

Vampire squid: detritivores in the oxygen minimum zone

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Vampire squid (*Vampyroteuthis infernalis*) are considered phylogenetic relics with cephalopod features of both octopods and squids. They lack feeding tentacles, but in addition to their eight arms, they have two retractile filaments, the exact functions of which have puzzled scientists for years. We present the results of investigations on the feeding ecology and behaviour of *Vampyroteuthis*, which include extensive *in situ*, deep-sea video recordings from MBARI's remotely operated vehicles (ROVs), laboratory feeding experiments, diet studies and morphological examinations of the retractile filaments, the arm suckers and cirri. Vampire squid were found to feed on detrital matter of various sizes, from small particles to larger marine aggregates. Ingested items included the remains of gelatinous zooplankton, discarded larvacean houses, crustacean remains, diatoms and faecal pellets. Both ROV observations and laboratory experiments led to the conclusion that vampire squid use their retractile filaments for the capture of food, supporting the hypothesis that the filaments are homologous to cephalopod arms. *Vampyroteuthis*' feeding behaviour is unlike any other cephalopod, and reveals a unique adaptation that allows these animals to spend most of their life at depths where oxygen concentrations are very low, but where predators are few and typical cephalopod food is scarce.

Keywords: *Vampyroteuthis infernalis*; cephalopoda; oxygen minimum zone; detritus; feeding

1. INTRODUCTION

Squid and octopuses are abundant cephalopod molluscs that inhabit the marine environment from coastal areas to the abyss. They have evolved a wide variety of strategies to pursue living prey like fish, crustaceans and other cephalopods [1]. Benthic octopuses use their extensive repertoire of camouflage patterns to sneak up on or ambush prey [2]. Cirrate octopods are believed to trap copepods by engulfment, using the web of thin tissue between their arms [3,4]. Squid have, in addition to their four arm pairs, a pair of highly extensible and contractile tentacles that allows them to grab prey in front of them [5]. Due to their speed and agility, some squid are able to catch fast-swimming prey like sardines and hake, while others are sit-and-wait predators who deploy their long tentacles to lure and ambush prey [6].

The 'vampire squid' *Vampyroteuthis infernalis*, the sole species in the Order Vampyromorpha, is a phylogenetic relic with features of both octopods and squid [7]; they have eight arms but lack feeding tentacles. They do have two long, extensible, retractile filaments [7,8], which are presumably a modified arm-pair [9] and are thought to have a sensory function in the detection of food items and/or potential predators [4,10]. The eight arms, which are joined by an extensive web, bear a longitudinal, distal row of up to 21 suckers and multiple finger-like projections called cirri [11]. Morphological phylogenetic reconstructions show the Vampyromorpha to be a sistergroup of the Octopoda, together forming the Octopodiformes [12].

Vampyroteuthis infernalis occurs circumglobally in temperate and tropical oceans, typically in waters with low levels of dissolved oxygen [13–16]. In waters over the Monterey Submarine Canyon, off Central California, we have found *Vampyroteuthis* throughout the depth range between 600 and 900 m and at oxygen concentrations centred around 0.4 ml l^{-1} . Mesopelagic oxygen minimum zones (OMZs) with concentrations less than 0.5 ml l^{-1} ($22 \mu\text{MO}_2$) occur commonly beneath areas of upwelling and high surface productivity; particularly where circulation is sluggish and source waters are relatively old [17]. In these upwelling areas, phytoplankton productivity is typically high and carbon availability often exceeds metazoan capability to consume it [18]. This results in high bacterial growth at depth as a result of the decomposition of organic carbon in sinking particulate matter, yielding very low oxygen concentrations [19]. While species from many taxa (including copepods, euphausiids, cnidarians, ctenophores, fish and squid) live entirely or part of the time (during diel or ontogenetic vertical migrations) within the most pronounced OMZs [20,21], many organisms are stressed or die under hypoxic conditions [22], and overall abundance and species diversity are reduced. OMZs have dramatic effects on the spatial distribution patterns of animals in the water column, and zones of enhanced biological and biogeochemical activity exist at the OMZ's upper and lower boundaries [23,24]. Metazoan species that permanently inhabit OMZs have specific adaptations to hypoxia [25].

Adaptations of *Vampyroteuthis* that enable a life in the OMZ include: suppression of aerobic metabolism, resulting in the lowest mass-specific metabolic rate measured for any cephalopod [26]; a respiratory protein (haemocyanin) with a relatively high affinity for oxygen [16] and neutral buoyancy that reduces energy expenditure

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for swimming. Vampire squid are dark and cryptically coloured, which reduces their visibility to predators [4], and they possess several bioluminescent displays, which are believed to be incorporated into anti-predation behaviour and perhaps to prey capture [15].

The variety of feeding strategies employed by deep-living squids appears to increase with greater depth as decreasing light, temperature and oxygen change the morphological and metabolic requirements for catching prey and avoiding predators [12,27]. While many of the physiological and morphological adaptations that allow the vampire squid to live in the OMZ are relatively well known, published information on the feeding habits of *Vampyroteuthis* is still very sketchy. Stomach contents reported from a few trawl-caught specimens include diatoms, copepods, a prawn, and cnidarian fragments [7,10]. In order to better understand how vampire squid are able to thrive under conditions that are highly adverse to most other cephalopods, we investigated their feeding behaviour and ecology.

2. MATERIAL AND METHODS

(a) *In situ observations and collection of individuals*

Between 1992 and 2012, 170 specimens of *Vampyroteuthis* were observed and recorded during remotely operated vehicle (ROV) dives in Monterey Bay [28]. These observations comprise a total of approximately 24 h of video footage all of which was annotated, reviewed and analysed for this study. Individual observations ranged from a glimpse of an animal in the distance to continuous recordings of more than 2 h duration. During analysis, we noted the specimen's posture when first encountered (the 'undisturbed' position), the position of the arms tips (forward or tucked in), the presence or the absence of extended filaments, the position of the filaments, the association of particles or other items with the filaments, and the presence of food in the mouth or mouth area. Individual video frames were digitized to further document-specific postures or behaviours of interest. Sample specimens collected by the ROVs confirmed that our observations included mature males, mated females, young individuals (juveniles with two anterior fins) and juveniles (with four fins) [9]. Specimens collected in Monterey Bay ranged in size from 22 to 150 mm mantle length (ML) and were found at depths between 600 and 800 m. Three individuals were observed during ROV dives in the Gulf of California (GoC) in February 2012 and two of these were collected (ML 135 mm and 210 mm). Three additional specimens were observed during a transit to Hawaii in March 2001; two in the region of the California Current and one in the eastern Central Pacific gyre.

(b) *Histology and scanning electron microscopy*

For histological sectioning, the distal 5 cm of two arms (with suckers and cirri) of a mature male, and retractile filaments (divided into five sections from proximal to distal) from several specimens of both genders, were dehydrated in a graded series of ethanol, embedded in paraffin and sectioned with a microtome. Sections were stained with haematoxylin and eosin. Additionally, to test for the presence of mucus secretion, sections of the arm tips were stained with mucicarmine stain, using tartrazine as the counter stain.

Several filaments were prepared for viewing under SEM. We used both standard critical point drying and

hexamethyldisilazane (HMDS) for chemical critical point drying (see the electronic supplementary material).

(c) *Analysis of digestive system contents*

Vampire squid have a crop, an enlarged oesophagus anterior to the stomach and caecum complex, apparently for food storage. For direct evidence of food identity, we used a stereomicroscope to examine: (i) the contents of six faecal droppings from five ROV-collected animals, all of which were produced shortly after the specimens were collected and therefore contained naturally ingested food items; (ii) five food boli that were each regurgitated by separate individuals immediately after capture by ROV; (iii) items from the crops of two ROV-collected animals (one from the GoC) that were examined shortly after capture and anaesthetizing with MgCl₂ or ethanol; (iv) the crop contents of 43 trawl-captured specimens. Thirty-six trawled specimens were collected between 1964 and 1971 by R/V *Velero IV* off southern California and northern Baja California; four were collected in 1967 in Mexican waters off northern and central Baja California by R/V *Velero IV*. These 40 specimens are accessioned in the collections of the Santa Barbara Museum of Natural History. Another three specimens were collected off the California coast in 2011 by cruises of the Scripps Institution of Oceanography as part of the California Current Ecosystem Long-Term Ecological Research (CCE-LTER) programme.

DNA samples were extracted from a selected piece of tissue from one regurgitated food bolus using the DNeasy Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. LCO1428 and HCO2198 primers [29] were used to amplify an approximately 500-base-pair section of the mitochondrial cytochrome c oxidase subunit I gene (COI) with the following PCR parameters: 38 cycles of 94°C for 1 min, 48°C for 1 min, 72°C for 1 min. The products were sequenced using BIGDYE TERMINATOR v. 3.1 Cycle Sequencing Kit on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). Using Basic Local Alignment Search Tool (BLAST) against the NCBI database, the forward sequence data showed highest sequence identity (97%, E-value 0) to *Gonatopsis borealis* (GenBank accession no. AF144725.1). We analysed sequences using a BLAST search against the NCBI database and determined they were from the squid family Gonatidae, most probably *G. borealis* (coverage = 100%, E = 0, maximum identity = 97%).

(d) *Laboratory feeding experiments*

Five animals collected by ROV were maintained initially in large Sealtite plastic bags and transferred from the ship to MBARI's dark, cold room (5.5°C) ashore. Plastic bags are used to prevent skin abrasion resulting from contact with aquarium walls, which leads to infections and reduced survival rates. Small specimens were maintained throughout their captivity in plastic bags, but large specimens were transferred within a day or two to circular kreisel tanks [15,30]. Animals were kept this way for periods up to 35 days. In the laboratory, vampire squid were provided regularly with fresh dead plankton, mostly copepods. When the animals were examined or fed, only red-light illumination was used. Experiments in the laboratory were performed with all five animals. Two small specimens were transferred to an aquarium about 2 h prior to experimentation. The larger animals remained in their residential kreisels. A high-resolution video camera was placed in front of the tank to record behaviour. During recording, white

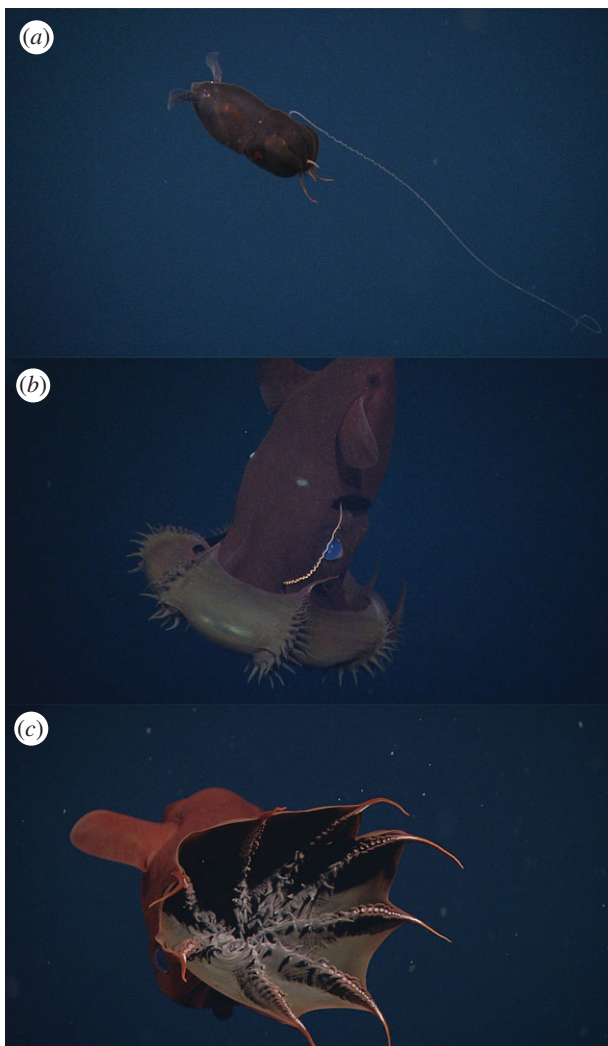


Figure 1. (a) A juvenile *Vampyroteuthis* with its filament extended, the short peduncle is visible at the base of the filament. (b) A vampire squid with a retrieved filament and the web partly curled, revealing the arms and cirri. (c) Oral view into the vampire squid's web showing the arms with cirri and the suckers present on the distal half of the arm.

light was used for illumination. Experiments consisted of the careful addition of food items (individual copepods or homogenized plankton collected by midwater trawl between 600 and 800 m) into the container with the subject. Food items were dispensed above the retractile filament and allowed to settle slowly.

3. RESULTS

(a) *Morphology of the arms, suckers, cirri and the retractile filament*

The arms of *Vampyroteuthis* are lined on their oral surface with a single median row of suckers (figures 1 and 4), running distally from midway between the beak and the end of the large web. Alternating with the suckers, on either side of the oral surface, are relatively large fleshy projections, the cirri (figures 1 and 4). Two rows of cirri on each arm extend proximally past the first suckers [7] (figures 1 and 4). There may be up to 10 pairs of primary cirri along the base of the arm before the first sucker, beyond which they alternate with the suckers [14] (figures 1 and 4). The paired, long retractile filaments emerge from pockets between the first and second arms and may be projected

for up to eight times the individual's total body length, usually one at a time (figure 1). When not extended, the filament is coiled within the pocket (figure 1). At the base of the filament is a short, broad and relatively rigid peduncle, from which the flexible primary filament extends. The retractile filament is widest at its base and narrows distally, becoming very thin towards the end.

The typical octopus sucker consists of a cup-like acetabulum, and a disc-like infundibulum that forms a wide lip to the cup; the two are separated by a strong sphincter muscle. On the inner sucker surfaces, there is a cuticular lining [31]. In *Vampyroteuthis*, the sphincter muscle, a distinct infundibulum and the cuticular lining are absent. At the base of the vampire squid's acetabulum and in the skin covering the sucker stalk, there are secretory cells that stain brightly for mucin secretion (figure 2). The secretory cells occur in three states: (i) goblet cell containing secretory granules (this is the same type of cell involved in mucus secretion); (ii) goblet cell in which the secretory granules are disappearing and mucous is forming; (iii) goblet cell in which mucous has been released, i.e. an empty cell. The type of secretory cells (goblet cells containing secretory granules) found on cirri is similar to those found at the base of the suckers' acetabulum and in the skin covering the sucker stalk. The rim of each sucker has a complex of radiating folds and hillocks, on the apices of which are pores, each with a bundle of cilia [32].

The primary filament is a uniform structure with large vacuolated cells forming a solid outer layer that covers the complete length of the filament (figure 3). In SEM pictures, the vacuolated cells are collapsed. Underneath the vacuolated cells, lies an epithelial layer of simple cuboidal cells, interspersed with large spherical, apparently sensory, cells. The latter have axon-like extensions that extend into the axial nerve, but lack obvious organelles commonly found in some sensory cells. Underneath the sensory cells is a thin muscle layer, followed by connective tissue (figure 3). In the centre of the filament is a relatively large axial nerve (figure 3), which runs to the ventral magnocellular lobe of the brain [8]. The other side of the filament, opposite the sensory side, is characterized by the presence of a distinctive thick muscle band (figure 3), probably involved in retraction of the filament.

Fine, flexible but stiff 'hairs' cover the exterior of the primary filament (figure 3) and were most abundant on the second half. These hairs are positioned on the junctions between the exterior vacuolated cells. The hairs are visible with SEM and on fresh tissue with a dissecting microscope, but are lost after histological sample preparation (figure 3). In SEM pictures, the hairs on the retractile filament do not stand up, contrary to their position under the light microscope (figure 3). These are artefacts of sample preparation.

Longitudinal histological sectioning reveals the asymmetric nature of the filament (figure 3). From outside to inside (aboral to oral), one side has the layer of vacuolated cells with the flexible but stiff hairs that lie on top of the vacuolated cells.

(b) *Ingested and digested items*

(i) *Vampyroteuthis captured by ROV*

In the crop of one recently captured specimen, we found three items that had apparently been ingested separately.

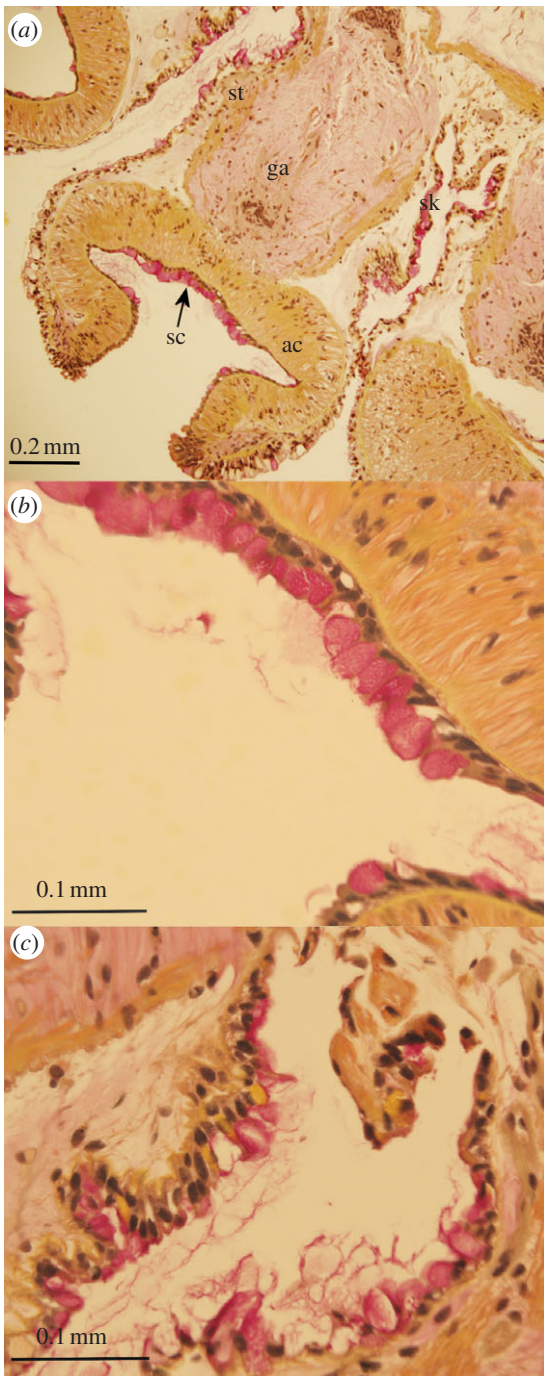


Figure 2. (a) Cross section through a sucker on the distal arm showing the muscular cup that forms the acetabulum (ac), the muscle of the sucker stalk (st), the skin covering the sucker stalk and outer sides of the sucker (sk), the sucker nerve ganglion (ga). (b) A close-up of the secretory cells in the acetabulum shown in (a). (c) Secretory tissue in the skin covering the sucker stalk.

Each was enclosed in a sticky mucus mass within which small (10–20 μm) red cells were incorporated. One item consisted of a long crustacean antenna that had several larvacean faecal pellets stuck to it. Another item was a ‘sinker’, the discarded mucus filtration house of a giant larvacean of the genus *Bathocordaeus* [28]. The sinker had larvacean faecal pellets and crustacean setae stuck to it. A third item was a bundle of crustacean setae, moults and a crustacean eye. The latter consisted of triangular units, the crystalline cones from crustacean

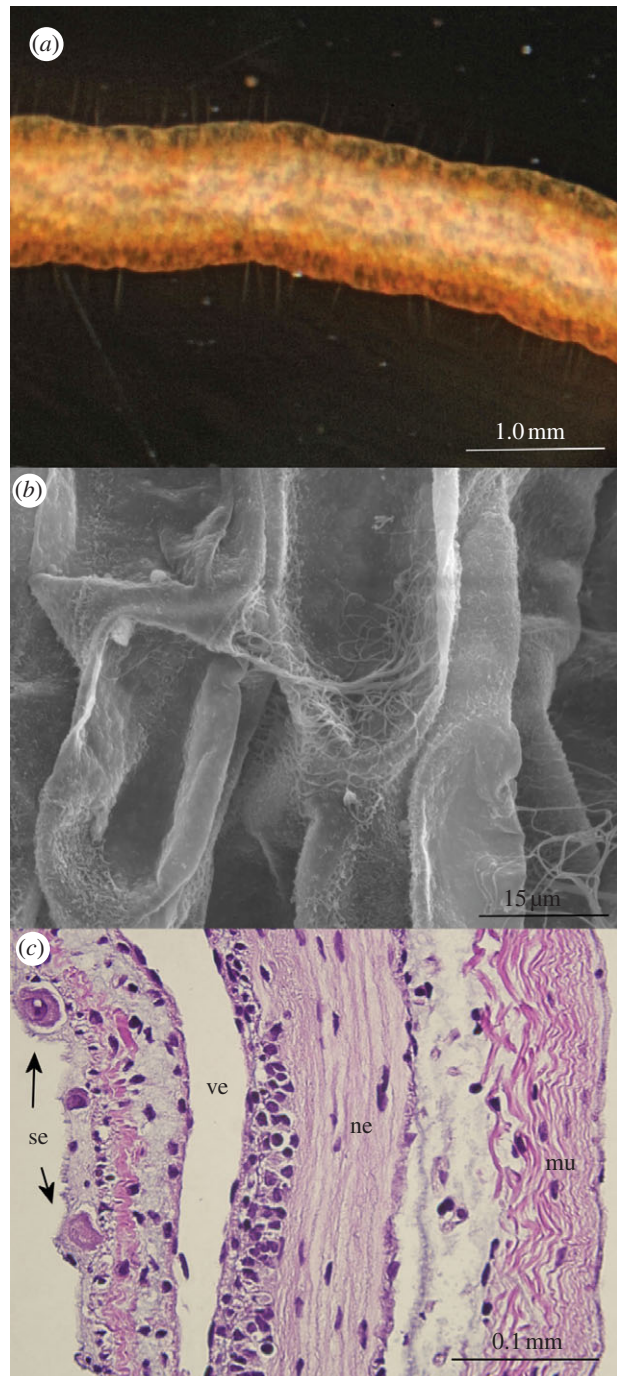


Figure 3. (a) A close-up of part of the retractile filament of *Vampyroteuthis infernalis* showing the hairs perpendicular to the axis of the filament, and the transparent outer layer of vacuolated cells. (b) A SEM micrograph of the retractile filament of *Vampyroteuthis infernalis* showing the hairs on the vacuolated cells. (c) Histological section of the asymmetric retractile filament showing the sensory cells (se) and a blood vessel (ve) on the one side and muscle tissue (mu) on the opposite side, the nerve is located centrally (ne).

eyes, which were also found in large numbers in droppings. The second specimen had typical marine snow contents in its crop, consisting of crustacean moults, diatoms, crustacean parts, copepods, eggs and faecal pellets.

Similarly, a regurgitated food bolus contained several food items that were held together by mucus, in which red cells were incorporated. It had several small pieces of flesh, identified by mt DNA COI sequence to be of

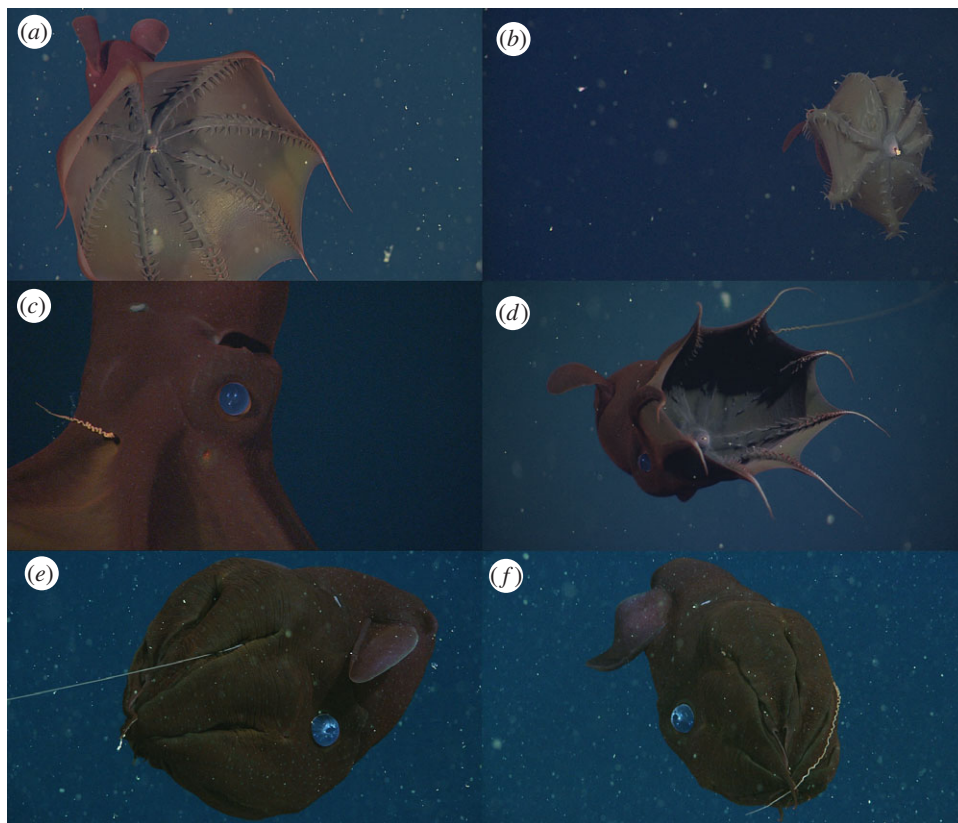


Figure 4. (a) An oral view of *Vampyroteuthis infernalis*. (b) A marine aggregate in the mouth of vampire squid. (c) A dorso-lateral close-up of the left side of the head of a vampire squid, showing the filament being retracted, a mucus trail is visible on the filament. (d) An oral view of a vampire squid showing a trail of mucus with associated food particles running to the mouth. (e) A vampire squid with a forward-extended filament and a piece of marine snow on the tip of the second right arm. (f) The same specimen shown in (e) now retrieving the filament along the tips of its arms.

the deep-sea squid *Gonatopsis borealis* (a species that occurs in the Monterey Submarine Canyon), in addition to diatoms, a radiolarian, a piece of a sinker, and larvacean faecal pellets.

Another animal was observed *in situ* to have food in its mouth just before collection by the ROV. This food item was collected along with the specimen and it turned out to be the inner filter of a small oikopleurid larvacean. In the shipboard laboratory, this animal regurgitated its crop contents consisting of a marine aggregate (figure 4). The regurgitations of two other specimens contained a sinker, gelatinous tissue from a salp or medusa, microscopic crustacean parts, faecal pellets from copepods, larvaceans and moults, all enclosed in sticky mucus with red cells.

The collected droppings of eight different animals were similar in appearance and in composition. They were red (because of the presence of the red cells also found in the ingested food and in the regurgitation) and contained intertwined fibres, crustacean antennae, setae and legs. Within this knot of different elongate structures, various other items were found, including radiolarians, metal-like flakes, a single fish scale, sand grains, diatoms, a *Doliolum* gonozooid, thousands of crystalline cones from crustacean eyes, crustacean moults, and what appeared to be copepod eggs.

The red cells present in the mucus of ingested food boli were also found in the mucus that was released by living animals, and are apparently produced by the cephalopod. These cells often gave the secreted mucus a reddish appearance. Such mucus was found floating in the tanks where the specimens were kept alive, and it was present in containers

after dissection of specimens. The cells were also found in faeces and are apparently not digested.

(ii) *Vampyroteuthis* collected by trawl

Of the 43 specimens collected by trawl, 36 had food in their crops. Ingested food was typically divided into: (i) marine aggregates, including sinkers and parts of other gelatinous organisms (salps, ctenophores and medusae), held together by sticky mucus and including complete organisms such as crustaceans (copepods, ostracods and amphipods), chaetognaths, foraminifera, radiolarians, diatoms and ciliates but also numerous faunal bits such as eggs, fish scales and fish bones, crustacean moults, eyes, antennae, setae, legs, urosomes and microscopic fragments of animals, dominantly crustaceans; (ii) one large fragment of a gelatinous organism (probably salp or medusa); (iii) complete crustaceans (ostracods, copepods and amphipods) inside an opaque jelly-like substance (only found in formalin-preserved material and potentially a preservation artefact); and (iv) complete or parts of crustaceans (ostracods, copepods and amphipods) without association with aggregates or jelly. In 19 animals, faecal pellets varying in size from 0.05 to 0.85 mm, and shape (spherical, cylindrical and ovoid) were present in the ingested food bolus, all consistent with the ingestion of marine aggregates and detrital matter.

(c) Behavioural observations

(i) In situ

Typically, *Vampyroteuthis* was initially found in a horizontal position, and 55 individuals (approx. 33%) had a

filament extended, including 2- and 4-fin juveniles. Only once was an individual observed with both filaments out. Extended filaments can reach within a broad arc forward, below and above the animal's head (figure 4). Vampire squid were also repeatedly observed with their filament drawn between the distal parts of their arms (figure 1). Thirteen individuals had the distal half of all eight arms tucked inward towards their mouth. One of these animals was collected and its crop contained food suggesting, as do our laboratory observations, that this posture is taken when food is being ingested.

In six individuals, particles or aggregates of particles were observed in the mouth (figure 4). In another animal, a mucus trail containing particles was observed stretching from an arm to the mouth (figure 4). Thirteen animals observed *in situ* had particulate material associated with the filament. In one specimen, we saw an apparent food item at the tip of the arms. On three occasions, we detected the presence of a large aggregate on the retractile filaments. We repeatedly observed smaller particles on the filaments during close-up recordings of the filament *in situ*.

(ii) Laboratory

The first indication that the retractile filament has a direct function in food collection came from an early observation made in the laboratory. Bits of homogenized plankton, previously added to the bag in which a specimen was kept, became stuck to a filament after contact in the bottom of the bag. The animal retrieved the filament and the food particles were removed when the filament was drawn across the oral surface of the arms. The individual particles that had been on the bottom of the bag became a food bolus within the oral web of the animal.

In recent more controlled experiments, homogenized plankton was squirted from a pipette, or individual copepods were released above the filament of a specimen in a kreisel. Food particles became attached on contact with the filament, and the animal immediately began retrieving the filament while passing it within its web, between the arms. The food was wiped off the filament, wrapped in mucus forming a food bolus, then was apparently transported along the web. When next we could see the mouth, the food was visible as a bolus between the chitin jaws. Filament retrieval and the removal of attached food particles were observed in four different specimens; in three of these, the behaviour was induced repeatedly.

Filament retrieval when food was not attached was induced *in situ* and in the laboratory by simply touching it. This led to a rapid withdrawal of the filament, directly towards the pocket with the filament becoming more tightly helical as it contracted. This response is much quicker than filament retrieval when food is transferred to the oral web cavity by passage between the arms, which took up to 5 min.

Mucus secretion from the arm tips of *Vampyroteuthis* associated with bioluminescence has been previously reported [15]. The mucus source here is most probably the secretory tissue in the arm suckers (figure 2). We unsuccessfully attempted to elicit a sucking response by touching the surface of the suckers. Both our laboratory and *in situ* observations indicate that food items attached to the filament are subsequently bound into an aggregate by mucus, presumably from the arm suckers. The cirri are thought to

transport a bolus to the mouth, owing to the similar function of cirri observed in the cirrate *Grimptoteuthis* sp. [4]

4. DISCUSSION

Most modern cephalopods are predatory carnivores. The results of our diet studies of *Vampyroteuthis* off central and southern California and in Mexican waters suggest a radically different feeding strategy. Typical prey of cephalopods which co-occur with *Vampyroteuthis* in the OMZ of the Monterey Submarine Canyon include bathylagid fishes, mysid shrimps, gonatid squids and a variety of cnidarians (MBARI, archived video data). The tissues of these animals were never found in large enough quantities in stomach contents to suggest active predation by the vampire squid. The food items that we found in digestive tracts, in droppings and regurgitations and that we saw being consumed during *in situ* observations were not representative of captured live prey. Instead, *Vampyroteuthis*' food consisted of agglomerated copepod parts, faecal pellets, diatoms, radiolarians and fish scales; often embedded in a mucus matrix. The most likely source of this eclectic mix is marine snow aggregates, including the feeding structures of larvaceans.

Aggregated marine snow particles and 'sinkers' (discarded mucus filters of the giant larvacean *Bathochordaeus*) are very common within the depth range occupied by *Vampyroteuthis* in Monterey Bay [28,33]. When we observed *Vampyroteuthis in situ* with food in and around its mouth, the food clump typically appeared as an amorphous mass of small particles wrapped in mucus. Food material in the crops was similar, and was in some cases clearly identifiable as sinkers. The nutritional value of these large particles is surprisingly high, with an average organic carbon content of 5.4 mg [28]. The sources of this carbon are the diatoms, crustaceans, faecal pellets and gelata that become trapped in the feeding filters while the larvaceans consume smaller suspended particles [34,35]. Various crustacean zooplankters feed on aggregates [35] and by feeding on aggregates, vampire squid also ingest the copepods, ostracods and amphipods that inhabit the aggregates, which may explain the source of complete crustaceans in the digestive systems. Utilization of marine aggregates is consistent with the low stable isotope signature measured in *Vampyroteuthis*' beaks [36], and also with the relatively weak musculature associated with *Vampyroteuthis*' beak and radula [10].

At depth, individual *Vampyroteuthis* were frequently encountered motionless except for gentle fin undulations, with one of their retractile filaments extended. Occasionally, small particles or larger aggregates were visible on the filaments, on the arms or in the mouth. During laboratory feeding experiments, we repeatedly observed particle-laden filaments being drawn between the arms in a behaviour that removed the particles and deposited them near the mouth in a mucus matrix. Both lines of evidence strongly suggest that one of the functions of the retractile filament is food collection. The short, stiff hairs on the filament probably function to secure particles, while the abundant sensory cells may signal the presence of food to the brain. The food is transferred to the arms, wrapped in mucus secreted from glands in the non-grasping suckers, and the cirri move the resulting mass along the arms to the buccal area for ingestion. The large crop allows for relatively large volumes of

dilute food to be taken in. The use of the filament in food collection allows not only the capture of larger aggregates, but also the capture of very small particles, which are wiped off by the arms and wrapped in mucus for transport to the mouth.

Previous authors have suggested that the retractile filaments allow the detection of living prey and predators. In aquarium experiments, Hunt [4] presented live *Artemia* nauplii to *Vampyroteuthis* with extended filaments. When the nauplii contacted the filament, the vampire squid swam around the location where the nauplii touched the filament and enveloped them within its webbed arms. These observations suggest a tactile sensory function for the filaments that is in keeping with our histological results showing abundant sensory like cells, but seemingly contradicts our observations that vampire squid feed mostly on detrital matter. The filament, however, is likely a multi-functional organ that is deployed to detect and capture detrital matter but at the same time may detect the presence of predators and perhaps small living prey. Detected food items that are too big to attach to the filament may be captured by engulfment with the web.

Although the feeding strategy of vampire squid is unlike any other cephalopod, aspects of it may be found in other related and unrelated organisms in the same environment. Meso- and bathypelagic cydippid ctenophores deploy long sticky filamentous tentacles for the acquisition of food [37] and coronate scyphozoans of the genus *Atolla* have a hypertrophied tentacle with a suggested role in feeding [38]. *Vampyroteuthis*' feeding strategy resembles that of munnopsid isopods which share the deep water column of Monterey Canyon. Munnopsids collect marine snow particles and aggregates with hair-like projections on their extremely long legs and antenna, then remove the food by drawing the appendages through their mouth parts [39]. Like *Vampyroteuthis*, the pelagic, cirrate octopus *Stauroteuthis* has questionable grasping capacity in its suckers. They are known to have a photophore at the base of each sucker, perhaps to lure copepods into a mucus web in between the eight arms [40]. The mucus in *Stauroteuthis* is produced by the enlarged posterior salivary glands [3], which are reduced in *Vampyroteuthis*.

A large percentage of marine snow particles, aggregates and sinkers are infused with bioluminescent organisms (e.g. radiolarians, small copepods, dinoflagellates, bacteria in faecal pellets) [41] and are capable of glowing during their descent to mesopelagic depths. So while *Stauroteuthis* may attract copepods with its own bioluminescence, vampire squid may benefit from the bacterial light that marine aggregates emit. First, *Vampyroteuthis* has large, highly developed eyes that are acutely sensitive to very dim light [42], and thus they can probably see the larger particles and aggregates as they transit the water column. Second, as many crustaceans are attracted to bioluminescent food sources [43], their presence enriches the aggregates on which they feed [35], thereby enriching the food of the vampire squid.

Vampyroteuthis has the lowest metabolic rate recorded for any cephalopod, comparable to that of a scyphomedusa of the same size [26,44], but this characteristic alone is insufficient for survival in the OMZ [20]. Permanent residents of the OMZ have a variety of respiratory and morphological adaptations [25]. For *Vampyroteuthis*, these

include the typical respiratory protein, haemocyanin, but with a heightened affinity for oxygen [16]. Like many other cephalopods, vampire squid are virtually neutrally buoyant, which is valuable for a sedentary species in the OMZ because it reduces the physiological costs of locomotion. Reduced musculature and relatively limited locomotory capabilities are further adaptations that allow *Vampyroteuthis* to succeed in the OMZ. Seibel *et al.* [45] estimate that only short duration burst swimming is available or necessary for predator avoidance, because cryptic coloration and bioluminescent countermeasures [15] probably contribute more to survival.

The primitive configuration of coleoid cephalopods is with 10 equal arms. Modern decapods with 10 unequal arms can be logically traced back to the earlier form. Octopods, with eight arms, have apparently lost one pair [46]. The broader filaments in hatchlings of *Vampyroteuthis* are more arm-like than in older individuals, which supports the interpretation that the filaments are the second pair of arms [47]. The central nerve of the filaments runs to the unusually large ventral magnocellular lobe, which is also found in *Mastigoteuthis* and *Joubiniteuthis*, squid with huge numbers of small suckers on their tentacles and/or arms. The size of their magnocellular lobes is probably related to the large amount of sensory information that is sent from the suckers to the brain in these squid. Although the function of this lobe is poorly known, it appears that the vampire squid's brain is receiving a great deal of information from the filaments (R. E. Young 2012, personal communication). We have shown that one of the functions of the filaments is food acquisition and handling, analogous to the arms and feeding tentacles of squid. Although the asymmetrical nature of the filaments, with muscle cells on the one side and sensory cells on the other, could be related to the coiling of the filament, the asymmetry corresponds with the morphology of arms and tentacles. Our findings of both function and form further support the hypothesis that the retractile filaments in *Vampyroteuthis* are actually modified arms.

Based on extensive *in situ* observations over 20 years, laboratory studies of live specimens and analyses of preserved material, it appears that *Vampyroteuthis* off central and southern California and on both sides of the Baja California peninsula is primarily a detritivore, that takes advantage of the OMZ in these regions. Predation pressure is probably less than in the surrounding mesopelagic layers because of the respiratory constraints imposed by diminished oxygen levels [48]. A significant component of *Vampyroteuthis*' diet, large, nutrient-rich detrital aggregates, are very abundant within this depth range [28,33]. These factors allow the vampire squid to assume a relatively passive lifestyle, under a reduced selective pressure to invest in muscular tissue, resulting in extremely low metabolic rates. The combination of its unique locomotory, physiological and feeding adaptations allows *Vampyroteuthis* to permanently inhabit and be very successful in the centre of the OMZ, an otherwise hostile environment where predators are few and a particular type of food is abundant.

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