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Supplementary material

Egg laying and embryo development of *Octopus huttoni* in response to temperature and season

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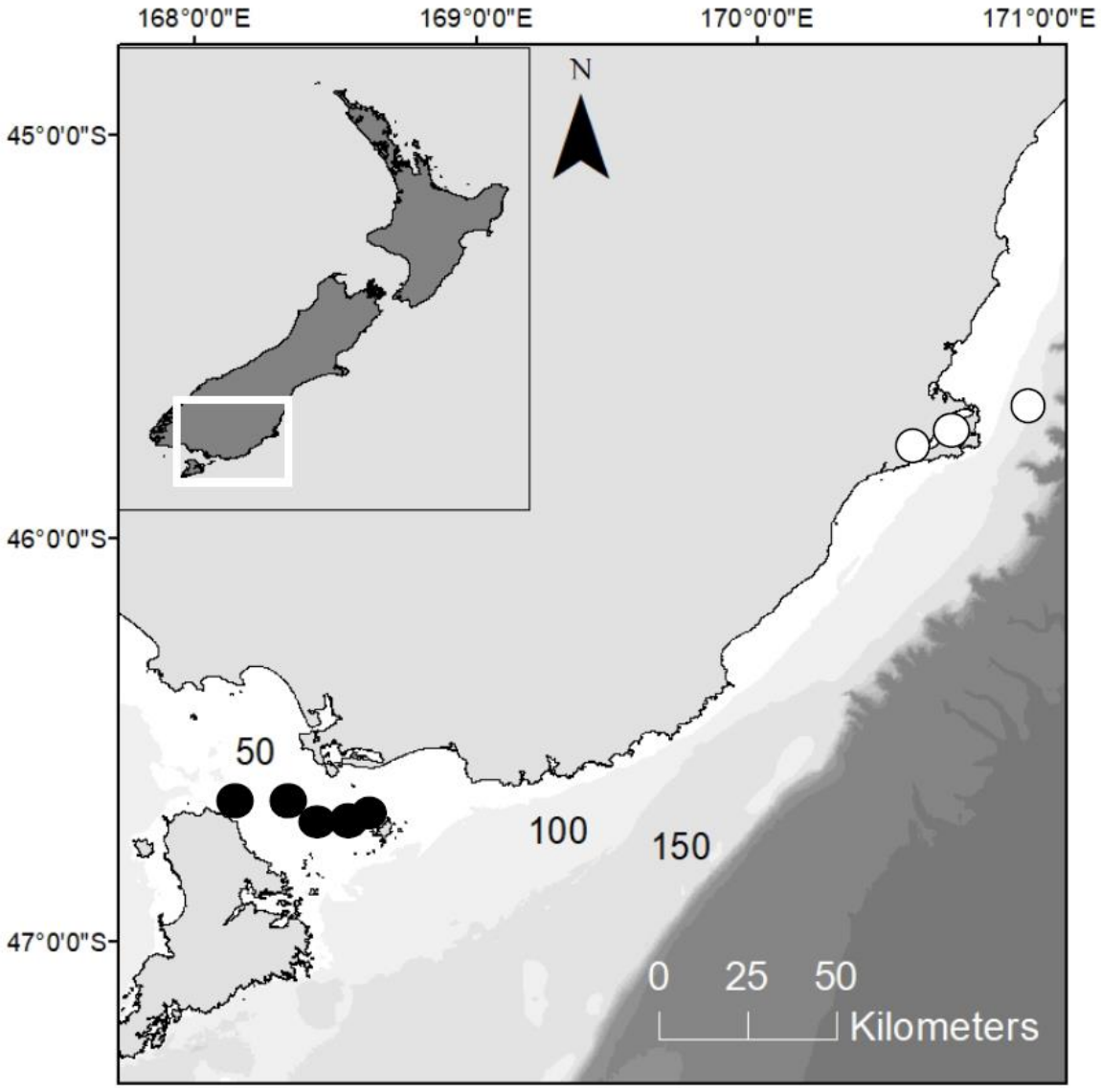

Fig. S1. Map of the three Otago sites (white dots) and five Foveaux Strait sites (black dots) where *Octopus huttoni* were collected. The numbers in black indicate maximum isobath depth for that shaded portion. Each shade increases 50 m in depth.

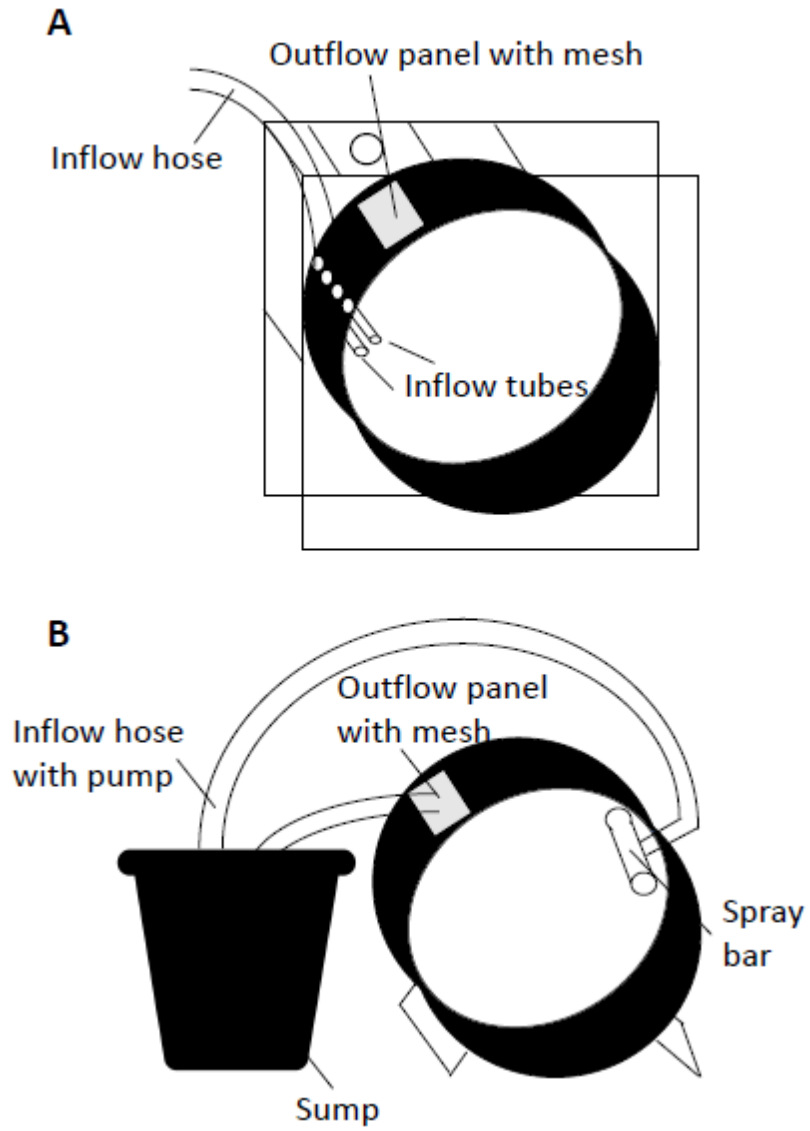

Fig. S2. Schematic of (a) the flow through plexiglass tank (9.6 L) with four inflow tubes, black plastic wrapped around the perimeter, and 300-µm mesh over the outflow panel. Schematic of (b) the recirculating pipe tank (11.6 L) with clear plexiglass sides and 300-µm mesh over the outflow panel.

Tank design is likely to be the most important factor affecting paralarval survival. If the flow was too high, the paralarvae would be hit by the water streams and scraped across the walls, causing injury to their arm and skin. Many of the dead paralarvae that were sampled were missing the tips of their arms or had a tear in the mantle. If the flow was too low, paralarvae would settle to the bottom and die. This damage could also be due to the high density of paralarvae per tank (100 paralarvae L^{-1}) as there is more risk of swimming into each other. Sánchez *et al*. (2013) successfully reared *O. vulgaris* at a density of 5 paralarvae L^{-1} and Perales-Raya *et al*. (2018) used 6 paralarvae L^{-1}. We suggest that larger tanks with lower densities of animals will minimise contact with surfaces and therefore reduce damage.

The type of inflow and the angle of the water stream is also important for paralarval rearing. A spray bar pointed down against the wall is suggested, as the smaller streams of water did not appear to damage the paralarvae skin as much as the large streams. The smaller holes on the spray bar should be placed close together so as to not produce any areas without constant flow as paralarvae can congregate there and die. As long as flow is constant throughout the tank without dead areas and not too strong, paralarvae should be well aerated.

Because the density of paralarvae in the tanks was so high (100–200 paralarvae L^{-1}) and the paralarvae were actively swimming as plankton, an aeration system is suggested for recirculating systems. The change to a tank with a spray bar and bubbler allowed the maintenance of paralarvae up to 19 days at ambient temperature instead of the 7 days from the temperature experiment. With the spray bar, there was a problem with the outputs getting clogged so that areas without flow would occur. In the future, slightly larger holes (1 mm) should be drilled in the spray bar to prevent easy clogging.

Density of prey also needs to be monitored because it was found that if too many prey (1 *Artemia* mL^{-1}) were added, there was a high risk of the paralarvae choking on the prey and dying. Occasionally, a paralarva would be swimming erratically or bursting across the bottom of the tank and when viewed under the microscope, it would have prey stuck in its siphon. It would then try and push it out by flushing its siphon and curl its arms up to try and pull it out. Another way to avoid this problem is to use prey slightly larger than *Artemia* nauplii (1 mm). This also applies to any small debris in the tank. All debris should be cleaned out of tanks daily to avoid this problem and to minimize the number of objects that could become projectiles and get lodged in the siphon. At times, prey were added in much smaller quantities or not at all for one day if there was still a significant number of prey left in the tank in order to avoid over-crowding. These modifications in tank design and rearing set up will aid future research that may be done on *O. huttoni* rearing.

Table S1. Description of octopus embryonic development Naef stages as described by Naef (1928) for *Octopus vulgaris*

Translated by Stina Kolodzey

Naef stage	Description
I	1200 cell stage, blastoderm developed
I–II	Entomesoderm development begins
II–III	Entomesoderm development continues
IV–VII	Distiction of germinal disc and growth of the yolk
VII–IX	Stages of unfolding
X–XII	Primary formation of the embryo
XIII–XV	Secondary unfolding and rearrangement of the head organs
XV–XVII	Development of the head, the crease, and the mantle
XVIII–XIX	Developing embryos
XX	Hatching

References

Naef, A. (1928) Die cephalopoden. Embryologie. In 'Fauna Flora Golf Neapel'. *Monografia della Stazione Zoologica di Napoli* **35**, pp. 1–357. (R. Friedländer & Sohn: Berlin, Germany.) doi:10.5962/bhl.title.11215

Perales-Raya, C., Nande, M., Roura, A., Bartolomé, A., Gestal, C., Otero, J., García-Fernández, P., and Almansa, E. (2018). Comparative study of age estimation in wild and cultured *Octopus vulgaris* paralarvae: effect of temperature and diet. *Marine Ecology Progress Series* **598**, 247–259. doi:10.3354/meps12218

Sánchez, F. J., Fuentes, L., Otero, J. J., Lago, M. J., Linares, F., Pazos, G., and Iglesias, J. (2013). Effect of tank volume on the growth and survival of reared *Octopus vulgaris* paralarvae. *Aquaculture Research* **44**(4), 667–670. doi:10.1111/j.1365-2109.2011.03049.x