GREAT SALT LAKE ARTEMIA

Artemia Guard Overview

Great Salt Lake Artemia ships only after rigorous Quality Assurance (QA) testing. However, once Artemia is used in the environment and the natural hatching process begins microbiological contamination is introduced.

Microbiological contamination is everywhere in the hatchery and needs to be kept under control to minimize the opportunity for pathogens to enter the culture system. **Artemia Guard** is a great solution for managing this bacterial load.

Generally, the gut of newly hatched larvae contains very few bacteria but is rapidly colonizing during the first few days. Once feeding begins, the intestinal microbiota of fish larvae is derived from the live feed ingested rather than bacteria present in the water.

Artemia hatching conditions are perfect for rapid bacterial growth, especially because of temperature and glycerol release during hatching. During the hatching process, nutrients (e.g., glycerol) are leached from the cysts into the hatching media, which supports further bacterial growth/bloom. Contamination by aerosols and equipment can quickly lead to high concentrations of bacteria.

Since growing fish and shrimp larvae may consume many nauplii, the consumption of bacteria via this live food source would make significant contributions to the intestinal microbiota of the larva.

Implement **Artemia Guard** in your process to manage the impact of bacteria in your hatchery.

Artemia **Handling** Instructions

- Package should be kept closed and sealed
- For optimal storage keep in cool, dry place
- Temperatures above 4 °C can influence the quality of the product
- Once opened, the product should be used immediately or stored at below 4 °C

Artemia **Hatching** Instructions

Tank Preparation

1. Take out all removable parts (pipes, etc.) and clean them separately with soap. Rinse and disinfect by immersion in a chlorine solution (150 ppm) or other commercial products such as Virkon, Sanocare PUR, etc. as directed
2. Brush the tank thoroughly with soap after rinsing
3. Repeat the exercise with bleach solution
4. Rinse extensively with water and fill the tank with filtered seawater. Make sure all cysts and cyst shells are removed (e.g., remaining in the outlet and in valves of the tank)
5. Disinfect the hatching water with 10 ppm active chlorine and aerate gently for ±1 hour
6. Deactivate any remaining chlorine by adding 8 ppm sodium thiosulphate ($Na_2S_2O_3$)

Hatching Artemia with Artemia Guard

DO NOT wash or rinse the cysts prior to incubation. **DO NOT** disinfect or decapsulate the cysts.

1. Hatching Tank: 1,000 L
2. Volume: 800 L
3. Diluted Seawater: 25-35 ppt salinity
4. Hatching Density: 2.5 g/L
5. TEMPERATURE: 28 °C to 30 °C. DO NOT exceed 30 °C
6. pH > 8.0: pH should be 8 – 8.5 during the entire hatching process
7. If necessary, add dissolved sodium bicarbonate or carbonate (preferably add bicarbonate half an hour before incubation)
   a) Immediately prior to adding cysts, add 120 ppm of NaOH
   b) In general, a second dosage of 120 ppm of NaOH will be necessary at T12
8. Continuous aeration (D.O. > 4 mg/L)
9. Continuous light (artificial or natural) – minimum 2,000 lux at the water surface
10. Once hatching is complete, allow the contents of the hatching tank to run through the separator and collect the out-flowing Artemia nauplii in a submerged net
11. After harvesting, rinse the nauplii

Sampling and **Dilution**

Sampling and dilution for bacterial analysis

1. Sample 50 ml hatching medium in a sterile falcon tube
2. Store the sample at 4 °C or immediately perform the next steps of the analysis
3. Separate nauplii and cysts from the hatching medium using a sterile sieve, transfer 25 ml of the medium onto the sieve and collect the water in a new sterile falcon tube
4. Close tube & store sample at 4°C or perform analysis immediately
   a) Collect the Artemia nauplii (1g or known volume) aseptically on a sterile sieve and rinse with sterile autoclaved

(sea)water. Repeat twice (= remove hatching medium)

b) Transfer nauplii aseptically to a sterile falcon tube + add sterile glass beads

c) Close tube and mix for 3 minutes. Store sample at 4 °C or perform analysis immediately

5. Start preparing dilution series by transferring 1 ml of the liquid to 9 ml of sterile medium
6. Repeat until you have reached your final dilution
7. Store dilution series at 4 °C or immediately perform the next steps of the analysis

Plating Instructions

1. Prepare the medium according to the instructions mentioned on the label of the medium
2. Pour plates and dry them in a laminar flow, subsequently store the plates on a dry and safe place
3. Determine dilutions and label the plates accordingly, decide on the amount of replicates per sample
4. Transfer 100 µl of each dilution with a sterile pipette on the plate
5. Use a Drigalski spatula to distribute the sample on the plate
6. Close the plate with parafilm
7. Incubate the plate at 30 °C for 24 hrs
8. Count the amount of colonies
9. Calculate the bacterial load present in the hatching medium and perform the next steps of the analysis

Quality and **Testing**

Harvesting and Processing

Our products are harvested using proprietary, state-of-the-art harvest equipment to ensure our freshly harvested cysts are treated properly and maintain optimal hatching.

Cysts are immediately treated at our pre-processing facilities located on the shores of the Great Salt Lake, where they are cleaned and conditioned. Subsequently, our cysts are transferred to freezer facilities for further conditioning, acclimatization and subsequent processing using proprietary processing techniques. These techniques are specifically tailored to every batch of cysts taking into account the time of harvest, level and stage dormancy, and ecological parameters.

Quality and Testing

Throughout this process, our batches are carefully sampled and tested multiple times in order to properly time each phase of production and maximize the hatching quality and shelf life of our products. After final processing and packaging, our batches undergo additional testing using proprietary imaging technology to ensure all hatching characteristics comply with our stringent quality standards.

Great Salt Lake Artemia applies a scientific approach to testing which ensures we can deliver a top-quality product with reliable hatching.

Our scientists have more than 100 years of combined experience researching and testing lake ecology, Artemia biology, and Artemia harvesting and processing. As such, our team developed the most sophisticated testing and production techniques based on the biological and ecological characteristics of the live Artemia embryo.

Our proprietary imaging technology developed by our team of scientists permits us to test each batch multiple times using large sample sizes in order to guarantee accurate and precise determination of hatching quality parameters.

Finally, we use an industry-appropriate statistical approach to assign a final grade to a batch of cysts. We do not apply average hatching quality to determine the appropriate grade for a batch of cysts. Rather, we use statistical lower confidence limits at the 99% level. This ensures that our cysts hatch at or above the certified rates. In short, if you buy 80% grade GSLA Artemia, you will experience hatch rates above 80%.

Artemia **QA Testing**

Great Salt Lake Artemia is committed to the biosecurity of our customers' operations and, to support that, we thoroughly test all our product. GSLA is proud to say we have over 15 years of testing and never had a positive test for any pathogen. We attribute this success to our commitment to Quality Control and our geographic isolation.

Our Test Procedures:

WSSV
White spot disease
White spot syndrome virus

IHHNV
Runt-deformity syndrome
Infectious Hypodermal and Haematopoietic Necrosis Virus

TSV
Taura syndrome
Taura syndrome virus

YHV
Yellow head disease
Yellow head virus

IMNV
Infectious myonecrosis disease
Infectious myonecrosis virus

PvNV
Penaeus vannamei nodavirus disease
Penaeus vannamei nodavirus

MrNV
White tail disease
Macrobrachium rosenbergii nodavirus

DIV1
Shrimp hemocyte iridescent virus disease (SHIV) 1
Decapod iridescent virus 1

MBV
Baculo virus disease
P. monodon baculovirus

Other **Testing**

- Bacterial Testing
  - Salmonella
  - E. coli
  - Bacillus cereus
  - Vibrio harveyi
  - AHPND/EMS
  - NHP-B
- Fungal Testing
- Test for chemicals, contaminants and heavy metals
- Overall, 82 tests for potentially harmful or disruptive agents

GREAT SALT LAKE ARTEMIA

Great Salt Lake Artemia (GSLA) is a collection of member companies working together to harvest and distribute the best Artemia products available.