

## RESEARCH NOTES

mortality in cases where slugs are swallowed whole may be ameliorated in nature if these predators are less important than crabs and fish, or if the nudibranchs are less preferred than other available prey.

The director and staff of the Bamfield Marine Station provided facilities and aid; S. Servant, J. Sunday and T. Stubbs aided with collections; L. Auffrey helped maintain predators; D.J. Arsenault provided statistical and logistical help; and A.R. Palmer provided editorial and financial support (NSERC 06395). To all I am grateful.

### REFERENCES

- GOSLINER, T.M. & BEHRENS, D.W. 1990. In: *Adaptive coloration in invertebrates* (M. Wicksten, ed.), 127–138. Texas A&M University Sea Grant College Program, College Station, Texas.
- ROSENBERG, G. 1989. *Evolution*, **43**: 1811–1813.
- THOMPSON, T.E. 1976. *Biology of opisthobranch molluscs*. Ray Society, London.
- SERVEDIO, M.R. 2000. *Evolution*, **54**: 751–763.
- FAULKNER, D.J. & GHISELIN, M.T. 1983. *Mar. Ecol. Prog. Ser.*, **13**: 295–301.
- JOHANNES, R.E. 1963. *Veliger*, **5**: 104–105.
- AVILA, C. 1995. *Oceanogr. Mar. Biol. Annu. Rev.*, **33**: 487–559.
- TULLROT, A. 1994. *Evolution*, **48**: 925–928.
- TULLROT, A. & SUNDBERG, P. 1991. *Anim. Behav.*, **41**: 175–176.
- DUFFY, J.E. & HAY, M.E. 2001. In: *Marine community ecology* (M.D. Bertness, S.D. Gaines & M.E. Hay, eds), 131–157. Sinauer Associates, Sunderland, Mass.
- EDMUNDS, M. 1974. *Defence in animals: a survey of anti-predator defences*. Longman Group, Harlow, Essex.
- ZAR, J.H. 1984. *Biostatistical analysis*. Prentice-Hall, Englewood Cliffs, New Jersey.
- PENNEY, B.K. 2002. PhD thesis, University of Alberta, Edmonton, Canada.
- HELLOU, J., ANDERSEN, R.J. & THOMPSON, J.E. 1982. *Tetrahedron*, **38**: 1875–1879.
- THOMPSON, J.E., WALKER, R.P., WRATTEN, S.J. & FAULKNER, D.J. 1982. *Tetrahedron*, **38**: 1865–1873.
- STACHOWICZ, J.J. 2001. In: *Marine chemical ecology* (J.B. McClintock & B.J. Baker, eds), 157–194. CRC Press, New York.
- BELOVSKY, G.E. & SCHMITZ, O.J. 1994. *J. Mammal.*, **75**: 816–832.
- PENNEY, B.K. 2002. *Oecologia*, **132**: 411–418.

*J. Moll. Stud.* (2004) **70**: 406–408

*Journal of Molluscan Studies* Vol. 70, No. 4 © The Malacological Society of London 2004, all rights reserved.

## Hatchlings of the deep-sea octopus *Graneledone boreopacifica* are the largest and most advanced known

Janet R. Voight<sup>1</sup> and Jeffrey C. Drazen<sup>2</sup>

<sup>1</sup>Department of Zoology, The Field Museum, 1400 S. Lake Shore Dr., Chicago, IL 60605, USA;

<sup>2</sup>Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039-9644, USA

Although parts of at least four deep-sea octopus egg clutches have been collected in the last 120 years,<sup>1–3</sup> free-living hatchlings have remained unknown. The remotely operated vehicle (ROV) *Tiburon* documented the hatching of an egg clutch brooded by a female of *Graneledone boreopacifica* Nesis, 1982 at 1,600 m depth on the Gorda Escarpment and collected 28 hatchlings. The 55-mm long hatchlings are the largest and most developmentally advanced octopus hatchlings known, although their external yolk sacs indicate that hatching was premature. The hatchlings carry up to 57 suckers per arm and the hectocotylus of male hatchlings carries the full adult complement of 42 or 43 suckers. The internal yolk sac displaces the small digestive gland to the ventral, posterior mantle cavity, as is typical of embryos of *Octopus vulgaris*.

All observations and collections reported were made with the ROV *Tiburon* on 22 July 2002 as it crossed the north-facing 1570–1600 m deep slope of the Gorda Escarpment.<sup>4</sup> The octopuses observed were identified as *Graneledone boreopacifica* based on their single, or zigzagged, suckers, clear supra-ocular papillae and the skin texture which corresponded to the faint papillae characteristic of North Pacific specimens of this genus from shallow in their depth range.<sup>5</sup>

A female adjacent to a rock outcrop in the brooding position<sup>3</sup> was collected and her exposed egg clutch briefly videotaped. As suction sampling began, mass hatching ensued (Fig. 1). After the ROV was recovered, a brief series of standard measurements was made on the moribund hatchlings (Fig. 2). The specimens,

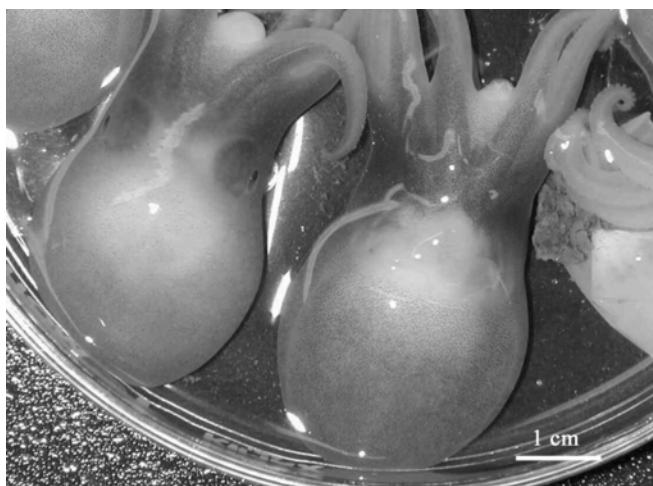
including the brooding female, an unhatched egg and egg cases were preserved for deposition at The Field Museum (Chicago, IL; FMNH 302123; FMNH 302124; FMNH 302125; FMNH 302302).

In the laboratory, the measurements were replicated after 9 months in alcohol storage. All arm suckers on one side of 17 individuals were counted under a dissecting microscope. The



**Figure 1.** A hatchling of *Graneledone boreopacifica* swims away as the suction sampler of the ROV *Tiburon* pulls at the egg clutch. Note the external yolk sac within the arm crown of the hatchling.

Correspondence: J.R. Voight; email: jvoight@fieldmuseum.org



**Figure 2.** Hatchlings, after their recovery from the ROV, in a standard Petri dish. Note the well-defined chromatophore organs are more abundant ventrally (the hatchling to the right) than dorsally, hatchling to the left. Also note the external yolk sacs extending beyond the deeply pigmented inter-brachial web.

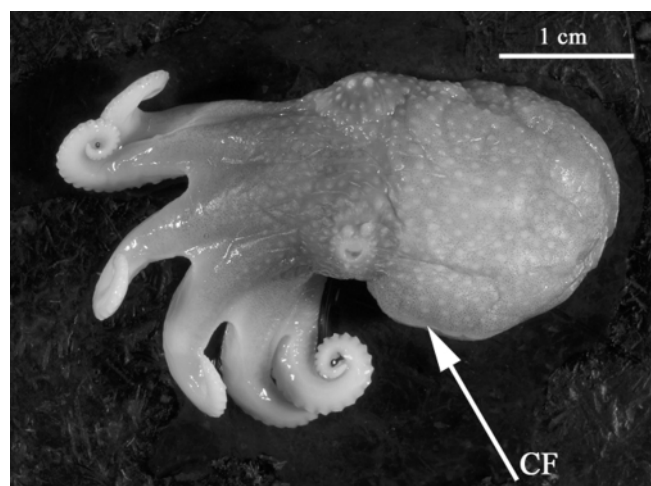
distribution of suckers among the arm pairs was compared using a G-test. The number of suckers on the putative hectocotylus of hatchlings was compared with that of 19 males of *Graneledone* collected from comparable depths in the N Pacific Ocean (J.R. Voight, unpublished). The ventral mantle of seventeen hatchlings was opened to reveal genital pores and gill lamellae. Two genital pores are present in females; one is present in males. The mantles of two specimens were bisected to determine the positions of the internal yolk sac and the digestive gland. The hood and crest lengths of the lower beaks of these individuals were measured.

To assess whether the large egg size reflects expansion of the chorion during development, the logarithmic-transformed lengths of early-stage eggs were plotted *vs* the log-transformed lengths of late-stage eggs using data from five shallow-water octopus species.<sup>6,7</sup>

During an 8-h dive at Gorda Escarpment, 63 adult octopuses, most apparently brooding eggs, were observed. The female collected showed only faint signs of senescence, e.g. cloudy eyes, wrinkled skin.<sup>3</sup> at least seven hatchlings swam away when she was collected; tens more hatched when sampling of the egg clutch began. The hatchlings had conspicuous whitish yolk sacs inside their webs (Fig. 1) and behaved as do benthic shallow-water octopus hatchlings that hatch prematurely (J.R. Voight, personal observation).

Clearly defined, apparently normal chromatophore organs were obvious on the fresh hatchlings (Fig. 2); they occurred both superficially and on the underlying dense musculature of the arms, mantle and head. No chromatophore organs were noticeably enlarged or differently coloured. Chromatophore organs were most abundant on the arms and web over the yolk sac; their abundance declined on the ventral mantle and became least dense on the dorsal mantle (Fig. 2). Chromatophore organs encircled the skin tubercles but appeared to be nearly absent from the circum-mantle skin fold. They were uniformly brick red when fresh; in preservation, the depth of colour differed. The papillated skin of the dorsal mantle was separated from the smooth ventral mantle skin by a circum-mantle skin fold (Fig. 3).

When fresh, the hatchlings' median mantle length was 28 mm (range 23–30); external yolk sacs were up to 11 mm long. Alcohol storage caused the specimens to shrink. After 9 months, median mantle length had shrunk to 18.3 mm; median mantle



**Figure 3.** Ethanol-preserved hatchling (FMNH 302125) illustrating change in morphology compared with fresh specimens in Figure 2 and the pronounced skin texture. The circum-mantle fold (CF) is posterior and ventral to the animal's left eye.

width shrank from the fresh value of 25 to 14.8 mm; head width from 16 to 12.6 mm and arm length from 26 to 22.8 mm. The percentage reduction from the fresh measurement ranged from 12 to over 40%.

Each normal arm of 17 hatchlings had from 43 to 57 suckers regardless of sex (Table 1). The third right arms of two hatchlings carried 42 and 43 suckers in contrast with the 49 suckers on their left third arms. The median number of suckers on the hectocotylus of 19 congeneric adult males from comparable depths in the N Pacific Ocean is 43 (J.R. Voight, unpublished). Only the left genital orifice was present in these specimens, confirming that they are male. Two genital orifices were discernible in females, and the small, but distinct reproductive organs also discriminated between the sexes.

Eight gill lamellae, including the minute terminal lamella, was the most common count, although three females had seven gill lamellae. The internal yolk sac covered the dorsal viscera. In one comparatively advanced specimen, the oesophageal dilation appeared to contain yolk; in another less mature embryo, the oesophagus was empty and buried deep in the internal yolk sac. In bisected mantles, the white and spongy digestive gland was evident ventral and posterior to the viscera, overlain by the renal organs.

The beaks were similar to those previously described,<sup>2</sup> but the lower beak was truly bifid. The two well-defined and dark-coloured points of the lower beak extended beyond the buccal mass to the first sucker of the dorsal arms. Denticles were present on the beak as were pronounced increments on the lower beak's lingual surface. Both beaks had lower crest lengths of 4 mm and lower hood lengths of 1.0 mm.

**Table 1.** Reported are the mean and ranges of normal arm sucker counts by arm for 17 hatchlings by sex.

	Arm 1	Arm 2	Arm 3	Arm 4
Mean females ( $n = 10$ )	52.8	53.6	51.3	49.6
Range	51–55	50–57	49–53	47–54
Mean males ( $n = 7$ )	51.4	52.1	49.7	48
Range	47–55	48–56	46–52	45–51

The second arm pair is significantly most likely to carry the greatest number of suckers ( $G = 14.64$ ,  $P < 0.005$ ); the fourth arm pair is most likely to carry the fewest suckers ( $G = 33.67$ ,  $P < 0.005$ ).

The intact egg, faintly translucent in preservation, measured  $39.6 \times 15.3$  mm. Removal of the chorion revealed the unabsorbed yolk to be 10 mm long and that the embryo had yet to undergo its second inversion. Available data from shallow-water *Octopus* species indicate that octopus eggs lengthen during development, as described by the equation:

$$y = 1.0257x + 0.0853 \quad (r^2 = 0.9701)$$

If this egg were ready to hatch, it is predicted to have been 32 mm long at spawning, if its chorion expands as does that of smaller eggs. Because the 40 mm long egg cited here contains an embryo that has yet to undergo its second reversal, it is not fully mature. Both the spawning and hatching egg lengths are predicted to exceed these estimates.

Although the length of the egg stalks embedded in the cement was highly variable, stalks that were intact and free of cement were consistently near 4.3 mm long. These intact stalks had a bulbous base as has been described in species of *Octopus*.<sup>8</sup>

Our observations of hatchlings of *Graneledone boreopacifica* further our basic knowledge of the species. These premature hatchlings are the largest and most developmentally advanced cephalopod hatchlings known to date, although normal hatching would be predicted to occur only after both the internal and external yolk sacs had been emptied.<sup>9</sup> The mantle length of fresh hatchlings of *Graneledone boreopacifica* exceeds that of adults of shallow-water tropical pygmy octopuses such as *Octopus wolffi*,<sup>10</sup> as does their weight in preservation, 2.5 g (after alcohol storage) versus for 2.0 g for *O. wolffi*.<sup>10</sup> Because these hatchlings have the typical broad and squat deep-sea octopus morphology,<sup>11</sup> only their arm lengths are surpassed by those of adult pygmy octopuses such as *O. bocki*.<sup>10,12</sup>

Examination of the reproductive organs reveals the sex of the hatchlings, a stage of maturity that has been termed sub-adult.<sup>13</sup> This status is typically reached at near 3.5 months of age, roughly one third of the lifespan, in *Octopus maya*, which hatch from eggs 17 mm long with a mantle length of 7.0 mm.<sup>7,14</sup> The development of the hectocotylus also indicates sexual maturation;<sup>15</sup> in shallow-water octopuses with large eggs and young, the hectocotylus becomes discernible roughly 3 months after hatching or after one-third of the lifespan in *O. joubini*<sup>15</sup> and *O. digueti*.<sup>16</sup> Although the development of the hectocotylus involves more than the arm suckers reaching their species-specific count, this character contributes to its differentiation.

These hatchlings of *Graneledone* had a large and conspicuous internal yolk sac. A previous report<sup>2</sup> stated that the digestive gland of an embryo attributed to *Graneledone* was full of yolk, but made no mention of a yolk sac. In the present specimens, because the internal yolk sac occupies the same space, has a similar shape and displaces the embryo's very small digestive gland to the posterior and ventral part of the mantle cavity, it could be mistaken for the digestive gland. Sagittal section, however, reveals the digestive gland to be positioned exactly as in embryos of *Octopus vulgaris*.<sup>9</sup> The previous report,<sup>2</sup> based on embryos from preserved eggs, found that clusters of cartilaginous elements were not generally apparent, although dorsally the head and mantle of some embryos had large processes; the skin texture that characterizes individuals of *Graneledone* is clear in these embryos (Fig. 3).

The morphological changes in preservation argue that preservation history must be recorded and reported with speci-

men data. In addition, because different parts of their bodies respond to preservation differently, calculation of the indices that are traditionally generated from these measurements<sup>17</sup> will magnify those differences.

Because egg length and ambient temperature are primary determinants of development duration in cephalopods<sup>18,19</sup> and female incirrate octopods brood their eggs,<sup>20</sup> producing large eggs dramatically increases maternal investment in each individual young. The costs of egg production and prolonged brooding may limit the number of eggs produced. The large size of these eggs and the maturity of the hatchlings support the hypothesis that maternal effort in this species maximizes the production of highly developed hatchlings at the cost of lower fecundity. This strategy has been suggested to minimize mortality early in the life cycle, possibly as an adaptation to their stable deep-sea habitat.

We thank the Captain and crew of the RV *Western Flyer* and the pilots of the *Tiburon* for their invaluable expertise. J.C.D. thanks MBARI and the Packard Foundation for postdoctoral support. They also provided partial cruise support with NURP Grant POF005001/AK-99-13 to R.A. Zierenberg. NSF DEB-0072695 and the Marshall Field Fund provided travel support for J.R.V. We thank G. Bello and B. Seibel for helpful discussions.

## REFERENCES

- ROBSON, G.C. 1932. *A monograph of the recent Cephalopoda. Part II. The Octopoda (excluding the Octopodinae)*. British Museum, London.
- O'SHEA, S. & KUBODERA, T. 1996. *Bull. Natl Mus. Tokyo, Ser. A*, **22**: 153–164.
- VOIGHT, J.R. & GREHAN, A.J. 2000. *Biol. Bull.*, **198**: 94–100.
- DRAZEN, J.C., GOFFREDI, S.K., SCHLINING, B. & STAKES, D.S. 2003. *Biol. Bull.*, **205**: 1–7.
- VOIGHT, J.R. 2001. *J. Moll. Stud.*, **67**: 95–102.
- WELLS, M.J. & WELLS, J. 1977. In: *Reproduction of marine invertebrates. 4. Molluscs: gastropods and cephalopods*. (A.C. Giese & J.S. Pearse, eds), 291–336. Academic Press, New York.
- VAN HEUKELEM, W. 1983. In: *Cephalopod life cycles*, **1** (P.R. Boyle, ed.), 311–323. Academic Press, London.
- ARAKAWA, K.Y. 1962. *Venus*, **22**: 176–180.
- BOLETZKY, S.V. 1975. *Zeit. Morph. Tiere*, **80**: 229–246.
- NORMAN, M.D. & SWEENEY, M.J. 1997. *Invert. Taxon.*, **11**: 89–140.
- VOIGHT, J.R. 1993. *Zool. J. Linn. Soc.*, **108**: 209–223.
- ADAM, W. 1945. *Arkiv Zoologi*, **37**: 1–25.
- STRANKS, T. N. & LU, C.C. 1991. In: *The marine flora and fauna of Albany, Western Australia*, **2** (F.E. Wells, D.I. Walker, H. Kirkman & R. Lethbridge, eds), 713–722. Western Australian Museum, Perth.
- HANLON, R.T. 1987. In: *Cephalopod life cycles*, **2** (P.R. Boyle, ed.), 291–305. Academic Press, London.
- FORSYTHE, J.W. 1984. *J. Zool.*, **202**: 393–417.
- DERUSHA, R.H., FORSYTHE, J.W. & HANLON, R.T. 1987. *Pac. Sci.*, **41**: 104–121.
- ROPER, C.F.E. & VOSS, G.L. 1983. *Mem. Natl Mus. Vict.*, **44**: 48–63.
- BOLETZKY, S.V. 1994. *Antarct. Sci.*, **6**: 139–142.
- LAPTIKHOVSKY V.V. 1999. *Ruthenica*, **9**: 141–146.
- BOLETZKY, S.V. 1987. In: *Cephalopod life cycles*, **2** (P.R. Boyle, ed.), 5–31. Academic Press, London.