**Supplemental Information: Association of deep-sea incirrate octopods with manganese crusts and nodule fields in the Pacific Ocean**

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**Supplemental Data**

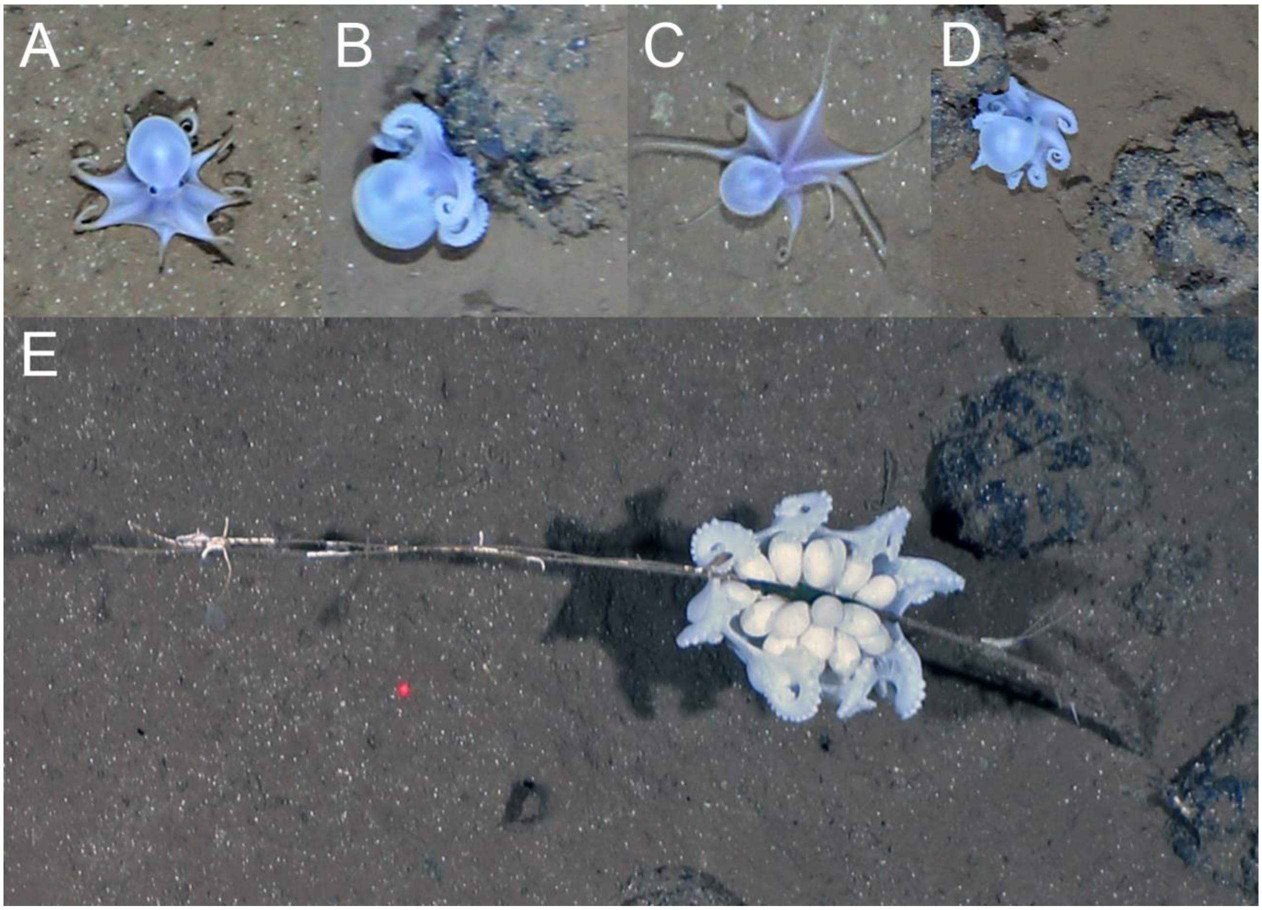


**Figure S1. Typical octopod behaviors observed during the current study.**
(A) Flat on seafloor, tentacles extended and curled, (B) Curled close to manganese nodule, (C) Flat on seafloor, penetrating sediment with undulating tentacles, (D) Curled between two adjacent manganese nodules, (E) Brooding eggs at the approximate mid-point of a dead sponge stalk, itself attached to an underlying manganese nodule.

**Table S1. Overview of data type, spatial location, size, habitat and behavior of all octopod individuals observed in the current study.**

| Date of observation | Time of observation (UTC) | Type of data collected | Octopus type | Mantle (length / diameter) cm | Seafloor type | Latitude | Longitude | Depth (m) | Nodule density (nodules $m^{-2}$) | Behavior |
|---|---|---|---|---|---|---|---|---|---|---|
| **Ka'ena Ridge Observation** | | | | | | | | | | |
| 2011.05.28 | 12:27:10 | Still images, Video | 'Casper' | 6.5/5 | Basalt, Fe-Mn crust | 21.570045 | -158.732044 | 3116 | Crust | Side of rock outcrop, curled tentacles |
| **Peru Basin Observations** | | | | | | | | | | |
| 2015.08.31 | 03:57:41 | Still image | 'Casper' | 3/3 | Not ploughed | -7.132668 | -88.448601 | 4126 | 6.2 | Flat, curled tentacles |
| 2015.09.01 | 02:54:22 | Still image, Video | 'Casper' | 4/3.5 | Ploughed | -7.070630 | -88.469907 | 4122 | 2.1 | Flat, curled tentacles |
| 2015.09.01 | 02:56:21 | Still image, Video | 'Casper' | 3/3 | Ploughed | -7.070670 | -88.469850 | 4139 | 0.6 | Flat, penetrating sediment |
| 2015.09.05 | 20:18:00 | Still images, Video | *Vulcanoctopus* sp. | ~9/7 | Not ploughed | -7.54 | -88.451 | 4099 | No data | Flat, penetrating sediment, creeping |
| 2015.09.06 | 04:30:56 | Still image | Unidentified incirrate | Not determined | Not ploughed | -7.057843 | -88.434452 | 4150 | 31.2 | Brooding on stalk |
| 2015.09.08 | 10:37:45 | Still image | 'Casper' | 3/3 | Not ploughed | -7.074089 | -88.456615 | 4156 | 9.2 | Side nodule, curled, traces |
| 2015.09.09 | 06:58:13 | Still images | 'Casper' | 5/3 | Not ploughed | -7.093517 | -88.441484 | 4151 | 10.2 | Groove between nodules, curled, traces |
| 2015.09.12 | 05:27:27 | Still image | 'Casper' | 2/2 | Not ploughed | -7.119392 | -88.451418 | 4139 | 7.3 | Flat, curled tentacles |
| 2015.09.15 | 11:06:36 | Still image, Video | Unidentified incirrate | Not determined | Not ploughed | -7.118954 | -88.441212 | 4181 | 10.4 | Brooding on stalk |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2015.09.15 | 17:16:14 | Still image, video | Unidentified incirrate | Not determined | Not ploughed | -7.125403 | -88.4514 | 4197 | No data | Flat, curled tentacles |
| 2015.09.16 | 05:41:23 | Still images, video | *Vulcanoctopus* sp. | 8/6 | Not ploughed | -7.069301 | -88.478893 | 4137 | 5.0 | Side nodule, curled |
| 2015.09.16 | 05:56:02 | Still images, video | 'Casper' | 4/3 | Not ploughed | -7.069962 | -88.477895 | 4136 | 5.3 | Flat, penetrating sediment |
| 2015.09.16 | 10:31:07 | Still image | 'Casper' | 4/3 | Ploughed | -7.086809 | -88.460951 | 4164 | 0.8 | Flat, curled tentacles |
| 2015.09.16 | 10:38:59 | Still images | 'Casper' | 5/5 | Not ploughed | -7.087799 | -88.460666 | 4164 | 9.1 | Flat, curled tentacles, on holothurian |
| 2015.09.16 | 11:00:56 | Still images, video | 'Casper' | 7/6 | Not ploughed | -7.090338 | -88.459986 | 4158 | 8.0 | Groove between nodules, curled, traces |
| 2015.09.18 | 03:41:19 | Still image | 'Casper' | 3/3 | Not ploughed | -7.054571 | -88.466957 | 4142 | 26.9 | Flat, curled tentacles |
| 2015.09.18 | 09:20:12 | Still image, video | 'Casper' | 5/4 | Not ploughed | -7.086112 | -88.455441 | 4147 | 6.7 | Flat, curled tentacles |
| 2015.09.19 | 07:16:30 | Still image, video | *Vulcanoctopus* sp. | 5/4 | Not ploughed | -7.082241 | -88.459931 | 4151 | 4.0 | Side of nodule, curled, traces |
| 2015.09.21 | 03:20:47 | Still image | 'Casper' | 6/4 | Not ploughed | -7.083633 | -88.457568 | 4153 | 3.1 | Flat, curled tentacles, bear nodule |
| 2015.09.24 | 07:50:42 | Still image, video | 'Casper' | 3/2.5 | Not ploughed | -7.118491 | -88.426379 | 4165 | 7.9 | Groove between nodules, curled, traces |
| 2015.09.25 | 06:55:18 | Still images, video | 'Casper' | 3.5/4 | Not ploughed | -7.078965 | -88.464116 | 4139 | 4.5 | Side of nodule, curled, traces |
| 2015.09.25 | 07:25:23 | Still images | 'Casper' | 6/5 | Not ploughed | -7.079321 | -88.461588 | 4145 | 3.8 | Flat, curled tentacles |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2015.09.25 | 09:03:15 | Still image, video | 'Casper' | 8.5/7 | Not ploughed | -7.080662 | -88.450815 | 4159 | 2.4 | Flat, curled tentacles, bear nodule |
| 2015.09.27 | 03:49:01 | Still image, video | 'Casper' | 3/2 | Not ploughed | -7.068486 | -88.534268 | 4123 | 7.5 | Groove between nodules, curled, traces |
| 2015.09.27 | 07:33:39 | Still images, video | 'Casper' | 5/4.5 | Not ploughed | -7.081846 | -88.517960 | 4114 | 10.4 | Groove between nodules, curled, traces |
| 2015.09.27 | 07:54:21 | Still images, video | 'Casper' | 4/3 | Not ploughed | -7.083206 | -88.516649 | 4109 | 12.5 | Groove between nodules, curled, traces |
| 2015.09.27 | 08:38:58 | Still image, video | 'Casper' | 3/2.5 | Not ploughed | -7.088194 | -88.516633 | 4109 | 8.5 | Flat, penetrating sediment |
| **Necker Ridge Observation** | | | | | | | | | | |
| 2016.02.27 | 22:33:08 | Still images, video | 'Casper' | 6.4/? | Basalt, Fe-Mn crust | 23.573759 | -164.028475 | 4290 | Crust | Flat, creeping |

## Supplemental Results and Discussion

*Octopod morphotypes*

Within the three survey locations four morphotypes of octopods were observed during the dives (Figure 1). Octopods 1A, 1C and 1D appear likely to be members of the same undescribed species with single series of arm suckers, referred to hereafter as 'Casper', whereas 1B appears to be a *Vulcanoctopus* sp. Table S1 gives the date and times of each observation, depth, octopod morphotype, mantle lengths, location and behavioral observations.

*Octopod spatial distribution*

The two ROV dives conducted in the central Pacific each observed a benthic incirrate octopod in association with exposed ferromanganese-encrusted basalt. The Kaʻena Ridge octopod was curled into a nook in a basalt outcrop (3,116 m depth, Figure 1A). The Necker Ridge octopod was encountered on top of a large, relatively flat outcrop of basalt, exposed to the open ocean (4,290 m, Figure 1C).

Within the DISCOL area (Peru Basin, SE Pacific), most octopods were observed in association with regions of seafloor with abundant nodule coverage (Table 1). Average nodule densities in these unploughed areas was 6.8 nodules $m^{-2}$ (SD = 3.29), with peak densities of 32 nodules $m^{-2}$. The experimental ploughing of the seafloor in 1989 (see *Survey locations* in the Supplemental Experimental Procedures section below) led to a burial of nodules into the sediment within the ploughed areas, where a reduced nodule density of 1.49 nodules $m^{-2}$ (SD = 1.53) was exposed at the surface in 2015. Incirrate density observed in unploughed regions was 2.8 individuals per ha, with a lower density of 2.1 individuals per ha observed within the ploughed areas. This difference in abundance indicates that it is likely that the mobile incirrate octopods prefer some aspect(s) of the more heterogeneous habitat of the nodule abundant areas of seafloor.

*Octopod behavior*

All of the octopods observed across the survey sites exhibited combinations of several behaviors (visually summarized in Figure S1, and outlined in Table S1). In the DISCOL area, many were observed huddled close to and between nodules (Figure S1D), much as the incirrate octopod observed at Kaʻena Ridge was huddled into a nook in a rock outcrop (Figure 1A). Two individuals were observed brooding eggs at the midpoints of dead sponge stalks (Figure 1E, S1F). In both cases the octopod was hanging perpendicular down from the stalk with its arms curled around the eggs with each egg individually attached to the stalk. Both batches consisted of ~30 eggs of 2.0 to 2.7 cm length.

Only the Necker Ridge octopod exhibited an escape behavior, backing away from the ROV. Extended experimental work conducted over 15 minutes close to one individual in the DISCOL area, with artificial illumination, thick plumes of resuspended material and noise did not appear to influence the behavior of the octopod.

*Octopod image and video availability*

All images and video data discussed within this paper are available online.
The DISCOL image and video data can be accessed via PANGAEA at:
https://doi.pangaea.de/10.1594/PANGAEA.865440.
The Kaʻena Ridge ROV video still images are available at: http://4dgeo.whoi.edu/webdata/virtualvan/html/VV-km1116/index.html.
The video footage of the Necker Ridge incirrate octopod are available at:
http://oceanexplorer.noaa.gov/okeanos/explorations/ex1603/dailyupdates/media/video/dive_1/ex1603_dive1_022716.html, with HD images at:
http://oceanexplorer.noaa.gov/okeanos/explorations/ex1603/logs/mar2/mar2.html

## Supplemental Experimental Procedures:

*Survey methodologies*

Three remotely operated vehicles (ROVs) were used to conduct transect surveys of the Kaʻena Ridge (*Jason 2*, ~26 km survey, 3,702–2,661 m depth, May 2011, [S1]), the DISCOL nodule area (*KIEL 6000*, ~2 km survey, 4,050-4,200 m, October 2015, [S2]) and the Necker Ridge (*Deep Discoverer*, 0.38 km survey, 4,222–4,291 m, February

2016). Still images and video were recorded of all incirrate octopods encountered during these surveys. Within the DISCOL nodule area ~50 km were surveyed by the Alfred Wegener Institute Ocean Floor Observation System (AWI-OFOS LAUNCHER) towed camera system, flown at a constant height of 1.5 m. Camera coverage was ~5.5 m of seafloor, imaged every 15 seconds by automated 24-megapixel still camera and continuously imaged in HD video. 16,000 still images were taken across ploughed and unploughed nodule areas (see 'survey locations' below). The images were inspected for incirrate octopods, and the mantle lengths of those present measured using the Papara(ZZ)I software application v2.0 [S3]. Any behavioral traits of individuals observed within the surveys were also noted. The nodule densities (nodules $m^{-2}$) within each image of ploughed and unploughed seafloor was determined with Papara(ZZ)I v2.0 (Table S1).

*Survey locations*

The Ka'ena and Necker Ridges are both submarine ridges within the Hawaiian Island archipelago. Ka'ena Ridge extends NW from the island of O'ahu. Its volcanic surface has developed a ferromanganese crust typical of seafloor of that age (~3–5 Ma), with maximum rind thicknesses ranging 7–12 mm for most samples [S1].The surface of the ridge is of high topographical complexity across a range of scales offering numerous habitat niches for sessile and mobile benthic fauna. The Necker Ridge, surveyed primarily for corals and geological samples during the 2016 *Okeanos Explorer* cruise, is similar in structure to the Ka'ena ridge.

In the late 1980s, in response to similar perturbations in metal prices, the DISturbance and COLonisation (DISCOL) experiment was instigated [S4]. A custom-designed plough was used to drive the majority of manganese nodules below the surface within 78 ploughtracks (0.5–8 km length, 4–10 m width). Subsequently the area was monitored to assess the faunal response to the nodule removal [S5]. Both mobile and sessile fauna biomass and biodiversity was reduced immediately following ploughing, though a return to pre-disturbance concentrations of many mobile fauna was observed within a decade. The removal of nodules resulted, however, in a near total removal of the associated sessile epifauna, including the stalked fauna. Sessile fauna was still absent from ploughed regions, after almost three decades, during the current study [S2]. In 2015, the *RV Sonne* revisited the DISCOL region twice — cruise SO242/1 concentrating on the remote survey of the seafloor using AUV technology to map the accurate locations of the historical plough tracks [S6] and SO242/2 following this up with ship-towed Ocean Seafloor Observation System (OFOS) and ROV surveys of ploughed and unploughed regions [S2]. Nodule densities in images containing octopods are given in Table S1.

**Author contributions:**

A.P., Y.M., H.-J.H, A.B. and U.P wrote the manuscript with input from all other authors. A.P., Y.M., H.B. and A.B. contributed Peru Basin data. D.E. contributed Ka'ena Ridge data. M.V. contributed Necker Ridge data.

**Supplemental References:**


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