An intensive, large-scale batch culture system to produce the calanoid copepod, *Acartia tonsa*

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Abstract

A major obstacle to the development of hatchery production for juveniles of many marine species is the difficulty in successfully feeding early larvae. Copepods contribute to the natural diet of most marine fish larvae and feature characteristics ideal for early larval feeds including small size and suitable nutrient content. However, the use of copepods as larval feeds is limited by the inability to consistently produce them in sufficient quantities to support large-scale fish culture. Here, an innovative design for an intensive, indoor batch culture system to produce the calanoid copepod *Acartia tonsa* (Dana 1849), a prime candidate for use as a live food item, is described. The system features integrated grow-out and egg-production units that can be operated sequentially by 2.5 full-time employees to produce a predictable daily output of nauplii for use as live feed. The system output was on average 22 million eggs d^{-1} (21,955,420 ± 8,709,668) with an average hatch rate of 49% (49.1 ± 14.8) over three seasons.

Key words: *Acartia tonsa*, live feeds, batch culture, copepod

Highlights

1. A batch culture system for intensive production of *Acartia tonsa* was developed
2. A continuous production of eggs is achieved by staggering stocking of cultures
3. The system output averaged 22 million eggs per day during the study period

1. Introduction

The feeding of early larvae poses major challenges to the development of marine finfish aquaculture. Rotifers and *Artemia* spp., the most commonly used live feeds in hatcheries, are too large for the early larvae of many marine fishes and/or do not fulfill their nutritional requirements (Shields et al., 1999; Toledo et al., 1999; Wilcox et al., 2006). Copepods are a primary prey item for many larval fish in the wild (Holt and Holt, 2000; Hillgruber and Kloppmann, 2001). They occur in sizes acceptable by all stages of most marine fish larvae (Detwyler and Houde, 1970; Fernández-Diaz et al., 1993; Toledo et al., 1999) and feature optimal levels and ratios of specific essential nutrients for use as larval diet (Helland et al., 2003; van der Meeren et al., 2008; Rayner et al., 2017).

Acartia tonsa (*A.tonsa*), Dana 1849, is a cosmopolitan, eurythermal, and euryhaline calanoid copepod found in subtropical and temperate latitudes. This copepod is of significant interest for marine larviculture because the small size of its first and second nauplii (Table 1) is compatible with the mouth gape of some of the smallest fish larvae (Detwyler and Houde, 1970; Fernández-Diaz et al., 1993; Toledo et al., 1999). Indeed, nauplii of *Acartia* have been used to culture the early larvae of marine species for which previous attempts with rotifers had been unsuccessful (Schipp et al., 1999; Toledo et al., 1999; Ogle et al., 2005). Further, *A. tonsa* produces eggs that can go dormant and remain viable in storage at 3ºC in the dark for several days (Drillet et al. 2006), thus allowing eggs to be stockpiled to help meet copepod demand in fish hatcheries.

Isolates of *A. tonsa* obtained from subtropical regions have been cultured successfully at temperatures between 20 and 30 °C and salinities between 20 and 35 ppt (Castro-Longoria, 2003; Leandro et al., 2006; Peck et al., 2015; Shayegan et al., 2016). At 25 ºC, female *A. tonsa*

reach the adult stage at 9-12 days post-hatch (dph) and begin broadcast-spawning negatively buoyant eggs (Ogle, 1979; Lemus, 2005; Leandro et al., 2006). Adult females can produce eggs for up to 30 days, although daily egg production typically peaks between 16 and 20 dph at 25 °C. Fecundity ranges between 3 and 7 eggs female^{-1} during the peak production period at an adult density of 0.5 adults mL^{-1}.

Adult *A. tonsa* are cannibalistic and eat their eggs and the first two stages of nauplii (Lemus, 2005; Drillet et al., 2014). Thus, for culture, eggs must be collected either by directly siphoning culture tanks (Støttrup et al. 1986) or using flow to entrain them in nets or a collection chamber (Toledo et al. 2005). Subitaneous eggs produced by *A. tonsa* hatch within 20 to 48 hours at 25 °C (Hansen and Drillet 2013). The size of newly-hatched N1 and N2 instars is compatible with the mouth gape of small marine fish larvae. However, *A. tonsa* molt out of these younger naupliar stages within a few hours after hatching (Lemus, 2005; Leandro et al. 2006). As a consequence, batches of eggs must be hatched at least every day to maintain a supply of N1 and N2 stages.

Van der Meeren and Naas (1997), Toledo et al. (1999), Lemus et al. (2004), Ogle et al. (2005), Uye (2005), and Skovgaard et al. (2015) described extensive methods for culture of *A. tonsa*. The composition and abundance of the zooplankton produced through extensive methods, however, is highly variable and unpredictable. In addition, extensive production does not prevent introduction of copepod or fish pathogens. Intensive culture provides for improved biosecurity, reduced footprint and water use, and increased control over vital rates, population structure, feeding, and other environmental factors. To date, attempts to produce *A. tonsa* intensively have relied on batch culture approaches. Adult *A. tonsa* are placed in tanks where they produce eggs that are then separated from the culture, hatched, and grown to the appropriate size for feeding

92 fish or restocking the adult culture (Støttrup et al., 1986; Marcus and Wilcox, 2007; Abate et al.,
93 2015). The tradeoffs for intensive production include increased technological and infrastructure
94 requirements and labor.

95 This paper describes a large-scale, intensive, batch culture system for continuous
96 production of *A. tonsa*. This production system was developed at the University of Southern
97 Mississippi's Thad Cochran Marine Aquaculture Center (TCMAC) in Ocean Springs, MS to
98 supply *A. tonsa* nauplii for larviculture of the red snapper (*Lutjanus campechanus*). Egg
99 production data obtained during operation of the system during the years 2013-2015 are
100 provided.

101 **2. System design**

102 The unit is designed as a batch–culture system organized into three phases: 1) production
103 of eggs by reproductive adults, 2) incubation of eggs, and 3) growout to the adult stage. The
104 system features two working elevations (decks). Gravity facilitates transfer of adult copepods
105 directly from growout tanks on the upper deck to the egg production tanks on the lower deck
106 where egg production, collection, and incubation occur (Fig. 1). A dedicated Heating Ventilation
107 and Air Conditioning (HVAC) unit provides temperature control, mechanical filtration (50- and
108 25-µm filters) and ultraviolet light (UV) irradiated air to the room.

109 *2.1. Seawater*

110 All phases of the culture are performed at 25 °C and 25 ppt. Seawater is produced from a
111 commercial marine salt (Bio-Sea Marinemix, AquaCraft, Inc., Hayward, CA) mixed with local
112 well water. Artificial sea salt is used to ensure consistent composition of the water, which could
113 not be achieved with local estuarine water. The seawater is filtered to 1-µm and passed through a
114 60-watt ultraviolet light at 2.0 L min^{-1} before being transferred to two 4,000-L acclimation tanks

where it is left to adjust to the temperature of the copepod culture room. Seawater is reclaimed after the egg production phase. Egg production tanks are drained to a floor sump through pipes. A float-operated pump transfers the water to a 40,000-L holding tank from which it is pumped to 19,000-L storage towers to be filtered and disinfected as described above for reuse. Under current operation conditions, ammonia levels in the system fall within the range of tolerance of *A. tonsa* (Jepsen et al., 2015) and never exceed 1 ppm. Culture water has been reused up to three times through the reclamation loop without inducing noticeable changes in production parameters.

2.2. Egg production and collection

Egg production occurs on the lower deck in six 1,900-L, cylindro-conical, black, high-density polyethylene tanks (Fig. 1). Each egg production tank is paired with an egg collecting unit featuring a 50-µm mesh net with 2-piece cod-end assembly and a 200-L cylindro-conical tank (Fig. 1). Egg production tanks are equipped with a removable center screen pipe containing 200-µm mesh windows to retain the adults and allow passage of eggs. Water drains by gravity from the egg production tank through the net in the egg collection tank and back to the egg production tank through an airlift pump at a rate of 16 L min^{-1}. The net in the egg collection tank is raised and lowered using a pulley suspended from the ceiling during harvests.

2.3. Egg hatching

Eggs are incubated in 18-L polycarbonate, conical incubators (Fig. 1). Each incubator features a vinyl airline attached to a rigid, polyvinyl chloride tube placed at the bottom of the center drain to aerate the culture and a bottom drain valve for harvesting nauplii.

2.4. Growout of nauplii to adults

The growout phase uses twenty-four blue, fiberglass cylindro-conical tanks at a working volume of 900 L aligned in two rows separated by an aisle on the upper deck (Fig. 1). A center standpipe isolates each tank. Each growout tank within a row is connected to the tank in the opposite row by a shared drain pipe that allows adult copepods from one or both of the tanks to be transferred by gravity to one of the egg production tanks below on the lower deck. All growout tank pairs drain to a common pipe which can deliver the adult copepods into any one of the six egg production tanks.

3. Operation

3.1. Master culture maintenance and production scale-up

Master cultures were obtained from a captive stock of *A. tonsa* held at The University of Texas Marine Science Institute, Port Aransas, TX in 2002. Cultures are maintained in 1-L glass bottles as continuous cultures without separation of the different life stages. Aeration at 0.5 bubbles sec^{-1} is achieved through a rigid tube inserted through a rubber stopper in the bottle mouth and extending down to one inch from the bottom of the culture bottle. The predominant culture stage and abundance is estimated visually each day to determine the feeding rate which ranges from 50,000 to 200,000 cells mL^{-1} of *Tisochrysis lutea* (*Tiso*), CCMP 1324 produced in artificial seawater (DOI to be assigned) daily. Once a week, each bottle is filtered through a 50-µm screen to collect and transfer the entire population to a clean 1-L bottle filled with new seawater.

Increasing the scale from master culture to full production requires a four-month period. The scale-up involves four sequential growout to egg production phases each consisting of: (1) egg production, collection, and refrigeration (cold-storage); (2) 48-hour egg hatch (as described in Section 2.3); and (3) 14-day growout to the adult stage (see Fig. 2).

In phase I, adult copepods from master cultures are transferred into a small-scale egg production unit for spawning. The unit includes a 1-L beaker and an inside, 0.5-L chamber where adult copepods are placed for spawning. The chamber features windows covered in 200-µm nitex mesh that allow eggs to pass through while adults are retained. The chamber is transferred to a new 1-L beaker with clean seawater daily. Eggs from the previous day's beaker are concentrated on a moist 50-µm nitex mesh screen and refrigerated at a density of up to 200,000 mL^{-1} in 75 mL glass jars until a minimum of one-hundred and fifty thousand eggs are accumulated (Fig. 2).

For phase II, 48 h nauplii from the eggs accumulated in phase I are stocked at a density of 1 mL^{-1} into 18-L buckets where they are grown out to the reproductive stage. The resulting adult copepods are stocked at 0.5 mL^{-1} into a 15-L chamber similar in design to the mesh-covered chamber described above and set inside a 30-L tank. Chambers containing the adult copepods are moved daily to a clean tank and the eggs are collected and stored as described for phase I until 1.5 million eggs are accumulated (Fig. 2).

In phase III, 48-hr nauplii hatched from the eggs accumulated in phase II are grown to adults at a stocking density of 1 mL^{-1} in two 200-L tanks. Adult copepods are then stocked into a 500-L egg production tank fitted with a center 200-µm mesh screen pipe. Eggs are entrained through the center drain to a 50-L tank where they are concentrated in a plankton net and collected twice daily. Water is circulated through the system by an airlift pump. Eggs are accumulated until a minimum of six million are collected within a two-week period (Fig. 2).

In phase IV, the six million eggs accumulated in the phase III are hatched at a density of 350,000 ± 50 eggs mL^{-1} over 48 h. Nauplii (N3-N4) are then split between two 900-L growout tanks in the production system at a density of 1 mL^{-1} and cultured for an additional 12 days. The

eggs produced are used to stock additional growout tanks over a seven-day egg production period. The eggs obtained from one egg production tank during the egg-production period allow stocking of up to twelve growout tanks. Because only two growout tanks and one egg production can be stocked in phase IV and the entire lifecycle requires three weeks (two-day incubation, twelve-day growout, and seven-day egg production), it takes six weeks from the initial stocking of the first growout tanks to stock the entire unit (Fig. 2).

3.2. Batch production process (Fig. 3)

3.2.1. Producing and collecting eggs

Egg production tanks are stocked with adult copepods at a density of approximately 0.5 ind mL^{-1}. Adults are fed 100,000 algae cells mL^{-1} twice daily. Eggs are harvested from the plankton net in each egg collection tank every 12 h by gently rinsing into a bucket. Eggs from all tanks are combined, poured through a 100-µm sieve to filter out detritus and fecal pellets, and gently rinsed with clean seawater through a 50-µm sieve. The combined harvest is consolidated in 500 mL of seawater from which three 1-mL samples are diluted (1/50 to 1/250 depending on the number of egg production tanks collected on a specific day), counted, and averaged. Eggs and nauplii are either stocked in an incubator to continue hatching for stocking other growout tanks or concentrated and stored in the refrigerator for delayed hatch. Egg collection begins when the culture is 14 days old, as this corresponds to the beginning of peak production by females, and ends on day 20. Because the hatch rate of eggs declines rapidly after 20 days of culture (Drillet et al.,2016; Hansen et al., 2016), egg collection is discontinued at that point (DOI to be assigned).

3.2.2. Incubating eggs

205 Newly spawned eggs collected daily are incubated at a stocking density of 350 ± 50 eggs
206 mL^{-1} with 5 bubbles sec^{-1} aeration. When eggs are hatched to produce N1 and N2 nauplii to feed
207 larval fish, the incubator is harvested up to three times over a period of 36 hours from the time of
208 egg collection.
209 If the hatched nauplii are used to stock growout tanks, specific developmental stages or
210 size fractions are not required. Thus, the incubation in conical incubators is extended to 48 h
211 post-stocking to account for variability in egg hatching time. Because those eggs that hatch early
212 will have developed to the N2-N4 feeding stages before 48 hrs have elapsed, incubators are fed
213 100,000 cells mL^{-1} *Tiso* at 24 h and 36h post stocking.
214 The harvest process involves draining through a tube into a 35 µm mesh sieve set in a
215 bucket to keep the nauplii submerged in water and prevent damage to the copepods. The
216 harvested nauplii and eggs are then rinsed into another bucket and unhatched, negatively-
217 buoyant eggs are allowed to settle. The top layer containing the nauplii are decanted into another
218 bucket where three 1-mL aliquots are removed, diluted in 50-100 mL, and counted using a
219 dissecting microscope.
220 *3.2.3. Growout management*
221 Aeration is provided through a silicate diffuser at 0.4 L min^{-1} upon stocking. At day 5
222 post-hatch, aeration is increased to 1.2 L min^{-1}. Copepods are fed *Tiso* once daily based on the
223 age of the culture (Table 1). Although the amount of *Tiso* fed generally increases with copepod
224 age, less is added on day 2 because residual *Tiso* remains relatively high through the first 24
225 hours. At 14 dph, ninety percent of the growout population has reached the adult stage. At that
226 stage, copepods are transferred from growout tanks to egg production tanks.

227 One egg production tank produces enough eggs to stock two 900-L tanks paired in
228 opposite rows in the growout system daily at 1 nauplius mL^{-1}. After 14 days, the adults from the
229 pair of growout tanks are used to replace the oldest of the six egg production tanks. Replacement
230 of the oldest egg production tank occurs daily to maintain young cultures that are constantly
231 producing a consistent number of eggs. When all egg production tanks are stocked, a combined
232 average of 22 million eggs d^{-1} can be achieved, which supplies approximately 11 million nauplii
233 d^{-1} to feed fish larvae and restock the system.
234 *3.3. Sampling and analysis*
235 Egg production is determined every twelve hours by collecting eggs from all production
236 tanks in use, consolidating them in 500 mL seawater, diluting a 1-mL sample from the mixture in
237 200 mL, averaging three counts from the dilution, and multiplying the average count by 100,000.
238 Daily egg production is the total number of eggs produced from all six egg production tanks
239 combined in two 12-hour collection periods. The Grand (yearly) Mean is based on all daily egg
240 collections during the production period.
241 Percent hatch is determined by harvesting and concentrating the nauplii from an
242 incubator and mixing into 4 L of seawater. Three 1-mL aliquots are immediately sampled,
243 diluted in 100 mL, and counted. The hatch rate is calculated by dividing the number of nauplii
244 collected over the entire hatching period by the total number of eggs stocked in an incubator and
245 multiplying by 100. The mean hatch rate of a production season is calculated across all
246 incubators stocked with fresh eggs collected within each 24-h period during the entire production
247 season.
248 Abundance and survival of the copepod growout cultures to the adult stage is assessed at
249 14 dph, prior to egg production transfer. An air diffuser is placed at the center of the growout,

and airflow is increased to mix the culture. Four 1-L samples are taken from around the tank and a fifth 1-L sample in the center. All samples are combined into a bucket and further mixed by pouring the mixture between two buckets. Five 100-mL samples are removed from the bucket (10% of the sample) and copepods are counted to derive an estimate of the total number of copepods in the culture by volumetric extrapolation. This estimate is used to calculate survival through the growout phase by dividing the abundance of adults by the initial stocking abundance of N3-N4 instars.

3.4. Operation of the system during the 2013-2015 period

The amount and perhaps the quality of microalgae available for feeding during the three production seasons varied due to limitations in the microalgae production unit including technical failures in the CO_2 delivery system, failures in the temperature control system, and contamination by protozoa that grazed on *Tiso* and reduced the amount available for the copepods. In 2014 and 2015, algae feedings were supplemented with *Rhodomonas lens* (*Rhodo*), Pascher & Ruttner, 1913, CCMP 739 because a preliminary study indicated that it increased egg production in the copepod production tanks when used as a supplement to *Tiso*. On average, 200,000 cells mL^{-1} *Tiso* and 50,000 cells mL^{-1} *Rhodo* were available for feeding adults in the egg production tanks in 2014. In 2015, copepod cultures were fed 200,000 cells mL^{-1} of *Tiso* and only 25,000 cells mL^{-1} of *Rhodo* because the *Rhodo* culture yield was lower than anticipated.

Average daily copepod production varied among years during the 2013-2015 period (Table 2). Egg production in 2013, 2014 and 2015 was 13,702,661 ± 6,194,111, 31,059,479 ± 7,984,523, and 21,104,121 ± 7,247,644 eggs d^{-1}, respectively. The average hatch of fresh eggs across the years 2013-2015 was 49.1 ± 14.8% (Table 2). Hatch in 2013 was 61.6 ± 17.5%; in 2014 and 2015 it was 32.7 ± 10.0% and 52.9 ± 14.5%, respectively. The average survival rate

from nauplii to the adult stage during 2013-2015 was 45.4 ± 11.8% (31.8 ± 14.1%, 52.3 ± 22.1%, and 52.1 ± 22.6% in 2013, 2014, and 2015, respectively Table 2).

4. Discussion and conclusions

During 2013-2015, this intensive copepod production system produced on average 22 million eggs d^{-1} (1,930 eggs L^{-1}) and 11 million nauplii d^{-1} (965 nauplii L^{-1}). Hatching of the eggs generated enough N1-N4 instars to support the initial feeding of 120,000 larvae of the red snapper (*Lutjanus campechanus*) stocked at 10 to 20 L^{-1}. The daily egg and nauplii production achieved with this unit exceeded by far those reported for an extensive system previously developed at TCMAC (Ogle et al. 2005). The extensive system only yielded a mean (±SD) of 3.3 million (2.9 million) nauplii d^{-1} and was limited by large fluctuations of daily production over time, perhaps due to the successional dynamics of extensive zooplankton cultures (Ogle et al. 2005). An additional benefit of the intensive unit described here is a much reduced temporal variability of production thanks to the controlled batch system. Therefore, this study demonstrates that consistent daily production of copepod nauplii can be accomplished on a large-scale in a biosecure, controlled environment with a much smaller footprint than an extensive system. Operating costs remain the main limiting factor as the unit requires personnel equivalent to 2.5 full-time positions and large amounts of live microalgae (6 trillion cells d^{-1}) when the system is fully stocked.

Simulations of intensive production scenarios conducted by Abate et al. (2015) predicted yields ranging from 7,500 to 25,000 eggs L^{-1} in systems stocked at adult densities of 1.5 to 5 mL^{-1} suggesting production per unit volume could be higher than in the present study. However, the estimates of Abate et al. (2015) were based on extrapolation of data obtained at a low density in the system described in Støttrup et al. (1986), which produced 200 eggs L^{-1}. These predictions

296 remain to be tested and must be viewed with caution because they involve culture densities much
297 higher than those used in most current empirical studies. Indeed, while some authors suggest that
298 copepod culture at such high density may be possible (Nilsson et al. 2018, Vu et al. 2017), others
299 did not recommend culturing *A. tonsa* at densities above 2.5 nauplii or adults mL^{-1} for growout
300 or egg production (Franco et al. 2016).

301 Egg production and growth rate can be optimized through controlled testing of multi-
302 species microalgae diets (Milione and Zeng, 2007). *Rhodo*, in particular, increases egg
303 production due to its suitable cell size, high lipid content, and favorable fatty acid profile
304 (Støttrup and Jensen 1990; Jónasdóttir 1994). During 2013-2015, the algal diet varied, but the
305 effects of the multi-species microalgae diet used in 2014 and 2015 should be interpreted with
306 caution. The addition of *Rhodo* may have contributed to improved egg production in 2014 and
307 2015, but the overall microalgae ration, which differed among years, also may have impacted
308 production results substantially. In 2013, the cell ration (100,000 *Tiso* cells mL^{-1} twice daily),
309 was only slightly above the 90,000 *Tiso* cells mL^{-1} threshold required for maximum egg
310 production (Støttrup and Jensen 1990), and may have declined below the threshold. Støttrup and
311 Jensen (1990) showed that reduction of *Tiso* concentration quickly reduces ingestion and Dagg
312 (1977) reported that complete deprivation of food for a period as brief as three hours reduces egg
313 production with substantial declines when deprivation lasts for nine hours. Microalgal rations for
314 the late phases of growout and for reproductively mature cultures appeared to be entirely
315 consumed within 12 to 24h of feeding, suggesting that increasing the ration during these culture
316 phases may allow for faster development rates and improved egg production.

317 Fluctuation in feeding rate and diet composition (in particular the percentage of *Rhodo*)
318 have also been shown to affect hatching success (Drillet et al., 2006), and therefore likely

affected hatch rate and/or survival in this system. Altogether, these results indicate that further efforts are warranted to optimize the feeding protocol, including the composition of the diet, the feeding rations, and feeding frequency accounting for consumption by the copepods.

We note that hatch rate was variable across years but was not correlated with either egg production or growout survival (see Table 2), indicating that egg production and hatch rate are independent traits that will both need to be considered during future efforts to optimize culture processes as they both contribute to the yield in nauplii. Production efficiency also could be improved through improved viability of cold-stored eggs. Cold-stored eggs in this study remained routinely viable for up to two weeks. Viability through storage may be increased by improving egg quality through optimization of the diet as discussed above and also through manipulations of environmental conditions during storage in particular anoxia as espoused by Hansen et al. (2016).

In conclusion, the vast amount of research on copepod physiology has led to methods for culturing multiple species at a small-scale. However, few studies have demonstrated mass-production due to a variety of factors including the overall high cost of operation involved in current protocols, the large quantities of live algae required for culture, the low performance of most copepod species at high density, the challenges in providing adequate hydrodynamic conditions in large systems, and the need for recycling culture water. This paper has focused primarily on the mechanics and logistics of operating a novel system to intensively produce the calanoid copepod, *A. tonsa*, continuously using batch cultures. The current system output is still limited to the supply of feeds for experimental fish cultures, but its capacity could be easily expanded by simply adding additional culture units. Copepod production could be further

341 improved by increasing the volume of culture units, varying the production parameters, and/or
342 optimizing the feeding protocol. However, those changes will require further research.

343 **Acknowledgements**

344 The authors would like to acknowledge Dr. Phillip Lee and Buck Schesny for assistance
345 with design of the system, David Butler for help assembling the system, and Ellen Flaherty for
346 contribution to operation of the system and data collection, and Paul Hundley and Sherry Lilley
347 (HTH aquaMetrics LLC) for assistance with Figure 1. This work was supported by the US
348 Department of Commerce (Seagrant National Marine Aquaculture Initiative award #
349 NA14OAR4170098) and the Mississippi Department of Marine Resources, Tidelands Trust
350 Fund Program (awards # S14-RS-USM/GCRL02 and USM/GCRL-04/FY15-M400-39).

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Figure Captions

Figure 1: Design of growout, egg production and incubation for a large-scale intensive, batch culture production system for the calanoid copepod, *Acartia tonsa* Dana (1849). A set of two growout tanks (row 1 and row 2) are connected to a common drain manifold and can be drained to any of the six egg production tanks. The design is operated in multiples of six to maintain continuous production.

Figure 2: Overview of the scale-up procedure for the production of *Acartia tonsa* from master cultures to a large-scale, intensive production system.

Figure 3: Overview of production procedure in a large-scale intensive system for the copepod, *Acartia tonsa*.

469 Table 1. Feeding schedule, tank volume, and life history stage by culture age of *Acartia tonsa*

470 Dana (1849) fed *Tisochrysis lutea* in an intensive batch culture system.

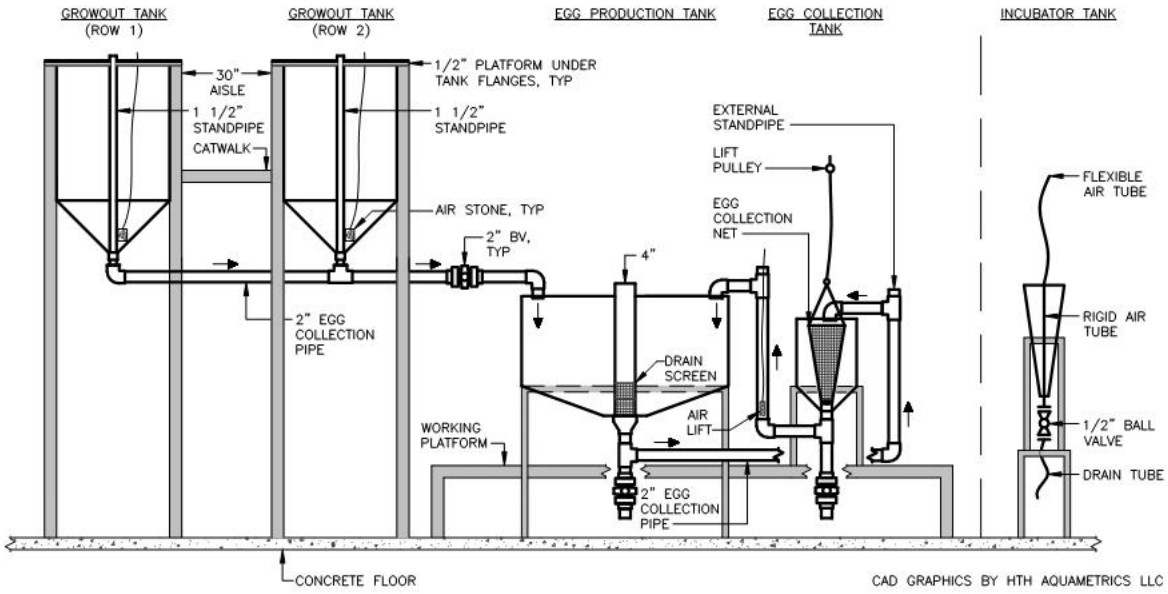
		2013-2015[b]		2009[c]		
Age (day post-hatch)	Life History stage[a]	Tank volume (L)	Cells mL^{-1} added (10^3)	Tank volume (L)	24-hr residual cells mL^{-1} (10^3)	Cells mL^{-1} added (10^3)
0	Egg-N2	18	100	900	0	0
1	N1-N3	18	100	900	0	50
2	N1 - N4	900	65	900	36 ± 2.5	16 ± 1.1
3	N3 - N6	900	20	900	44 ± 2.8	19 ± 2.8
4	N3 - C2	900	50	900	45 ± 3.8	46 ± 3.8
5	N5 - C3	900	80	900	21 ± 2.7	114 ± 2.7
6	C1 - C3	900	170	900	15 ± 3.0	232 ± 3.0
7	C1 - C5	900	200	900	18 ± 4.7	333 ± 4.7
8	C2 - C5	900	200	900	28 ± 9.6	341 ± 9.6
9[d]	C3 - C5	900	200	1,900	77 ± 5.4	359 ± 4.88
10	C4 - Adult	900	200	-	-	-
11	C4 - Adult	900	200	-	-	-
12	C5 - Adult	900	200	-	-	-
13	C5 - Adult	900	200	-	-	-
14 – 20	Adult	1,900	200[e]	-	-	-

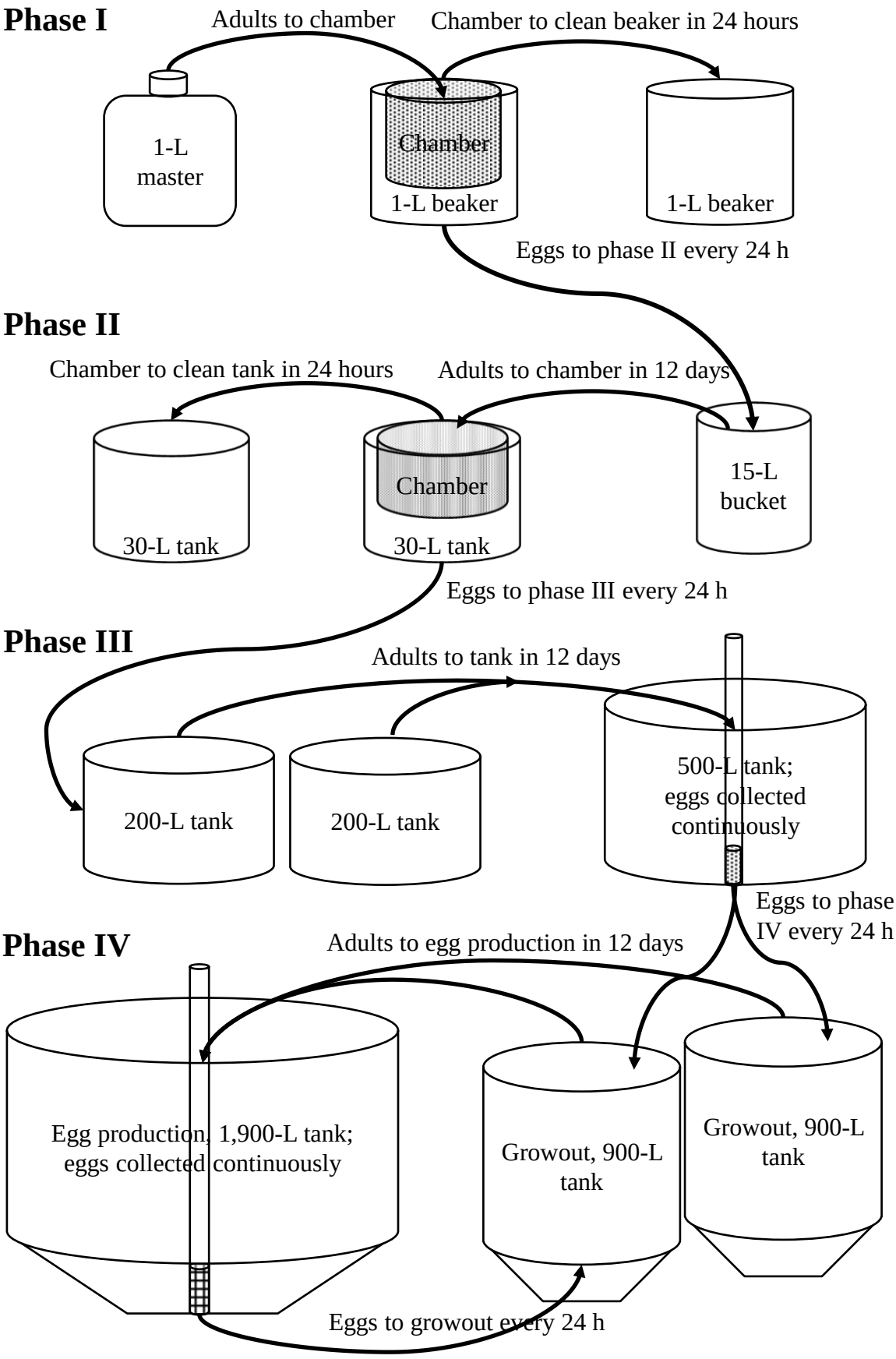
471

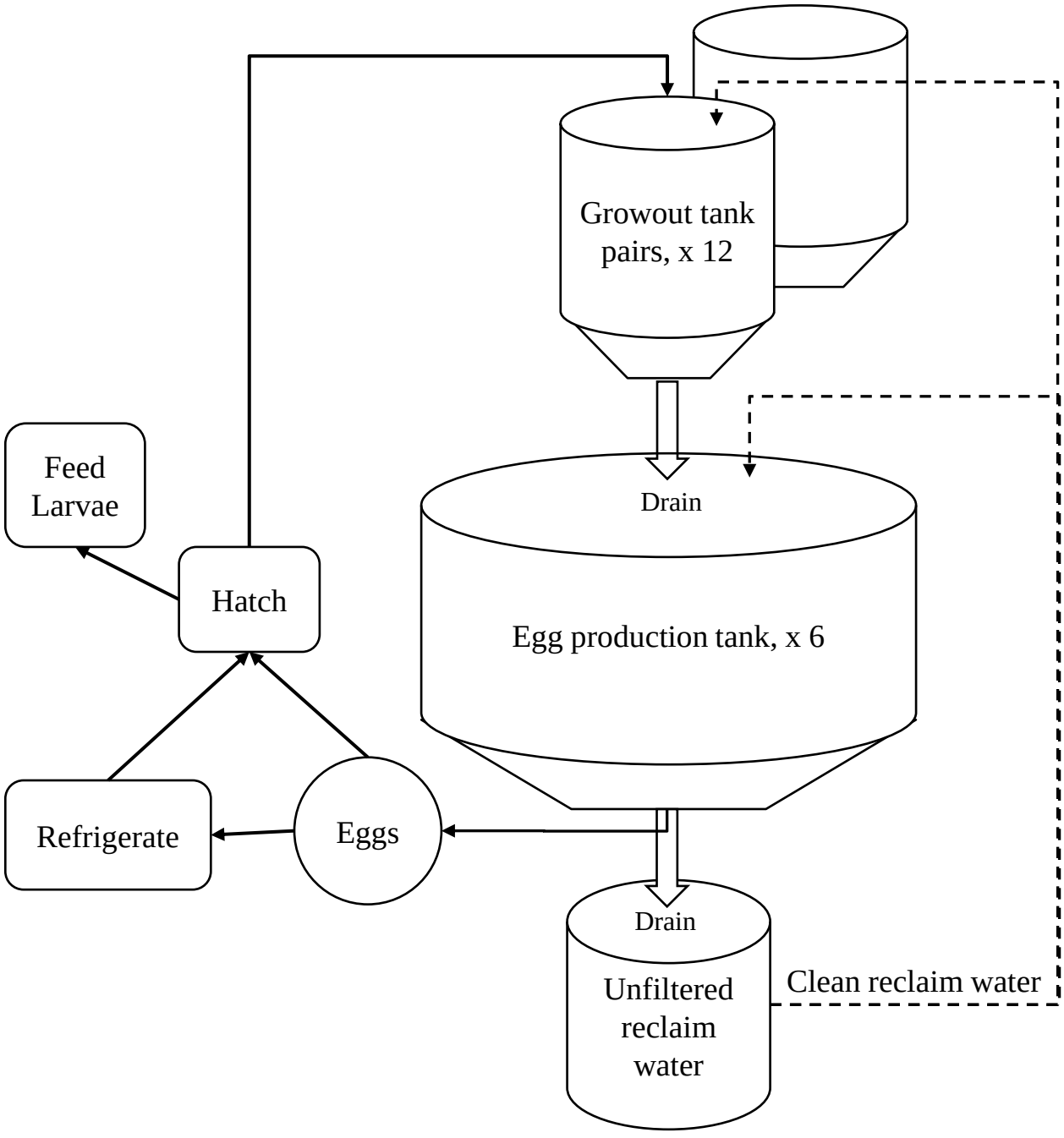
472 [a] Size of stages (µm ± SD) for 2013-2015. *Egg*, 78.2 ± 5.2; *N1* (L x W), 104.4 ± 4.9 x 56.9 ±
473 4.3; *N2*, 131.5 ± 18.3 x 70.4 ± 10.5; *N3*, 153.0 ± 8.4 x 83.9 ± 7.4; *N4*, 165.8 ± 4.5 x 88.0 ± 7.8;
474 *N5*, 205.0 ± 12.3 x 108.3 ± 7.4; *N6*, 238.5 ± 17.0 x 114.1 ± 7.7; *C1*, 404.1 ± 35.4 x 112.7 ±
475 11.1; *C2*, 532.2 ± 44.1 x 131.8 ± 8.8; *C3*, 613.6 ± 44.4 x 154.7 ± 10.6; *C4*, 701.5 ± 54.5 x
476 180.6 ± 14.6; *C5*, 811.6 ± 46.7 x 209.6 ± 15.7; *Adult* ♂, 841.7 ± 48.5 x 217.4 ± 14.5; *Adult* ♀,
477 933.0 ± 36.7 x 232.3 ± 10.6. Stage description and criterion for determination are described in
478 Conover (1956) and Sabatini (1990).
479 b. Eggs were stocked at equivalent to 1.0 mL^{-1}.
480 c. 2009 cultures were in a greenhouse with water temperature of 28-30°C; eggs were stocked at
481 equivalent to 1.7 mL^{-1}; one growout tank was used per egg production tank; growout occurred
482 over 8 days.
483 d. 2009 egg production occurred during days 9-15 and stages were not measured.
484 e. Quantity and composition of diet in egg production tanks varied among years. See Section 3.4.
485

Table 2. Production statistics for *Acartia tonsa* Dana (1849) in an intensive, mass production system during 2013-2015. Egg production is the average number of eggs produced daily ± standard deviation (SD) across the total production period for a given year. Egg hatch is the average proportion of eggs hatched ± SD from fresh eggs incubated over a 48-h period. Growout survival is the average survival from stocked N3-N4 nauplii to the adult copepod stage ± SD. n = number of samples used to calculate the average.

Year	Egg production	Hatch (%)	Growout survival (%)
2013	13,702,661±6,194,111 (n=162)	61.6±17.5 (n=40)	31.8±14.1 (n=310)
2014	31,059,479±7,984,523 (n=119)	52.9±14.5 (n=24)	52.3±22.1 (n=238)
2015	21,104,121±7,247,644 (n=123)	32.7±10.0 (n=12)	52.1±22.6 (n=193)
Grand mean (3 years)	21,955,420±8,709,668	49.1±14.8	45.4±11.8


GROWOUT TANK
(ROW 1)
GROWOUT TANK
(ROW 2)
EGG PRODUCTION TANK
EGG COLLECTION
TANK
INCUBATOR TANK
30"
AISLE
1 1/2"
STANDPIPE
CATWALK
1/2" PLATFORM UNDER
TANK FLANGES, TYP
1 1/2"
STANDPIPE
EXTERNAL
STANDPIPE
LIFT
PULLEY
FLEXIBLE
AIR TUBE
AIR STONE, TYP
2" BV,
TYP
4"
EGG
COLLECTION
NET
2" EGG
COLLECTION
PIPE
RIGID AIR
TUBE
DRAIN
SCREEN
AIR
LIFT
WORKING
PLATFORM
1/2" BALL
VALVE
DRAIN TUBE
2" EGG
COLLECTION
PIPE
CONCRETE FLOOR
CAD GRAPHICS BY HTH AQUAMETRICS LLC


Phase I
Adults to chamber
Chamber to clean beaker in 24 hours
1-L master
Chamber
1-L beaker
1-L beaker
Eggs to phase II every 24 h
Phase II
Chamber to clean tank in 24 hours
Adults to chamber in 12 days
30-L tank
Chamber
30-L tank
15-L bucket
Eggs to phase III every 24 h
Phase III
Adults to tank in 12 days
200-L tank
200-L tank
500-L tank; eggs collected continuously
Eggs to phase IV every 24 h
Phase IV
Adults to egg production in 12 days
Egg production, 1,900-L tank; eggs collected continuously
Growout, 900-L tank
Growout, 900-L tank
Eggs to growout every 24 h


Growout tank pairs, x 12
Drain
Egg production tank, x 6
Feed Larvae
Hatch
Refrigerate
Eggs
Drain
Unfiltered reclaim water
Clean reclaim water