First in situ observation of Cephalopoda at hadal depths (Octopoda: Opisthoteuthidae: Grimpoteuthis sp.)

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Abstract
The Cephalopoda are not typically considered characteristic of the benthic fauna at hadal depths (depths exceeding 6000 m), yet occasional open-net trawl samples have implied that they might be present to ~ 8000 m deep. Previous in situ photographic evidence has placed the deepest cephalopod at 5145 m. The discrepancies between the two have meant that the maximum depth for cephalopods has gone unresolved. In this study we report on unequivocal sightings, by HD video lander, of a cephalopod at hadal depths. The demersal cirrate octopod Grimpoteuthis sp. was observed at both 5760 and 6957 m in the Indian Ocean. These observations extend the known maximum depth range for cephalopods by 1812 m and increase the potential benthic habitat available to cephalopods from 75 to 99% of the global seafloor.

Introduction
The total bathymetric range of marine organisms is often difficult to resolve accurately because sampling effort becomes less frequent with increasing depth. One important group with ambiguous records of maximum depth is the molluscan class Cephalopoda. Establishing the maximum depth of the very mobile cephalopods is further hampered because few bottom trawls have been performed at hadal depths (> 6000 m), and contemporary hadal sampling has favoured baited traps and baited camera systems to which cephalopods, being predatory, are rarely attracted. While the cephalopods are not considered to be characteristic for hadal depths, a few historical reports suggest they may indeed be present below 6000 m (Belyaev 1989). In 1949, in the Kuril-Kamchatka Trench, an octopod was captured by an open trawl that sampled to 8100 m (Uschakov 1952). Also, an octopod egg chorion from the subfamily Bathypolypodinae, which are known to attach their eggs to the seafloor, was found in the intestine of the snailfish Pseudoliparis (Careproctus) amblystomopsis from the same trench at 7210–7230 m (Birstein and Vinogradov 1955) which also indicated a hadal distribution (Akimushkin 1963). Finally, in 1975 a specimen of Grimpoteuthis sp. (Opisthoteuthidae) presumably from 7280 m was obtained by the American J. E. Pillsbury expedition in the Cayman Trench (Belyaev 1989). Note, however, that the J. E. Pillsbury report was never published, possibly indicating lack of confidence that the octopod was captured at the maximum depth of the open trawl. Grimpoteuthis spp. are known to swim, at least occasionally, far above the bottom. These specimens may, therefore, have been collected during either descent or retrieval of the open trawl nets. The egg could have originated from shallower depths and descended down the trench slopes following detachment; a process that concerned scientists at the time with regards to validating trawl samples (Houot 1972). In the absence of direct in situ observation by submersible, or remote camera, these accounts have, therefore, been considered questionable. Indeed, Hoving et al. (2014) inferred that these findings may be ‘biased’, which insinuates a shallower origin, or at least lack of unequivocal proof of hadal occurrence. The deepest “unbiased” benthic cephalopod record in the literature is a cirrate octopus that was photographed on the abyssal plains 325 miles off Barbados (Jahn 1971). This was a single photograph of an octopod belonging to the family Cirroteuthidae at a depth of 5145 m. This 50-year-old photograph has, until now, remained the
deepest unequivocal maximum depth record for the Cephalopoda (Hoving et al. 2014). Octopoda such as these are, however, relatively well known at deep abyssal depths close to this record (e.g. Vecchione 2017).

Generally, the Cephalopoda are restricted to abyssal depths or shallower (ca. < 5000 m). The deepest sighting of an incirrate octopod, the ‘Casper morphotype’, is currently 4290 m; (Purser et al., 2016). The deepest unequivocal record of oegopsid squids to date is a ‘bigfin squid’ (Family: Magnapinnidae) observed at 4735 m in the western Atlantic (Vecchione et al. 2001).

Here we report on two direct in situ observations of the cirrate octopod *Grimpoteuthis* sp. from 5760 and 6957 m in the Java Trench (Indian Ocean). The former represents a depth extension of 615 m from that of Jahn (1971) while the latter is the first ever in situ imaging of a cephalopod unequivocally at hadal depth, and extends the deepest reliable record of this class of molluscs by 1812 m.

### Materials and methods

The observational deployments reported here were conducted during the Indian Ocean leg of the Five Deeps Expedition (2018–2019). Three identical autonomous baited camera and trap landers with conductivity, temperature and depth (CTD) sensors were deployed seven times between depths of 5760 and 7176 m in April 2019 (Fig. 1). The landers, known as Skaff, Flere and Closp were deployed from the support ship DSSV Pressure Drop (Jamieson et al. 2019). The HD cameras (IP Multi SeaCam 3105; Deep Sea Power and Light, US) recorded continuously throughout the deployments and CTD (SBE 49 FastCAT, SeaBird Electronics, US) measurements were taken every one second. The landers were baited with mackerel (Scombridae) and the cameras were positioned ~ 30 cm above the seafloor looking horizontally across the seafloor.

Mantle length (ML) and total length (TL) were digitally estimated from frame grabs taken when the individuals were in line with the lander bait arm. The bait arm served as a reference scale in the field of view.

### Results and discussion

Cephalopods were observed in two of the seven lander deployments. The depths and locations where cephalopods were seen were 5760 m (11.2483°S/114.8800°E) and 6957 m (11.1200°S/114.9283°E). The bottom time of the two positive deployments was 6 h 41 min and 4 h 59 min respectively. The environmental conditions were 1.34 °C and 1.51 °C respectively with a salinity of 34.7. The substrata at both locations were soft sediments.

At the 5760 m site, a single *Grimpoteuthis* sp. (130 mm ML, 430 mm TL) approached the lander 2 h 25 min after it reached the seafloor (Fig. 2). The octopod spent 23 min in view, generally not interacting with the bait or lander but apparently foraging around on the seafloor. It exited the field of view for 6 min, returned for 7 min and exited. It was observed in close proximity to a cusk eel (*Bassoeactus* sp.) and a decapod crustacean (*Plesiopeneaus* sp.). At 6957 m, a single *Grimpoteuthis* sp. (110 mm ML, 350 mm TL) approached the lander 7 min after the lander set down. There it spent 5 min, approaching the lander, then generally not interacting with the bait or lander but rather “foraging” on the seafloor around the surrounding area similarly to the observation described above. It did, however, pick up a munnopsid isopod (~ 3 cm body length), held it for a few seconds, and dropped it back onto the seafloor before exiting the field of view. It was out of view for 21 min before being observed passing by the lander, several metres away.

We assume here that the two sequential views of cirratures in both observations represent two views of the same animal rather than additional cirratures in the vicinity of the lander. No other cephalopods were observed. As both individuals approached from the horizon of the camera field of view, and in both cases left and returned to the lander, it suggests that artificial illumination was not a deterrent. Furthermore,
given both individuals had short residence times relative to the duration of the illumination suggests artificial illumination was also not a significant attractant. The behaviour that we presume to be foraging consisted of brief “hops” from place to place, separated by centimetres, on the bottom. Upon contact with the bottom, the arms and webs were spread until the octopod again lifted off the bottom a few seconds later. No observed behaviours suggested that either of these individuals were resting or moribund.

The observations of the cirrate octopod of the genus *Grimpoteuthis* at 6957 m extends the reliably documented maximum depth range for cephalopods by 1812 m (Fig. 3a). Some of the deepest cephalopods captured to date are indeed species of *Grimpoteuthis* (Collins 2003; Collins and Villanueva 2006), but these new observations extend the range for this genus by over 2000 m (Fig. 3b). It also unequivocally places a cirrate octopod as a member of the hadal community.

Both cirrate octopods observed in this study are from the same area, therefore it is difficult to confirm whether their hadal presence is global or the result of chance encounter with an abnormally deep population. However, these new in situ observations provide evidence that may substantiate the reports from the former-Soviet trawling campaigns (Ushakov 1952; Birstein and Vinogradov 1955; Akimushkin 1963), which, if are correct, puts the deepest records slightly deeper again at 8100 m. It also provides credence to the *Grimpoteuthis* sp. from 7280 m in the Cayman Trench (cited in Belyaev 1989). Indeed, it appears that cephalopods, at least *Grimpoteuthis*, are perhaps more prevalent in the upper hadal trenches than previously anticipated.

Cirrate octopods are not uncommon in photographic surveys of deep habitats (Roper and Brundage 1972; Vecchione 2017). However, photo-based taxonomy of cephalopods is difficult because many of their generic and specific characters require close morphological examination, often internal, and are not visible in imagery (Vecchione and Roper 1991). The salient point in this study is the unequivocal bathymetric range extension of the molluscan class Cephalopoda, represented by the family Opisthoteuthidae, to well inside the hadal zone—to at least ca. 7000 m. The significance of this bathymetric range extension is perhaps best demonstrated by converting the depth to area of benthic habitat available for these demersal cephalopods. A distribution of 0–5145 m,
gives octopods access to ~75% of the total ocean floor. This new extension to 7000 m adds another 24%, bringing the theoretically accessible area of global seafloor to 99%; all but the deepest few thousand metres that diminish exponentially in area with increasing depth (Stewart and Jamieson 2018).

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Data availability The data that support the findings of this study are copyright of Discovery Channel 2020 and restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Discovery Channel 2020.

Compliance with ethical standards

Conflict of interest Alan Jamieson declares that he has no conflict of interest. Michael Vecchione declares he had no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms for the study have been followed.

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