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The First In Situ Observation of the Ram's Horn Squid *Spirula spirula* Turns "Common Knowledge" Upside Down

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The ram's horn squid *Spirula spirula* (Linnaeus, 1758) is the only extant cephalopod with an internal calcareous, chambered shell that is coiled, making it the sole living representative of the once speciose order Spirulida [1]. As also supposed for its Cenozoic and Cretaceous ancestors [2], the function of the septate, many-chambered shell of *Spirula* has been considered as primarily for buoyancy. Behavioral observations of this species have been confined to those made in aquaria involving freshly net-caught specimens [3]. Invariably, during those aquaria observations, the posterior end containing the open planispiral shell pointed towards the top of the tank, while the upward-oriented terminal fins moved with a rapid "waving or fluttering motion", presumably attempting to keep the animal submerged [4]. A large photophore is present between the two fins on the posterior end of the body, and this has been observed to emit a "pale, yellowish-green light" that can glow "uninterruptedly for hours" [3]. We report here the first in situ observations of *S. spirula* in its natural habitat, illustrating the importance of such observations for a correct understanding of the ecology of deep-water organisms.

A single individual of *S. spirula* was observed between 837–860 m depth on 27 October 2020 during dive 402 of the remotely-operated vehicle (ROV) SuBastian (R/V Falkor Voyage FK200930, Leg 2, Schmidt Ocean Institute). The squid was first observed at 01:34:01 UTC (11:34:01 local time; 12.12960371° S, 143.97562933° E, 837 m), at a height of ~1100 m above the seafloor with a depth of 1941 m. The observed location lies generally within the entrance to Wreck Bay on the continental slope of the far northern Great Barrier Reef, Queensland Australia. Environmental parameters were recorded using a Sea-Bird SBE 49 FastCAT conductivity-temperature-depth meter (CTD) attached to the ROV and were as follows: Temperature 5.33 °C, salinity 34.44 and dissolved oxygen 4.1 ml/L (184.8 μM).

The squid appears on video for a total of 4 min 57 s (Appendix A) and, when first encountered (i.e., as far away as can be seen in the video recording) and throughout most of the subsequent observations, it was oriented nearly vertically with its head upward (Figure 1).

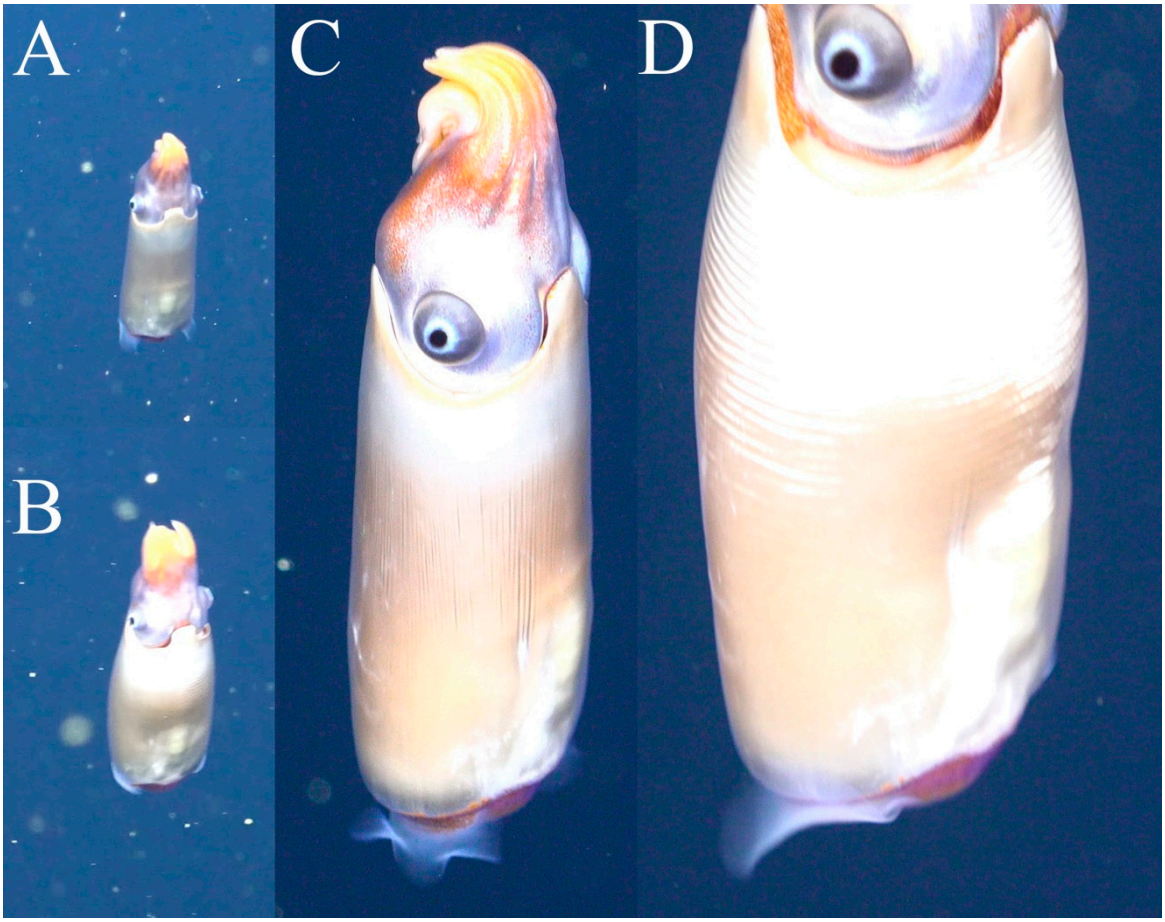

Figure 1. Framegrabs from the 4K video record of *Spirula spirula* observed in situ: (**A**) resting position with no part of the interior shell visible external to the mantle; (**B**) inflated mantle prior to jetting escape showing the lack of mantle inflation in the area of the internal shell; (**C**) resting position with curled arms; and (**D**) hyperinflated mantle at start of jetting escape.

This was surprising because the assumption for *Spirula* had been a head-down orientation (or horizontal orientation) given the position of its internal shell, with gas-filled chambers, near the posterior end of the mantle, especially in light of previous shipboard observations in aquaria [3]. However, the presence of the large photophore on the tip of the mantle apex, between the fins, and the propensity for midwater squid to aim bioluminescent light downward in order to achieve counterillumination, both suggest that a head-up orientation (with the photophore directed downward) should not be surprising. Counterillumination is used by squid to disrupt silhouettes against downwelling light from above. As observed in the video, *Spirula* was capable of remaining virtually motionless with the exception of actively beating fins. It is reasonable to suggest that such rapid fin motion would be the easiest aspect of *Spirula* to be seen by potential predators from below. The single photophore may therefore act to break up the solid shape of the squid while masking the motion of the beating fins. Although the pale, yellowish-green color reported for the emitted light [3] is not usual for counterillumination, some squid have been reported to change the color of their bioluminescence depending on the ambient temperature, with colder temperatures correlated with blue light and warmer with green [5]. Because *Spirula* is known to undertake diel vertical migration from a daytime depth of 550–1000 m to a nighttime depth of 100–300 m [6], and because it is also known to occur mostly in tropical waters with high surface water temperatures [4], it would not be surprising if the previously reported color of bioluminescence [3] was due to the water in the shipboard aquaria

being somewhat warm and that its bioluminescence at daytime depths may be bluer. Unfortunately, the present observation was unable to record the color of any light emitted by the animal.

At one point in the video, the beating fins of *Spirula* could be observed clearly [7]. Although small, they seem to aid in keeping the orientation and position of the squid nearly still. Waves along each fin travelled in opposite directions to each other. The behavior of the fins and their use in attitudinal control of the squid has been observed in histioteuthid squids [8], though the orientation of the histioteuthids was head down. In both histioteuthids and the present observations of *Spirula*, the relatively small rounded fins enable the squids to remain still and make very slow turns when needed.

These observations are reminiscent of advances in our understanding of functional morphology of mastigoteuthid squids. Based on anatomy and shipboard observations they were predicted to assume an arms-up posture [9]. However, when actually seen in situ they are almost always in the opposite, arms-down, posture and maintain that by unusual active fin motions [10].

The *Spirula* attempted to jet escape five times during the encounter. A partially gas-filled shell would seem to be a hinderance to rapid movement such as jetting, particularly when changing depth (and pressure). The shell of *Spirula* is thin and calcareous, and it is therefore reasonable to imagine that it could be damaged by vigorous mantle contractions or rapid changes in depth/pressure. Apparently, when rapid escape is needed, jetting is fairly vigorous, though throughout the encounter it appeared that the squid tired. Body angle during escape was never more than ca. 30° from the vertical. The first time it jetted, it dove about 5 m. The second time was more vigorous, and it dove about 10 m, after which time it remained still for nearly 30 s. It dove again about 5 m the third time, and the fourth diving attempt was weak and the squid did not move far (1 m). During this fourth and weakest attempt at diving, the *Spirula* also changed trajectory twice (the only time it was observed to do so). It appeared to be tiring out. It remained stationary (again with only the fins beating) for over 2 min before it (presumably) gained enough energy to jet away one last time, inking as it did so. The ink was more cohesive than observed histioteuthid inks although it did not appear as a pseudomorph. Although it was not possible to measure the squid using the ROV footage, this specimen was likely around 4 cm, a typical length for *Spirula*. Traveling 27 m is the equivalent of 675 body lengths, substantial for such a small animal.

When *Spirula* was not jetting, the funnel could not be observed to move with normal exhalation, as is the usual case with squids. It was difficult to observe water entering the mantle collar or being exhaled through the funnel. The eyes, though protruding and prominent, were never observed to move independently of the body as they are observed to do in other midwater squids. The general impression left from this observation is that stillness is the primary defense of *Spirula*. Unlike in many photographs and drawings of *Spirula* captured in trawl nets, the shell is completely internal, not protruding above the mantle musculature or skin either dorsally or ventrally.

A paralepid barracudina, a group known to prey on squid, was observed at the end of the *Spirula* observation.

Although live observations of *Spirula* are very rare, there have been important advances in our understanding of its shell structure and function [11,12], life history and biogeography [13,14] and phylogeny and systematics [15–17]. Still, live observations offer researchers a unique opportunity to directly examine the behavioral responses of organisms about which behavior otherwise has to be merely surmised.

There is a single undocumented report in the literature [18], described as a personal communication from Dr. M. R. Clarke, that "one of the 'Discovery' specimens, which was in good condition, swam head upwards for about an hour before returning to its usual posture." Even while escaping, the *Spirula* in the present observation was never more than 30° from the vertical and the head was always upwards. The buoyant shell being situated below the heavier head and arms would presumably reduce stability in the animal, but would allow for more rapid changes in direction during escape, similar to the relaxed stability designs of many combat aircraft. The righting moment would presumably become too great

to overcome if the *Spirula*'s orientation became too close to horizontal, explaining why in captured, injured animals the head usually points down with the buoyant shell-containing posterior end up.

This 5 min video sequence represents the only known in situ observation of the enigmatic ram's-horn squid, *Spirula spirula*. It demonstrates the valuable insights that can be gained by serendipitous live observations while exploring relatively remote areas of the ocean. These particular observations further have implications for paleobiology because *Spirula*, like nautilids, is considered a living model for the function of the coiled phragmacone (in this case internal) in numerous fossil species. In order to better understand the behavior and ecology of midwater and deep-sea animals, we must continue to explore the vast expanses of our deep oceans.

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Appendix A

The squid appears on video at 01:33:22 UTC in the distance; vehicle depth is 834 m. As the vehicle approaches the squid is observe oriented head up (mantle down) at 01:33:55 UTC and has not noticeably moved from that orientation. At 01:33:59 UTC, the squid hyperinflates the mantle and jets downward (appears to be angling a few degrees from vertical moving away from the vehicle); vehicle depth is 838 m. It travels about three body lengths in one second and swims off the bottom of the camera screen. The ROV dives and catches up to the squid (which is again still and in the same head up position) at 01:34:10 UTC; vehicle depth 842 m.

At 01:34:22 UTC (843 m) the ROV trims out and the camera zooms in to get the best shot yet of the encounter. The squid's orientation is nearly vertical, still angled slightly away from the ROV. The ventral side is facing the camera, the tips of the arms are curled dorsally, both eyes and fins are visible as is a bright reflection of the internal planispiraled shell along the ventral midline near the end of the mantle. The fins were beating about 2.5 times per second. The squid once again hyperinflates the mantle and jets away at 01:34:27 UTC, once again disappearing the bottom of the camera screen in less than one second (it moves between 2 and 3 body lengths before going offscreen).

Once again, the ROV dives and comes upon the squid in the same orientation at 848 m (01:34:40 UTC), the squid continues diving this time disappearing once again off camera. The ROV catches up at 01:34:51 UTC (853 m). Another close-up video begins and shows the beating fins about 2.5 times per second again. At 01:35:13 UTC, another hyperinflation and rapid jet downward. The ROV catches up with the squid at 01:35:24 UTC (858 m). It is again still and remains oriented head up.

The next hyperinflation and jet occurs at 01:35:43 UTC (859 m) but there is a slight change of the angle first one way and then another. It did not move very far this time and the ROV finds it again at 01:35:52 UTC (860 m). The video comes into sharp focus for another good view at 01:36:10 UTC (861 m). The frequency of the fin beating has increased (to about 3 per second). The arms are again curled dorsally (as they have been for most of the encounter).

At 01:36:31 UTC (861 m) we get the best shot of the fins. They are small compared to the body size and the waves along the edge of the fins are running opposite each other in such a way as to keep the squid stable (fixed in attitude and orientation). The action of the fins is reminiscent of how histioteuthid squids use their fins to slowly rotate and control attitude in the water (however, histioteuthids are usually head down when such fin motions occur).

Best video at 01:37:31 UTC (861 m). Camera is zoomed in, fin beats are slowed a little to 2.5 times per second. It appears arm pair 1 (the dorsal most arms) are curled more than the other three pairs of arms (2–4) with no clear view of the tentacles. The squid is oriented vertically and turned slightly so the right side is facing more towards the camera. The right eye is protruding and does not appear to move independently of the body throughout. There appears to be one band of chromatophore coloring the mid-section of the mantle, with paler coloration on the anterior and posterior mantle. Chromatophore darkening is also observed over much of the arms and dorsal side of the head. The siphon is never observed to move, so it is difficult to gauge respiratory cycles.

At 01:38:19 UTC (861 m) the squid hyperinflates and disappears off the bottom of the camera. This jetting is close up and as the mantle collar expands, there is a bright orange layer showing just on the inside of the mantle collar. It is unclear if this is real or an artifact of the ROV lighting. An ink appears onscreen at 01:38:29 UTC (861 m) at the approximate location where the squid jetted away and is presumably from the *Spirula*. The ink was more cohesive than observed histioteuthid inks although it did not appear as a pseudomorph (it may have been disturbed by the ROV as well).

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