

Supplemental information

Vampire squid reproductive strategy is unique among coleoid cephalopods

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Supplemental Experimental Procedures

Ovaries were dissected from 43 vampire squid accessioned in the Santa Barbara Museum of Natural History. These specimens were collected between 1962 and 1972 off southern California at depths ranging from 677-3292 m. The wet weight of specimens was determined to the nearest 1 g and the mantle length was measured to the nearest mm. Specimens were assigned adult when ripe eggs or postovulatory follicles were present in the ovary (n=27), or subadult when ripe eggs or POFs were absent (n=15). For one specimen it was unclear whether it was subadult or adult. The four specimens which had a wide size range of oocytes in addition to a batch of oocytes included one adult, two subadults and the specimen for which the maturity stage was unclear. To determine the fecundity, ovulation and spawning patterns of individual vampire squid, the oocytes and postovulatory follicles in the ovary were measured and counted. This was done by weighing the ovaries to the nearest 0.0001 g. Then all large eggs (>2 mm) were counted and measured to the nearest 0.1 mm. The remaining smaller oocytes were re-weighed, and a subsample of approximately 15-20% of the total volume was taken for further analysis. In this subsample all oocytes and postovulatory follicles were counted and a random sample of 100-200 follicular sheaths of normal oocytes, atretic oocytes and postovulatory follicles were measured. The total number of smaller oocytes and follicles was divided by the weight of the subsample, and multiplied by the ovary weight (excl. eggs > 2mm) to obtain the total number of smaller

oocytes and follicles in the ovary. Potential fecundity was the sum of the developing and atretic oocytes, ripe eggs and postovulatory follicles. Oocytes were called atretic when 1) envelopes were shrinking, 2) the cytoplasm was bubbly and separated from cytoplasmatic membrane, and 3) when the nucleus was disintegrating. In four of the 43 specimens the number of post ovulatory follicles could not be established unambiguously because of intensive oocyte atresia (degeneration).