Correspondence

Vampire squid reproductive strategy is unique among coleoid cephalopods

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Coleoid cephalopods are thought to go through only one reproductive cycle in their life. We here report that vampire squid (*Vampyroteuthis infernalis*) show evidence of multiple reproductive cycles. Female vampire squid spawn their eggs, then return to a resting reproductive state, which is followed by the development of a new batch of eggs. This reproductive cycle is likely to be repeated more than twenty times. This combination of reproductive traits is different from that of any other extant coleoid cephalopod.

Extant coleoid cephalopods (all living cephalopods, except the chambered nautilus) are semelparous — an individual undergoes only a single reproductive cycle after which it dies. This reproductive strategy is different from iteroparity, whereby an individual has multiple reproductive cycles [1]. Although some cephalopod species may grow between spawning events or spawn multiple egg batches [2], extant coleoid cephalopods do not have a gonadal resting phase. In iteroparous fishes, the gonadal resting phase includes the period when spawned adults have no growing oocytes or ripe eggs in the ovary [3]. The notion that all coleoid cephalopods are semelparous was recently challenged by observations of the first known carcass of a mature female of the oceanic squid *Kondakovia longimana* [4]; however, conclusive evidence of iteroparity in a coleoid cephalopod had been absent to date.

We investigated the reproductive strategy of the vampire squid (Figure 1). This coleoid cephalopod occurs at open ocean depths between 500 and 3000 m and is able to inhabit low oxygen environments [5]. Vampire squid feed opportunistically on zooplankton and detritus [6]. Young [7] observed that in adult females, developing eggs were present in various sizes (up to 3 mm), "indicating a lack of a development cycle and a lack of a restricted breeding period", but the exact reproductive strategy of vampire squid remained unknown. We quantified reproductive parameters in 43 female specimens of *Vampyroteuthis* collected in net tows off southern California (Supplemental information). Subadult specimens (mantle lengths 65–98 mm; n = 15) had only undeveloped oocytes in the gonad (Figure 1). In adult specimens (mantle lengths 82–132 mm; n = 27), 108–3,827 post-ovulatory follicles were present as evidence of previous spawning. One batch of 9–140 oocytes (2.2–4.5 mm) was observed within the ovaries of each of five adult specimens, four of which had ripe, spherical eggs (~4 mm) (Figure 1). In another adult specimen, the batch (1.7–2 mm) was degenerating. In four specimens, oocytes of a wide size range were present in addition to the batch. The fate of these intermediate-sized oocytes is unclear. In 20 adult specimens, the gonad was in a resting state, i.e. there was no batch of ripe or developing eggs. The length frequency distribution of oocytes in the gonads of these resting individuals resembled that of subadult specimens (Figure 1). The mean relative ovary weight (in % body mass) in our specimens was low; 0.19 ± 0.12% (range 0.07–0.51%) for subadult individuals; 0.21 ± 0.06% (range 0.09–0.32%) for resting adult vampire squid. Ovaries with ripe eggs weighed 0.20–0.97% of body mass.

Because post-ovulatory follicles are resorbed slowly, the spawning history of most of our adult specimens could be reconstructed. The most advanced female (mantle length 102 mm, body mass 448 g) had released at least 3,800 eggs, yet still retained ~6,500 viable oocytes for future spawning, but the gonad was in a resting state. Assuming an average batch size of 100 eggs suggests that this female had spawned at least 38 times and had oocytes for another ~65 spawning episodes. The potential fecundity (Supplemental information) of all specimens varied from 5710 to 20,711 (n = 41).

The reproductive strategy of *Vampyroteuthis* is unlike that of any other extant coleoid cephalopod. Females invest relatively little energy into gonad development, ovulation is discontinuous and eggs are spawned in multiple events. Between spawning events, the gonad returns to a resting condition. The length of this resting period in vampire squid is unknown but it is not until a batch of eggs has been spawned, and post-ovulatory follicles degenerate to a quarter of their initial size, that a new batch of oocytes starts to develop. Given that vampire squid have low metabolic rates and ingest low calorie food [5,6], the time to accumulate enough resources for the next spawning episode may be substantial.

In the epipelagic octopods *Argonauta* and *Tremoctopus*, which live at >20°C and have continuous ovulation, it takes 3 to 5 days for an egg batch to ripen [8]. Oogenesis in vampire squid is likely to be much slower at the low temperatures (~2–7°C) of the meso- and bathypelagic zone they inhabit. Our data were not suitable to analyze seasonality, but the time between reproductive events, i.e. the resting period plus the duration of oogenesis, may be at least one month. If we assume 38–100 spawning events (as in our most advanced female), the duration of the adult stage is at least 3–8 years in this specimen, with the total life-span exceeding these numbers. These life-span estimates agree with recent observations on deep-sea octopods, which have the longest brooding time known for any animal (53 months) [9]. Deep-sea octopods and vampire squid may therefore have record life-spans among extant coleoid cephalopods.

The selective pressures that have resulted in iteroparity in *Vampyroteuthis* remain unclear. The metabolic and trophic characteristics of the species suggest a slow pace of life [5,6], and they may not be able to take in enough energy to support the high, short-term fecundity characteristic of semelparity. We hypothesize that only iteroparity can yield a sufficiently high lifetime reproductive output for this species. Life history theory predicts that iteroparity is favoured when adult survival is increased [10]. With the ability to inhabit low oxygen environments, where predators are fewer [6], vampire squid may benefit from increased adult survival rates, allowing for multiple reproductive cycles at low reproductive cost.

SUPPLEMENTAL INFORMATION

Supplemental Information including experimental procedures can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.02.018.

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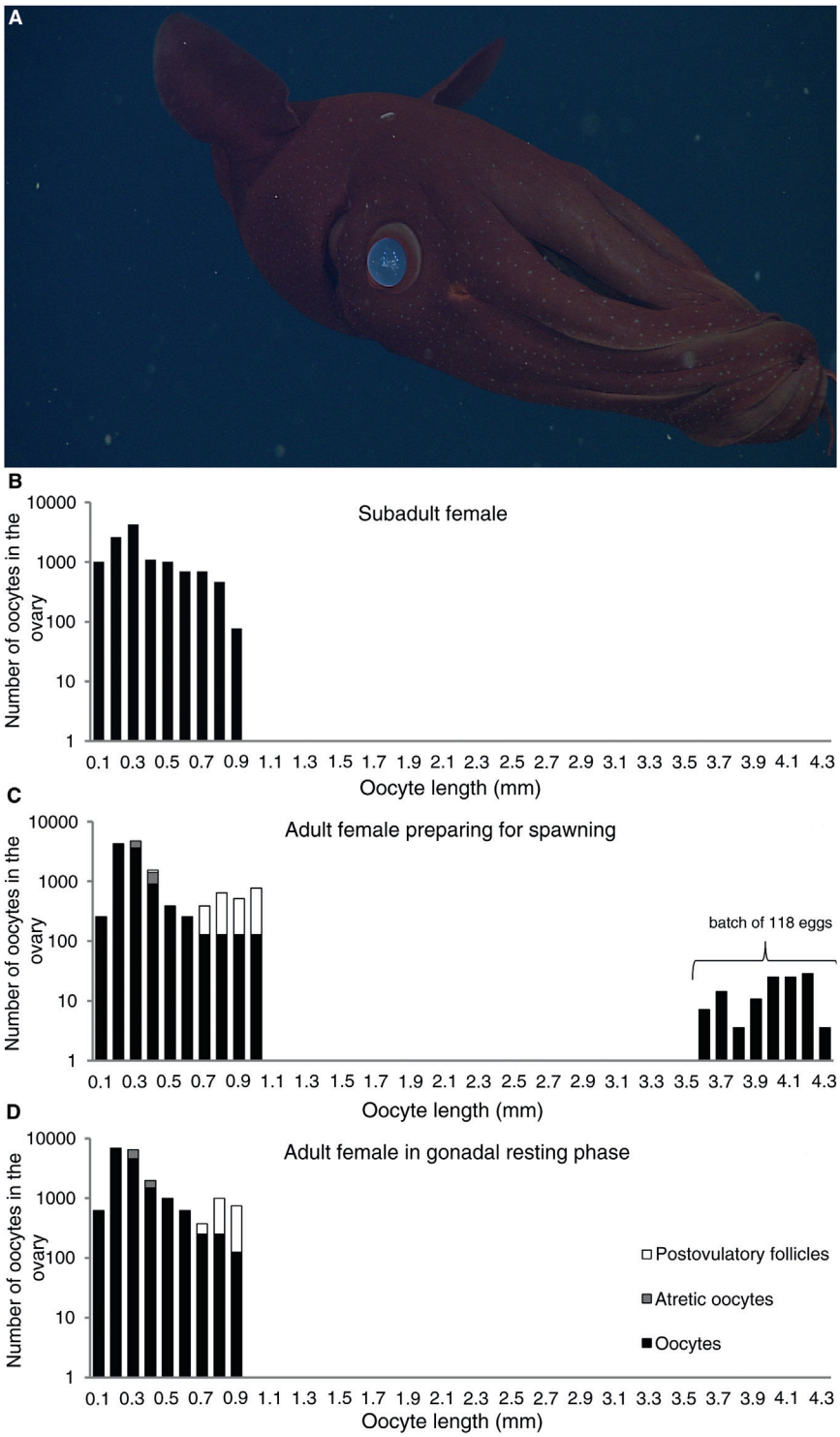

Figure 1. Evidence for multiple reproductive cycles in vampire squid.
(A) A female vampire squid *Vampyroteuthis infernalis*; image © 2013 MBARI. (B) Length frequency distributions of oocytes, postovulatory follicles and ova in a subadult female (mantle length 98 mm), showing one large stock of undeveloped oocytes; (C) *idem*, but in an adult female (mantle length 90 mm) preparing for spawning, with undeveloped oocytes and ripe eggs in the ovary; (D) *idem*, but in an adult resting female (mantle length 115 mm), with undeveloped oocytes and postovulatory follicles evidencing a previous spawning event. Black bars: viable oocytes and ripe eggs; white bars: postovulatory follicles; grey bars: atretic oocytes.

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