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Clusters of deep-sea egg-brooding octopods associated with warm fluid discharge: An ill-fated fragment of a larger, discrete population?

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ABSTRACT

Benthic octopods cluster on bare rock on Dorado Outcrop, a ~3000 m deep basalt exposure. The outcrop hosts intermittent discharge of relatively cool (up to 12.3 °C) hydrothermal fluid that carries about half as much oxygen as bottom seawater (~54 μM vs. 108 μM). We analyzed 231 h of video footage and still images taken by sub-sea vehicles in 2013 and 2014 that documented the clustered octopods, members of the poorly-known genus *Muusoctopus*. The largest cluster (102 octopods) occurred in a 19 m² area of fluid discharge, where the basalt was sediment-free; individual octopods were also seen across the outcrop. The clustered octopods appeared to be brooding eggs and a total of 11 egg clutches were confirmed. None of the 186 eggs closely examined showed embryonic development. The intermittent fluid discharge may clear the basalt of sediment and attract gravid octopods which then spawn. However, the increased temperature and limited oxygen of the discharging fluids may threaten the octopods' survival. Octopods in/near areas of discharging fluid had significantly higher estimated respiration rates (3.1–9.8 contractions/min) than did octopods away from discharging fluid (0.8–6.0 contractions/min). Warm fluids likely increase the octopods' metabolic rate and thus their oxygen demand but provide only limited oxygen. The resultant physiological stress is hypothesized to eventually kill eggs near fluid discharge. We hypothesize, because these eggs do not survive, the population is sustained by a larger pool of undetectable females that brood their eggs inside cool conduits of this and perhaps other, unstudied basalt outcrops.

1. Introduction

Clusters of brooding deep-sea octopods are rarely observed. The few exceptions are on deep (> 2000 m depth) basaltic outcrops (Voight and Grehan, 2000). Such outcrops are exposed above the thick layer of seafloor sediment and serve as conduits for warm fluid discharging from the crust into the overlying seawater (Fisher and Wheat, 2010). Baby Bare Outcrop (Mottl et al., 1998), Dorado Outcrop (Wheat et al., 2017), and the crust located adjacent to the Sea Cliff hydrothermal field (D. Clague, pers. comm.) are all examples of fluid conduits. These sites of fluid discharge do not support animals typical of hydrothermal vents (Mottl et al., 1998; Lee et al., 2015; JRV, pers. obs.), as the discharging fluids lack the reduced chemicals that sustain chemosynthesis (Jannasch and Mottl, 1985). Female octopods at these three sites brood eggs attached to exposed rock (Voight and Grehan, 2000; JRV pers. obs.), as is typical of most benthic octopods (Hoving et al., 2014).

Robison et al. (2014) report that a female deep-sea octopod of *Graneledone* Joubin, 1918, brooded its eggs for over 53 months on a

rock outcrop near 1400 m depth. Robison et al. (2014) deduced that brooding was continuous and the female did not eat; over time she developed cloudy eyes and slack, grayish skin characteristic of senescent octopods (Voight and Grehan, 2000; Anderson et al., 2002; Voight, 2008). The exceptionally long egg development was attributed to the large eggs and low ambient temperatures (Boletzky, 1994; Robison et al., 2014). Reports of clustered, egg-brooding octopods at greater depths have been anecdotal, lacking documentation of the physical environment (Voight and Grehan, 2000; Drzen et al., 2003).

Ambient temperature can strongly affect the metabolic rate of octopods, as they are poikilothermic animals (Vidal et al., 2014). Experiments to deduce the impact of temperature on octopods have focused on shallow-water animals (Giménez and García, 2002; Juárez et al., 2015), because such animals may be exposed to a wide temperature range (e.g., seasonality) throughout their lives (Pörtner, 2001). In contrast, deep-sea octopods have been restricted to constant temperatures of about 2 °C throughout evolutionary time (Strugnell et al., 2009, 2011). Deep-sea octopods are likely to be stenothermic (Pörtner

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et al., 2000) and have quite low upper critical temperatures, as do other deep-sea animals (Peck et al., 2004).

We report here the first biological study of Dorado Outcrop at 3000 m depth, a site of warm crustal fluid discharge. Abundant, muscular and comparatively large benthic octopods brood their eggs in warm discharging fluid. We posit that the octopods and their eggs, being adapted to well-oxygenated, isothermal deep-sea conditions are unlikely to survive long-term exposure to these fluids. We suggest that they represent surplus members of an undetectable population brooding eggs within the outcrop.

2. Setting and methods

2.1. Site description

Dorado Outcrop (9.08°N, 87.09°W) lies on 23-mya seafloor on the Cocos Plate, ~250 km west of Costa Rica. This 2-km-long, 0.5-km-wide, 150-m-tall (at the highest peak) outcrop lies oriented from northwest to southeast at a depth of 3100 m at its base (Wheat et al., 2017). Dorado is part of a cool ridge flank hydrothermal system with areas of low-temperature (maximum 12.3 °C) fluid discharge from sediment-free basaltic crust (Lee et al., 2015; Wheat et al., 2017). Discharged fluid shimmers upon mixing with seawater. Much of the discharge occurs on the southwest face of the southern end (Marker K, Fig. 1) and at one site on the northwest end of Dorado Outcrop (Marker A, Fig. 1) (Wheat et al., 2017). Most of Dorado Outcrop is covered by lightly sedimented basalt flows.

Dorado Outcrop was explored on two research cruises, one using the remotely operated vehicle (ROV) *Jason-II* and the autonomous

underwater vehicle (AUV) *Sentry* (AT26-09; December 2013), and the other using the human occupied vehicle (HOV) *ALVIN* (AT26-24; December 2014). The cruises were dedicated to studying discharging fluids; the methods and results of the cruises are detailed by Wheat et al. (2017). Germane to this study, *in situ* fluid temperature and dissolved oxygen concentration were monitored. Long-term temperature loggers were deployed in 2013 and recovered in 2014. Short-term loggers recorded temperature and dissolved oxygen at 5-minute intervals over three to four days; two were deployed twice in 2014 directly in discharging fluids at Markers R and W. Probes attached to the submersibles monitored instantaneous temperature and oxygen conditions. The sensor tips were placed as deep as possible into cracks and crevices with the aim of locating the warmest and least diluted discharging fluids.

To summarize from Wheat et al. (2017), temperatures of the discharged fluid ranged from 2.5 °C to 12.3 °C, the maximum having been measured in cracks and crevices. The temperature deep within the outcrop was estimated to be 15 °C; the ambient bottom water was measured at 1.8 °C. Oxygen concentrations of the discharged fluid were about half of the concentrations measured in bottom water (54.5 μM vs. 108 μM) and were inversely correlated with temperature (Fig. 2). Otherwise, the fluids were chemically like bottom seawater. The discharged fluids mixed quickly with bottom seawater, increasing their oxygen content and lowering their temperature.

Both the volume and temperature of fluid flow vary over time. For example, the long-term temperature logger at Marker K recorded twice daily fluctuations that ranged from just above ambient to 12.3 °C and the short-term loggers recorded twice daily fluctuations between ~5 and 12 °C. At Marker A, *Jason-II*'s temperature probe recorded a high

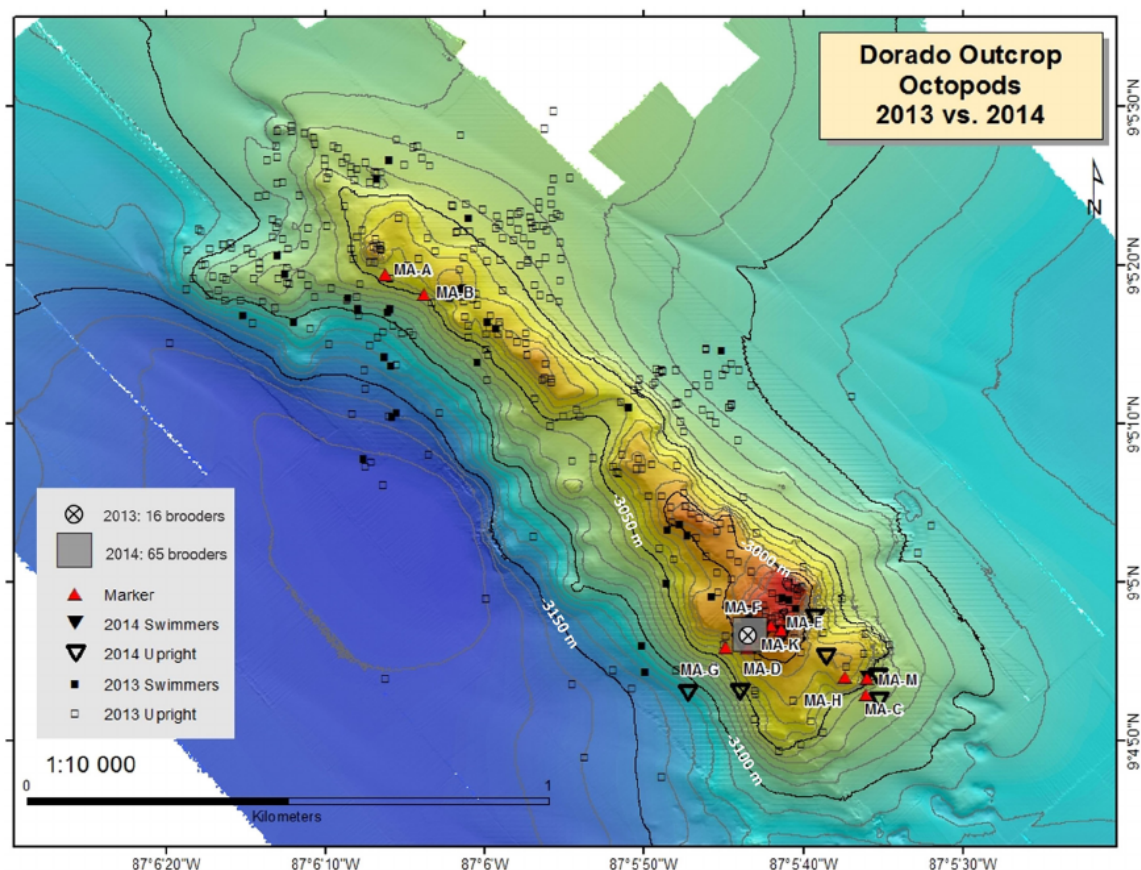


Fig. 1. Dorado Outcrop bathymetric map. Contours are 10 m intervals. Site markers are red triangles labeled with black and white text; Markers R and W plot on top of Marker K at this scale, thus those labels are not shown. Small squares and upside-down triangles represent 2013 and 2014 octopod observations, respectively. Solid black symbols designate swimming octopods and open symbols represent upright octopods. The number of apparently brooding octopods in the ~16 m² area around Markers R, W, and K is depicted by a light-grey circle with a black-x and a dark-grey square for 2013 and 2014, respectively. Map created in ArcGIS.

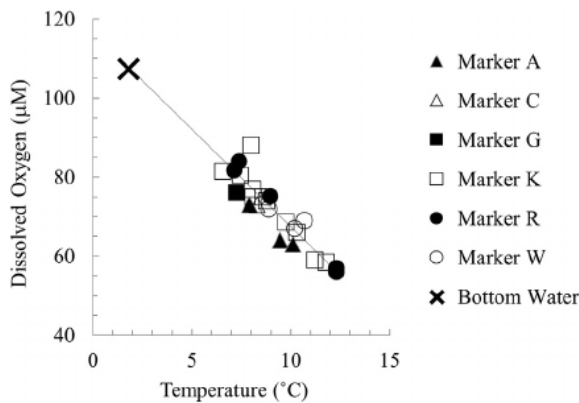


Fig. 2. Scatter plot displaying the inverse relationship of temperature and dissolved oxygen content in discharged fluid from Dorado Outcrop at Markers: A, C, G, K, R, and W. *In situ* sensor data were measured using ALVIN's thermistor and an oxygen sensor. Bottom water temperature is averaged from four measurements made on three, non-consecutive dives. The solid line is the linear regression of all data points ($r^2 = 0.954$).

temperature of 11.6 °C in discharged fluid but ALVIN documented a change from zero flow to active discharge of 7.6 °C fluid over six days.

2.2. Image surveys

Jason-II recorded 96 h of seafloor video during four dives (J2-751, J2-752, J2-756, J2-757) and Sentry recorded 90 h of video during eight dives (213–220). ALVIN recorded 45 h of bottom video during ten dives (4775–4784). Sentry, Jason-II, and ALVIN were precisely navigated; latitude and longitude data are in degree, decimal minute to six decimal places.

Jason-II was equipped with three cameras with pan and tilt features. Camera equipment included a hybrid HD camera, an Insite Mini-Zeus HD camera, and an eight-video channel configuration to record several of the selected cameras simultaneously. Jason-II had sixteen 17,700 lm light-emitting diodes (LED) lights and two 250-watt incandescent lights. Still images are available via the Jason-II Virtual Control Van (WHOI, 2013). Sentry was equipped with a Prosilica GE-4 11MP Digital still camera.

ALVIN was equipped with two Insite Mini Zeus HD cameras, each with pan and tilt, and two Kongsberg OE14-522 HD cameras with pan/tilt/zoom. Not all cameras were functional during every dive. ALVIN had twelve lighting channels, LED lights, situational and emergency lighting, and down-looking survey lighting. Still images from ALVIN are available on the frame grabber (WHOI, 2014). Differences among the quality, lighting, and angle of the cameras complicated direct comparison of the Jason-II, Sentry, and ALVIN images and videos.

2.3. Analysis of octopod biology and ecology

Octopods on Dorado Outcrop were identified by comparing their images to those of the two benthic octopod genera observed from the submersible ALVIN in the Eastern Pacific Ocean, *Graneledone* and *Muusoctopus* Gleadall, 2004, discussed as *Benthoctopus*, e.g. by Voight (2008). None were collected. The grainy skin and single sucker row of *Graneledone* contrast to the smooth skin and two rows of suckers of *Muusoctopus*; their brooding posture also differs. Brooding octopods of *Graneledone* hold their arms to the egg mass, keeping their dorsal mantle away from the substrate (Robison et al., 2014). Brooding *Muusoctopus* position themselves inside crevices with their mantles toward the egg clutch with the oral, sucker-bearing side of their arms exposed (Voight and Grehan, 2000; Voight, 2008; Neptune Canada, 2010; NOAA, 2013).

Octopod size was estimated using objects of known size in the same

image as the octopod, such as a milk crate, or ALVIN's robotic manipulator. When visible on ALVIN video, a pair of laser lights, spaced 100 mm apart, was used to confirm the scale-derived estimates. Mantle length (the distance from the midpoint between the eyes and the mantle tip) and mantle width (the greatest breadth of the mantle) were estimated using the scaled objects.

Video and still images of each octopod documented its location, substratum, posture, and proximity to the shimmering fluid. The location in latitude and longitude of the vehicle when each image was secured was automatically recorded. The octopods' postures were categorized as upright, brooding, or swimming. Their proximity to shimmering fluid was assessed using video footage, particularly when it was narrowly focused. Once octopods were recognized in low-resolution still images, we made detailed observations of the simultaneous high-definition ALVIN video footage. ALVIN footage provided the most detailed observations of the octopods in areas of shimmering flow.

The octopods' population density was calculated from the number of octopods per area, with area defined by one of two methods. The first, applied only when the entire area was not captured in a single image, used the latitude and longitude of Marker R and the final position of ALVIN before its ascent. The second employed the site marker (~0.2 m diagonal) as a scale to calculate the areas imaged around Markers R, W, and K.

To determine whether specific brooding spots were reused, we re-located specific surface sites using navigational data and seafloor features, such as cracks, associated sessile organisms, and discoloration of the bare basalt. We identified ten and 11 specific locations within small (1.1 m² and 0.75 m²) areas near Markers R and W, respectively. We re-located those exact sites in ALVIN and Jason-II video to document octopod presence or absence; we term this method the 'popular spot count'.

We estimated octopod respiration rates (in contractions per minute) if the video was suitable. One respiration was defined either as 1) a full open-close-open cycle of the funnel or, when the funnel could not be observed, 2) a full cycle of mantle contraction and relaxation. Respiration rate was determined for individuals videoed for at least 38 s for funnel views and 13 s for mantle contractions. All respiration rates calculated from funnel observations were made on octopods in or near fluid flow; respiration rates based on mantle fluctuation included octopods both in or near and away from fluid flow. We removed a single outlier defined by the upper and lower bounds for each of the groups ($Q_n \pm IQR \times 1.5$; where Q_n is the quartile and IQR is the interquartile range). We compared the respiration rates using two-sample t-tests.

To determine if the octopods were brooding eggs, we watched ALVIN video clips repeatedly in slow motion, looking for eggs between the octopods' arms. Octopod eggs were recovered in 2014 on a sampling tube that had been deployed in 2013; they were photographed with a Nikon hand-held digital camera. Egg length was estimated relative to the outer diameter of the sampler tubing (12.7 mm). We counted the eggs in this clutch and *in situ* clutches using still images.

Octopods attach the stalks of their eggs to a substrate with a secretion of the oviducal gland, termed oviducal cement (Froesch and Marthy, 1975); the cement remains in place for some unknown time after the eggs are either removed or hatch. The green cement was readily visible in close-up images and enabled us to identify where octopod eggs had been attached (Froesch and Marthy, 1975; Voight and Drazen, 2004). We counted patches of oviducal cement that were *in situ*.

2.4. Laboratory analysis

Green oviducal cement was recovered on a rock sample collected in 2014. It was air-dried, stored in a sealed plastic container and occasionally handled to prepare thin sections of the rock. We removed flakes of the cement from the rock and analyzed them on an FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM) and energy-dispersive spectrometer attachment at The University of Akron, OH.

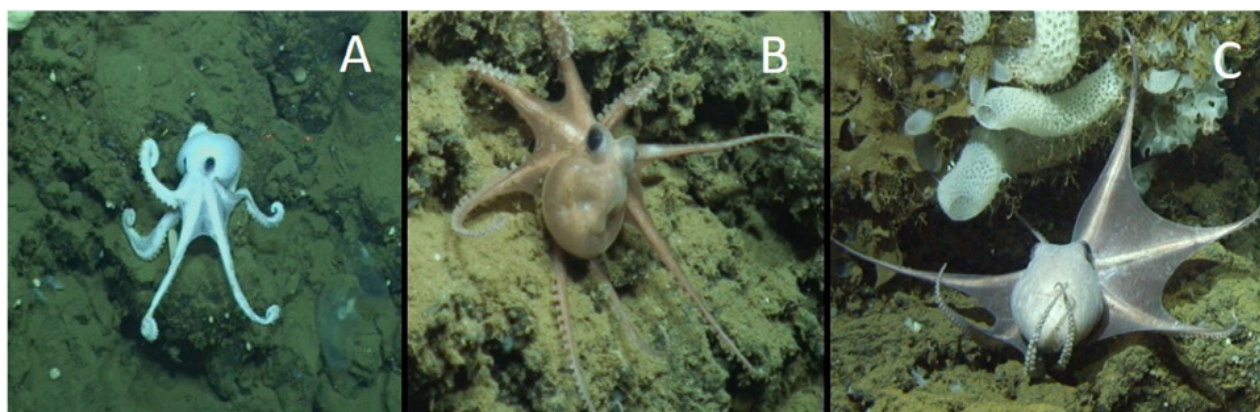


Fig. 3. Morphotypes of octopods observed on the outcrop: A) light, bi-colored octopus with thin mantle and arms; B) uniform orange/brown coloring with narrow arms and bulging eyes; and C) light purple with large eyes and mantle, and relatively short arms. Still images are from *ALVIN*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Octopod identification

All octopods were identified as species of *Muusoctopus*, with smooth skin and two rows of arm suckers; none of the octopods resembled *M. hydrothermalis*. Most individuals are thought to correspond to one species, but we distinguish three morphotypes (Fig. 3). The first, similar in some regards to one species reported by Purser et al. (2016), had a light-colored dorsum with a hint of reverse counter-shading, a narrow head, and long arms (Fig. 3a). The second had large, prominent eyes, an even orange-brown color, and thin, long arms with conspicuous suckers (Fig. 3b). The third type was the most abundant, composing over 99% (603 of 606) of the observations. These octopods were comparatively dull purple with large, not overly prominent eyes and short arms relative to their mantle (Fig. 3c). Mantle lengths of four of these octopods, including two replicates, were estimated to average 128 ± 19 mm. Mantle widths of three individuals and two replicates were estimated to average 99 ± 11 mm (Supplementary data Table 1).

3.2. Spatial distribution, postures, and population density

We observed 606 octopods (269, 189, and 148 with *Sentry*, *Jason-II*, and *ALVIN*, respectively) in swimming, upright, and brooding postures

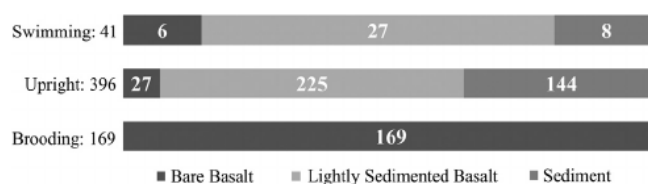


Fig. 5. A bar graph showing the number of octopods observed in different postures on the different substrate classifications. The bars are the octopod postures (swimming, upright, and brooding) as a function of each substrate type (bare basalt: dark-grey; lightly sedimented basalt: light-grey; sediment: grey) in 2013 and 2014 by *Sentry*, *Jason-II*, and *ALVIN*.

(Fig. 4.a-c); some may have been counted more than once. In both years, octopods were observed across the entire outcrop, on all substrates (Figs. 1 and 5). The octopods clustered on the bare basalt on the southwest face of the southern end (Fig. 1; Supplementary data Video 1) near the largest flux of discharging fluids. Submersible probes documented the full range of temperatures and dissolved oxygen concentrations near these octopods. Clustered octopods were observed primarily in the brooding posture; octopods elsewhere on the outcrop were generally in the upright posture.

The population density of octopods on bare basalt around Markers R, W, and K ranged from 1 to 12/m² between days and years, and many

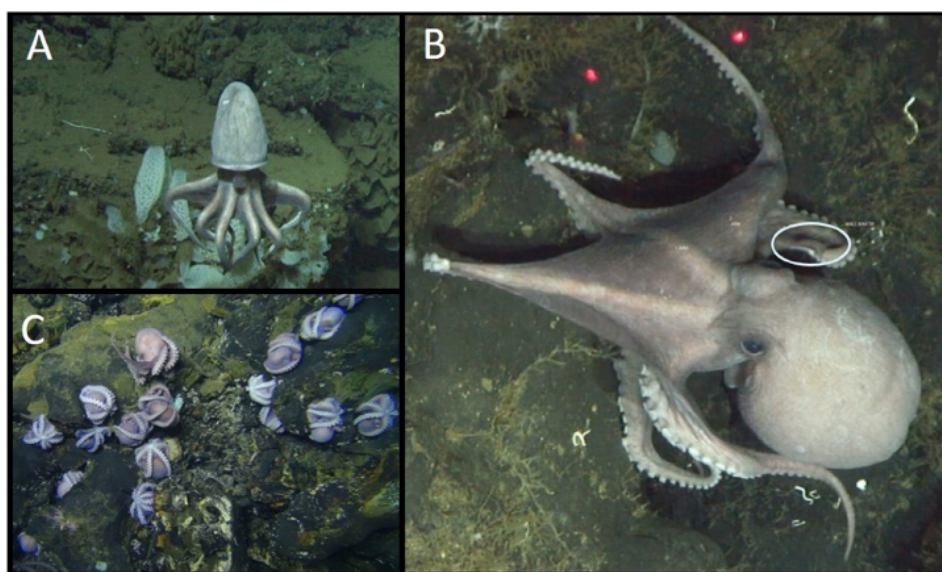
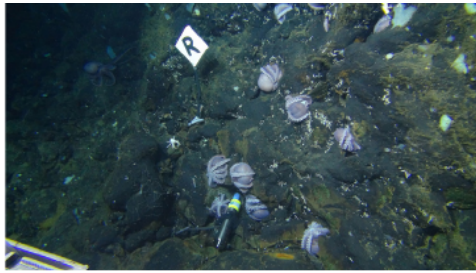


Fig. 4. Octopods were observed in three postures: A) swimming; B) upright (male- the ligula at the tip of the third right arm is circled) with a pair of lasers spaced 100 mm apart visible in the top-center; and C) brooding. C shows 16 octopods; 15 individuals are brooding and one is upright (top octopus on the left side of the image). Still images are from *ALVIN*.



Video S1. Overview of area with an average of 5 apparently brooding octopuses per square meter. The videos are spliced segments from a larger transect in which *ALVIN* remained still for long periods of time; video segments are from: dive 4779 Port S001_S001_T001.mov 05:04:58–5:14:15. Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.dsr.2018.03.011>.



Fig. 6. Octopod arms extending from a crevice on Dorado Outcrop. *ALVIN*'s manipulator arm and temperature probe, with a shrimp, are on the left and center of image, respectively. Patches of green oviducal cement are circled in black above the manipulator arm and above and left of the octopod arms. Contrast of this figure is enhanced. Image is from *ALVIN*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of the popular spots near Markers R and W were reused. In the small area near Marker R, the population density of octopods ranged from 2 to 12/m². Two of ten popular spots were occupied in 2013 and between three and eight popular spots were occupied during every 2014 dive (supplementary data Fig. 1; Table 2.a-b). At Marker W, the population density of octopods varied between 1 and 7/m². Of the 11 popular spots, one was occupied in 2013 and during all 2014 dives. In 2014, octopods occupied from three to nine of the 11 popular spots during all dives. In the ~16 m² area around Markers R, W, and K, the number of brooding octopods increased from 16 in 2013 to 65 in 2014, an increase in population density from ~1 to ~4/m² (Fig. 1). The highest density was observed during an *ALVIN* transect, which found 102 octopods in a 19 m² area, a population density of ~5/m². The population density increase (1–4/m²) between years is consistent with the population density change (1–12/m²) observed between any two sequential *ALVIN* dives. Our estimates are minimal; many unexplored crevices could have harbored additional octopods. For example, during close examination of rock faces in 2013, arms of otherwise unseen octopods emerged from ten distinct holes or crevices (Fig. 6).

3.3. Octopod respiration rates

Our estimates of respiration rate based on funnel versus mantle contraction of animals in or near flow were not significantly different ($t = 1.71$; d.f. = 49; $p = 0.09$). Neither were estimated respiration rates of octopods we considered to be in discharging fluids different

from respiration rates of those that were only near discharging fluids ($t = 0.76$; d.f. = 31; $p = 0.45$). Respiration rates of octopods in or near fluid flow were significantly higher than those of octopods away from fluid flow ($t = 8.79$; d.f. = 69; $p = 7.21 \times 10^{-12}$). Additional t-tests found no significant effect of the observations' duration on respiration rate ($t = 0.58$; d.f. = 31; $p = 0.57$) and no difference in the respiration rate of females documented to be brooding eggs and those not brooding eggs ($t = 0.20$; d.f. = 56; $p = 0.84$). Upright, moving octopods and those far away from fluid flow were estimated by mantle fluctuations to respire 0.8–6.0 times per minute ($n = 13$). Octopods in and near warm fluid were estimated to respire 6.7–9.7 times per minute ($n = 18$) based on funnel open/closing and 3.1–9.8 per minute ($n = 33$) based on mantle contractions. Individual respiration rates are presented in Supplementary material Table 3.a and b.

3.4. Egg and clutch size

Detailed observations of individuals in the brooding posture on Dorado's southwest face revealed the presence of egg clutches in 2013 and 2014; the few octopods in the brooding posture elsewhere on the outcrop were not documented to have eggs. In 2013, only one egg clutch was observed, tucked in a vertical crevice near Marker R. In 2014, we documented 11 egg clutches in the same immediate area. We could not determine that each of the 95 octopods in the brooding posture during an *ALVIN* transect (Supplementary Video 1) was in fact brooding eggs. In 2014, one egg clutch was in the same spot as the clutch observed in 2013; whether it was the same clutch is unknown. Although most egg-brooding octopods were observed near fluid discharge, they were not necessarily in contact with the shimmering fluid.

Eggs observed *in situ* were often merely glimpsed between the octopods' moving arms. However, in some cases as the submersible used its scientific instruments, it displaced an octopod to reveal an entire clutch. Eggs in three clutches (observed *in situ*) numbered from 33 to 65 (Fig. 7.a, b; Supplementary data Fig. 2.a; Table 4). All eggs observed were transparent; no embryos or eyespots were visible. Nor did any of the brooding females show signs of late-stage senescence, such as cloudy eyes or slack skin. Eggs in the single recovered clutch were clear (Fig. 7.c; Supplementary data Fig. 2.b). The ~40 eggs averaged 28.6 ± 1.7 mm long ($n = 4$) and 6.8 ± 0.5 mm wide ($n = 5$) (Supplementary data Table 5). The eggs were spawned between the sampler's deployment on 12/21/2013 and its recovery on 12/01/2014.

3.5. Oviducal cement

At least 23 patches of oviducal cement were observed *in situ* on Dorado Outcrop in 2014. Oviducal cement was only observed on bare substrate, ledges or near crevices on the outcrop, regardless of the presence of fluid flow. Analysis of the oviducal cement recovered on a basalt sample from 2014 at Marker R with an ESEM showed no special structure. The elemental composition, in order of abundance, after O and C, included Si, Al, Fe, and Ca. The cement, preserved dry on the rock surface for over two years, showed no signs of decay; we cannot estimate how long oviducal cement can remain *in situ* in the deep sea.

3.6. Interactions with other animals

Video footage shows both sessile and mobile octopods interacting with other organisms, notably shrimp and, inferring from their small size and swimming, amphipods. No octopod was observed preying on either taxon; instead, brooding octopods pushed the shrimp and amphipods away. In one case, *ALVIN* moved a brooding octopod, exposing her egg clutch and perhaps damaging some eggs. Within seconds, shrimps emerged from cracks and crevices in the outcrop to swarm the exposed clutch. Within minutes, tens of shrimp were evident, as were abundant amphipods (Supplementary Video 2). One shrimp disappeared into the egg cluster and emerged with what appeared to be

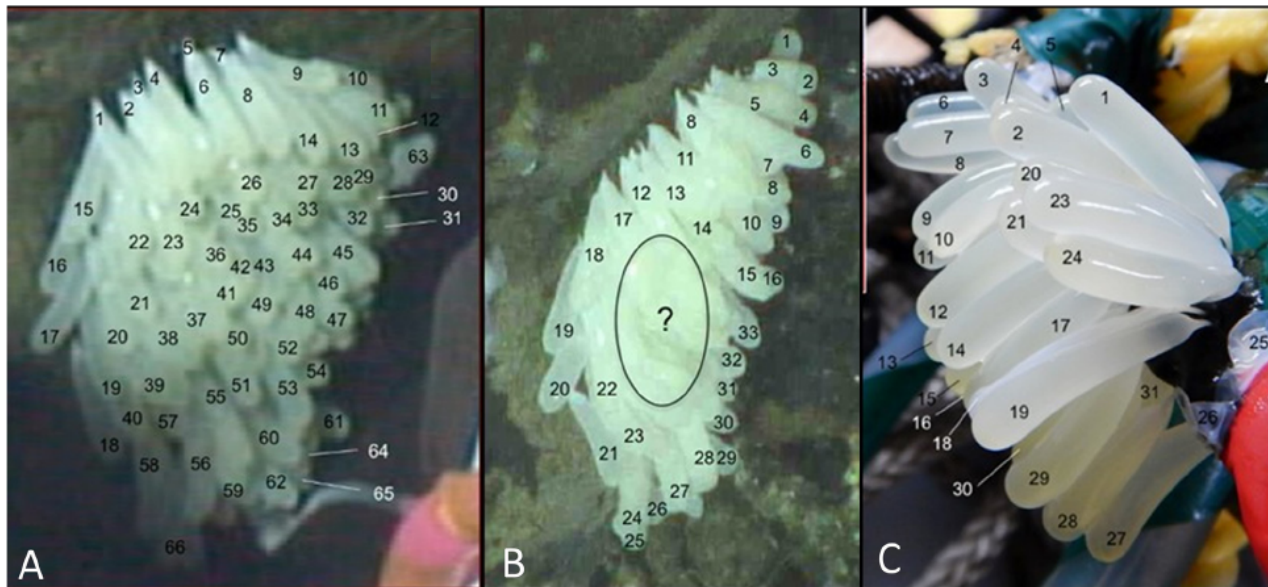


Fig. 7. A) and B) Exposed *in situ* egg clutches on the outcrop near the hydrothermal discharge area (Marker K). C) Octopod eggs recovered on a sampling tube. The numbers are egg counts. Images A and B are from ALVIN; Still image C was taken by AMH.



Video S2. Interaction of the octopods with shrimp. The egg clutch is exposed when ALVIN's manipulator pushes the female to access a hole with the temperature probe. The video shows green oviducal cement to the right of the exposed egg clutch on the grey-stained and cracked rock surface. Video segment is from dive 4780 Port S001_S001_T001.mov 00:43:30–1:30:01. Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.dsr.2018.03.011>.

part of an egg in its anterior appendages which it then appeared to eat. After three minutes, ALVIN withdrew, and the octopod returned to the brooding posture. On ALVIN's return 30 min later, she was still on the egg clutch; she and two adjacent brooding octopods were actively deflecting abundant shrimp and amphipods with their arms. Two days later when ALVIN revisited the site, the focal octopod and her eggs were gone; only oviducal cement bearing remnants of egg stalks were observed. Two other spots in the immediate vicinity that had previously hosted octopods with clutches had been abandoned; only cement and egg stalk remnants were observed.

4. Discussion

Octopods brooded their eggs on sediment-free rocks on the 3000 m deep Dorado Outcrop at population densities of up to $\sim 12/\text{m}^2$; discharge of warm fluid from within the outcrop likely kept the outcrop surface free of sediment. Although exposure to this warm fluid is likely to speed up egg development (Drazen et al., 2003; Robison et al., 2014; Salinas-de-León et al., 2018), it poses two problems. First, the fluid's higher temperatures increase the metabolic rate of the ectothermic octopods and thus their need for oxygen (Seibel and Childress, 2000; Valverde and García, 2004; Seibel and Drazen, 2007; Oellermann et al., 2015). Second, the fluid delivers only half the oxygen present in bottom

seawater (Wheat et al., 2017). We argue that the elevated temperatures and low oxygen concentrations in discharging fluids generate severe and potentially lethal physiological stress for these octopods and their eggs.

Although to our knowledge, physiological studies have not been conducted on any species of *Muusoctopus*, the animals are members of an ancient deep-sea clade (Strugnell et al., 2009, 2011), and thus are likely to be stenothermic (Pörtner et al., 2000). Pörtner et al. (2000) predict that because energetic costs of maintaining eurythermy are high, stenothermy is the preferred strategy for animals living in constant temperatures over evolutionary time; it evolves as selection minimizes the animals' aerobic capacity and energy expenditure.

We cannot test the effect of elevated temperatures on the octopods or their assumed stenothermy. We document that they respire significantly faster in/near warm fluid than in ambient fluids. Increases in respiration can be related to oxygen stress and, for some species, the rate increase is limited to doubling (Borer and Lane, 1971; Wells and Wells, 1985; Valverde and García, 2004). Stroke volume, which we could not measure, may be the far more critical variable for assessing oxygen uptake (Wells and Wells, 1985). In regard to stroke volume, we can only note anecdotally that one female exhaled so violently that her gill extended out of her mantle aperture. Increasing respiration effort itself requires significant energy expenditure and increases the animals' oxygen debt (Daly and Peck, 2000; Valverde and García, 2004).

Given the likelihood that the octopods are stenothermic and exposure to the discharging fluid results in physiological stress, why do they appear to preferentially brood their eggs in the warm fluid?

We hypothesize that gravid female octopods encounter exposed rock they find suitable for egg attachment when fluid discharge is minimal or non-existent. As noted, fluid discharge can increase from virtually none to a steady warm stream in as little as six days. Once they begin to spawn on the outcrop, the females may be unwilling to leave, as their eggs are permanently attached. Brooding females may in fact experience a more moderate environment than do their eggs; oxygen-rich seawater is likely to partially bathe the females, and brooding females consume comparatively little oxygen (Parra et al., 2000). In contrast, the eggs, being closely attached to the rock, are apt to be fully immersed in the warm discharging fluid that rises along the rock face. Furthermore, the eggs are growing.

The duration of egg development is hypothesized to be lengthy, increasing the odds that the eggs will eventually encounter fluid

discharge. Newly spawned 15 by 5 mm eggs of deep-sea octopods of *Graneledone* require an estimated 53 months to develop as they expand to 33 by 16 mm (Robison et al., 2014). Early-stage eggs of *Muusoctopus* average 28.6 by 6.8 mm. Their larger size predicts that their development will require at least 53 months, although the actual duration is unknown. Embryonic development will only heighten the problems posed by low oxygen concentrations in the warm discharging fluid. During development, metabolically active embryonic tissues and their oxygen consumption increase exponentially (Parra et al., 2000). Late-stage embryos of *Octopus vulgaris* consume roughly twice as much oxygen as the brooding female (Parra et al., 2000).

The egg clutches observed *in situ* (Fig. 7a and b) were attached to nearly-vertical surfaces. Elsewhere on the outcrop, nearly-vertical, exposed rock surfaces without fluid discharge showed only sporadic oviducal cement and no active brooders. Why is oviducal cement so much more abundant in areas of fluid discharge than on seemingly comparable, fairly close-by, nearly-vertical rock surfaces? One factor may be the increased turnover of the brooding octopods at sites near fluid discharge. If the lethal effects of fluid discharge terminate brooding early, the spots become available more frequently for other clutches.

Despite the scarcity of oviducal cement elsewhere, we suspect that octopods do brood elsewhere on the outcrop, but rather than on its surface, they may brood within its basaltic mass. Octopods of *Muusoctopus* have been known to brood eggs in enclosed spaces (Voight and Grehan, 2000; Voight, 2008; Neptune Canada, 2010; NOAA, 2013), although Laptikhovsky (2001) argued that they brood “in the open ground” based on how eggs were attached to trawled rocks. The adiabatic rise of hydrothermal fluids from conductive heat loss discussed by Wheat et al. (2017) leads us to predict that a network of conduits and tunnels channel Dorado's warm fluids to local areas. Most of the inner outcrop is thus cool, enabling the octopods to brood inside rock crevices in what may be near-ambient temperature. Conduits for flow within the basaltic crust are heterogeneous, especially in the upper layers where pillow lavas reside (Neira et al., 2016). The conduits that are not linked to fluid discharge may create ideal octopod egg-brooding sites, accessible through narrow openings in pillow basalts that render the octopods undetectable, even to sub-sea vehicles. If such internal brooding sites offer slightly elevated temperatures (by conduction) without low oxygen concentrations, such sites may shorten the duration of development and reduce the risk of predation (e.g., Boletzky, 2003 and references within, Uriarte et al., 2012; Salinas-de-León et al., 2018).

One could argue that the contrast between the few (16) brooding octopods observed in 2013 and their abundance in 2014 (65) is evidence of synchronous brooding. Synchronous brooding, however, has significant liabilities. Inevitably, competition among mature females for brooding sites and for food among dispersing hatchlings would increase. In addition, the population's vulnerability to stage-specific catastrophic events, such as those that could impact the brooders and their immobile eggs, would increase. Although shallow-water populations can show a pulse of brooding activity due to seasonal temperature increases (e.g., Voight, 1992), without such a temperature shift, no octopod population is documented to brood in synchrony. Given the liabilities associated with synchronous brooding, it seems unlikely to have created the difference in the number of brooding octopods observed between 2013 and 2014. In addition, the change in population density observed between the years was comparable to changes observed between successive days in the popular spot count. Transient females may move through the area, seeking an appropriate brooding site, generating frequent, short-term changes in population density.

5. Conclusion

If the warm sites on Dorado Outcrop are lethal to the next *Muusoctopus* generation, what sustains the population? We suspect that the population (or metapopulation) is much larger than our observations indicate. If the availability of hard substrate on which to brood

eggs limits benthic deep-sea octopods (Voight, 2000; Voight and Grehan, 2000), then where hard substrate is available, large populations can exist. Multi-beam echo sounder data gathered within 75 km of Dorado Outcrop reveal at least five additional, as yet unexplored outcrops and seamounts (Hutnak et al., 2008). Given that millions to tens of millions more seamounts and outcrops exist worldwide (Wessel et al., 2010), some of these seafloor features must have exposed surfaces, and may, like Dorado, have conduits and networks within their framework that can provide high quality, sediment-free areas for octopod egg brooding.

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Competing interest statement

The authors have no conflicts of interest.

Hazard to animal subjects

The research was a result of video already collected on AT26-09 (2013) and AT26-24 (2014). Interactions with animals were a consequence of the hydrogeologic sampling using a submersible. No animals were collected or harmed.

Contribution

Anne M. Hartwell initiated the investigation, analyzed the footage, drafted the manuscript, and, with comments provided by and discussed with the co-authors, edited the manuscript.

Janet R. Voight was a constant correspondent with Anne Hartwell. Her expertise, particularly on deep-sea octopods, bounded the fundamentals of the paper and was instrumental in shaping the discussion.

C. Geoffrey Wheat was the Chief Scientist on the two expeditions that collected the video and other data upon which this manuscript is based. He provided direction of and constraints for the geologic and hydrothermal setting of the outcrop. He freely shared all unpublished data and contributed to the manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.dsr.2018.03.011>.

References

- Anderson, R.C., Wood, J.B., Byrne, R.A., 2002. Octopus senescence: the beginning of the end. *J. Appl. Anim. Welf. Sci.* 5 (4), 275–283. <http://dx.doi.org/10.1207/>

- S15327604JAWS0504.02.
- Boletzky, S.V., 1994. Embryonic development of cephalopods at low temperatures. *Antarct. Sci.* 6 (2), 139–142. <http://dx.doi.org/10.1017/S0954102094000210>.
- Boletzky, S.V., 2003. Biology of early life stages in cephalopod molluscs. *Adv. Mar. Biol.* 44, 144–204.
- Borer, K.T., Lane, C.E., 1971. Oxygen requirements of *Octopus briareus* Robson at different temperatures and oxygen concentrations. *J. Exp. Mar. Biol. Ecol.* 7, 263–269.
- Daly, H.I., Peck, L.S., 2000. Energy balance and cold adaptation in the octopus *Pareledone charcoti*. *J. Exp. Mar. Biol. Ecol.* 245, 197–214.
- Drazen, J.C., Goffredi, S.K., Schlining, B., Stakes, D.S., 2003. Aggregations of egg-brooding deep-sea fish and cephalopods on the Gorda Escarpment: a reproductive hot spot. *Biol. Bull.* 205, 1–7. <http://dx.doi.org/10.2307/1543439>.
- Fisher, A.T., Wheat, C.G., 2010. Seamounts as conduits for massive fluid, heat, and solute fluxes on ridge flanks. *Oceanography* 23 (1), 74–87. <http://dx.doi.org/10.5670/oceanog.2010.63>.
- Froesch, D., Marthy, H.J., 1975. The structure and function of the oviducal gland in octopods (Cephalopoda). *Proc. R. Soc. Lond. B: Biol. Sci.* 188, 95–101. <http://dx.doi.org/10.1098/rspb.1975.0005>.
- Giménez, F.A., García, B.G., 2002. Growth and food intake models in *Octopus vulgaris* Cuvier (1797): influence of body weight, temperature, sex and diet. *Aquac. Int.* 10 (5), 361–377. <http://dx.doi.org/10.1023/A:1023335024053>.
- Gleadall, I.G., 2004. Some old and new genera of octopus. *Interdiscip. Inf. Sci.* 10, 99–112.
- Hoving, H.J.T., Perez, J.A.A., Bolstad, K.S., Braid, H.E., Evans, A.B., Fuchs, D., Piatkowski, U., 2014. The study of deep-sea cephalopods. *Adv. Mar. Biol.* 67, 235–359. <http://dx.doi.org/10.1016/B987-1-12-800287-2.00003-2>.
- Hutnak, M., Fisher, A.T., Harris, R., Stein, C., Wang, K., Spinelli, G., Silver, E., 2008. Large heat and fluid fluxes driven through mid-plate outcrops on ocean crust. *Nat. Geosci.* 1, 611. <http://dx.doi.org/10.1038/ngeo264>.
- Jannasch, H.W., Mottl, M.J., 1985. Geomicrobiology of deep-sea hydrothermal vents. *Science* 229 (4715), 717–725. <http://dx.doi.org/10.1126/science.229.4715.717>.
- Joubin, L., 1918. Etudes préliminaires sur les céphalopodes recueillis au cours des croisières de SAS le Prince de Monaco, 6e Note: *Vitreledonella richardi* Joubin. *Bull. Inst. Oceanogr.* 340, 1–40.
- Juárez, O.E., Galindo-Sánchez, C.E., Díaz, F., Re, D., Sánchez-García, A.M., Camaal-Monsreal, C., Rosas, C., 2015. Is temperature conditioning *Octopus maya* fitness? *J. Exp. Mar. Biol. Ecol.* 467, 71–76. <http://dx.doi.org/10.1016/j.jembe.2015.02.020>.
- Laptikhovsky, V., 2001. Fecundity, egg masses and hatchlings of *Benthooctopus* spp. (Octopodidae) in Falkland waters. *J. Mar. Biol. Assoc. U. K.* 81, 267–270. <http://dx.doi.org/10.1017/S0025315401003733>.
- Lee, M.D., Walworth, N.G., Sylvan, J.B., Edwards, K.J., Orcutt, B.N., 2015. Microbial communities on seafloor basalts at Dorado Outcrop reflect level of alteration and highlight global lithic clades. *Front. Microbiol.* 6, 1–20. <http://dx.doi.org/10.3389/fmicb.2015.01470>.
- Mottl, M.J., Wheat, G., Baker, E., Becker, N., Davis, E., Feely, R., Moyer, C., 1998. Warm springs discovered on 3.5 Ma oceanic crust, eastern flank of the Juan de Fuca Ridge. *Geology* 26, 51–54. <http://dx.doi.org/10.1130/0091-7613>.
- Neira, N.M., Clark, J.F., Fisher, A.T., Wheat, C.G., Haymon, R.M., Becker, K., 2016. Cross-hole tracer experiment reveals rapid fluid flow and low effective porosity in the upper oceanic crust. *Earth Planet. Sci. Lett.* 450, 355–365. <http://dx.doi.org/10.1016/j.epsl.2016.06.048>.
- Neptune Canada, 2010. August 18). Kraki the Octopus Broods at ODP 889. Retrieved July 25, 2017, from <https://www.youtube.com/watch?v=rp4Ua2DieGo>.
- NOAA, 2013. US Department of Commerce, National Oceanic and Atmospheric Administration (December 17). NOAA Ocean Explorer: For Fun: Octopus Friday Image Gallery. Retrieved July 25, 2017, from <http://oceanexplorer.noaa.gov/forfun/octopusfriday/welcome.html>.
- Oellermann, M., Lieb, B., Pörtner, H.O., Semmens, J.M., Mark, F.C., 2015. Blue blood on ice: modulated blood oxygen transport facilitates cold compensation and eurythermy in an Antarctic octopod. *Front. Zool.* 12 (1), 1–16. <http://dx.doi.org/10.1186/s12983-015-0097-x>.
- Parra, G., Villanueva, R., Yufra, M., 2000. Respiration rates in late eggs and early hatchlings of the common octopus, *Octopus vulgaris*. *J. Mar. Biol. Assoc. U. K.* 80, 557–558.
- Peck, L.S., Webb, K.E., Bailey, D.M., 2004. Extreme sensitivity of biological function to temperature in Antarctic marine species. *Funct. Ecol.* 18 (5), 625–630. <http://dx.doi.org/10.1111/j.0269-8463.2004.00903.x>.
- Pörtner, H.O., Van Dijk, P.L.M., Hardewig, I., Sommer, A., 2000. Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: Davison, W., Williams, C. Howard (Eds.), *Antarctic Ecosystems: Models for Wider Ecological Understanding*. Caxton Press, Christchurch New Zealand, pp. 109–122.
- Pörtner, H., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* 88, 137–146. <http://dx.doi.org/10.1007/s001140100216>.
- Purser, A., Marcon, Y., Hoving, H.J.T., Vecchione, M., Piatkowski, U., Eason, D., Boetius, A., 2016. Association of deep-sea incirrate octopods with manganese crusts and nodule fields in the Pacific Ocean. *Curr. Biol.* 26 (24), R1268–R1269. <http://dx.doi.org/10.1016/j.cub.2016.10.052>.
- Robison, B., Seibel, B., Drazen, J., 2014. Deep-sea octopus (*Graneledone boreopacifica*) conducts the longest-known egg-brooding period of any animal. *PLoS One* 9 (7), 1–4. <http://dx.doi.org/10.1371/journal.pone.0103437>.
- Salinas-de-León, P., Phillips, B., Ebert, D., Shivji, M., Cerutti-Pereyra, F., Ruck, C., Marsh, L., 2018. Deep-sea hydrothermal vents as natural egg-case incubators at the Galapagos Rift. *Sci. Rep.* 8 (1), 1–7. <http://dx.doi.org/10.1038/s41598-20046-4>.
- Seibel, B.A., Childress, J.J., 2000. Metabolism of benthic octopods (Cephalopoda) as a function of habitat depth and oxygen concentration. *Deep Sea Res. Part I: Oceanogr. Res. Pap.* 47, 1247–1260. [http://dx.doi.org/10.1016/S0967-0637\(99\)00103-X](http://dx.doi.org/10.1016/S0967-0637(99)00103-X).
- Seibel, B.A., Drazen, J.C., 2007. The rate of metabolism in marine animals: environmental constraints, ecological demands and energetic opportunities. *Philos. Trans. R. Soc. Lond. B* 362, 2061–2078. <http://dx.doi.org/10.1098/rstb.2007.2101>.
- Strugnell, J., Voight, J.R., Collins, P.C., Allcock, A.L., 2009. Molecular phylogenetic analysis of a known and a new hydrothermal vent octopod: their relationships with the genus *Benthooctopus* (Cephalopoda: Octopodidae). *Zootaxa* 2096, 442–459.
- Strugnell, J.M., Cherel, Y., Cooke, I.R., Gleadall, I.G., Hochberg, F.G., Ibáñez, C.M., Vecchione, M., 2011. The Southern Ocean: source and sink? *Deep Sea Res. Part II: Top. Stud. Oceanogr.* 58, 196–204. <http://dx.doi.org/10.1016/j.dsr2.2010.05.015>.
- Uriarte, I., Espinoza, V., Herrera, M., Zúñiga, O., Olivares, A., Carbonell, P., Rosas, C., 2012. Effect of temperature on embryonic development of *Octopus mimus* under controlled conditions. *J. Exp. Mar. Biol. Ecol.* 416, 168–175. <http://dx.doi.org/10.1016/j.jembe.2012.03.003>.
- Valverde, J.C., Garc'a, B.G., 2004. Influence of body weight and temperature on post-prandial oxygen consumption of common octopus (*Octopus vulgaris*). *Aquaculture* 233, 599–613. <http://dx.doi.org/10.1016/j.aquaculture.2003.11.025>.
- Vidal, E.A., Villanueva, R., Andrade, J.P., Gleadall, I.G., Iglesias, J., Koueta, N., Albertin, C.B., 2014. Cephalopod culture: current status of main biological models and research priorities. *Adv. Mar. Biol.* 67, 1–98. <http://dx.doi.org/10.1016/B978-0-12-800287-2.00001-9>.
- Voight, J.R., 1992. Movement, injuries and growth of members of a natural population of the Pacific pygmy octopus, *Octopus digueti*. *J. Zool.* 228, 247–264. <http://dx.doi.org/10.1111/j.1469-7998.1992.tb04606>.
- Voight, J.R., 2000. The distribution of octopuses of *Graneledone* (Cephalopoda: Octopodidae) in reference to deep-sea features. *Malacologia* 42, 63–74.
- Voight, J.R., 2008. Observations of deep-sea octopod behavior from undersea vehicles. *Am. Malacol. Bull.* 24, 43–50. <http://dx.doi.org/10.4003/0740-2783-24.1.43>.
- Voight, J.R., Grehan, A.J., 2000. Egg brooding by deep-sea octopuses in the North Pacific Ocean. *Biol. Bull.* 198, 94–100.
- Voight, J.R., Drazen, J.C., 2004. Hatchlings of the deep-sea octopus *Graneledone boreopacifica* are the largest and most advanced known. *J. Mollusca. Stud.* 70, 400–402. <http://dx.doi.org/10.1093/mollus/70.4.400>.
- Wells, M.J., Wells, J., 1985. Ventilation frequencies and stroke volumes in acute hypoxia in *Octopus*. *J. Exp. Biol.* 118, 445–448.
- Wessel, P., Sandwell, D.T., Kim, S.S., 2010. The global seamount census. *Oceanography* 23, 24–33. <http://dx.doi.org/10.5670/oceanog.2010.60>.
- Wheat, C.G., Fisher, A.T., McManus, J., Hulme, S.M., Orcutt, B.N., 2017. Cool seafloor hydrothermal springs reveal large global geochemical fluxes. *Earth Planet. Sci. Lett.* 476, 179–188. <http://dx.doi.org/10.1016/j.epsl.2017.07.049>.
- WHOI, 2013. Jason Virtual Control Van. Retrieved November 29, 2017, from <http://4dgeo.whoi.edu/webdata/virtualvan/html/VV-at26-09/index.html>.
- WHOI, 2014. ALVIN Frame Grabber. Retrieved November 29, 2017, from <http://4dgeo.whoi.edu/alvin>.

