From *http://dianawalstad.com*

HATCHING and GROWING BRINE SHRIMP (*Artemia*)[1]

By Diana Walstad
(updated January 2020)

The usual method for feeding fish nauplii (live baby brine shrimp) wastes expensive eggs and requires preparing fresh saltwater every day. More than one fish breeder has decided that feeding live brine shrimp just is not worth the trouble. Maybe not. But before discounting this wonderful live food altogether, see article's new sections on feeding decapsulated eggs, cold-stored shrimp, and harvesting dishes. Fish get the brine shrimp's nutritional value with less hassle.

For old-timers like me who like to do things the hard way, I also describe my procedure for culturing brine shrimp for a few days before feeding them to the fish. The shrimp are still small enough for newborn livebearers, but having been fed, grow 3-4 times their hatched size. They are sought after and devoured eagerly by juveniles and even adult guppies (**Fig 1**). Many a stressed fish will come out of the doldrums to track down these universally delectable critters. Everybody loves shrimp!

Fig 1 Live Brine Shrimp are hors d'oeuvres for these 3-month-old guppies and essential for the rapid growth of fry. I feed baby guppies live brine shrimp 2-3 times per day during their first two weeks.

In this article, I describe many of the factors that increase successful egg hatching. Some like salinity and metal toxicity have rarely been discussed.

However, before moving on to these more complex topics, I would first like to describe basic hatching.

---

[1] Acknowledgements: Gerald Pottern, charter member of the Raleigh Aquarium Society and an authority on live foods, assisted with the original 2017 article. Karen O'Connell (PhD in Genetics and CEO of the brine shrimp service provider Demeter Biosciences) provided further guidance for the 2019 revision.

Abbreviations: hr = hour(s); g = grams; GSL = Great Salt Lake; 24 hr LC50 = Lethal Concentration killing 50% within 24 hours; mg = milligrams; mm = millimeters; ppm = parts per million; ppt = parts per thousand; qt = quart; PUFAs = poly-unsaturated fatty acids; T. = tablespoon (15 ml); tsp = teaspoon (5 ml); wt. = weight

BASIC HATCHING

This section describes a "bare-bones" method for hatching brine shrimp. It is for wary readers that have never hatched eggs before. Necessary, because the Internet contains a deluge of "how-tos" that often require carpentry, air pumps, air stones, and complex tubing arrangements. Before getting tangled up in the minutiae or my complex culturing method, I recommend that readers simply try out the following recipe. It involves hatching eggs in a shallow dish without aeration or paraphernalia. Any rank beginner or older child should be able to do this.

Basic Recipe Ingredients

- shallow bowl
- 1 cup (240 ml) tapwater
- ½ level tsp. (3.5 g) table salt
- pinch of baking soda (raises pH above 8.0)
- 1 drop of aquarium water conditioner
- pinch of brine shrimp eggs
- light

Box shows what's required. Add the salt and baking soda to water and stir. If possible, add an aquarium water conditioner.[2] Pour solution into a shallow dish and set the dish near a good light source. Sprinkle the brine shrimp eggs on the water surface. Try not to jostle the dish such that the eggs get caught up on the rim.

The next day, you should see an orange cloud of hatched shrimp (**Fig 2**).

Fig 2 Basic Hatching
I hatched eggs overnight in two shallow dishes set next to a sunny window. The orange clouds are the nauplii. For the dish on the right, I used *iodized* table salt; left dish, *non-iodized* table salt. Each dish held 1 cup of saltwater.

My basic method is not magical. Indeed, almost any hatching method will hatch *some* eggs. The brine shrimp (*Artemia*) is incredibly adaptive to oxygen, salinity, temperature, ammonia, etc. If the water temperature is 65ºF instead of 82ºF, the eggs will take longer to hatch, but they will still hatch. The main vulnerability to hatching brine shrimp is metal toxicity. If you have never gotten eggs to hatch, this is probably the reason (*See* page 11 for more on metal toxicity).

Scaling up the basic hatching method for a larger quantity often requires an air pump and associated tubing. Large-scale tropical fish breeders often hatch a teaspoon of eggs in a gallon of vigorously aerated saltwater each day.

However, before moving on to air pumps, gang valves, and air-line tubing, hobbyists might want to consider a hatchery dish.

HATCHERY DISH

A hatchery dish[3] makes hatching eggs for an occasional fish spawn very convenient. It allows small, frequent feedings of nauplii soon after they hatch. This solves the harvest timing problem where 24 hr is too soon and 48 hr is too late (*See* page 10).

---

[2] The conditioner neutralize any heavy metals (e.g., copper, zinc) in tapwater. Most aquarium water conditioners contain chelators (e.g., EDTA) that will bind and detoxify heavy metals. Another option to counteract heavy metals is to substitute distilled water or aged aquarium water for the tapwater.

[3] I bought my hatchery dish from https://www.brineshrimpdirect.com/, but they are also available on Amazon.com.

Because the dish is shallow and flat, no aeration is required. Moreover, the clever design greatly simplifies harvesting by automatically separating nauplii from the egg shells and unhatched eggs.

One can follow the manufacturer's instructions, but those instructions make no adjustments for alkalinity and metal toxicity. Hobbyists who prepare their saltwater from hard, alkaline tapwater may get much better harvests than those with soft acidic water containing heavy metals.

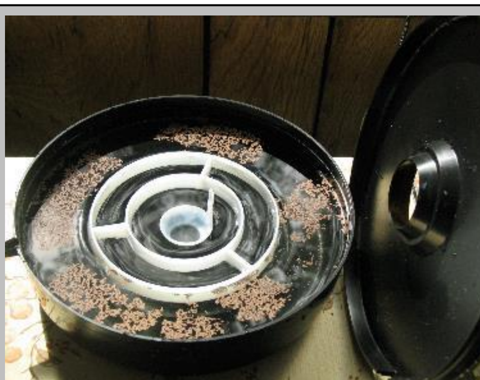

Fig 3 Seeded Eggs. White rings keep the eggs walled off from the center. Nauplii must swim under the rings to reach the center cup, leaving shells and unhatched eggs behind.

Nauplii swim towards the light at the lid's center hole and swim over the top rim of the harvesting cup.

Fig 4 Harvest Cup

A day after seeding, I collect freshly hatched nauplii several times during the day. The cup has a mesh bottom that makes it easy to drain off the saltwater.

I add 1 & 1/2 tsp table salt, a pinch of baking soda, and 3 drops of an aquarium water conditioner to 3 cups of tapwater. I then stir until the salt goes into solution. This produces a low salinity (13 ppt) that speeds up egg hydration and possibly hatching.

I place the dish in its final resting place before pouring in the saltwater. (While the tops of the white rings should be *above* the water, the rim of the harvest cup should be *below* the water.)

The manufacturer's little cup (included with the kit) holds slightly less than 1/8 tsp of eggs. I use it to sprinkle eggs around the outer edge (**Fig 3**). Then, I cover the dish. Eggs begin hatching for me at 24 hr at a room temperature of 65-70ºF. Most hatching is over by 48 hr. I like to seed in the early morning so that I can harvest nauplii throughout the next day. Then, on morning of the following day, I collect all the late hatchers and set up the dish for a new batch.

Harvesting consists of simply pulling up the harvesting cup, draining out the saltwater, and blotting the cup's bottom on a paper towel (**Fig 4**). Then, I just dip the cup directly into the fish tank. So easy!

Surprisingly, I found that the dish must be thoroughly cleaned or perhaps sterilized in between hatches. Several hobbyists have reported an excellent first hatch with their brand new dishes, but subsequent ones were poor. Indeed, I was getting variable nauplii yields, apparently dependent on the vigor of my dish cleaning. Now, I sterilize the dish briefly in between hatches and get consistently good results. [4]

Any excess nauplii are easily stored. I transfer them—along with a little of their saltwater—into a small container to be refrigerated. Refrigerated nauplii will stay alive for 1-2 days.

[4] After sponge cleaning the dish, I add a sterilizing solution (1 tablespoon of chlorox in 3 cups warm water) to the dry dish containing rings and harvest cup. I let dish sit 10 minutes while I prepare a fresh salt solution. Then, I rinse out the chlorox solution and set up the dish as usual.

COLD STORAGE

Harvested nauplii can be refrigerator-stored in a small volume of their saltwater for a couple days. Cold-stored nauplii allows more frequent feedings of a high-quality food.

Refrigeration dramatically decreases the shrimps' metabolic rate such that shrimp do not progress to the Instar II molt stage, even after 48 hr [1]. They lose almost none of their food value. For example, nauplii kept at 4°C for 24 hr lost only 2.5% of their dry weight; those kept at 25°C lost 30% of their dry wt. and 26% of their total lipids [1, 2]. Nauplii stored at 4°C for 24-48 hr were just as good a live food source as freshly hatched nauplii—in terms of the survival, growth, and reproductive development of mysid juveniles (an invertebrate called the Opossum Shrimp) [1].

Live nauplii can be refrigerated at densities of 2,000 to 8,000 per ml of saltwater. Higher densities probably require aeration [1]. I calculated that 1/8 tsp of eggs produces about 60,000 nauplii. I store harvested nauplii in about ¼ cup (59 ml), which provides a low density of only 1,000 nauplii/ml. Before feeding the fish, I rinse the nauplii with tapwater.

DECAPSULATED EGGS

Before embarking on any procedures for hatching eggs or culturing nauplii as live food for fish, I would urge readers to consider "DC-eggs" (decapsulated brine shrimp eggs). DC-eggs, particularly the dry form, are a very convenient and nutritious food for fry and young fish. Moreover, DC-eggs are small enough to be eaten by the tiny fry of many egg-laying fish. (Nauplii are 0.4 mm in size; DC-eggs, 0.2 mm [3].)

DC-eggs, which have had their shells removed by chemical treatment, rival fresh nauplii as a food source. They contain all the embryo's concentrated nutrients, plus valuable fatty acids.[5] Because the nauplii's hatching and emergence from the shell requires considerable metabolic energy, DC-eggs contain 30% more energy than freshly hatched nauplii [3].

DC-eggs are sold as wet or dry. Dry DC-eggs have been shown to be a better fish food than wet ones [4, 5]. Wet DC-eggs, sometimes sold as "E-Z Hatch" eggs, are perishable and not as nutritious as dry DC-eggs. In my opinion, there is little point to hatching DC-eggs.

Dry DC-eggs have been shown repeatedly to support faster fish growth than nauplii. For example, investigators [4] put 4 groups of guppy fry (one-day-old, 50 fry/group) on different diets. Groups were fed exclusively one of the foods shown in **Table 1** for 40 days. (Dry DC-eggs were simply wet DC-eggs that had been dried for 24 hr at 35°C.) Results show that the growth of fish fed dry DC-eggs increased from 1 mg/fish to 150 mg/fish. This 150-fold increase was significantly better than that for fish fed the other three foods.

Table 1 Effect of Various Foods on Guppy Growth [4]

Fish Diet of:	Final Fish Dry Wt. (mg)
Dry DC-eggs	150 (±36)**a**
Wet DC-eggs	50 (±6)**c**
Nauplii	94 (±6)**b**
Dry food	42 (±7)**c**

Average starting dry wt. for each fish was 1 mg. Results show mean and standard deviation. Differing **letters** indicate values that differed significantly ($p<0.05$); same letter means difference was not significant.

[5] Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are often represented by DHA (Docosahexaenoic Acid or 22:6*n*-3) and EPA (Eicosapentaenoic acid or 20:5*n*-3). They are components of the "Fish Oil/Omega-3" nutritional supplements that people take. While vital for marine larva, they are not for freshwater organisms, which can sysnthesize their own long-chain fatty acids [6].

Dry DC-eggs also performed well in another study [5] comparing the growth, etc. of guppy fry. Final dry weights of 28-day-old juveniles were: 37 mg for dry DC-eggs; 25 mg for wet DC-eggs; and 24 mg for nauplii. (Dry DC-eggs were significantly better than the other 2 foods.) In contrast, there was no significant difference between these 3 foods on the growth of *older* guppies.

Investigators [5] noted that the feeding behavior of fish could influence results. For example, the fry of Black Neon Tetras took a week to adapt to wet DC-eggs, resulting in some death initially. Because the eggs quickly sank and the fry did not feed off the bottom, some fry starved.

In a study [7] of African catfish, growth rates and survival were significantly better for fry raised on dry DC-eggs than on nauplii or on a commercial diet. For example, survival at 4 weeks was 85% for nauplii, 93% for dry DC-eggs and 33% for a commercial dry food.

However, live brine shrimp are still good food. Combining nauplii with a dry food appears to work especially well. Investigators [8] testing the growth of juvenile Zebrafish fed 3 different diets for 2 months reported the following fish dry wt. results: 237 mg for dry DC-egg; 106 mg for nauplii; and 232 mg for nauplii + a commercial pellet food. There was no significant difference in growth results for dry DC-eggs versus nauplii + pellets.

Dry DC-eggs, where the brine shrimp embryo is dead and not metabolizing away valuable nutrients, are more nutritious than wet DC-eggs, even when freshly prepared [4].

Occasionally, I feed my guppies purchased DC-eggs. Floating on the surface and sold as "non-hatching," I know that these eggs are the desired dry form of DC-eggs.

MY CULTURING METHOD

My culturing method consists of growing shrimp out for a few days before feeding them to the fish.

The advantages are that I get larger shrimp (**Fig 5**). And because all eggs are given plenty of time to hatch, less eggs are wasted.

I provide food for the brine shrimp when starting out with freshly prepared saltwater. Afterwards, they can feed on microorganisms growing in the culture bottle. Within a couple weeks, the reused saltwater will become colonized by bacteria and micro-algae, possibly from the shrimps' native habitat.[6]

The bottle with the oldest culture (3-day-old) is sufficient to feed a couple hundred fry and juvenile guppies. (Adult guppies also go wild for these shrimp.) I start a new batch of eggs each day, but the system is flexible. For example, I can store harvested shrimp in the refrigerator for a later feeding.

Fig 5 Three-Day-Old Brine Shrimp are considerably larger than unhatched eggs (dark dots in photo).

[6] Investigators [9] showed that 1 gram of San Francisco Bay Brand eggs, freshly obtained from an air-tight can contained 230,000 bacteria, about 1 bacterium per egg. Investigators also showed that the bacteria originated from the egg shell's surface (i.e., not from inside the egg). These saltwater-adapted bacteria would not carry disease to freshwater aquariums, because they could not survive the osmotic change.

For simply hatching shrimp, one can work with a high egg density (1 tsp eggs per 2-4 qt of saltwater). However, my method involves setting up an ecosystem and reusing the salt water. Thus, it is very important not to overpopulate the bottles. Adding any more eggs than 1/8 tsp/2 qt can easily cause a "culture collapse" (i.e., shrimp begin dying, fouling the water, and causing ever more death).

After harvesting, I set up the bottle with the filtered and reused saltwater, but I wait a day before adding eggs. This "rest day" seems to result in better hatches.

I reuse saltwater for many months. I noticed that the water does gradually accumulate ammonia, but it does not seem to greatly affect the harvests.[7]

HATCHING FACTORS

<u>Eggs:</u> I have gotten essentially the same results using eggs from either SFB (San Francisco Bay) or the GSL (Great Salt Lake).[8] (For feeding delicate *marine* animals, I would only use eggs from SFB.[9]) Brine shrimp eggs that I bought in 1990 and stored in the freezer still hatched after 25 years.

A small amount of eggs will feed lots of fry. The number of nauplii produced per gram of cysts varies between <100,000 and >300,000 [11]. One gram (~1/2 tsp) of eggs from the GSL contains 280,000 eggs and reportedly will produce—assuming a 90% hatch rate—about 250,000 nauplii [12].

I add 1/8 level tsp of brine shrimp eggs to each bottle containing 2 qt (~2 liters) of saltwater. This volume of eggs with its 95% hatch rate produces about 60,000 nauplii, enough for a generous daily feeding of 200 guppy fry and juveniles.

Fig 6 My Brine Shrimp Setup consists of four ~2 qt bottles positioned under a clamp light with a 13 watt CFL bulb. Aeration consists of very gentle bubbling.

<u>Bottles:</u> I slice off the tops of ordinary, 2 qt plastic bottles. I keep 4 bottles going all the time (**Fig 6)**. Bottles contain either: (1) no eggs for a "rest day"; (2) 24 hr cultures; (3) 48 hr cultures; or (4) 72 hr cultures. Each day, I harvest the bottle with the 72 hr culture.

I cover the bottles with pieces of saran wrap to minimize water evaporation. When water levels gets low, I just add tapwater.

<u>Food:</u> I feed my brine shrimp, especially after a new setup. [The newly prepared saltwater contains no food, because it has not yet been colonized by microorganisms (bacteria and micro-algae).]

---

[7] Brine shrimp are much more resistant to ammonia than fish; the lethal concentration (24 hr LC50) for newly hatched *Artemia* is 840 ppm [10]. Indeed, I have measured 50 ppm NH_3 in bottles producing excellent harvests.

[8] Eggs and nauplii from the GSL are slightly larger than those form SFB. GSL egg diameter and nauplii length are 0.24 mm and 0.48 mm, respectively; SFB, 0.22 mm and 0.43 mm [6].

[9] GSL eggs and nauplii are deficient in vital fatty acids (i.e., PUFAs) compared to those from SFB. For feeding marine larva, this distinction is critical, but it is not for freshwater fish [6].

Fig 7 Feeding Shrimp Spirulina algae powder. (I actually add about half of what is shown here.)

For growing out shrimp, people have used the following foods: Spirulina algae powder, yeast, boiled egg yolk, rice bran, and powdered fish food. Foods high in lipid (i.e., fats) and protein are recommended for young brine shrimp. Yeast is actually a relatively poor food, because its tough cell wall proteins are hard for shrimp to digest [13]. Food should be small particles, not chunks. (Shrimp are filter feeders; they cannot ingest large particles.)

For feeding, I sprinkle about 25 mg of finely powdered Spirulina algae once a day onto the water surface (**Fig 7**).

Whether to feed or not depends on the bottle's status. If the shrimp are thriving, they will eat up everything in the bottle and clear the water. In this situation, I would feed the shrimp. In contrast, if the eggs or the shrimp encounter problems and start dying, they will release nutrients into the water and stimulate bacterial growth. The water will turn cloudy. Adding food will only foul the water and worsen the problem.

<u>Aeration, Oxygen, Iron and Shrimp Color:</u> Most hatching setups show vigorous aeration, but this has drawbacks for culturing shrimp. Brine shrimp—like newborn fish—will lose energy and die if they are constantly fighting a cyclonic current.

In my setups, gentle aeration seems to work, perhaps because the bottles contain iron, are not over-populated, and contain micro-algae that help oxygenate the water. I regulate the bubbling such that the water surface is barely disturbed. No foam is generated and eggs/nauplii are not thrown up onto the sides. Large air bubbles are released from a glass tube at a rate of about 1-2 per second. The glass tube (5 mm O.D. X 12 inches long) is attached to airline tubing, a 4-outlet gang-valve, and a small, inexpensive air-pump.

High oxygen levels (6-8 ppm) are optimal, but brine shrimp are adapted to low oxygen. In nature, they have been found alive at very low oxygen (1 ppm) [14]. One investigator reported a 91% survival rate for brine shrimp cultured at 3.4 ppm O_2 when supplemented with iron (FeEDTA); in contrast, control shrimp with no iron had a 70% survival rate. [15]. Under oxygen stress, the shrimp use iron to synthesize increased hemoglobin. Hemoglobin, the oxygen-transporting molecule, is a major protein for shrimp, constituting ~20% of its soluble protein [16]. Iron-rich hemoglobin gives shrimp its characteristic color ranging from pale pink to red [15] (**Fig 8**).

Fig 8 Ideal Harvest

This harvest at 3 days shows a pulsating orange cloud of healthy shrimp.

<u>Temperature</u>: Hobbyists are frequently advised to use higher temperatures (>82°F) to speed up the hatching process for the usual 24 hr harvest. However, many slow-hatching eggs still get thrown out with a 24 hr harvest. I hatch eggs at my “house temperature,” which ranges from 65°F to 82°F. Because I wait 3 days before harvesting, all viable eggs are given time to hatch.

<u>Light:</u> Hatching is purportedly triggered by intense light [2]. Although some fish breeders recommend continuous light, investigators [17] showed no difference in hatching efficiency using either a 2, 12, or 24 hr photoperiod. Likewise, light seems to have no effect using hatchery dishes. (Eggs hatch well whether the dish is covered or not.) For shrimp culturing, however, I provide 12 hr of light per day to encourage micro-algae.

<u>Salt</u>: Ordinary table salt works fine for basic hatching. I use whatever salt is convenient.[10] Despite dire warnings to the contrary, I was unable to detect any difference in hatching using iodized salt versus non-iodized salt (*See* Fig 2).

For culturing shrimp, though, I think it is important to include marine salts designed for saltwater tanks. Marine salts provide the minerals (e.g., potassium) and micronutrients [e.g., Fe (iron)] required by both shrimp and micro-algae. **Table 2** lists those in Instant Ocean®. Moreover, commercial preparations automatically raise and buffer the pH. Tapwater that is soft may not have the minerals and alkaline pH required to sustain a brine shrimp culture.

Table 2 Composition of Instant Ocean® [22]
prepared for ocean’s salinity (~35 ppt)

Ingredient	**PPM** (or mg/l)
Cl^-	18,400
Na^+	10,200
SO_4^{-2}	2,500
Mg^{+2}	1,200
K^+	370
Ca^{+2}	370
Bicarbonate	140
Borate	25
Phosphate	1
Mn^{+3}	1
Molybdate	0.7
Iodine	0.07
Zn^{+2}	0.02
Fe^{+3}	0.01
Cu^{+2}	0.003

<u>Salinity:</u> Brine shrimp (*Artemia*) possess the best osmoregulatory system in the animal kingdom [14] and can tolerate salinities ranging from 1 to 200 ppt [18]. This is essential to their survival in saltwater lakes where the salinity is high and fluctuates seasonally. Salinity in the GSL, the origin of most eggs sold, often ranges from 50 to 270 ppt [19]. Highly saline waters are an environmental niche for a very tasty animal that has no protective armor. Fish and other potential predators cannot survive at the ultra-high salinities where brine shrimp are found.

I like to use lower salinities (10-20 ppt) for two reasons.

Firstly, the initial step in hatching is the egg’s absorption of water. Hydration becomes increasingly difficult—because of osmotic forces—at higher salinities. Eggs cannot hydrate, and therefore hatch, at salinities above 85 ppt [14].

Secondly, in contrast to adult shrimp, nauplii and young shrimp are stressed metabolically by high salinity [18]. As the embryo emerges from its protective shell, it depends on a temporary neck gland to excrete excess salt [20, 21]. The development of the adult’s fully functioning—and more effective—salt excretion system, mostly in the coxal gills, takes 4-7 days [20].

---

[10] Readers should note that the same volume of table salt contains more actual salt (NaCl) than large granulated salts. For example, 1 T. of table salt is equivalent (weight-wise) to 1.25 T. of Kosher salt.

Brine shrimp are in osmotic balance in ~30% saltwater (~10 ppt salinity) [20] where the salinity of their internal fluid (hemolymph) better matches that of the outside media. If the water is more saline, the shrimp must get rid of the excess salt via energy-requiring pumps.[11] If salinity is less than 10 ppt, the shrimp will have to pump in salt. {Nauplii die after about an hour in freshwater (0 ppt) [3].}

Pumping salt requires energy, energy that could be used for the nauplii's survival and growth. Thus, I started getting much better harvests with a lower salinity (19 ppt) than one only moderately higher (28 ppt). High salinities of ≥40 ppt produced very poor harvests—unhatched eggs, many dead shrimp, cloudy water, and debris.

To measure salinity (ppt), I use a simple floating aquarium hydrometer. I convert the specific gravity readings (g/ml) to salinity (ppt) using an on-line calculator [23].

For my culture method, I now use a relatively low salinity of 13-17 ppt (density 1.010 to 1.013 g/ml). My saltwater recipe (described below) produces it.

<u>Saltwater:</u> I prepare concentrated (2 X) salt water by adding ¼ cup of Instant Ocean® salt, ¼ cup of table salt and enough tap water to fill a gallon jug. I bubble air in the jug to dissolve the salts. I dilute this solution 1:1 in the culture bottle and then add ~8 drops (0.4 ml) of an ordinary aquarium water conditioner. The resulting saltwater has a final salinity of 16 ppt.

I use table salt simply to conserve the more expensive marine salts. I reuse saltwater in the bottles, occasionally topping off with tap water, as long as harvests are good. When yields drop off, usually after a few months, I clean the bottles and start over with fresh saltwater.

<u>Aquarium Water Conditioner</u>: Most AWC (aquarium water conditioners) neutralize heavy metals along with other toxins. The label should say so. I assume that AWCs contain a chelator (e.g., EDTA) that will bind, and thus detoxify, heavy metals.

Brine shrimp hatching is exquisitely sensitive to heavy metals, so I use an AWC whenever I prepare fresh saltwater. Specifically, I add 1 drop (0.05 ml) of Instant Ocean's Marine Conditioner per cup of saltwater. [This is 38% more than the manufacturer's recommended dosage of 5 ml per 10 gal, but AWC is virtually harmless, so a little extra will not hurt.] Thus, the 2 qt bottles get 8 drops (0.4 ml) and the hatchery dish with its 3 cups saltwater gets 3 drops (0.15 ml). AWC should protect shrimp from any traces of metals in tapwater. It will also protect shrimp from metal micronutrients (e.g., copper, zinc, manganese) contained in marine salt preparations.

<u>Harvesting:</u> For harvesting, I pour the entire bottle contents through a very fine net into a pitcher. (Nets fine enough for sieving out nauplii are hard to find; the ones advertised often turn out to be for adult brine shrimp. In a pinch, I fit a cloth square from an old bed sheet into a fish net.)

---

[11]Excess sodium is removed via the "sodium pump" (Na^+/K^+-ATP-ase) where for every ATP (i.e., energy) consumed, the cell excretes three Na^+ ions and brings in two K^+ ions. Chloride removal also requires energy. This salt excretion is located in "Chloride Cells" similar to those found in marine fish [20]. Mg^{++} and K^+ are required in the media for osmoregulation [20], which is why I recommend marine salt, which contains both minerals, for preparing saltwater.

Fig 9 Shells float to the surface; nauplii and any unhatched eggs sink to the bottom.

I transfer the shrimp with tapwater into a cup. To get rid of the shells[12] I pour the cup's contents into a tall, narrow cylinder [**Fig 9**]. After about 5-10 minutes, the empty egg shells float to the surface where I can pour them off. I like to see a lot of shells at the top, very few unhatched eggs at the bottom, and a big orange cloud of nauplii near the bottom.

I pour the shrimp and any unhatched eggs back into the cup for feeding the fish. Sometimes, I feed the fish half in the morning and put the rest in the refrigerator with a little saltwater for a later feeding.

After harvesting, I clean the inside of the bottles with a long-handled brush, scraping off attached algae and biofilm coatings. Inevitably, this reduces the nutrient load in the bottle. I pour the old filtered saltwater back into the cleaned bottle and set the bottle up with light and aeration. However, I wait one day before adding eggs. Giving the bottle a rest day seems to result in better harvests.

<u>Timing the Harvest:</u>
Aquarium hobbyists are instructed to use the nauplii soon after hatching before they lose nutritional value. The problem is that many eggs take longer than 24 hr to hatch, so those eggs are wasted. Investigators [25] found that only about 50% of the eggs hatched within 24 hr, with some eggs taking 2-3 days to hatch (**Fig 10**).

However, if one waits for a complete hatch, many of the older shrimp will have lost their food value. With the conventional method of harvesting at 24 hr, it is virtually impossible to get an optimal harvest.

Culturing the shrimp for a few days after hatching solves the harvest timing problem. Moreover, the *overall* nutritional value of brine shrimp increases once the brine shrimp—at about 8 hr—start feeding. For example, the average protein concentration was found to increase from 42% in nauplii to 60% in adults [14].

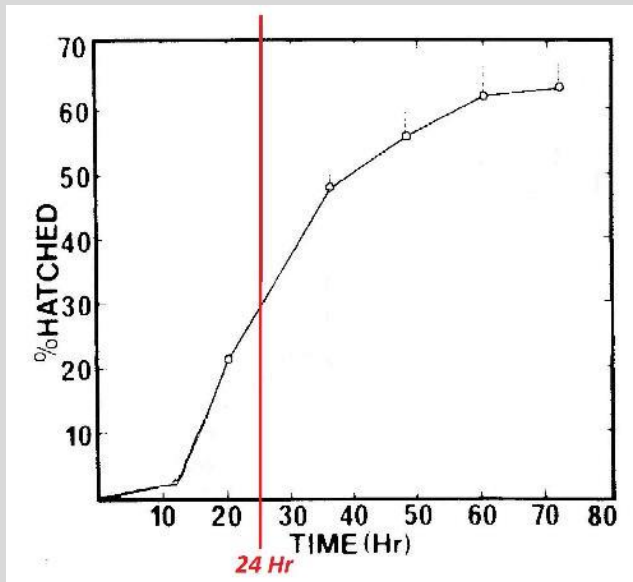

Fig 10 Egg Hatching over Time [25]

At 24 hr, the hatch rate shown was ~30%. By 60 hr, hatching had almost reached its maximum rate of ~60%. Thus, at 24 hr, only half (50%) of the eggs had hatched (30% ÷ 60% = ½). Experiments were conducted at 28°C (82.4°F) using artificial seawataer, which typically means 35 ppt. I think that the slow hatching shown here—despite the warm temperature—is due to the relatively high salinity.

---

[12] Because empty cyst shells are undigestible, they may cause death or poor growth when ingested indiscrimminately by delicate fry [6]. While they have not caused obvious problems in my guppy fry, one investigator [24] noted that half of 7-day-old fry of Striped Bass contained only egg shells and died, while those that contained nauplii survived.

Investigators [26] showed that shrimp populations—whether starved or fed—began declining 3-5 days after hatching [**Fig 11]**. Once shrimp die, they foul the water. My harvest timing represents a "sweet spot." The 3 days of culturing allows the hatching of all eggs and the growth of nauplii, but the harvest comes before the shrimp start dying at 4 days.

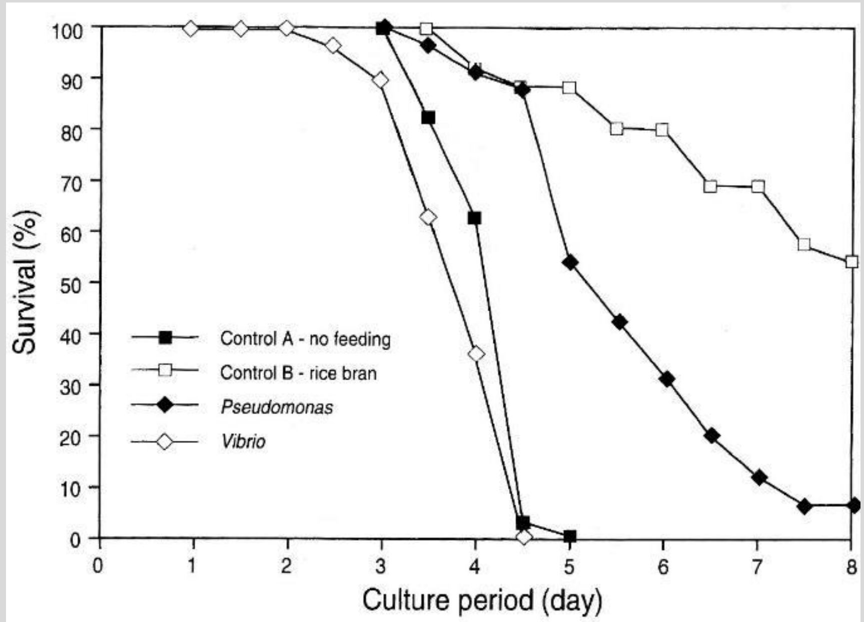

Fig 11 Effect of Food Types on Shrimp Survival [26]
Unfed shrimp ('Control A') start dying at 3-4 days. Those fed rice bran ('Control B') do the best; over 50% are still alive at 8 days. Next best food here is 'Pseudomonas' bacteria. In contrast, shrimp fed on 'Vibrio' bacteria are worse off than starved shrimp.

<u>Metal Toxicity</u>: Metal toxicity probably explains why some hobbyists never get a decent hatch. These hobbyists should try using an aquarium water conditioner. Or they can use aged aquarium water from an established tank for preparing their saltwater. {I found that aged water from my planted tanks has enough DOC (dissolved organic carbon) to easily prevent metal toxicity [27].}

Zinc and copper are occasionally present in tapwater at levels that—while not harmful to humans—are toxic to aquatic animals. These metals leach via plumbing (e.g., copper pipes) into the water supply.

The brine shrimp embryo is exquisitely vulnerable to heavy metals as it emerges from its protective cyst. The smallest traces will kill it. **Fig 12** shows zinc's inhibition of brine shrimp egg hatching. The zinc concentration blocking 50% of hatching was found to be only 0.07 ppm [25]. [13] The investigators also showed that copper was 10 times more toxic than zinc.

My well water contains 0.8 ppm zinc, possibly from exposure to galvanized metal from the well's pumping system. Unsurprisingly, eggs will not hatch in freshly prepared saltwater unless I add a water conditioner.

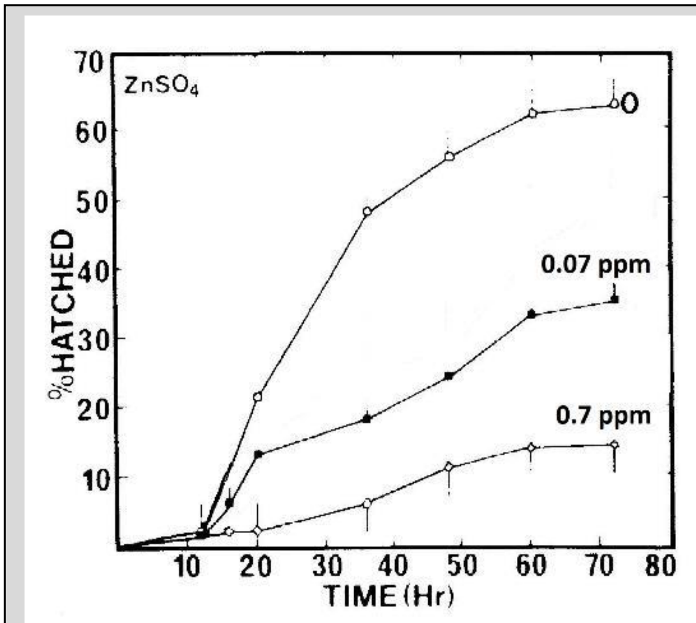

Fig 12 Effect of Zinc on Egg Hatching [25]

[13] Nauplii and adult brine shrimp are much less sensitive to heavy metals than embryos [25]. For example with nauplii, the 24 hr LC50s for zinc and copper were 18 and 20 ppm, respectively [28].

DISCUSSION

Hatching brine shrimp eggs (or "cysts") in clean saltwater is fairly straight-forward. Feeding fish purchased DC (decapsulated) eggs is as easy as feeding flake food. Many studies have shown that dry DC-eggs are more nutritious—as a sole diet—for young fish than nauplii. These eggs, combined with a commercial diet of pellets, are probably all that fish require for good growth. That said, I am still hatching brine shrimp eggs every day.

Raising nauplii to adulthood is the ultimate challenge. I succeeded once 20 years ago in raising about 50 shrimp to reproductive adulthood in a liter bottle.[14] Afterwards, I decided that raising nauplii to adulthood was fascinating but impractical for simply feeding fish.

My method of culturing shrimp for a few days has worked well for many years. **Table 3** shows some key factors that are often not discussed by hobbyists. However, there are many more, because the bottle's ecosystem is very complex. It represents an interplay of salinity, oxygen, iron, micro-algae, bacteria, light, etc.

Tabel 3 Some Key Factors for *Culturing* Brine Shrimp

- Use an aquarium water conditioner to counteract any metal toxicity in tapwater
- Use gentle aeration and relatively slow water movement
- Use low salinity (10- 20 ppt)
- Include marine salt, which contains nutrients Ca, Fe, K, etc
- Don't overpopulate the bottle with eggs

For a long time, I assumed that the more algae the better. So I did not clean the bottles, allowing the bottles to accumulate attached algae and dark floating clumps. Shrimp yields declined to zero. Perhaps the bottles contained cyanobacteria (i.e., "Blue-green algae")? (Many cyanobacteria release toxins that kill zooplankton [30].) Or they accumulated harmful bacteria? Since I started cleaning the bottles in between harvests, recycled saltwater lasted longer. Also, harvest dish results (*See* page 3) support the benefits of cleaning.

Bacteria can be helpful or harmful. They can be live food for shrimp and recycle organic waste. However, not all bacteria types are equally good food. Fig 11 (*See* p. 11) shows that shrimp survived longer when fed exclusively on Pseudomonad bacteria than Vibrio bacteria. In a separate study, investigators showed significantly greater survival for shrimp cultured in old, reused shrimp water with its associated bacteria than in seawater with biofilter bacteria [31].

In my bottles, iron helps brine shrimp survive in a crowded, low-oxygen environment. Marine salts and the initial feeding of Spirulina algae provide iron. The decomposition of cyst shells, composed of iron rich material [15], probably provides iron in older bottle cultures.

Readers should not expect to get a perfect harvest every time. Each bottle contains its own ecosystem. Bottles can go downhill fast, but they can also recover quickly. Bacteria and micro-algae have population doubling times of minutes and hours. I have had cultures crash after a long electrical power outage. Afterwards, I just filtered out the debris and reused the saltwater as usual.

My four bottle system allows me to experiment with culture conditions. I can easily test a variable in one bottle and see how it compares with the other bottles.

---

[14] Adult brine shrimp will not survive at the high densities used for hatching. The maximum density under the best natural conditions (summer-time in Mono Lake, Calif.) is 6-8 adults per liter [29]. The density can be increased to 1,000/liter under optimal lab conditions [15]. My culture bottles contain about 30,000 nauplii/liter, far too crowded to allow growth to adulthood.

My culture bottles are not completely balanced. Nutrient input from egg and food additions seems to be higher than its removal by shrimp harvesting and bottle cleaning. I base this on ammonia levels that gradually rise. (Fortunately, ammonia is not that toxic to shrimp. *See* Footnote #7)

Brine shrimp have long been recognized as a superior food for fry and young aquarium fish. Due to their value as a live food for farmed fish and marine invertebrates, the demand (and high price) for brine shrimp eggs is predicted to continue [32]. Aquaculturists the world over have not yet found a better food source for raising their farmed lobsters, scallops, crabs, tiger shrimp, and ornamental fish.

Unlike other foods, one can hatch stored eggs when convenient; no need to keep a live culture going when there are no fish to feed. Dry decapsulated eggs represent the ultimate in nutrition and convenience. I believe that they deserve wider acceptance by tropical fish keepers.

Aquarium hobbyists that choose to hatch brine shrimp eggs can help themselves by learning how to optimize hatching conditions. Better this than simply blaming poor hatches on "bad eggs." Allowing the shrimp to grow 2-3 days is admittedly tedious but worth considering. For me, it adds to the fun and challenge of keeping aquarium fish.

REFERENCES

1. Leger, P *et al*. 1983. International study on *Artemia* XXIV. Cold storage of live *Artemia* nauplii from various geographical sources: Potentials and limits in aquaculture. *Aquacultural Engineering* 2: 69-78.
2. Sorgeloos P *et al*. 2001. Use of brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture* 200: 147-159.
3. Malla S and Banik S. 2015 Production and application of live food organisms for freshwater ornamental fish larviculture. *Adv. Biores*. 6: 159-167.
4. Baboli MJ *et a*l. 2012. The potential dried decapsulated *Artemia urmiana* cysts on growth and survival guppy fry, *Poecilia reticulata*. *Int. J Agr Crop Sci* 4: 1391-1393.
5. Lim IC *et al*. 2002. Use of decapsulated *Artemia* cysts in ornamental fish culture. *Aquaculture Research* 33: 575-589.
6. Leger P *et al*. 1986. The use and nutritional value of *Artemia* as a food cource. *Oceanogr Mar Biol Ann Rev* 24: 521-623.
7. Adewolu MA *et al*. 2009. Growth performance and survival of hybrid African catfish larvae (*Clarias gariepinus* X *Heterobranchus bidorsalis*) fed on different diets. *The Zoologist* 7: 45-51.
8. Tye M *et al*. 2014. Nonhatching decapsulated *Artemia* cysts as a replacement to *Artemia* nauplii in juvenile and adult zebrafish culture. *Zebrafish* DOI: 0.1089.
9. Austin B and Allen DA. 1982. Microbiology of laboratory-hatched brine shrimp (*Artemia*). *Aquaculture* 26: 369-383.
10. Chen J-C *et al*. 1989. Joint action of ammonia and nitrite on *Artemia* nauplii. *Aquaculture* 77: 329-36.
11. Bengston DA, Leger P and P Sorgeloos. 1991. Use of *Artemia* as a food source in aquaculture. In: Browne RA, Sorgeloos P and Trotman CNA (Eds.), *Artemia Biology,* CRC Press (Boca Raton, FL), pp.255-285.
12. https://saltlakebrineshrimp.com/hatching-brine-shrimp/ (Accessed October 2019)
13. Marques A *et al*. 2005. Effects of bacteria on *Artemia franciscana* cultured in different gnotobiotic environments. *Appl. Environ. Microbiol*. 71: 4307-4317.
14. Persoone G and Sorgeloos P. 1980. General aspects of the ecology and biogeography of *Artemia*. In: Persoone G *et al* (Eds). *The Brine Shrimp Artemia* (Vol 3). Universa Press (Wetteren, Belguim), p 36.
15. Versichele D and Sorgeloos P. 1980. Controlled production of *Artemia* cysts in batch cultures. In: Persoone G *et al* (Eds). *The Brine Shrimp Artemia* (Vol 3). Universa Press (Wetteren, Belguim), pp 231-246.
16. Moens *et al*. 1989. The hemoglobins of *Artemia*; oxygen carriers with multidomain globin chains. In: MacRae TH *et al* (Eds). *Biochemistry and Cell Biology of Artemia.* CRC Press (Boca Raton FL).
17. Asil SM *et al*. 2012. The influence of light (intensity and duration) on the cysts hatching parameters and nauplii growth of *Artemia urmiana* (Günther 1890). *World J. Zoology* 1:60-64.

18. D'Agostino A. 1980. Physiology and nutrition of *Artemia*. In: Persoone G, Sorgeloos P, Roels O and Jaspers E (Eds). *The Brine Shrimp Artemia* (Vol 2). Universa Press (Wetteren, Belguim), pp 55-82.
19. Wikipedia (accessed October 2019)
20. Holliday CW *et al*. 1990. Salinity-induced changes in branchial Na^+/K^+-ATPase activity and transepithelial potential difference in the brine shrimp *Artemia salina*. *J. Exp. Biol*. 151: 279-296.
21. Charmantier G and M Charmantier-Daures. 2001. Ontogeny of osmoregulationin in crustaceans: The embryonic phase. *Amer. Zool*. 41: 1078-1089.
22. Trieff NM. 1980. Toxicity of metals, oils and other organics on *Artemia*. In: Persoone G *et al* (Eds). *The Brine Shrimp Artemia* (Vol 1). Universa Press (Wetteren, Belguim), pp 253-262.
23. https://www.hamzasreef.com/Contents/Calculators/SalinityConversion.php/
24. Karen O'Connell (Personal communication, October 2019)
25. MacRae TH and Pandey AS. 1991. Effects of metals on the early life stages of the brine shrimp, *Artemia*: A developmental toxicity assay. *Arch. Environ. Contam. Toxicol*. 20: 247-52.
26. Gorospe J and Nakamura K. 1996. Associated bacterial microflora in *Artemia*-rice bran culture. *Israeli J. Aquaculture* 48: 99-107.
27. Walstad, Diana. 2013. *Ecology of the Planted Aquarium* (3rd Edition). Echinodorus Publishing (Chapel Hill, NC), pp 14-16.
28. Gajbhiye SN and R Hirota. 1990. Toxicity of heavy metals to brine shrimp *Artemia*. *J. Indian Fisheries Assoc*. 20: 43-40
29. Mohebbi F. 2010. The brine shrimp *Artemia* and hypersaline environments microalgal composition: a mutual interaction (Review). *Inter. J. Aquat. Sci*. 1: 19-27
30. Gross EM. 1999. Allelopathy in benthic and littoral areas: Case studies on allelochemicals from benthic cyanobacteria and submersed macrophytes. In: Dakshini, I *et al* (Eds), *Principles and Practices in Plant Ecology: Allelochemical Interactions*, pp. 179-199.
31. Verschuere L *et al*. 1999. Microbial control of the culture of *Artemia* juveniles through preemptive colonization by selected bacterial strains. *Appl. Environ. Microbiol.* 65: 2527-33.
32. Lavens P and Sorgeloos P. 2000. The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture* 181: 397-40.

Diana Walstad is the author of *Ecology of the Planted Aquarium* (2013). For articles and more information about her books, see: http://dianawalstad.com