Sri Manjunatha Hatchery

Facility: Alagayapalem, Ulavapadu, Karedu, Andhra Pradesh 523292

FIELD VALIDATION TESTIMONIAL

We conducted a field trial of **Nevizh Microbial Solutions** (VibQuench®, DriftAct®, and HeteroMight®) in our shrimp hatchery under normal operating conditions.

During the trial, we observed:

- Consistent stability of microbial balance in the tested tanks
- Reduced dependency on chemical sanitization
- Improved regulation of ammonia levels
- Comparable larval survival and development

Further, we found that the system remained stable during a temporary contamination event, which demonstrates good resilience under practical conditions. Also, the trial experimentation was highly informative, and strengthens our expertise towards the effective implementation.

On the whole, the approach was significantly aligned with our hatchery operations, as it did not require additional infrastructure, which offers a very practical and sustainable option.

Thanks, and best wishes to Nevizh team.

Jayanth Varma Alluri
Managing Director
Sri Manjunatha Hatchery

Field Evaluation of Nevizh™ Microbial Solutions for Vibrio and Ammonia Management in Shrimp Hatchery Systems

Key Outcomes (4-Day Hatchery Trial)

- ✅ **Stable beneficial Vibrio dominance achieved**
- ✅ **No chemical sanitization required in test tanks**
- ✅ **Ammonia rise controlled (0.2–0.3 ppm vs 0.4–0.6 ppm in control)**
- ✅ **Comparable larval survival up to Z3**
- ✅ **System remained stable even under accidental contamination stress**

Field Evaluation of Nevizh™ Microbial Solutions for Vibrio and Ammonia Management in Shrimp Hatchery Systems

Objective:

To evaluate the efficacy of Nevizh Pvt. Ltd.'s microbial solutions for vibrio management and ammonia control in shrimp hatchery conditions at Sri Manjunatha Hatcheries, Ulavapadu, Prakasam Dist., Andhra Pradesh.

The trial aimed to:

- Demonstrate the ability of VibQuench® to establish and maintain a dominant population of beneficial Vibrio species capable of suppressing pathogenic strains through quorum quenching.
- Assess the performance of DriftAct® in supporting microbial balance without reliance on chemical sanitization.
- Evaluate the effectiveness of HeteroMight® in reducing ammonia levels via biological assimilation under hatchery conditions.
- Compare water quality parameters and microbial dynamics between control tanks (standard hatchery protocol) and test tanks (standard protocol + Nevizh™ microbial solutions).
- Validate that microbial interventions can maintain larval health and survival (up to Z2–Z3 stage) comparable to conventional methods, while reducing dependence on chemical treatments

Product composition and principle:

VibQuench® : Probiotic formulation containing beneficial vibrio sp. (yellow colony forming units in TCBS agar) that could quench the quorum of pathogenic vibrio.

DriftAct® : Vibrio phage formulation capable of sustained release in hatchery rearing tanks.

HeteroMight® : Probiotic formulation containing two heterotrophic ammonia assimilating bacteria. The heterotrophic bacterial combination can directly assimilate ammonia, nitrite and nitrate into biomass when carbon source is provided.

Methodology

Note: This study was conducted entirely under commercial hatchery conditions and not in a laboratory environment. All procedures, monitoring methods, and interventions were designed to be compatible with standard hatchery operations without the need for advanced laboratory infrastructure.

The objective was to demonstrate real-world applicability, ensuring that the outcomes observed can be directly replicated by hatchery operators using practical, on-site methods.

All monitoring in this study was carried out using hatchery-compatible tools and field test kits. No spectrophotometers or advanced microbiology equipment were used.

This ensures that the microbial management approach demonstrated in this trial is fully implementable at the hatchery level without additional capital investment.

Experimental Design

Four larval rearing tanks were selected at the nauplii stocking stage:

Control Tanks: Tank 5 (old batch), Tank 11 (new batch) – followed hatchery's standard protocol

Test Tanks: Tank 3 (old batch), Tank 9 (new batch) – standard protocol + Nevizh™ microbial solutions

Water Volume Progression

Initial: ~4.5 tons at nauplii stage

Final: ~7 tons at Z2–Z3 stage

Treatment Protocol (Test Tanks Only)

Vibrio Management: Application of VibQuench® and DriftAct®

Ammonia Management: Application of HeteroMight® with carbon supplementation (Sucrose – Table sugar).

Control Tank Practices

Use of yucca extract for ammonia management.

Use of chemical sanitization agents (documented as sanitization inputs) for microbial control.

Monitoring Parameters

Vibrio population dynamics (colony characteristics and counts)

Ammonia levels

Larval survival and stage progression (up to Z2–Z3)

Duration

Continuous observation from nauplii stocking to Z3 stage (~4 days).

Product dosage timings

VibQuench® and HeteroMight® were brewed under below conditions:

Eg: for 10 Ton water level, take 10 liters of treated sea water in a bucket, aerate with air stone, add 30 grams jaggery and add 50 ml of probiotic (Corresponds to 5 ppm with respect to 10 Ton water level in culture tank). Brew for 6 hours (plus or minus 1 hour) and apply the whole content into the culture tank.

Table 1:

Day (Stage)	Products applied	Comments
14 April 2026 (Nauplii)	VibQuench® DriftAct®	Nauplii socking at 4.5 Ton water level by afternoon, product applied by 21.30 to both the Test tanks: Just before 1st pumping of algae (time: 22.45). This ensures that VibQuench® probiotic establish dominance before arrival of high number of bacteria via algae feeding. Soon after algae pumping is completed 15 ml of DriftAct® applied to Test tanks (time: 23.15). This ensures that the incoming vibrio population from algae pumping gets disrupted.
15 April 2026	VibQuench® DriftAct®	VibQuench® added to both test tanks (Tank 3 & 9) at 09.00 at 5.5 ton of tank level. Each of the test tank received one vial of DriftAct® diploid in sustained release mode.
16 April 2026	VibQuench® DriftAct® HeteroMight®	VibQuench® added to both test tanks (Tank 3 & 9) at 09.00 at 6 ton of tank level. It was subsequently identified that the water used for brewing originated from a secondary nauplii batch with elevated Vibrio load (predominantly green CFU). At this stage, system stability in the test tanks was maintained by previously established microbial populations from VibQuench® and DriftAct® applications. As emergency response, one full vial (broken) of DriftAct® applied in both the test tanks: This additional intervention was precautionary and may not have been necessary under standard conditions. Same time (14.00) fresh brewing of VibQuench® and HeteroMight® (As ammonia start to show signs of raising in all four tanks) was setup separately, considering 6 ton of tank volume. Application done to both test tanks by 21.00. Sugar (Carbon source) dosed at only 2 ppm for both the test tanks as ammonia levels has not reached critical levels yet.
17 April 2026	VibQuench® HeteroMight®	For 7 ton tank volume, fresh brewing of VibQuench® and HeteroMight® was set up at 11.00. Application done to both test tanks by 17.00. Sugar (Carbon source) dosed at 10 ppm (70 gram per tank) in test tanks (Tank 3 & 9) as ammonia level in all four tanks are considerably high.

Results & Observations

1. Vibrio Population Dynamics

Test tanks treated with VibQuench® showed:

Stable establishment of beneficial Vibrio dominance.

Controlled and balanced microbial ecosystem.

Control tanks:

Required chemical sanitization

Observed shift in colony profile (yellow → predominantly green colonies, ~20–40 CFU range post-treatment), indicating microbial imbalance

Note: Vibrio population dynamics followed with TCBS agar. Spread plating done using 0.1 ml of direct hatchery tank water (without larvae). Only neat plating done without any serial dilutions, as the aim is to qualitatively follow the dynamics and not to quantitate.

Table 2:

Timing of plating	Time Point Number	Observation in Control tanks	Observation in Test tanks	Comments
14 April 2026 : 20.00; Before initial algae pumping.	1V	Too Numerous To Count (TNTC): Only yellow colonies (confluent). Colonies are circular, entire, convex, yellow-pigmented, approximately 1 to 2 mm in diameter.	Too Numerous To Count (TNTC): Only yellow colonies (confluent). Colonies are circular, entire, convex, yellow-pigmented, approximately 1 to 2 mm in diameter.	All tanks (test and control) had similar vibrio profile.
14 April 2026: 20.00; Plating of VibQuench® Brewing.	2V	N/A	N/A	Yellow lawn, as expected. Brewing is proper.
14 April 2026: 23.00; Just after algae pumping	3V	TNTC of yellow colonies (confluent) in tank 5 and TNTC of	TNTC of yellow colonies (confluent) in both tank 3 & 9	

and just before DriftAct® application.		green and yellow cfu in tank 11. Note: heterogeneous (green to yellow-green) appearance in tank 11 plate may indicate mixed populations or density effect: sucrose fermentation cannot be reliably interpreted due to lack of isolated colonies.		
15 April 2026: 09.30.	4V	Tank 5 & 11: Dense growth with apparent heterogeneity in colony distribution and appearance; possible mixed population;	Tank 3 & 9: Predominantly yellow, confluent growth observed;	Test tanks plates show more homogeneous colonies compared to control tanks, possibly indicating the establishment of VibQuench® probiotic.
16 April 2026: 2nd round nauplii plating: refer table 1, row 3.	5V	N/A	N/A	The second round on nauplii stoking (unrelated tank to both test and control), whose water accidently used for brewing VibQuench® (refer table 1, row 3) shows heterogeneous cfu having wide morphological variations and distributed sucrose fermentation profile.
17 April 2026: 14.50; to get an idea of vibrio dynamics after emergency	6V	Tank 5 & 11: Abrupt drop in vibrio population due to chemical intervention. Also noted is the	TNTC in both tank 3 & tank 9 plates with only yellow colonies, with a particular morphology dominant (80 to 95%	Test tank (3&9) clearly shows healthy microbial (vibrio) dynamics. Both show a singly

intervention: refer table 1, row 3 and after chemical sanitization in tank 5 & 11.		surviving population are mostly sucrose non-fermenting (green cfu). Tank 5 plate showed 50 to 60 cfu with near 80% dominated with small, regular, convex, green cfu. Tank 11 plate showed 20 to 30 cfu with near 80% dominated with small, regular, convex, green cfu.	on the plates).	morphology dominating the other, possibly indicating the establishment of VibQuench® probiotic. Control tanks were given chemical treatment as the existing protocol could not biologically alter or intervene the vibrio dynamics : mostly affected by vibrio inflow from nauplii and algae.

2. Ammonia Management

Test tanks with HeteroMight®: (Tank 3 & 9)

Maintained lower and stable ammonia levels.

Demonstrated effective biological assimilation of nitrogenous waste.

Control tanks: (Tank 5 & 11)

Existing protocol including the use of Yucca extract showed limited effectiveness in ammonia control.

Note: Ammonia levels (Total ammonia nitrogen – TAN) followed using our semi-quantitative ammonia test kit, HeteroMight® Track. Results presented in below table & chart. Note: below detection limit (BDL) is depicted as zero ppm of TAN. Always, treated sea water used as internal control: should read BDL.

Table 3:

Timing of plating	Time Point Number	Observation in Control tanks	Observation in Test tanks	Comments
14 April 2026: 20.00; Before initial algae pumping.	1A	BDL; 0 ppm in both the tanks.	BDL; 0 ppm in both the tanks.	No detectable ammonia present.
14 April 2026: 23.40; After initial algae pumping.	2A	BDL; 0 ppm in both the tanks.	BDL; 0 ppm in both the tanks.	No detectable ammonia present.
15 April 2026: 08.00.	3A	BDL; 0 ppm in both the tanks.	BDL; 0 ppm in both the tanks.	No detectable ammonia present.
15 April 2026: 18.30.	4A	0.1 ppm in both the tanks.	0.1 ppm in both the tanks.	Ammonia levels just building.
16 April 2026: 02.00.	5A	Less than or equal to 0.2 ppm in both the tanks.	Less than or equal to 0.2 ppm in both the tanks.	Ammonia levels just building.
16 April 2026: 12.50.	6A	Tank 5: 0.25 ppm. Tank 11: 0.25 ppm.	Tank 3 and 9: less than or equal to 0.2 ppm.	Ammonia level is building up.
16 April 2026: 20.20.	7A	Tank 5: 0.25 ppm. Tank 11: 0.25 ppm.	Tank 3 and 9: less than or equal to 0.2 ppm.	Ammonia level is building up.
17 April 2026: 09.00.	8A	Tank 5: 0.4 ppm. Tank 11: 0.35 ppm.	Tank 3: 0.3 ppm. Tank9: 0.3 ppm.	Ammonia level is building up.
17 April 2026: 19.00.	9A	Tank 5: 0.6 ppm. Tank 11: 0.4 ppm.	Tank 3: 0.3 ppm. Tank9: 0.2 ppm.	Test tanks (3 and 9: Treated with HeteroMight®) showed slower ammonia increase compared to control tanks (5 and 11) over time. The control tank (Tank 5) is clearly in stress zone.

Figure 1: Ammonia dynamics in control and test tanks

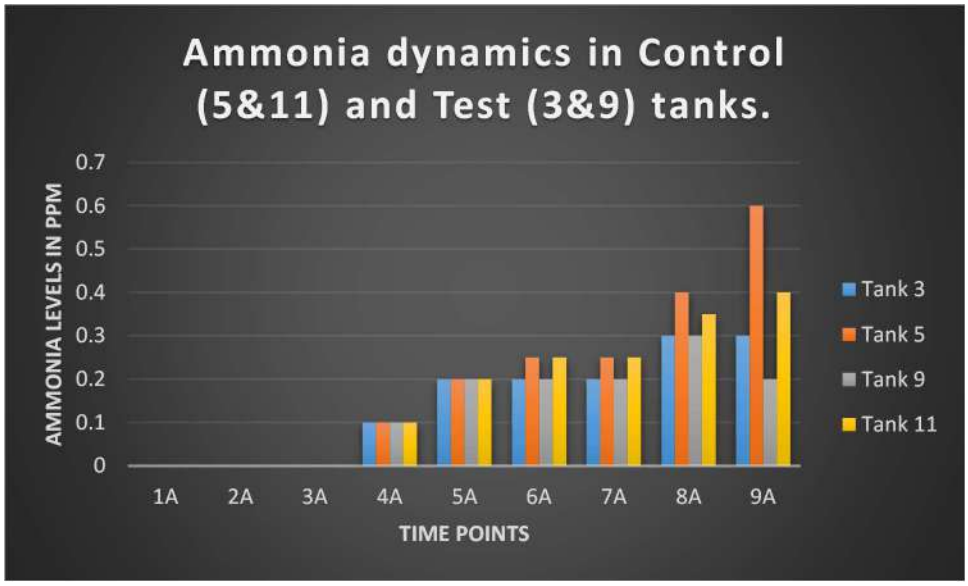
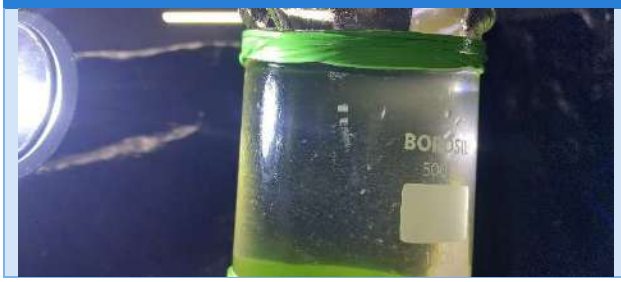
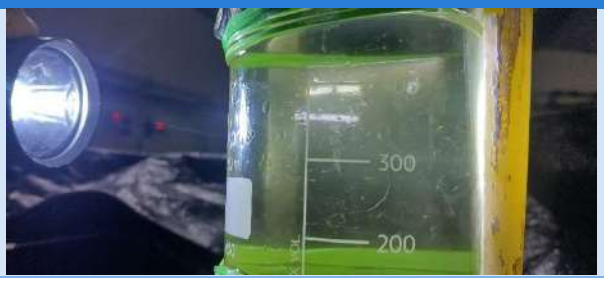


Figure 2: Ammonia levels as tested at time point "9A"

Below is the image of the tested samples at time point number 9A: From right to left: Sea water – internal control, Tank 3, Tank 5, Tank 9 & Tank 11:

Figure 3: Biofloc formed on 17 April 2026 in test tank 9

Below is the image of the bioflocs formed in test tank 9 vs control tank 11 (without bioflocs) for comparison:

Bioflocs in test tank 9	Control tank 11
	

3. Chemical Usage Comparison

Test Tanks:

No chemical sanitization used

Fully microbially managed system

Control Tanks:

Dependent on chemical sanitization inputs for microbial control

4. Larval Performance

At Z2 stage:

Animal counts were comparable across all tanks.

No negative impact observed from microbial-only approach.

Counting of animals to compare survival (equal nauplii stocking) in all four tanks done on 16th April 2026: 7 pm. One counting done Mr. Mohanraj, Nevizh Pvt. Ltd. and another counting done by section in-charge, Mr. Chanti. Counting done by taking 400ml of culture water in a glass beaker, manually counted by lighting up with torch light. Results presented in below table.

Table 4:

Tank Number	Counted by Mr. Mohanraj	Counted by Mr. Chanti
3	157	160
5	150	170
9	157	145
11	158	170

Note: Since this is manual counting, allow for a variation of plus or minus 10 to 20 animals per beaker.

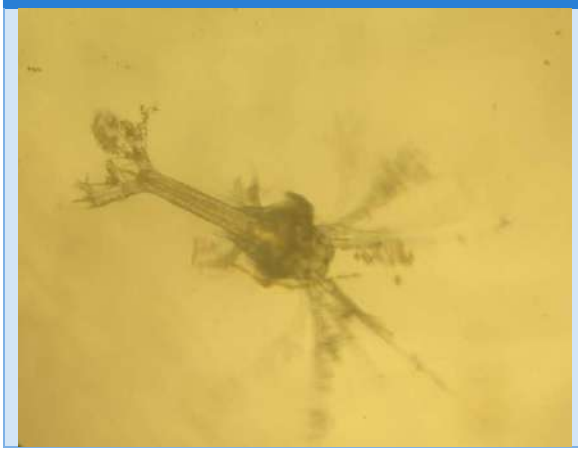
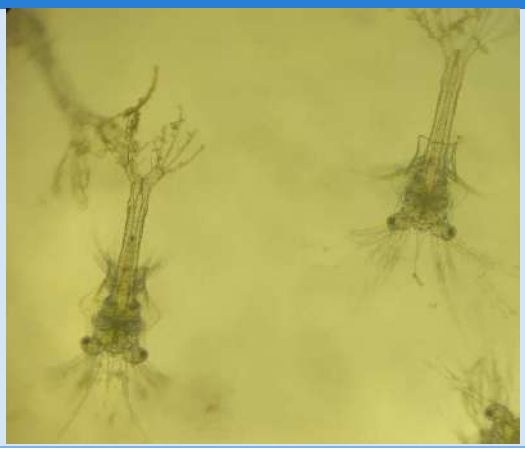
Demonstrates equivalence in survival and development without chemical dependency using Nevizh™ microbial solutions.

5. A note on attachments of debris on Larval surface

Temporary attachment of microbial flocs on larval surface (nauplii/zoea etc.,) is a normal outcome of an active microbial management system. These attachments are superficial, non-pathogenic, and are naturally cleared during moulting. Observations confirm that larvae transition (into various stage) with clean surfaces and no adverse impact on survival or development.

Below is representative image of such transition (More details can be seen in the Raw Data section):

Figure 4: Larval Surface Attachment and Clearance During Stage Transition

Attachments before transition	Reduced attachment after transition
	

Note: During the Zoea stage (16th April 2026), a deviation from the standard feeding protocol occurred in one of the observation periods, where the scheduled combined feed (powder + liquid) was not fully administered in the morning, and feeding was adjusted to primarily liquid feed with reduced powder input.

Despite this variation, larval counts and stage progression in the test tanks remained comparable to control tanks, indicating that the microbial management system, maintained stability and did not negatively impact larval performance under minor operational inconsistencies.

Conclusion

The trial demonstrated that Nevizh Pvt. Ltd.'s microbial solutions can serve as an effective and sustainable approach for shrimp hatchery management under practical conditions.

Application of VibQuench® and DriftAct® enabled the establishment of a stable and beneficial microbial community, characterized by the dominance of beneficial *Vibrio* populations. This contributed to suppression of potentially pathogenic strains without the need for chemical intervention.

HeteroMight® showed effective control of ammonia through biological assimilation, maintaining lower and more stable TAN levels compared to conventional inputs such as yucca extract.

The integrated microbial approach reduced reliance on chemical sanitization while maintaining comparable larval survival and development up to the Z2–Z3 stage, indicating no compromise in production performance.

An unintentional introduction of high *Vibrio* load during the trial provided an opportunity to assess system robustness. Despite this stress event, the treated tanks maintained stable microbial dynamics without collapse or pathogenic overgrowth, demonstrating resilience of the established microbial ecosystem under real-world conditions.

Overall, the study supports a microbial-first hatchery management strategy that:

- Reduces dependency on chemical inputs
- Promotes ecological balance within the culture system
- Provides a lower-stress environment for larval development
- Maintains consistent production performance

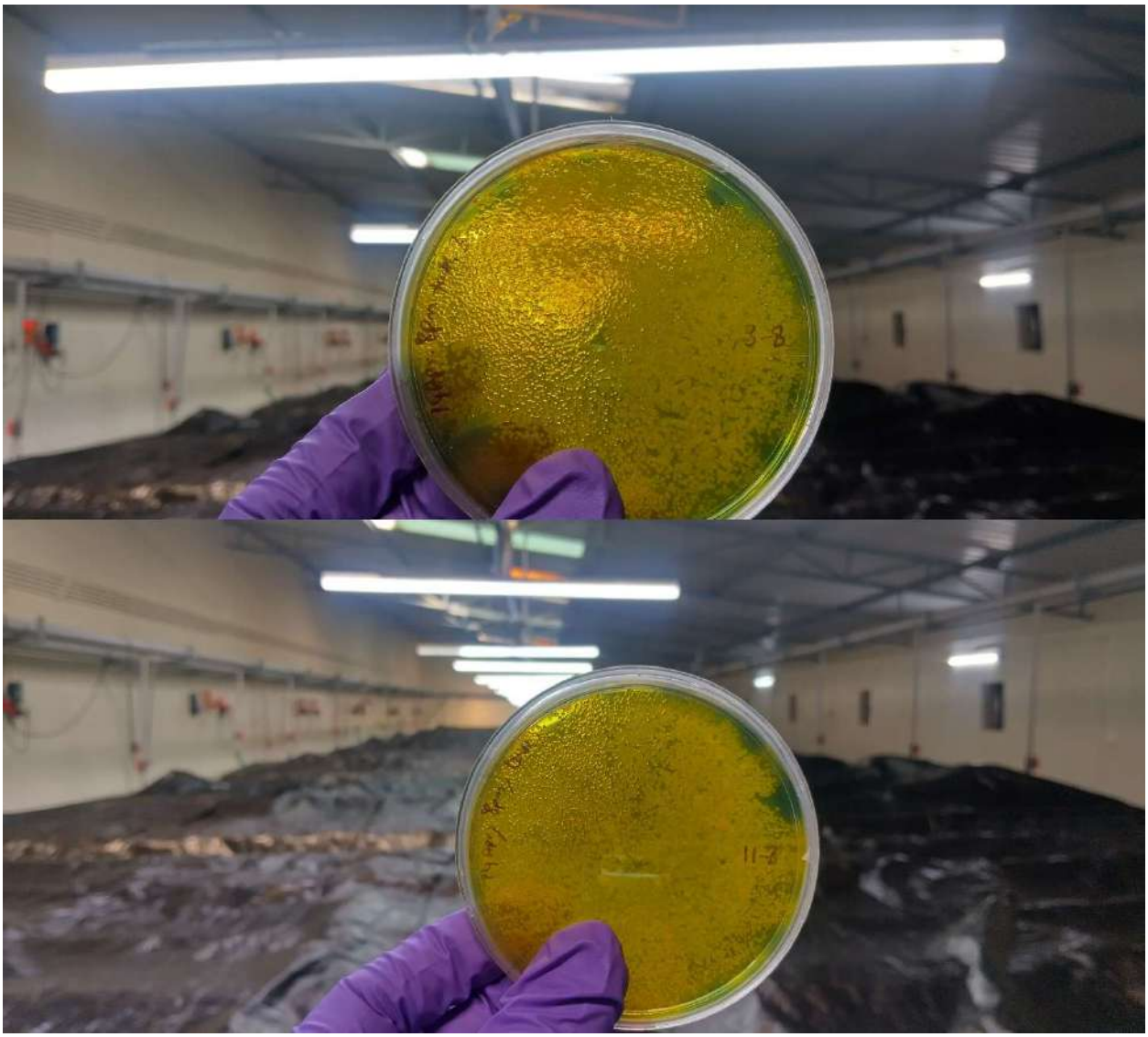
Raw Data:

Below is the raw data:

Brewing setup:

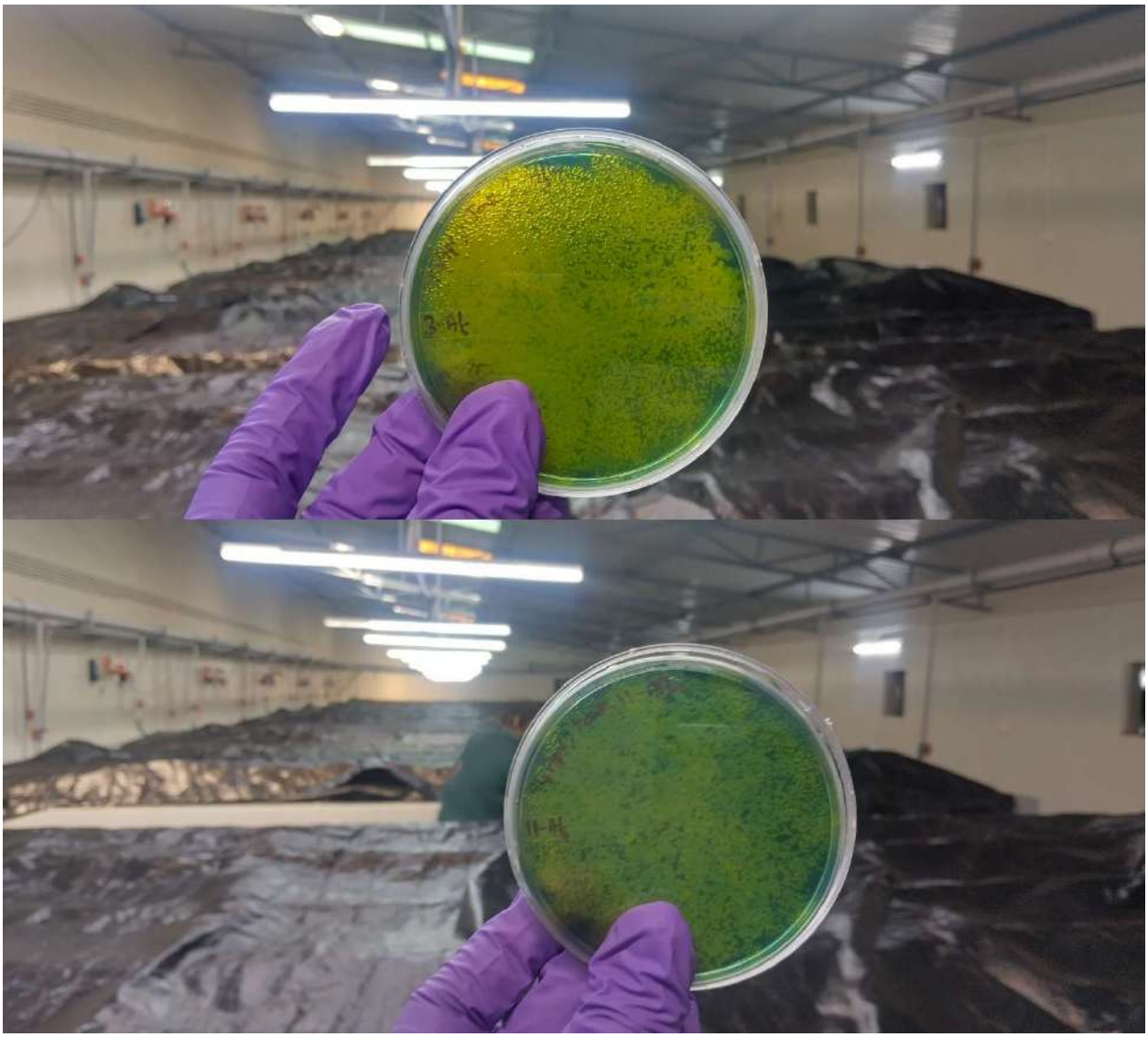
Raw data pertaining to Vibrio Population Dynamics:

Time point number 1V (Refer Table 2):

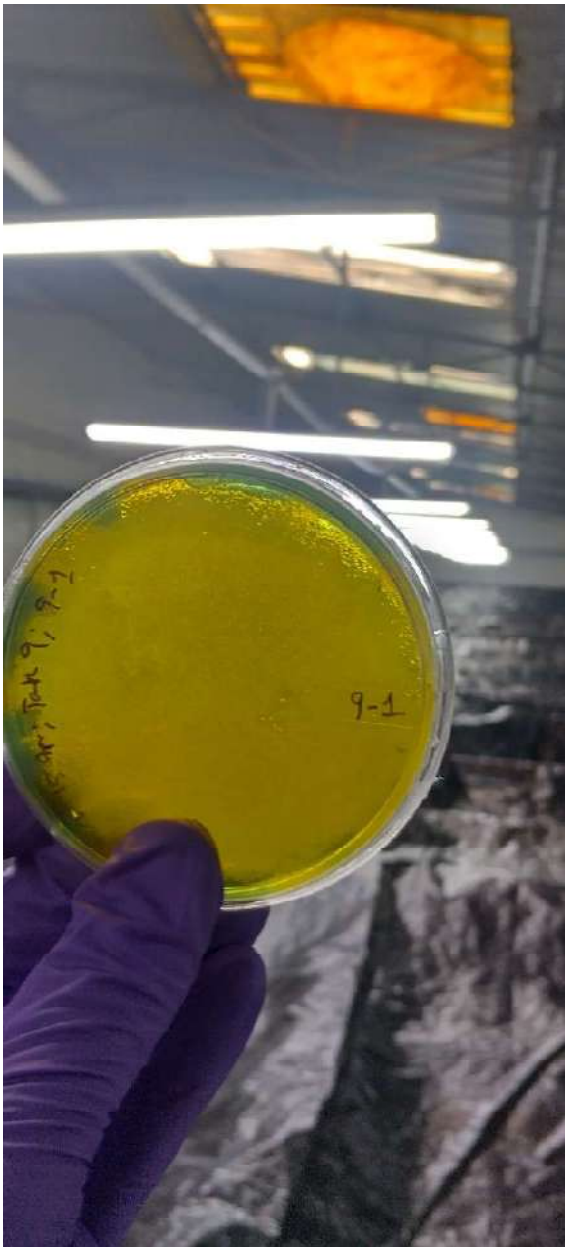

3-B
11-3

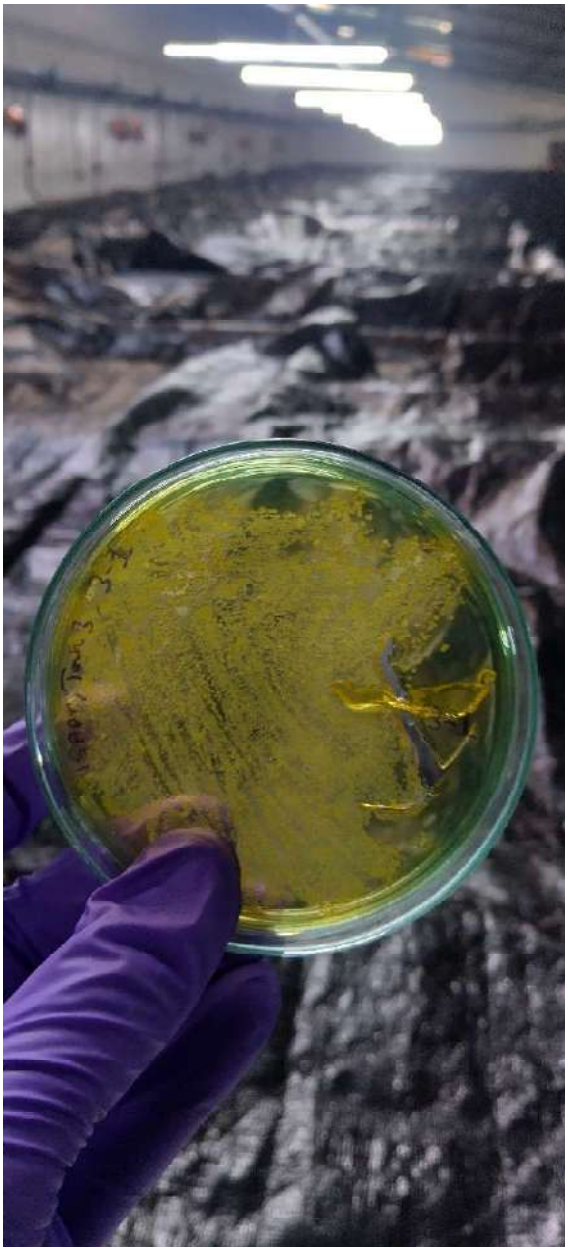
Time point number 2V (Refer Table 2):

Time point number 3V (Refer Table 2):



Time point number 4V (Refer Table 2):

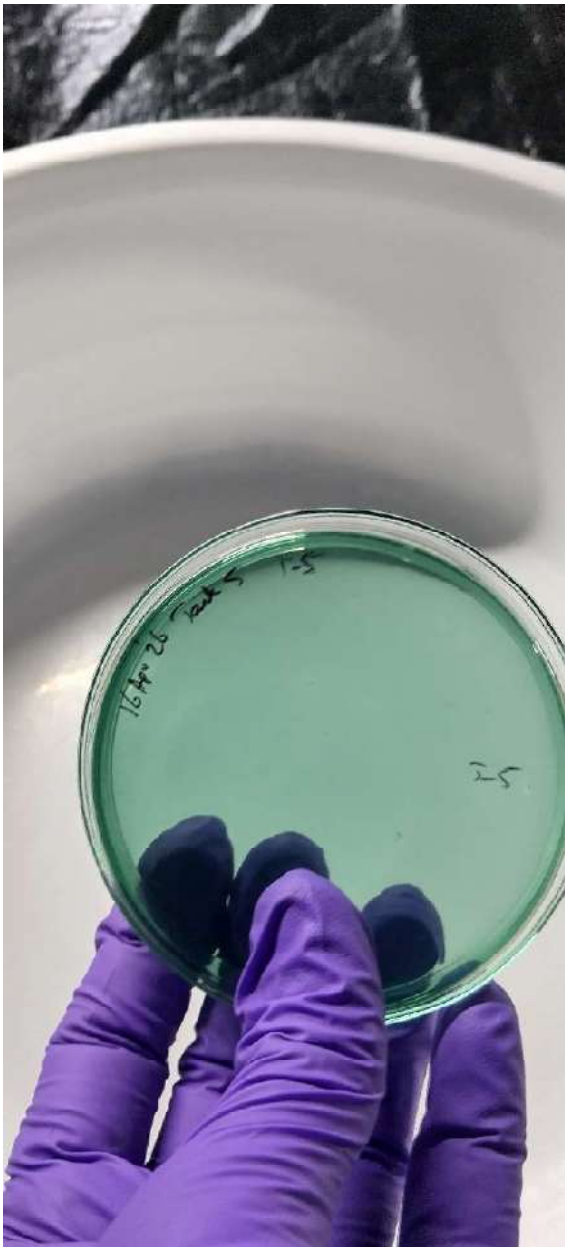

9-1



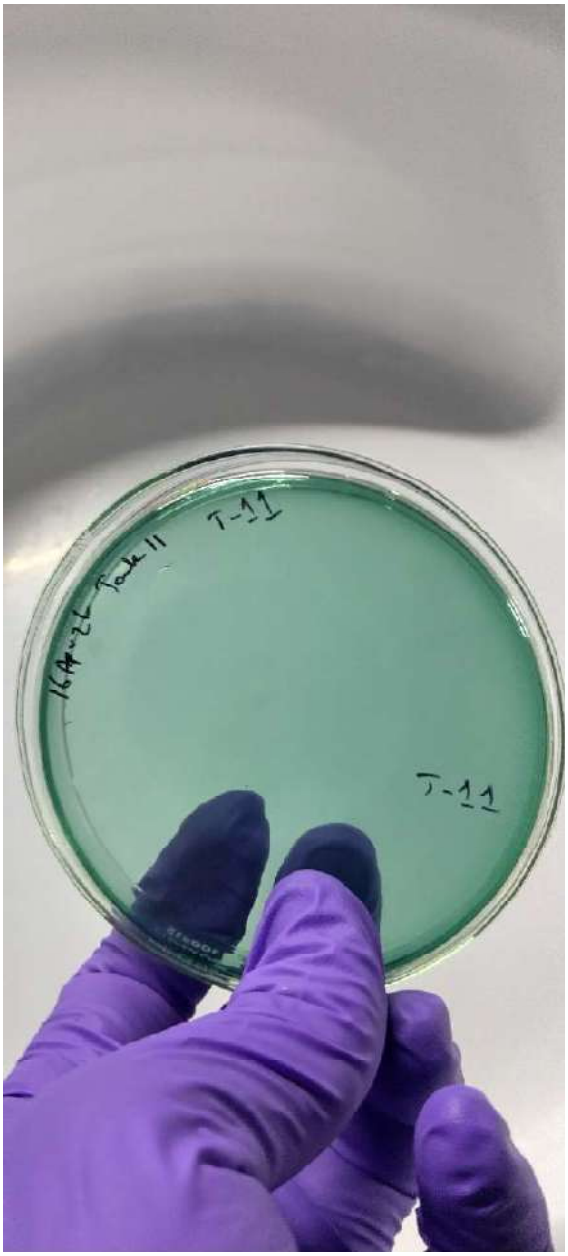
Time point number 5V (Refer Table 2):

Time point number 6V (Refer Table 2):


16 April 26 Tank 9 T-9
T-9

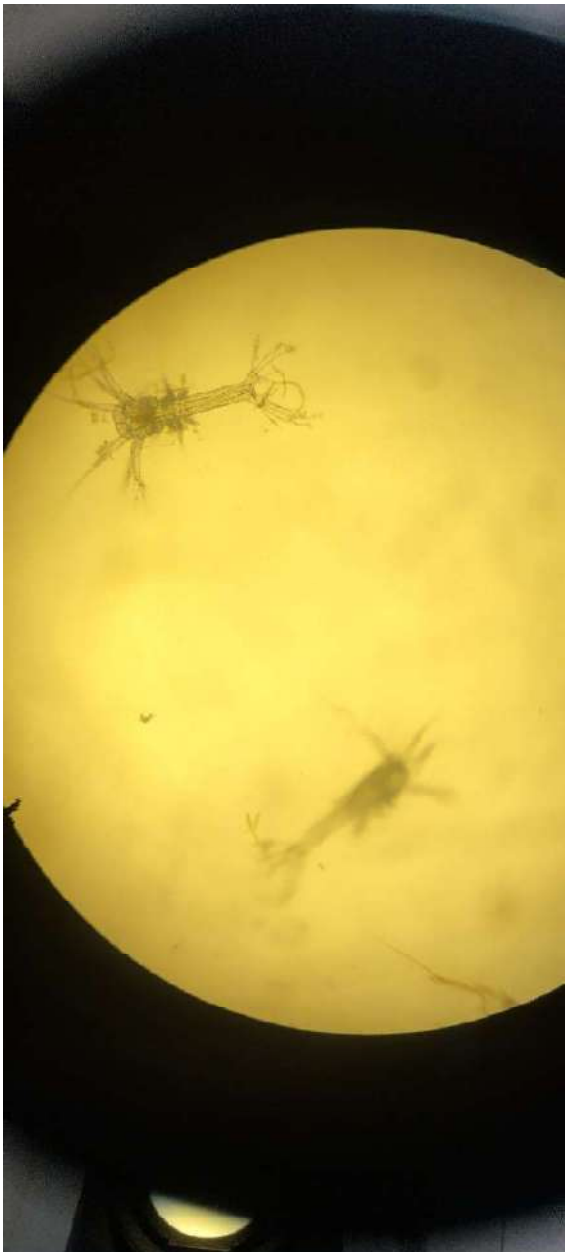


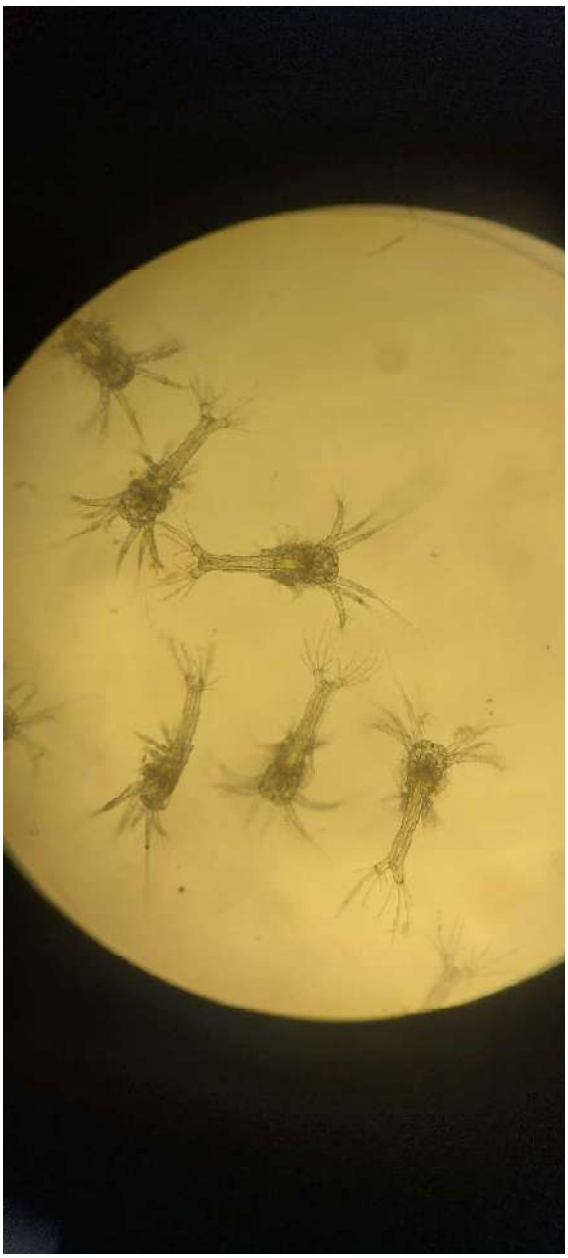

T-3



Larval Condition Observations – Representative Images

Test Tank (Tank 3 / 9): Nauplii Stage





Control Tank (Tank 5 / 11): Nauplii Stage

Test Tank (Tank 3 / 9): Zoea Stage

Control Tank (Tank 5 / 11): Zoea Stage