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An Intensive, Large-Scale Batch Culture System to Produce the Calanoid Copepod, *Acartia tonsa*: Appendix A

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1 Supplementary appendices to accompany:

2 Sarkisian, B. L., Lemus, J.T., Apeitos, A., Blaylock, R.B., and Saillant, E. A. 2018. An intensive,
3 large-scale batch culture system to produce the calanoid copepod, *Acartia tonsa*. Aquaculture
4 DOI: 10.1016/j.aquaculture.2018.11.042

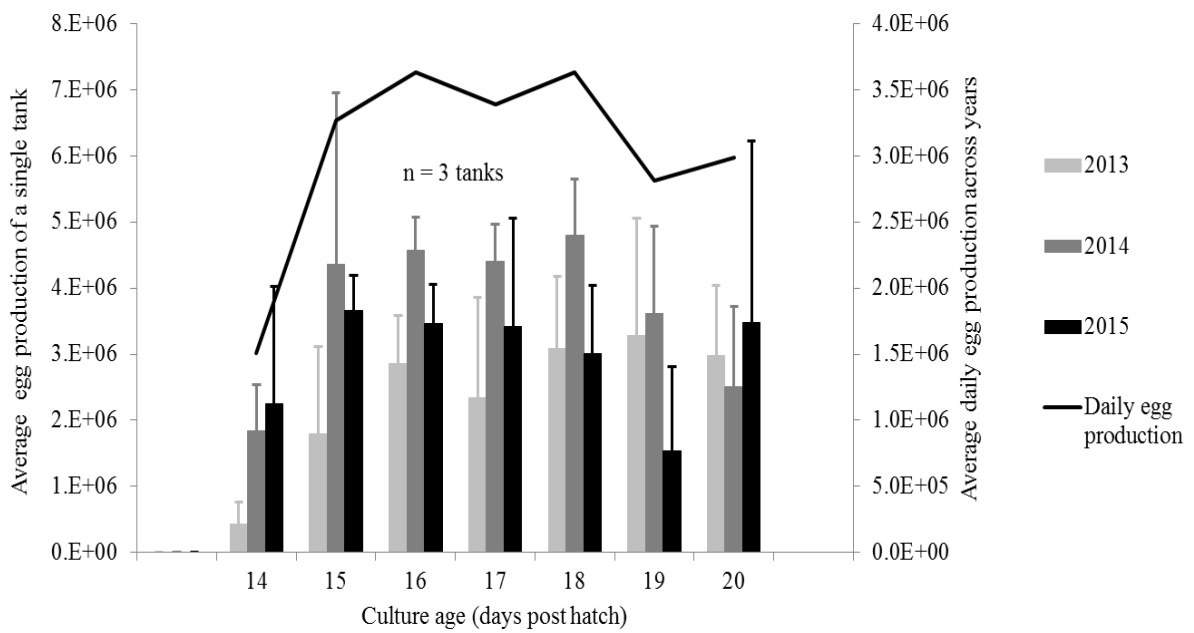
5 Appendix A. Microalgae culture

6 Algae production is carried out in a batch culture system in a room adjacent to the
7 copepod production system. A batch algae system is the most cost-effective method to provide
8 for high copepod demands and a small volume footprint (150 L, 6 trillion total cells day⁻¹).
9 Tahitian–strain *Isochrysis lutea*, CCMP 1324 is used for the main source of algae to feed
10 copepod cultures. Algae production is carried out in 19 L plastic bags set on racks with
11 fluorescent lights between the racks. Light intensity is held at 12,000 – 17,000 lux by use of 32
12 watt, 2,900 lumen, 4,100 Kelvin mercury-fluorescent bulbs (GE 66349 - F32T8/SPP41/ECO,
13 General Electric, Boston, MA) over a 24-h photoperiod. The bags are inoculated with 2 L of
14 algae (in exponential phase at a minimum density of 30 x 10⁶ cells mL⁻¹) and 16 L of seawater.
15 Bags are fed F/2 Algae Food (Fritz Aquatics, Mesquite, TX), 2.5 mL of part A to 2.5 mL part B
16 solution every other day. After a ten to twelve day grow-out period, the bags are harvested as
17 feed for the copepods at a density of 40 - 50 x 10⁶ cells mL⁻¹.

18 For the years 2014 and 2015, *T-Iso* feedings were supplemented with *Rhodomonas lens*,
19 Pascher & Ruttner, 1913, CCMP 739 (*Rhodo*) was produced in a separate room. This algae
20 requires a specific photoperiod (10 h light : 14 h dark cycle using the same techniques as for T-
21 Iso), so it was cultured in another building. *Rhodo* bags were inoculated and supplied with
22 nutrient media the same way as *T-Iso*, reaching a density of 15 - 20 x 10⁶ cells mL⁻¹. On any
23 given day, only a total of 25 L of *Rhodo* (0.4 trillion total cells) was available to supplement
24 copepod feedings.

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26 Appendix B. Egg production from a single tank over three trials in 2013, 2014, and 2015. Bars
27 represent an average of daily egg production over a seven-day collection period \pm SD. Average
28 daily egg production across years is represented by a curved line. The procedure to derive daily
29 production and averages is the same as that described in section 3.3 but the single tank was
30 counted separately.



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