi, J. & Bualia, L. (2009) Designing a resilient network of marine protected areas for Kimbe Bay, Papua New Guinea. Oryx, 43, 488–498.

Hsu, S.C., Lin, F.J., Jeng, W.L., Chung, Y.C., Shaw, L.M. & Hung, K.W. (2004) Observed sediment fluxes in the southwesternmost Okinawa Trough enhanced by episodic events: flood runoff from Taiwan rivers and large earthquakes. Deep-Sea Research I, 51, 979–997.

Ishibashi, J., Sano, Y., Wakita, H., Gamo, T., Tsutsumi, M. & Sakai, H. (1995) Helium and carbon geochemistry of hydrothermal fluids from the Mid-Okinawa Trough back arc basin, southwest of Japan. Chemical Geology, 123, 1–15.

Jannasch, H.W. & Mottl, M.J. (1985) Geomicrobiology of deep-sea hydrothermal vents. Science, 229, 717–725.

Jannasch, H.W. & Wirsen, C.O. (1979) Chemosynthetic primary production of East Pacific seafloor spreading centers. BioScience, 29, 592–598.

Kishida, K., Sohrin, Y., Okamura, K. & Ishibashi, J. (2004) Tungsten enriched in submarine hydrothermal fluids. Earth and Planetary Science Letters, 222, 819–827.

Kojima, S. (2002) Deep-sea chemosynthesis-based communities in the Northwestern Pacific. Journal of Oceanography, 58, 343–363.

Kojima, S., Ohta, S., Yamamoto, T., Miura, T., Fujiwara, Y. & Hashimoto, J. (2001) Molecular taxonomy of vestimentiferans of the western Pacific and their phylogenetic relationship to species of the eastern Pacific I. Family Lamellibrachiidae. Marine Biology, 139, 211–219.

Kojima, S., Fujikura, K. & Okutani, T. (2005) Genetic differentiation of two vesicomyid bivalves, Calyptogena okutanii and Calyptogena nankaiensis, between seep areas off the central Honshu and hydrothermal vent fields in the Okinawa Trough. Venus, 64, 45–53.
Konno, U., Tsunogai, U., Nakagawa, F., Nakaseama, M., Ishibashi, J., Nunoura, T. & Nakamura, K. (2006) Liquid CO₂ venting on the seafloor: Yonaguni Knoll IV hydrothermal system. Okinawa Trough. Geophysical Research Letters, 33, L16607.

Levin, L.A., Etter, R.J., Rex, M.A., Gooday, A.J., Smith, C.R., Pineda, J., Stuart, C.T., Hessler, R.R. & Pawson, D. (2001) Environmental influences on regional deep-sea species diversity. Annual Review of Ecology and Systematics, 32, 51–93.

Little, C.T.S. & Vrijenhoek, R.C. (2003) Are hydrothermal vent animals living fossils? Trends in Ecology and Evolution, 18, 562–588.

MacDonald, I.R., Boland, G.S., Baker, J.S., Brooks, J.M., Kennicutt, M.C. & Biggar, R.R. (1989) Gulf of Mexico hydrocarbon seep communities. II. Spatial distribution of seep organisms and hydrocarbons at Bush Hill. Marine Biology, 101, 235–247.

Masuzawa, T., Honda, N., Kitagawa, H. & Kusakabe, M. (1992) Sulfate reduction using methane in sediments beneath a bathyal “cold seep” giant clam community off Hatsushima Island, Sagami Bay, Japan. Earth and Planetary Science Letters, 110, 39–50.

Masuzawa, T., Nakatsuka, T. & Honda, N. (1995) Geochemistry of pore waters from a bathyal Calyptogena community off Hatsushima Island, Sagami Bay, Japan. Biogeochemical Processes and Ocean Flux in the Western Pacific (ed. by H. Sakai and Y. Nozaki), pp. 407–421. Terra Scientific Publishing Company, Tokyo.

McArthur, A.G. & Tunnellcliffe, A. (1998) Relics and antiquity revisited in the modern vent fauna. Modern Ocean Floor Processes and the Geological Record (ed. by R.A. Mills and K. Harrison), pp. 271–291. Special Publication of the Geological Society of London, London.

McClain, C.R. & Hardy, S.M. (2010) The dynamics of biogeographic ranges in the deep sea. Proceedings of the Royal Society B, 277, 3533–3546.

Narita, H., Harada, K. & Tsunogai, S. (1990) Lateral transport of sediment particles in the Okinawa Trough determined by natural radionuclides. Geochemoical Journal, 24, 207–216.

Nelson, D.C. & Fisher, C.R. (1995) Chemoautotrophic and methanotrophic endosymbiotic bacteria at deep-sea vents and seeps. The Microbiology of Deep-Sea Hydrothermal Vents (ed. by D.M. Karl), pp. 125–167. CRC Press, Boca Raton.

Nyholm, S.V., Robidart, J. & Girgius, P.R. (2008) Coupling metabolite flux to transcriptomics: insights into the molecular mechanisms underlying primary productivity by the hydrothermal vent tubeworm Ridgea piscesae. The Biological Bulletin, 214, 255–265.

Ramírez-Llodra, E., Tyler, P.A., Rowden, A.A., Levin, L., Smith, C., Clark, M.R., Escobar, E., Aksel Bergstad, O., Baker, M.C., Rogers, A., Van Dover, C.L. & Menot, L. (2011) Man and the last great wilderness: human impact on the deep sea. PLoS ONE, 6, e22588.

Sakai, H., Gamo, T., Kim, E.-S., Shitashima, K., Yanagisawa, F. & Tsutsui, M. (1990) Unique chemistry of the hydrothermal solution in the mid-Okinawa Trough backarc basin. Geophysical Research Letters, 17, 2133–2136.

Sakai, H., Kanayama, S., Tsunogai, U., Ishibashi, J. & Wakit-a, H. (1994) Gas geochemistry of submarine hydrothermal systems at Okinawa backarc basin and Ogasawara arc. Mineral Magazine, 58A, 797–798.

Sarrazin, J. & Juniper, S.K. (1999) Biological characteristics of a hydrothermal edifice mosaic community. Marine Ecology Progress Series, 185, 1–19.

Sibuet, M. & Olu, K. (1998) Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Research II, 45, 517–567.

Sørensen, T. (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its applications to analysis of the vegetation on Danish commons. Biologiske Skrifter, 5, 1–34.

Tognelli, M.F., Silva-García, C., Labra, F.A. & Marquet, P.A. (2005) Priority areas for the conservation of coastal marine vertebrates in Chile. Biological Conservation, 126, 420–428.

Toki, T., Tsunogai, U., Gamo, T., Kuramoto, S. & Ashi, J. (2004) Detection of low-chloride fluids beneath a cold seep filed on the Nankai accretionary wedge off Kumanō, south of Japan. Earth and Planetary Science Letters, 228, 37–47.

Toki, T., Tsunogai, U., Gamo, T. & Tanahashi, M. (2007) Geochemical studies of pore fluid in surface sediment on the Daini Atsumi Knoll. Journal of Geochemical Exploration, 95, 29–39.

Tsunogai, U., Ishibashi, J., Wakita, H., Gamo, T., Watanabe, K., Kajimura, T., Kanayama, S. & Sakai, H. (1994) Peculiar features of Suiyo Seamount hydrothermal fluids, Izu-Bonin Arc: differences from subaerial volcanism. Earth and Planetary Science Letters, 126, 289–301.

Tsunogai, U., Ishibashi, H., Wakita, H., Gamo, T., Watanabe, K., Kajimura, T., Kanayama, S. & Sakai, H. (1996) Fresh water seepage and pore water recycling on the seafloor: Sagami Trough subduction zone, Japan. Earth and Planetary Science Letters, 138, 157–168.

Tsunogai, U., Yoshida, N. & Gamo, T. (2002) Carbon isotopic evidence of methane oxidation through sulfate reduction in sediment beneath cold seep vents on the seafloor at Nankai Trough. Marine Geology, 187, 145–160.

Tunnellcliffe, V. (1991) The biology of hydrothermal vents: ecology and evolution. Oceanography and Marine Biology: An Annual Review, 29, 319–407.

Tunnellcliffe, V. (1992) Hydrothermal-vent communities of the deep sea. American Scientist, 80, 336–349.

Tunnellcliffe, V., McArthur, A.G. & McHugh, D. (1998) A biogeographical perspective of deep-sea hydrothermal vent fauna. Advances in Marine Biology, 34, 355–442.

Van Dover, C.L., German, C.R., Speer, K.G., Parson, L.M. & Vrijenhoek, R.C. (2002) Evolution and Biogeography of deep-sea vent and seep invertebrates. Science, 295, 1253–1257.
Van Dover, C.L., Smith, C.R., Ardron, J. et al. (2011) Environmental management of deep-sea chemosynthetic ecosystems: justification of and considerations for a spatially-based approach. ISA Technical study; no. 9., International Seabed Authority, Kingston, Jamaica. Available at: http://www.isa.org.jm/files/documents/EN/Pubs/TS9/index.html
Van Dover, C.L., Smith, C.R., Ardron, J., Dunn, D., Gjerde, K., Levin, L., Smith, S. & Contributors, T.D.W. (2012) Designating networks of chemosynthetic ecosystem reserves in the deep sea. Marine Policy, 36, 378–381.
Watanabe, H. & Kojima, S. (in press) Vent fauna in the Oki-nawa Trough. Subseafloor Biosphere Linked to Global Hydrothermal Systems; TAIGA Concept (ed. by J. Ishibashi, K. Okino and M. Sunamura). Springer, Tokyo, Japan.
Watanabe, H., Fujikura, K., Kojima, S., Miyazaki, J. & Fujiwara, Y. (2010) Japan: vent and seep in close proximity. The Vent and Seep Biota Aspects from Microbes to Ecosystems, Topics in Geobiology, vol. 33 (ed. by S. Kiel), pp. 379–402. Springer + Business Media B.V., Netherlands.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Correlations between species richness and (a) depth, (b) water temperature, (c) latitude and (d) longitude.

**Figure S2** Species richness in relation to sampling effort (number of dives) from 42 deep-sea chemosynthetic ecosystems around the Japanese archipelago (a) and dendrogram with SIMPROF test from the sites that have less than 15 sampling efforts (b).

**Table S1** Benthic animal datasets for 155 species from 42 deep-sea chemosynthetic ecosystems around the Japanese archipelago.

**BIOSKETCH**

Ryota Nakajima is a postdoctoral researcher at JAMSTEC with major interests in the conservation and ecology of the deep sea and coral reef. He is involved in a scientific project on integrative observation and assessment of biodiversity loss in changing seas, as part of the research programme entitled ‘Integrative Observation and Assessment of Asian Biodiversity’, which was initiated for 2011–2015 with the support of the Environment Research and Technology Development Fund (S9) of the Ministry of the Environment, Japan. The project aims to evaluate the present status and future perspectives of marine biodiversity for coral reefs, kelp coasts, seagrass beds, pelagic waters and the deep sea, and to identify ecologically or biologically significant areas (EBSA). This paper is the fruit of EBSA identification for deep-sea chemosynthetic ecosystems around the Japanese archipelago.

Author contributions: R.N., T.Y., H.W., K.F., H.Y. and Y.S. designed the research; R.N., T.Y., H.W., K.F., K.T., H.Y. collected the data; R.N. and T.Y. conducted the analysis; and R.N. wrote the manuscript with the help of all co-authors.

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