“Spotted Wolffish (Anarhichas minor) – Refining and Validating Best Practices for Juvenile Production Technologies and Initiating an Aquatic Animal Health Surveillance Program”, on behalf of Memorial University of Newfoundland’s Ocean Sciences Centre (OSC) and Amar Seafood Ltd.

To:
Canadian Centre for Fisheries Innovation (CCFI)
P.O. Box 4920
St. John’s, NL
A1C 5R3

Final Report
December 31, 2023

NEWFOUNDLAND & LABRADOR, CANADA
MEMORIAL UNIVERSITY

Department of Ocean Sciences
Ocean Sciences Centre
0 Marine Lab Rd.
St. John's, NL Canada A1C 5S7
Tel: 709 864 3708 Fax: 709 864 3220

December 31, 2023

Mr. Keith Hutchings
Canadian Centre for Fisheries Innovation
P.O. Box 4920
St. John's, NL
A1C 5R3

Dear Mr. Hutchings and Ms. Kielly;

Please accept this "Final Report" for project ***"*Spotted Wolffish (*Anarhichas minor)* – Refining and Validating Best Practices for Juvenile Production Technologies and Initiating an Aquatic Animal Health Surveillance Program*",*** on behalf of Memorial University of Newfoundland's Ocean Sciences Centre (OSC) and Amar Seafoods Ltd.

This project utilized the extensive experience and expertise of research personnel/Scientist at the Department of Ocean Sciences and its dedicated marine technical and management staff at the Dr. Joe Brown Aquatic Research Building (JBARB) in conjunction with technical, management and research expertise of Amar Seafoods Ltd. through Merinov in Quebec and PEI and its Norwegian affiliates.

This project aligned with our provincial "***Multi Species Strategy***" as well as in alignment with Amar Seafoods Ltd. priorities. This work allowed us to increase our knowledge and production outlook for wolffish and make significant advances towards this species reaching its commercialization goals as you will read through - out the report.

Specifically, this proposal addressed:

- Initiation of a "Broodstock Program".
- Refining and validating best practices for "Semi Commercial Scale Juvenile Production Technologies".
- Initiate an "Aquatic Animal Health Surveillance Program".
- Technology and Knowledge Transfer
- Rural Sustainability for our Industry

Currently, there is no commercial wolffish landbased hatchery in Newfoundland and therefore the Dr. Joe Brown Aquatic Research Building (JBARB) continues to play a major role in these types and semi commercial scale projects.

Experienced hatchery managers understand very well that it is essential to continually optimize protocols in order to guarantee the health status of their juveniles and performance during subsequent rearing. Indeed, the production of marine finfish like wolffish is a perpetually evolving process, and as new solutions to problems arise, the technology to produce healthy marine juveniles and grow to market size with land based technology is always advancing.

As this wolffish project concludes, the next logical step is the building of a commercial hatchery. The JBARB will remain a dominant player in research and development, in applying semi-commercial hatchery technology(s) to wolffish aquaculture, and in the training of highly qualified staff, once such a facility is constructed. We anticipate that the wolffish held (with exception of broodstock) at JBARB and knowledge gained from this project will be transferred to the Amar Seafoods staff and to their land based licensed facilities. We will continue to partner / collaborate with Amar Seafood's Ltd. multi-disciplinary team in Quebec, PEI and Norway. This partnership has allowed national and international capacity and brought together a team of talented people to work on this species. This project has aided in the execution by Amar Seafoods Ltd. of moving this file to commercial wolffish production.

This project has also complimented ongoing research and development activities at the OSC, which are already providing information/data that are essential for the viability and continued growth of our multi species (Lumpfish, American Oysters, Sea Cucumber, Sea Urchins, Atlantic Halibut) aquaculture industry. We believe, collectively, that funding projects such as this one will help to solidify Atlantic Canada as world leaders in aquaculture innovation and safe farming practices. Also, at the same time maintaining the prosperity that we see as a direct result of the marine finfish and shellfish farming operations. Thanks for your vision and continued support.

We feel this project titled "Spotted Wolffish – Refining and Validating Best Practices for Juvenile Production Technologies and Initiating an Aquatic Animal Health Surveillance Program" was highly innovative, commercially relevant and a benefit to the Canadian aquaculture sector. We are very excited here at the Ocean Sciences Centre to continue a partnership with Amar Seafoods Ltd. and continue to help play a key role in aquaculture, which is the fastest growing food sector in the world.

The Ocean Sciences Centre staff works closely with its industry partners, as demonstrated through current and past projects that have been undertaken here at Memorial University of Newfoundland.
Again, many thanks for CCFI's vision and support. You have made a difference.

Truly;

Danny Boyce
JBARB Facility and Business Manager
Ocean Sciences Centre
Memorial University of Newfoundland

Javier Santander, PhD
Associate Professor
Marine Microbial Pathogenesis and Vaccinology Lab
Department of Ocean Sciences
Memorial University of Newfoundland,

Captain Knut Trellevik
President Amar Seafoods Ltd

1.0 PROJECT MILESTONES

	Project Milestone	Date of Completion
M1	Establishment of Broodstock Program in NL	December 31, 2023
M2	Refinement of Juvenile Production Technologies	December 31, 2023
M3	Initiation of Aquatic Animal Health Surveillance Program	December 31, 2023

2.0 PROJECT ACTIVITIES AND TIMELINES

Activity #	Activity Title	Description of Activity	Start Date	Finish Date
M1- A1	Broodstock Establishment	Daily Husbandry	March 1, 2022	Dec 31, 2023
M2- A2	Juvenile Production Technologies	Juvenile Low Temperature Tolerance Feeding Trial (100 - 300 gm fish)	March 1, 2022	Dec 31, 2023
M2- A3	Juvenile Production Technologies	Juvenile Production – Maximize Growth (3-10 gm fish)	July 1, 2022	Dec. 31, 2023
M2- A4	Juvenile Production Technologies	Juvenile Production- Maximize Growth (100-300 gm fish)	June 1, 2022	Dec. 31, 2023
M2- A5	Juvenile Production Technologies	Stocking Density Evaluation	March 1, 2022	Dec. 31, 2023
M2- A6	Juvenile Production Technologies	Grading, Water Levels and Flow Direction / Velocity	March 1, 2022	Dec. 31, 2023
M2-A7	Camera Feeding System	Artificial Intelligence Feeding System	April 1, 2022	Dec. 31, 2023
M2- A8	Fish Transport	Fish Transport - QC to NL	June 1, 2022	Dec. 31, 2023

M3- A9	Aquatic Animal Health Surveillance Program	Aquatic Animal Health Surveillance Program - Fish Health - Genomics, Transcriptomics, Microbiota Profile and Pathogen Surveillance	March 1, 2022	Dec. 31, 2023

Month	March 2022	April-June 2022	July-Sept. 2022	Oct.-Dec. 2022	Jan.-Dec 2023
Quarter	1	2	3	4	5
Project Activities and Timelines					
A1-Broodstock Establishment - Daily Husbandry					
A2-Juvenile Production Technologies - Low Temperature Tolerance Feeding Trial (100-300 gm fish)					
A3- Juvenile Production Technologies - Maximize Growth (3-10 gm fish)					
A4 - Juvenile Production Technologies – Maximize Growth (100-300 gm fish)					
A5 - Juvenile Production Technologies - Stocking Density Evaluation					
A6 - Juvenile Production Technologies - Grading, Water Levels and Flow Direction / Velocity					
A7 - Artificial Intelligence Feeding System					
A8 - Fish Transport. QC to NL					
A9 - Aquatic Animal Health Surveillance Program - Fish Health - Genomics, Transcriptomics, Microbiota Profile and Pathogen Surveillance					

3.0 PROJECT ACTIVITY PROGRESS

3.1 M1 – A1 Establishment of a Broodstock Program

Broodstock Maintenance and Daily Husbandry

Currently, Amar Seafood Ltd. are in the process of developing a broodstock program in NL at the Department of Ocean Sciences (JBARB). Going forward the plan is to retain a number of Amar's the larger fish (2-3 kg) from the general domesticated population to use as future broodstock (Figure 1) for egg production. At current, we are holding a large group of domesticated juveniles whom will mature in the coming year and will be the future mature broodstock for NL program.

Male (L) and Female (R) Spotted Wolffish Broodstock

On June 14, 2023, we received, 98 – 4-6 kg broodstock from AMAR Seafood PEI to JBARB NL. They are currently housed in 2 tanks (B7&B8- refer to table below) and we expect spawning during Winter 2024. Transport went excellent with zero mortality.

Large domesticated spotted wolffish broodstock at JBARB in B2.

Table 1: Fish distribution From November 1 – December 5, 2023

Tank #	Fish Start	Fish End	Average weight (g) Start	Average weight (g) end	Feed % bw/day	Feed per day (kg)	SGR %/day	Density (kg/m2) start	Density (kg/m2) end
B1	409	415	2286.8	2343.9	0.5	4.86	0.07	27.35	28.44
B3	641	631	1169.7	1308.4	0.5	4.13	0.32	40.53	44.63
B7	50	51	4402	4402	0.5	1.12		19.65	20.04
B8	48	47	4929	4929	0.5	1.16		21.12	20.68
Total	1148	1144							

***We had to expend more monies into truck transport (7.5K), facility rentals (10K tanks) and some extra labor (15K) on this project, than anticipated. This was in part to securing a domesticated broodstock for the future of the spotted wolffish program in NL, which is very exciting. We will spend less on Equipment/ Materials category and re-allocate monies (40K) from the Valox Camera System (60K) line within the budget as identified previously. No extra funds are required, but a mere reallocation within project.*

Continuation of the Broodstock Management Program in NL for Spotted Wolffish in critical. The "Security" of a domestic spotted wolffish broodstock for NL is the number one priority. In absence of a broodstock, there are limited commercial opportunities.

3.2 M2 – A2 Refinement of Juvenile Production Technologies

3.2.1 Trial #1: Juvenile Low Temperature Tolerance Feeding Trial (100-300 gram fish).

Trial #1 Low Temperature Feeding Threshold

Objective:

In an attempt to determine a low feeding threshold for juvenile spotted wolffish a mini trial was carried out.

Design:

One hundred juvenile wolffish with an average weight of 81.1g were transferred from their stock tank to a trial tank. This trial tank had a diameter of one meter and a water height of 0.4 meters giving a volume of 314L of water. Initial stocking density was at 4.70 kg/m^2. There was a one week acclimation period. During acclimation and throughout the trial the fish were fed a 2.0/3.0 mm Skretting feed pellet at 1% body weight per day.

Sampling/Procedures:

Initial weights were taken on 15 fish prior to any temperature adjustment. Weight samples, were taken on 15 fish weekly thereafter. After weight samples were taken, feed amounts were adjusted accordingly. After a one-week acclimation period, temperature was decreased by two degrees each week (Table 1). Once the temperature reached 4 degrees the period in between temperature adjustments was longer as it appeared to take the fish longer to acclimate to their new temperature and begin feeding again. This trial continued until the fish stopped feeding and was terminated after a period of three weeks where there was little to no growth.

Table 1: Rearing temperatures throughout the trial period

Date	Temperature
Acclimation week – Week 1	9°C
Week 1-Week 2	8°C
Week 2 – Week 3	6°C

Week 3- Week 6	4℃
Week 6 – Week 9	3℃
Week 9-Week 11	2.5℃

The fish were hand fed twice daily as well as equipped with automatic vibrating feeders that dispensed food every 60-90 minutes. During hand feedings, feed response was noted and recorded. Feed response was rated from 0-4 (Table 2).

Table 2: Feed Response rating

Feed Response	**Numerical Value**
none	0
Low	1
ok	2
good	3
great	4

After the first three weeks, mesh socks were placed at the end of the outflow pipe and were checked each morning for the presence of feed this was done to ensure that the fish were being fed to excess and any limitation in growth was not due to under feeding.

Results:

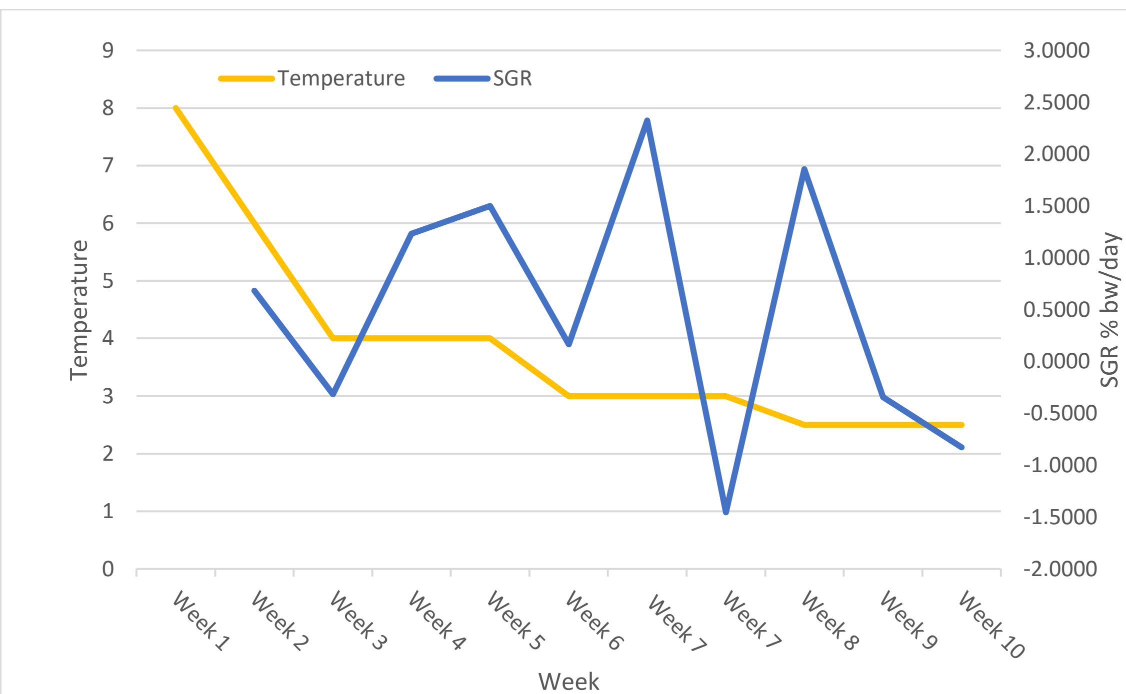


Figure 1: Specific growth rate (-) measured in % body weight per day over the duration of the trial plotted against the temperature (-).

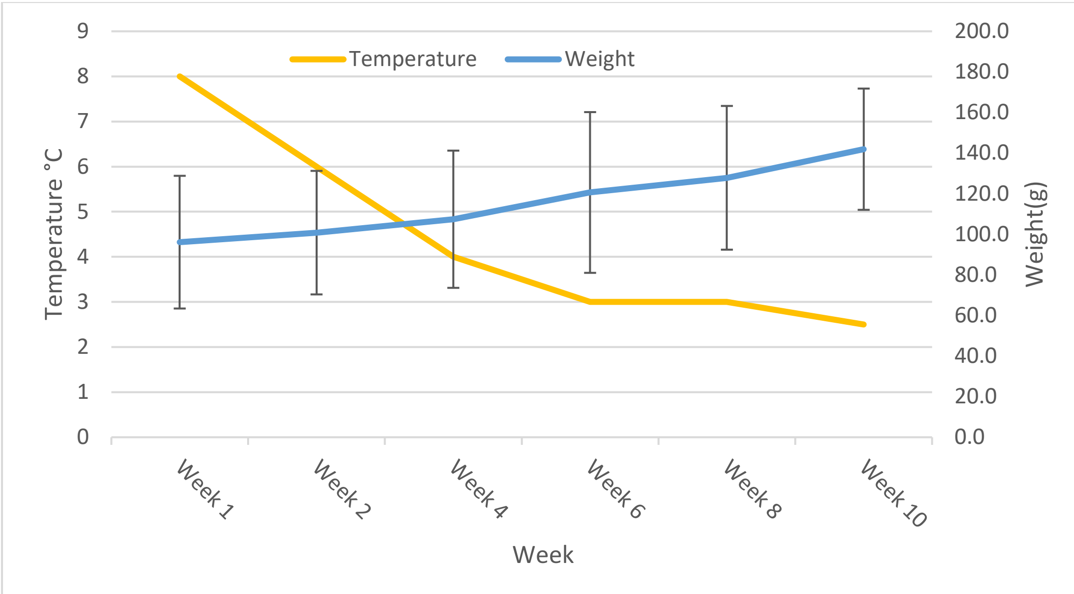


Figure 2: Average weight (-) ± standard deviation in grams over the duration of the trial plotted against the temperature (-).

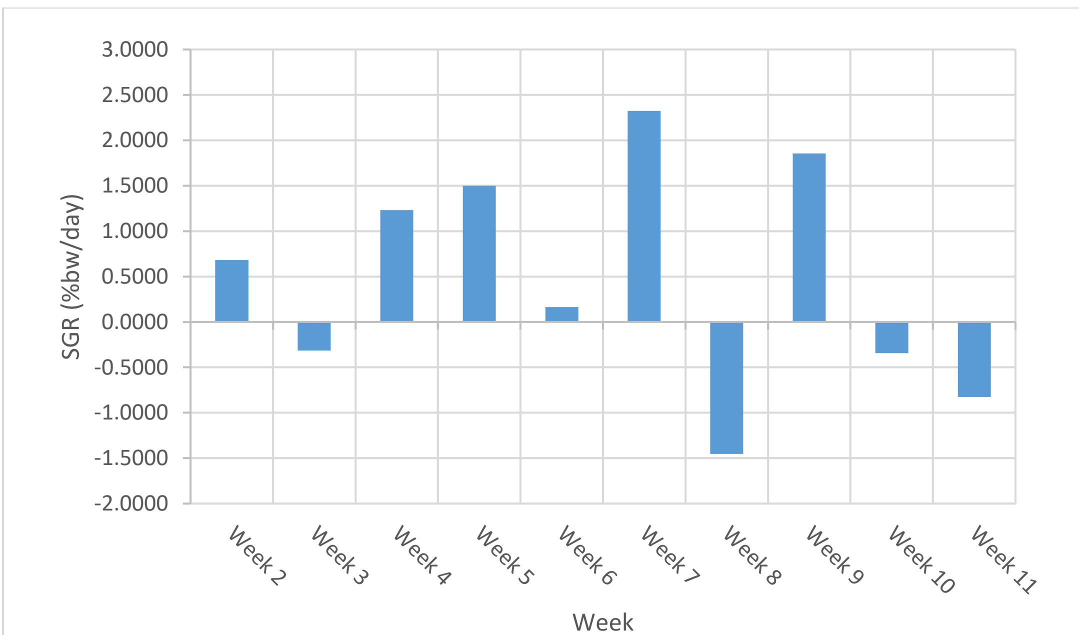


Figure 3: Specific growth rate (SGR) measured in % body weight per day over the duration of the trial.

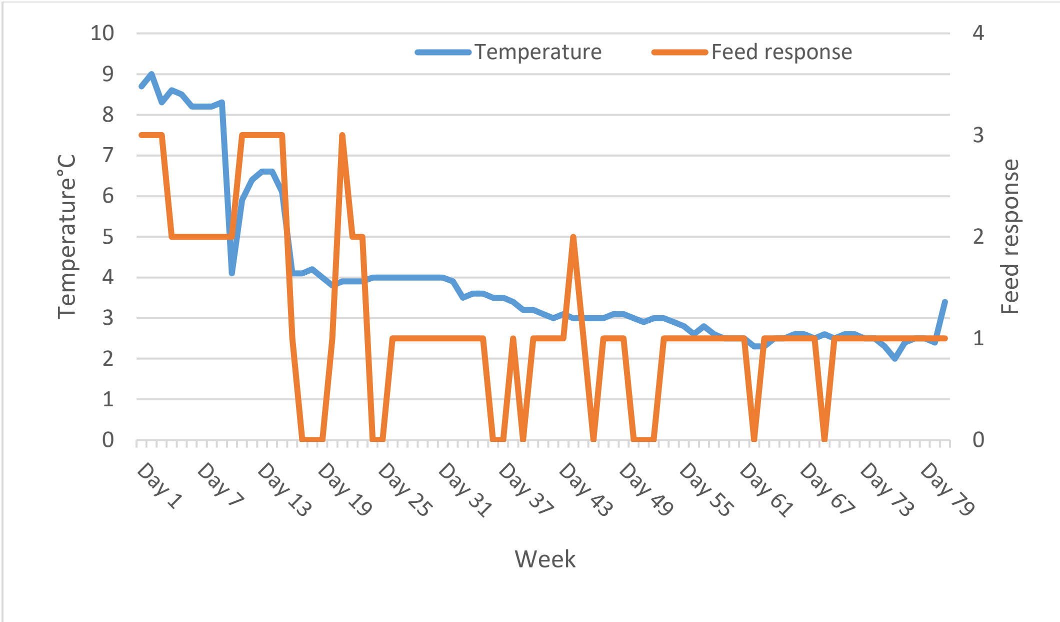


Figure 4: Feed response (-) over the duration of the trial plotted against the temperature (-).

Conclusion:

The results of this trial indicate that as the temperature decreases so does the feed response of the fish. It also appears that with each temperature decrease the time required for acclimation was longer than with previous adjustments.

The specific growth rate throughout the trial was inconsistent (Figures 1 & 3). Some weeks SGR was positive other weeks the SGR was negative. This may indicate that this species will undergo some compensatory growth once given the opportunity to acclimate to a change in rearing conditions.

However, despite the inconsistent specific growth rates, over the course of the trial average weights did continue to increase (Figure 2). There was a noticeable decrease in feed response and feed intake once the tank temperature reached 4℃ and once the temperature had dropped below 4℃ the feed response was none to low each day (Figure 4) and specific growth rates were negative for the last two sampling periods. The results of this trial indicate that rearing temperature for spotted wolffish of this size, should be kept above 6℃ at minimum.

3.2.2 Trial #2: How feed response relates to water temperature and fish size.

Objective:

Continuing from the observations made with the first temperature trial where it was observed that juvenile spotted wolffish will have a decreased feed response once rearing temperatures drop to 4°C a second trial was set up to determine if this result would be consistent between groups independent of fish weight.

Design:

Six hundred juvenile wolffish of two different sizes were divided into 6 trial tanks. One group (large) had an initial average weight of 175.8 ±30g and the second group (small) had an initial weight of 129.5 ±20.5g. These trial tanks had a diameter of one meter and a water height of 0.4 meters giving a volume of 314L of water. The fish were given a one-week acclimation period at 8°C to adjust to the tanks. The fish were fed a 2.0/3.0 mm Skretting feed pellet at 1% body weight per day during acclimation and throughout the trial period.

Sampling/Procedures:

After the acclimation period, initial weights were taken on 15 fish per tank. Weight samples, were taken on 15 fish every ten days thereafter. After weight samples were taken, feed amounts were adjusted accordingly. Once weights were taken after the acclimation period was over, temperature was decreased by one degree every 10 days (Table 3) in 4 of the six tanks. Two of the tanks were kept between 7.5 and 8°C (control) to use as a comparison. Once the rearing temperature reached 2.5 degrees, the fish were kept at this temperature for the duration of the trial. The trial was terminated due to a period where feed response and growth was minimal.

Table 3: Rearing temperatures throughout the trial period

Date	Temperature
Day 1 -10	8°C
Day 10-20	7°C
Day 20-30	6°C
Day 30-40	5°C
Day 40 - 50	4°C
Day 50 - 60	3°C
Day 60-70	2.5°C

The fish were hand fed twice daily as well as equipped with automatic vibrating feeders that dispensed food every 60-90 minutes. During hand feedings, feed response was noted and recorded. Feed response was rated from 0-4 (Table 4).

Table 4: Feed Response rating

Feed Response	Numerical Value
None	0
Low	1
Ok	2
good	3
great	4

After 30 days, mesh socks were placed at the end of the outflow pipes and were checked each morning for the presence of feed this was done to ensure that the fish were being fed to excess and any limitation in growth was not due to under feeding.

Results:

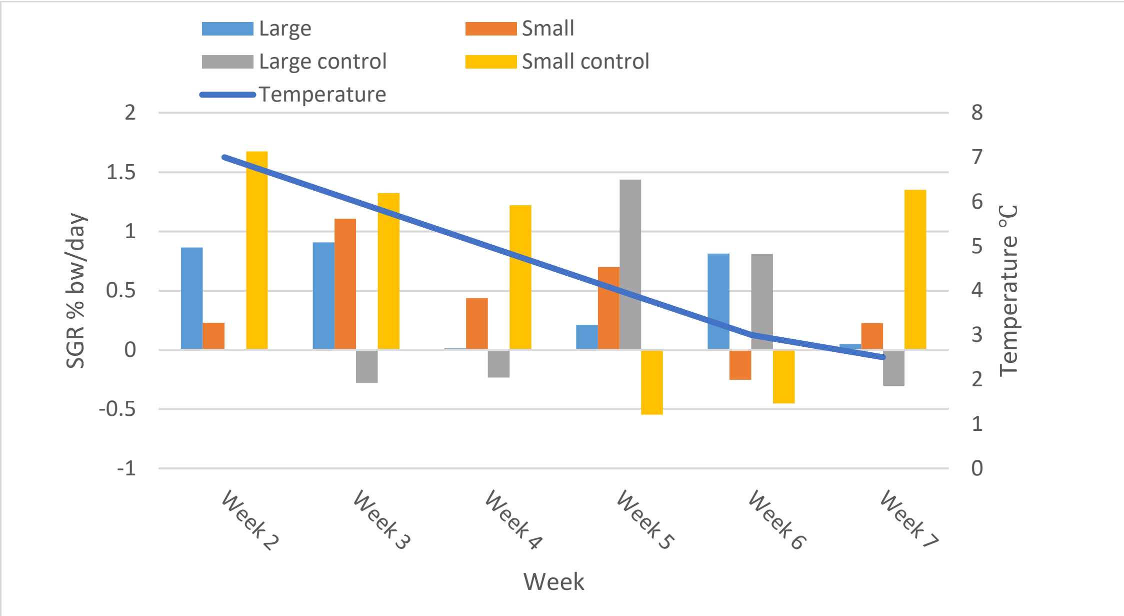


Figure 5: Specific growth rate measured in % body weight per day over the duration of the trial plotted against the temperature.

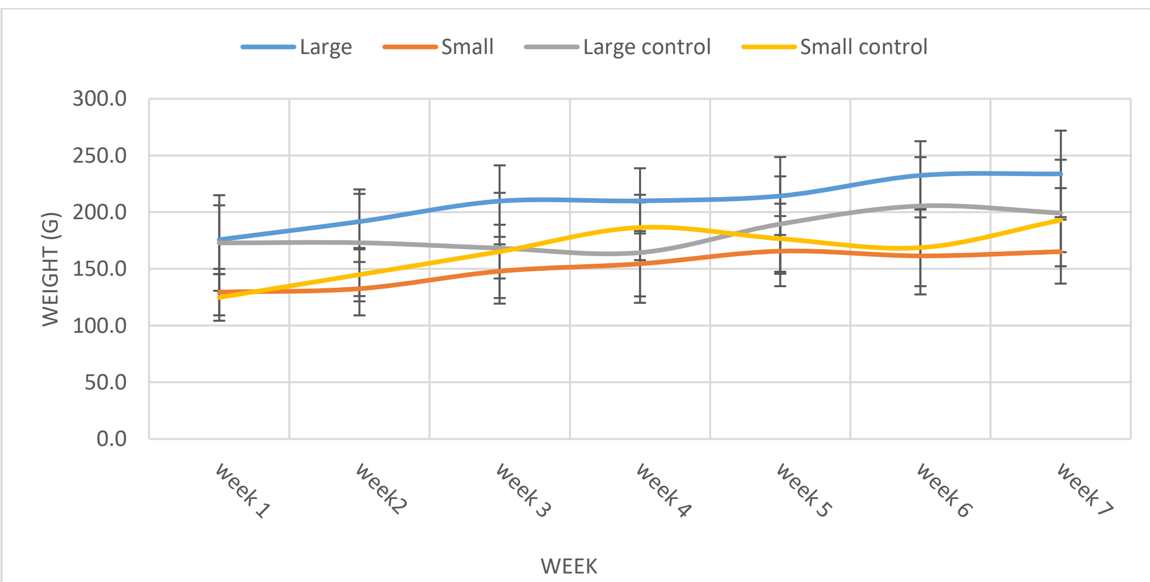


Figure 6: Average weight ± standard deviation of the large and small groups in grams over the duration of the trial plotted against the temperature.

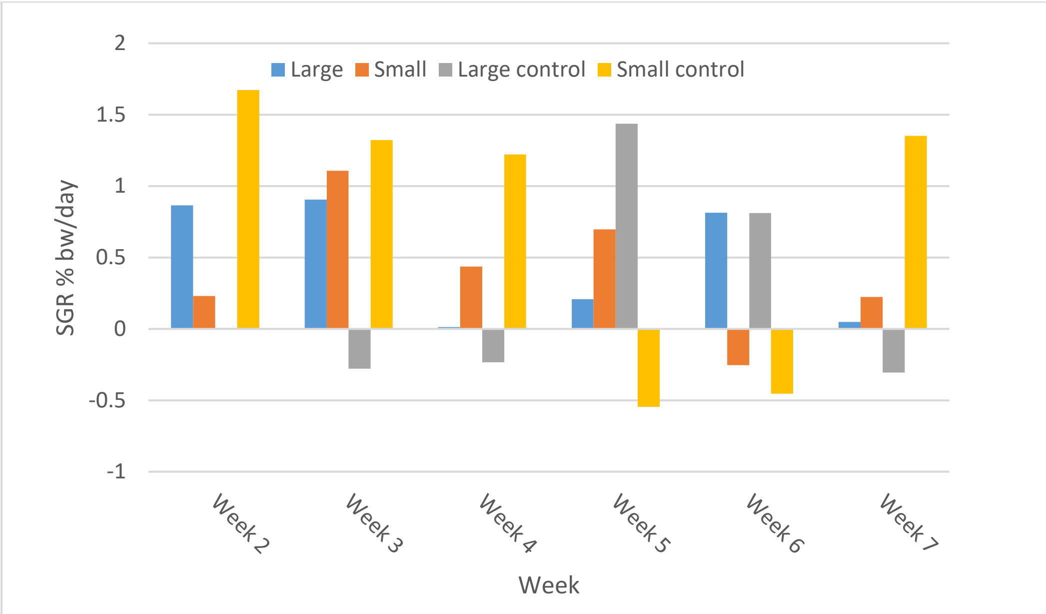


Figure 7: Specific growth rate (SGR) measured in % body weight per day over the duration of the trial.

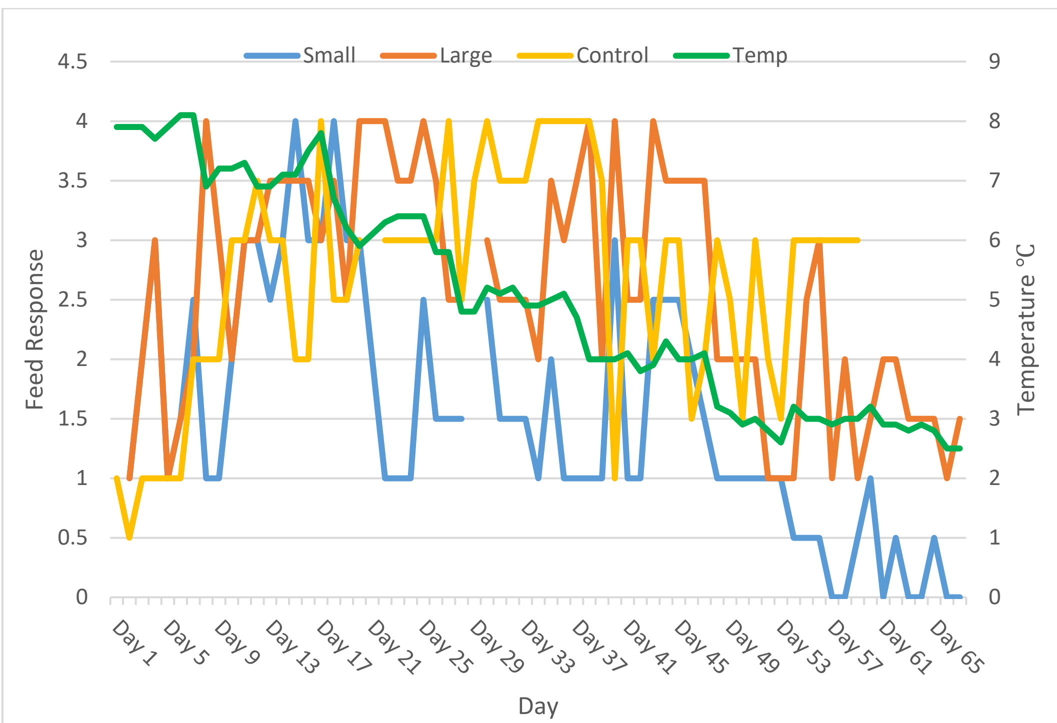


Figure 8: Feed response over the duration of the trial plotted against the temperature for both groups of fish (-).

Conclusion:

The results of this trial are consistent with the results obtained in the first mini trial indicating that as the temperature decreases so does the feed response of the fish. It also appears that with each temperature decrease there is an acclimation period before the fish begin feeding.
The specific growth rate throughout the trial was inconsistent (Figures 5 & 7). Some weeks SGR was positive, other weeks the SGR was negative. This may indicate that this species will undergo some compensatory growth once given the opportunity to acclimate to a change in rearing conditions.

However, despite the inconsistent specific growth rates, over the course of the trial average weights did continue to increase (Figure 6). There was a noticeable decrease in feed response and feed intake once the tank temperature reached 4℃ for the larger fish. However, this decrease in appetite was noted at 6℃ for the smaller group of fish. Once the temperature had dropped below 4℃ the feed response was none to low each day for the smaller fish (Figure 8). The results of this trial indicate that smaller juvenile spotted wolffish may be more sensitive to lower rearing temperatures than their larger counterparts it is recommended that for juvenile spotted lumpfish of this size rearing temperatures should be kept above 6℃ at minimum and if possible even higher for smaller fish.

3.3.3 Trial # 3: How growth and feed response are affected by temperature and feeding.

Objective:

This trial was conducted to determine if feed response and growth are affected by different rearing temperatures (8℃ vs 10℃) and/or feeding method (wet or dry) over a 30 day trial.

Design:

360 juvenile wolffish averaging 120 ± 28.6g were divided into 6 trial tanks with an initial stocking density of 8.48 kg/m^2. These trial tanks had a diameter of one meter and a water height of 0.4 meters giving a volume of 314L of water. The fish were given a one-week acclimation period, three tanks were set at eight degrees celsius and three were set at ten degrees celsius. After the acclimation period four tanks were fed the regular dry pellets, one tank at 8 degrees and one tank at 10 degrees were fed with a “wet” feed. Fifteen minutes prior to feeding, feed pellets were soaked in distilled water. The fish were fed a 2.0mm Skretting feed pellet at 1% body weight per day.

Sampling/Procedures:

Initial weights were taken on 20 fish per tank. Weight samples were taken on 20 fish every 14 days thereafter. After weight samples were taken, feed amounts were adjusted accordingly. The fish were hand fed three times daily (9am, 12pm & 2pm). During hand feedings, feed response was noted and recorded. Feed response was rated from 0-4 (Table 5). Differences in feed response, growth and specific growth rates were measured and compared between the tanks held at 8 degrees and 10 degrees as well as a comparison between the tanks fed wet versus dry food.

Table 5: Feed Response Rating

Feed Response	**Numerical Value**
none	0
Low	1
ok	2
good	3
great	4

Results:

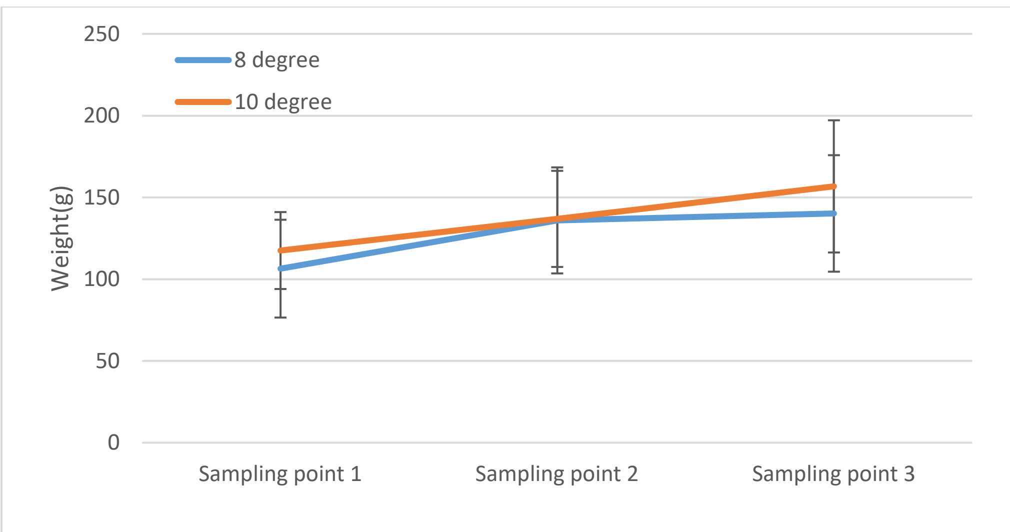


Figure 9: Average weight ± standard deviation of the 8 degree and 10 degree groups in grams over the duration of the trial.

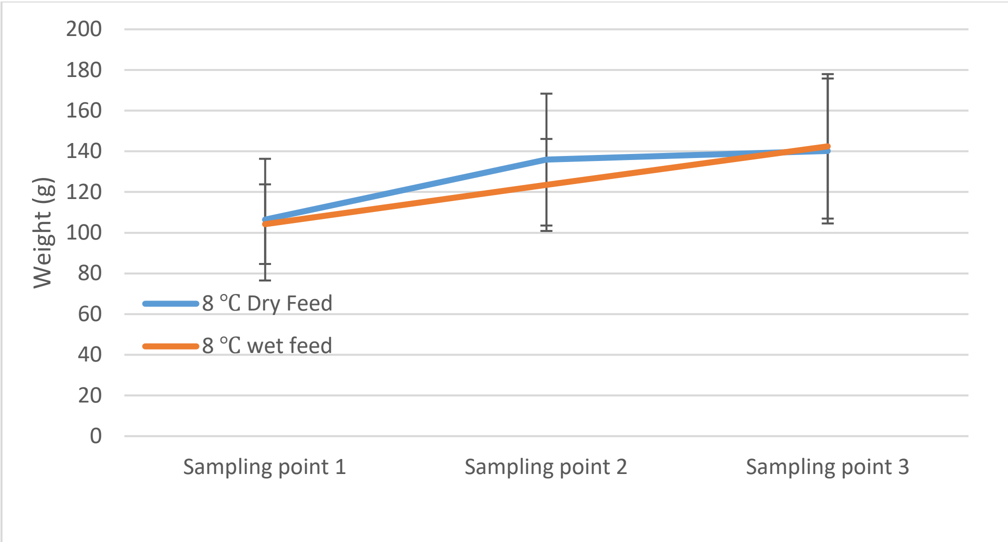


Figure 10: Average weight ± standard deviation of the 8 degree wet feed and 8 degree dry fed groups in grams over the duration of the trial.

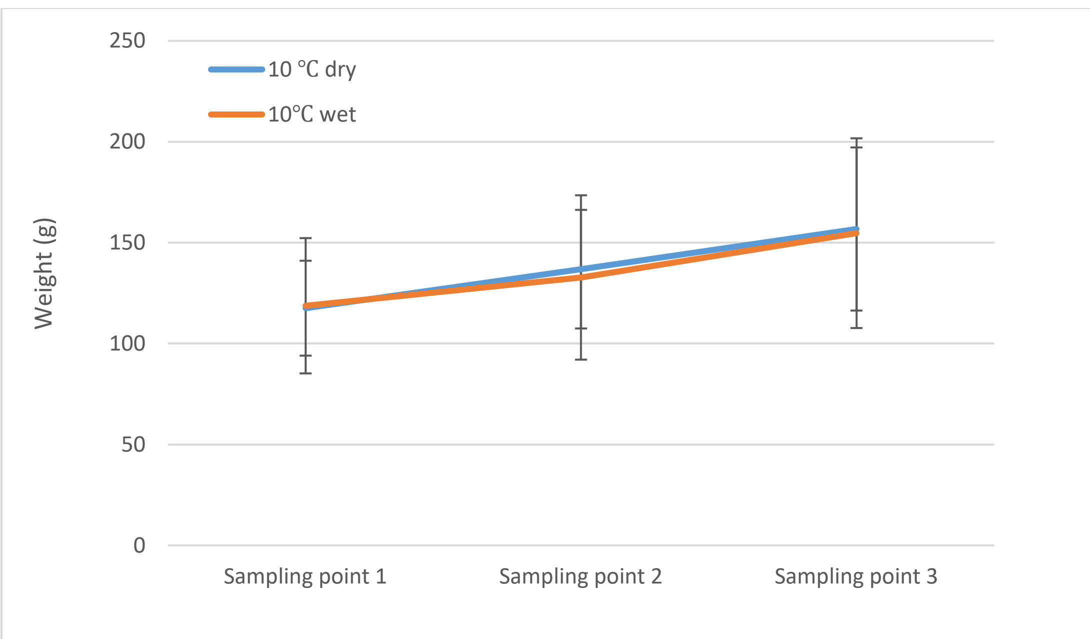


Figure 11: Average weight ± standard deviation of the 10 degree wet feed and 10 degree dry fed groups in grams over the duration of the trial.

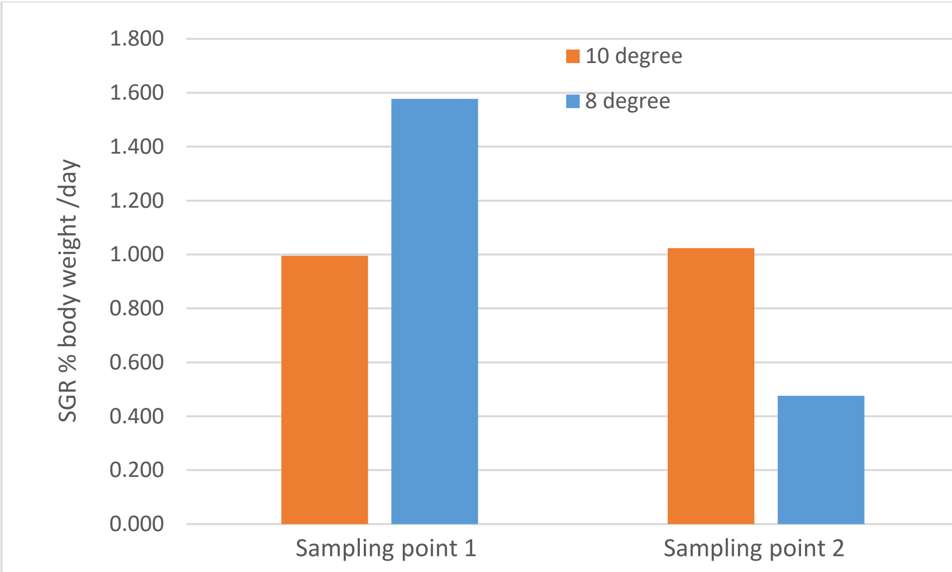


Figure 12: Specific Growth rate of the 8 degree and 10 degree groups in percent body weight per day.

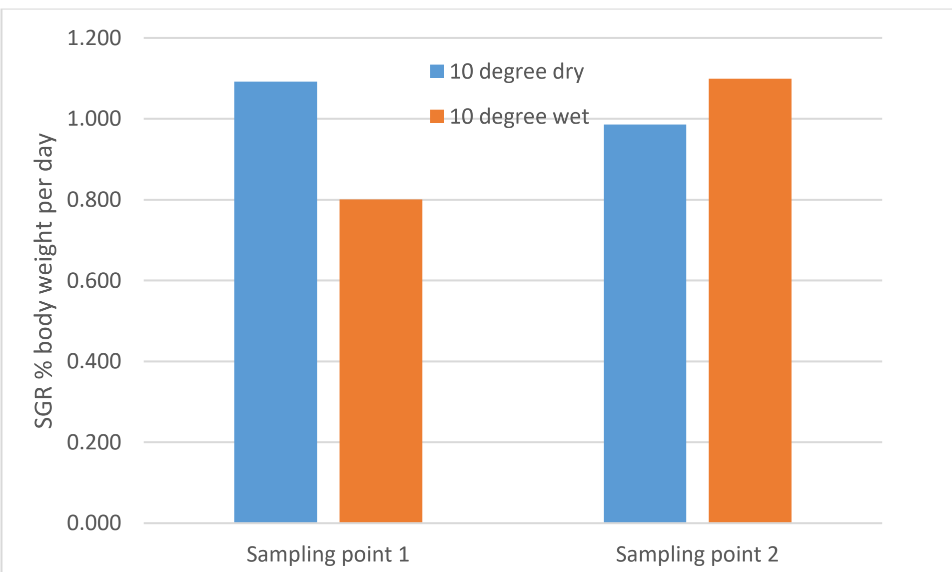


Figure 13: Specific Growth rate of the 10-degree wet and 10-degree dry fed groups in percent body weight per day.

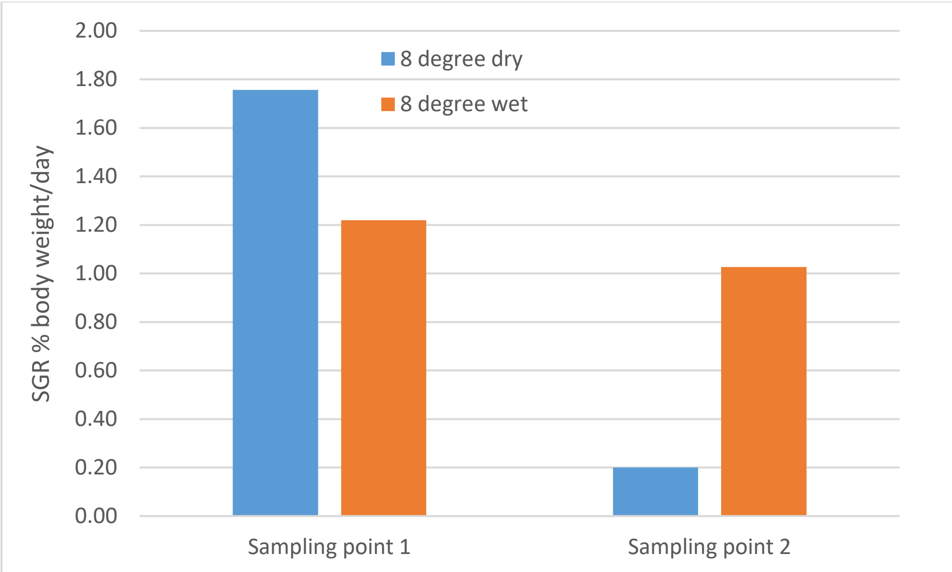


Figure 14: Specific Growth Rate of the 8-degree wet and 8-degree dry fed groups in percent body weight per day.

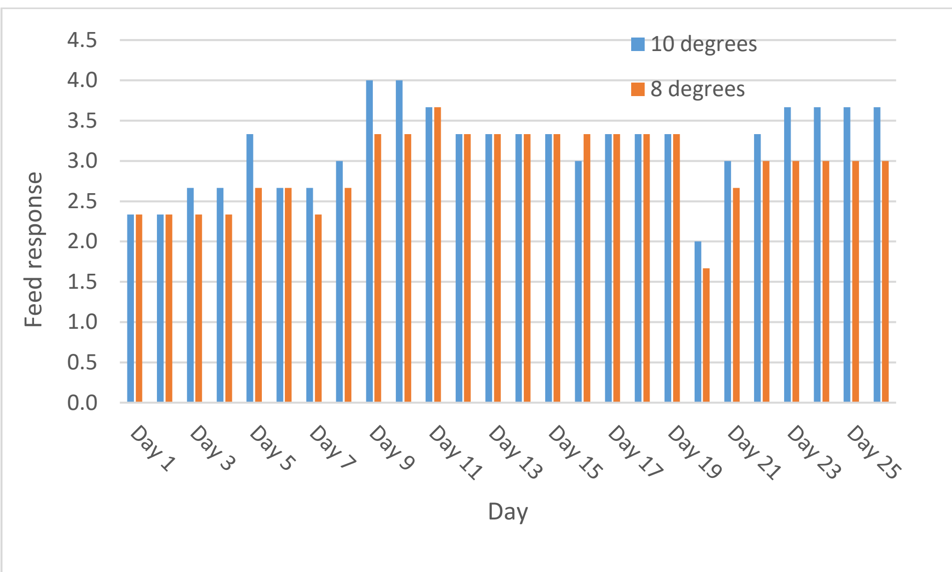


Figure 15: Feed response of the 8-degree and 10-degree groups.

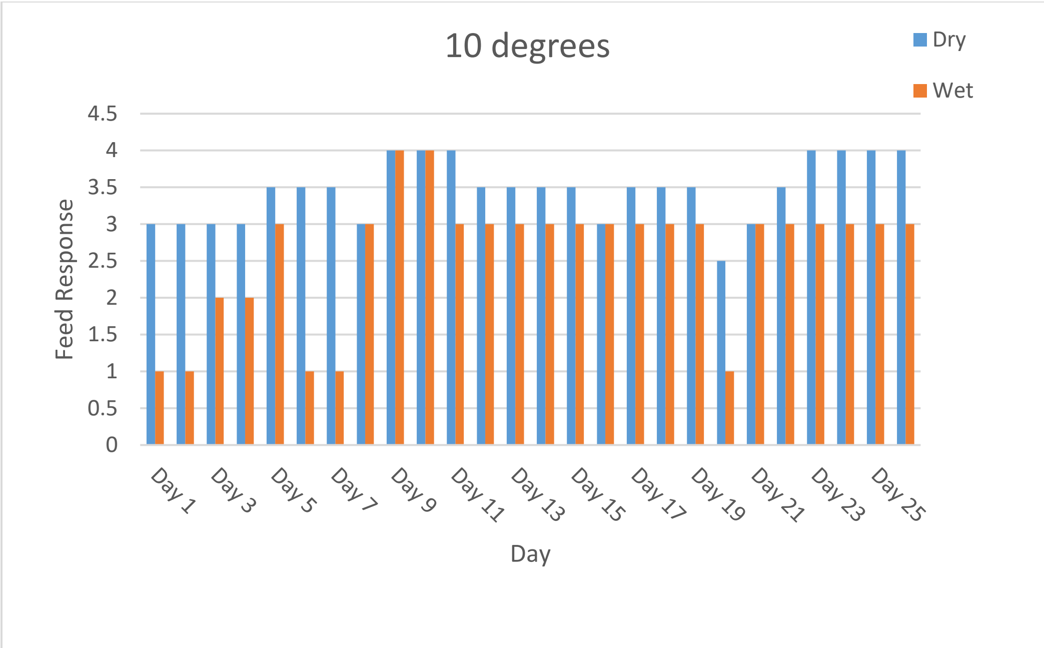


Figure 16: Feed response of the wet and dry fed groups held at 10 degrees celsius.

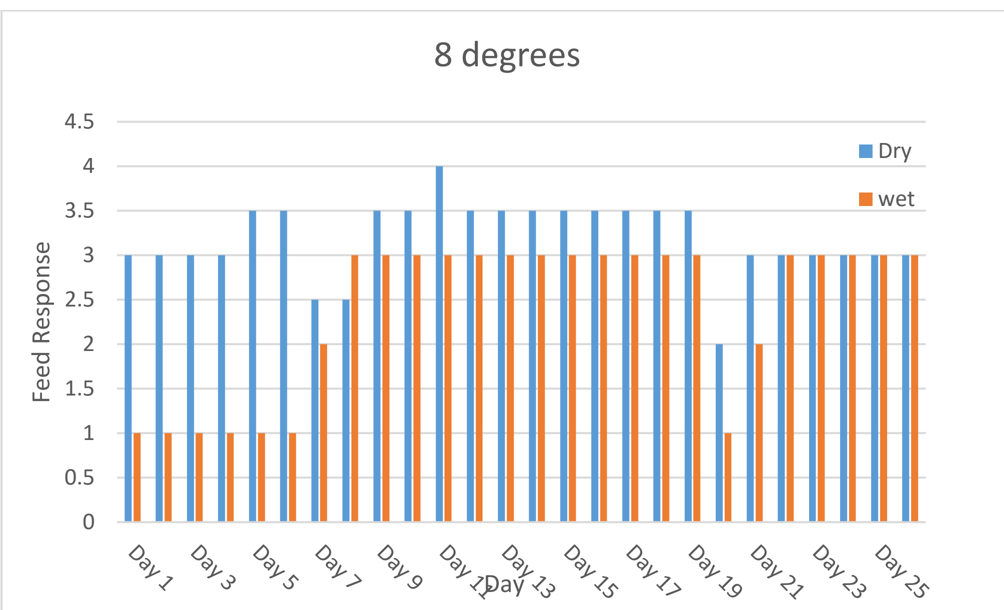


Figure 17: Feed response of the wet and dry fed groups held at 8 degrees celsius.

Conclusion:

The results of this trial indicate juvenile wolffish can be reared at either 8 °C or 10 °C without any major differences in the growth of the fish (Figure 9). When the trial ended, the fish reared at 10 °C weighted slightly higher than the fish reared at 8 °C , weighing 156 grams versus 140 grams respectively.

There also appeared to be no difference between the fish that were wet fed versus the fish that were dry fed fish at 8 degrees (Figure 10) at the end of the trial, the wet fed group weighed 142 grams and the dry fed group weighed 140 grams. This was a similar result to the fish at 10 degrees (Figure 11). At the end of the trial the fish that were fed the wet feed were 154 grams versus 156 grams for the fish that were dry fed.

As for other trials conducted, specific growth rates were inconsistent despite the continuous growth rates (Figures 12-14). This leads us to be further convinced there is some sort of compensatory growth effect that happens with this species. It is hypothesized that this inconsistent specific growth rate is actually the normal growth pattern for this species. To confirm this, a longer trial period would be required.

Feed response was good in both the 8 versus 10 °C group as well as in the dry versus wet fed groups. The fish held at 10 degrees showed a slightly increased feed response compared to the fish held at 8 °C (Figure 15). When comparing the wet fed group to the dry fed group, there was a slightly higher response in the dry fed group at both 8 and 10 °C (Figures 16 & 17). Initially it appeared that the fish took a few days to adjust to the wet food as feed response was low for a few days initially but increased as the fish acclimated to the palatability.

3.3 M2 –A3 Refinement of Juvenile Production Technologies

3.3.1 Maximization of Growth in 3-10 gram fish

To be completed in 2023 if eggs and hatched larval are available. Focus has been on the larger juveniles studies as updated in M2-A2, A4, A5, A6.

3.4 M2 – A4 Refinement of Juvenile Production Technologies

3.4.1 Maximization of Growth in 100-300 gram fish.

The growth of wolffish in all tanks is monitored monthly. Weights and total length (Figures 18 - 20) is measured on thirty fish per tank. From this data, their specific growth rate and condition factor are calculated. Each month culture conditions are analysized / re-evaluated and changes are made if necessary to maximize growth.

Figure 18: Set up for weighing and measuring fish

Figure 19: Measuring total length

Figure 20: Weighing wolffish

Initially rearing temperatures were held between 7-8 °C however, from our low temperature feed tolerance trials as well as observations, it has been noted that the fish show a greater feed response if rearing temperatures are maintained between 8 –9 °C. It has also been observed that response is also increased if oxygen levels are maintained above 100% saturation. Further trials will be carried out to examine the effect of oxygen saturation on feed response.

It is also hypothesized that stocking density also plays a role in optimizing growth rates, throughout 2023 further trials will be carried out to determine the effect stocking density has on feed response and specific growth rates.

3.5 M2 – A5 Refinement of Juvenile Production Technologies

3.5.1 Evaluation of Stocking Densities.

Throughout 2023, we have been monitoring and evaluating various stocking densities (Table 6). We have held juveniles from (Figure 21) 300-1100 grams in densities ranging from 40-80 kg/m2 without issue. The key is to keep oxygen above 90% saturation post feeding at the outlet water.

Table 6: Monthly populated data sheet. December 2023.

Tank #	Fish # Start	Fish # End	Average Weight (g) Start	Average Weight (g) End	Biomass Start (kg)	Biomass End (kg)	Feed % BW/Day	Feed Per Day per tank (kg)	Density kg/m2 Start	Density kg/m2 End
B1	409	415	2286.8	2343.9	935.3	972.7	0.50%	4.86	27.35	28.44
B3	641	631	1169.7	1308.37	749.8	825.6	0.50%	4.13	40.53	44.63
B7	50	51	4402	4402	220.1	224.502	0.50%	1.12	19.65	20.04
B8	48	47	4929	4929	236.6	231.663	0.50%	1.16	21.12	20.68
Total	1148	1144								

Figure 21: Juvenile holding at 40 kg/m2 in a 25 tonne tank.

3.6 M2 – A6 Refinement of Juvenile Production Technologies

3.6.1 An evaluation of the following rearing protocols.

3.6.1.1 Grading of Juveniles

Upon arrival at the Department of Ocean Science all fish were assessed, weighed and grouped in tanks based on sizing. Upon receipt of the wolffish it was noted that there were three size groupings. After a period of acclimation, it was necessary to size grade the fish to ensure feeding efficiency.

A standard box grader was used to size grade the two larger groups and fish were grouped as either over or under 100g.

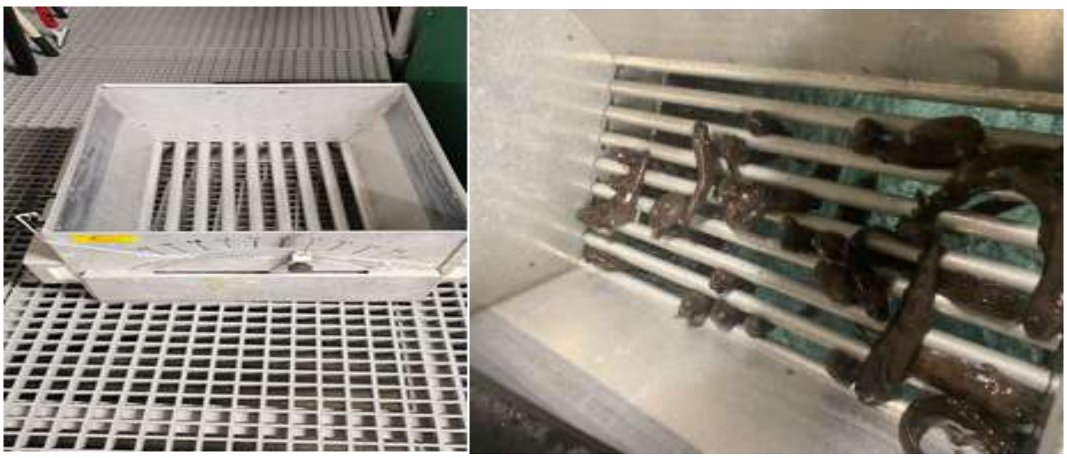


Figure 22: Box grader used to grade smaller wolffish

As the fish continued to grow and become quite large it was no longer feasible to use a box grader (Figure 22) as the fish were too big and very, few could be placed in the box grader at a time. At this point it was necessary to develop a more efficient system. A shallow water table was set up with a continuous flow of water. Fish were added to the grading/water table (Figures 23-24) in batches and they were then hand graded based on size, weighed and distributed to tanks. This grading/water table worked quite well as it minimized the stress on the fish and provided an opportunity to observe the fish noting any deformities or other concerns that may be present.

Figure 23: Grading table set up with flow through water

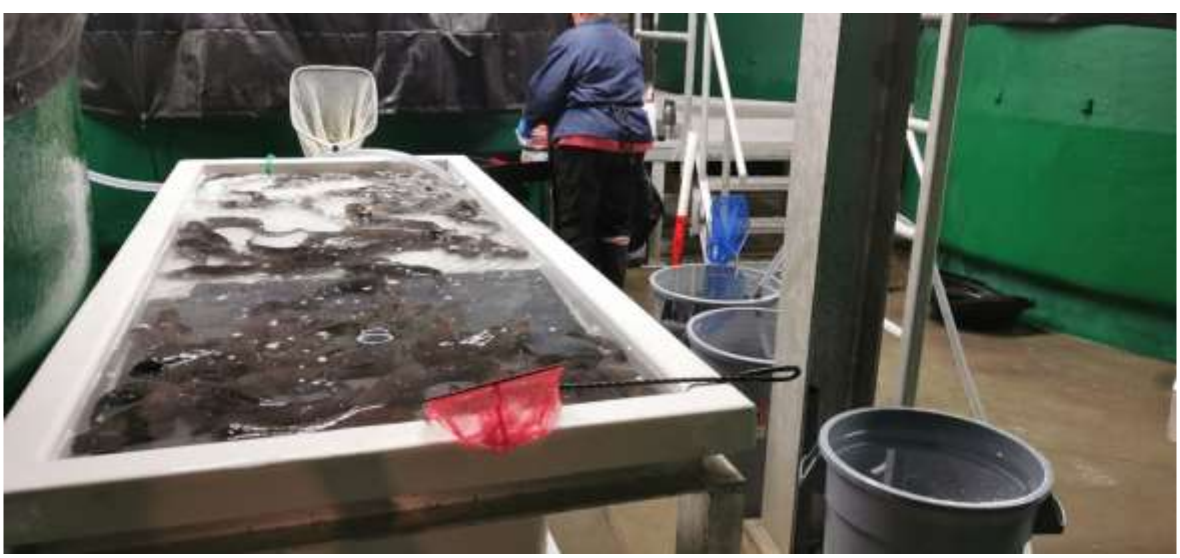


Figure 24: Fish being hand graded on water table

3.6.1.2 Water level height

We feel that an adequate water height for juveniles (100-1500 gams) stocked between 40-80 kg/m2 in our tanks of this size is 0.6-0.75 of a meter.

3.6.1.3 Flow Velocities and Tank Turn Over

We feel the tank should have a complete water exchange at a minimum every 2 hrs or preferably every 1 hour. Tank direction and velocity is clockwise, with a water inlet pipe submerged and angled to aid in waste removal, but not impacting fish swimming velocity. Ensure that inlet and outlet pipes are sized correctly to the tank to minimize a vortex in the middle of tank or dead zones. We also use surface overflows and protein skimmers on the surface to aid in water clarity.

Oxygen should be maintained at 80-100% saturation post feeding at outlet level. If the water leaving tank is 90% saturated, you will be fine. Light levels in the 100-200 lux range for juveniles with a photoperiod of 16L:8 D regime for juvenile rearing production.

Other things to consider in times of water loss / emergencies are back up supports to ensure oxygen levels are adequate with no water flows. This will not be achieved with a single or multiple oxygen stone in a larger tank with these fish that just lay on the bottom.

We have found that water movement is required, plus back up oxygen supply. Therefore a side loop or sump pump configuration to ensure water movement within tank. You need to be able to move the static water within the tank during emergencies. The excess feces and uneaten food may be re-suspended and water clarity may not be ideal, but you fish will survive for a period of time until water is restored o facility and or tank.

3.6.1.4 Response to acute temperature spike

Throughout the year incoming ambient water temperature is typically fairly stable and remains below 10 degrees Celsius. However, there was a period during this season when there was a spike in the incoming ambient water temperature. Without the ability to chill the incoming water the fish were inadvertently exposed to a high temperature event. While, this event was not planned valuable information was gathered from the occurrence. The incoming ambient sea water temperature spiked from 3°C to 14.2°C over a period of 48 hours. This jump in ambient temperature resulted in a drastic increase in tank water temperatures. Tank water temperatures have a setpoint of 9 degrees as a result of the temperature spike water temperatures in the tank increased from 9.0°C to 14.9°C (Figures 25-28 & 34-35) in approximately 3 hours resulting in an acute temperature stressor. This increase in water temperature also created a quick drop in oxygen levels. (Figures 29-32 & 36-37). Ambient water temperatures remained above 12 degrees celsius over an 11 day period. During the higher temperature period, the wolffish lost interest in feed and showed no feed response.

In order to combat lower oxygen levels brought about by the higher temperatures, additional supplemental oxygen was added to the tanks using portable oxygen generators.

The acute temperature stressor resulted in a mortality spike of 3.4% overall during the 11 day period of increased temperatures and low feed response. The mortalities resulting from this event were seen two days post event (Figures 33, 38 & 39) (Table 7). The percent mortality ranged from 0.77% in B7 to 7.9% in LS 3 (Table 7). We lost 3.4% of the total population.

As temperature stabilized, the mortality stopped and the wolffish were able to tolerate the higher temperature without further mortalities. However, they were not able to tolerate the acute 6 °C increase and lost equilibrium with the rapid increase. While the wolffish were able to tolerate the higher temperatures, it was not until temperatures returned to a normal temperature range of 9°C that the fish began to show a return to normal feeding behaviours. The frequency distribution shows that the size of the mortalities ranged from 19-919g with the bulk of them being less than 219g (121 fish) (Figure 39).

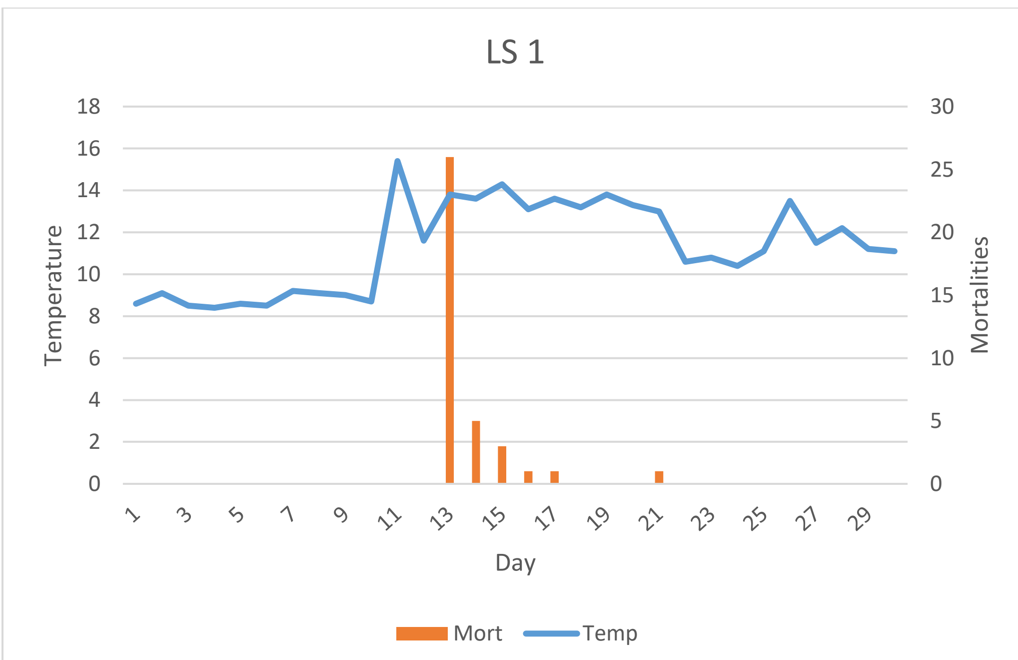


Figure 25: Tank temperature throughout the month with mortalities caused by temperature spike in LS 1.

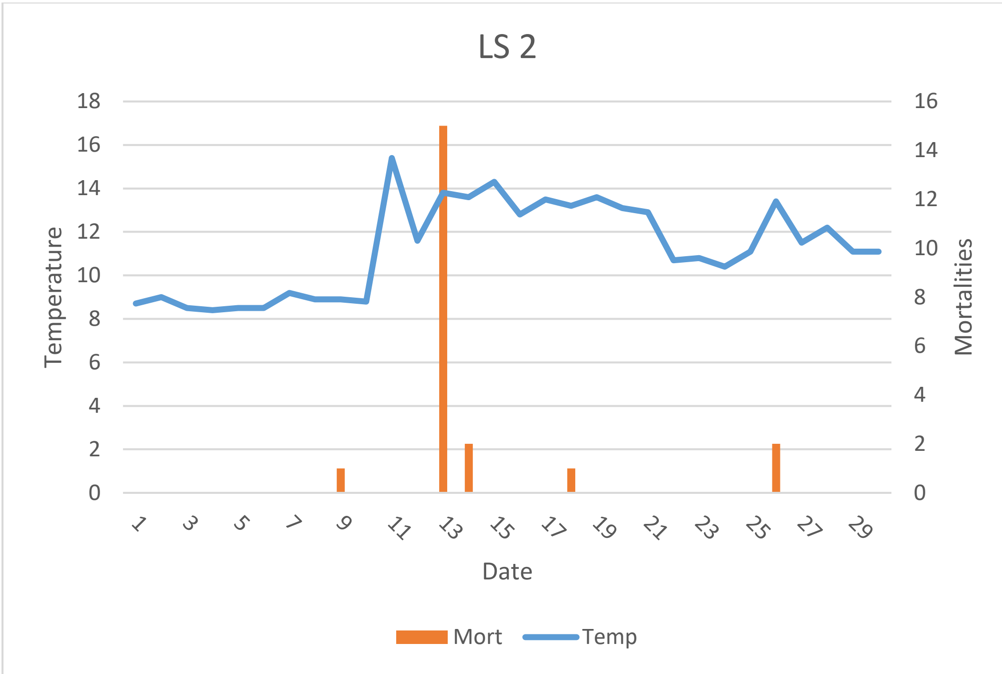


Figure 26: Tank temperature throughout the month with mortalities caused by temperature spike in LS 2.

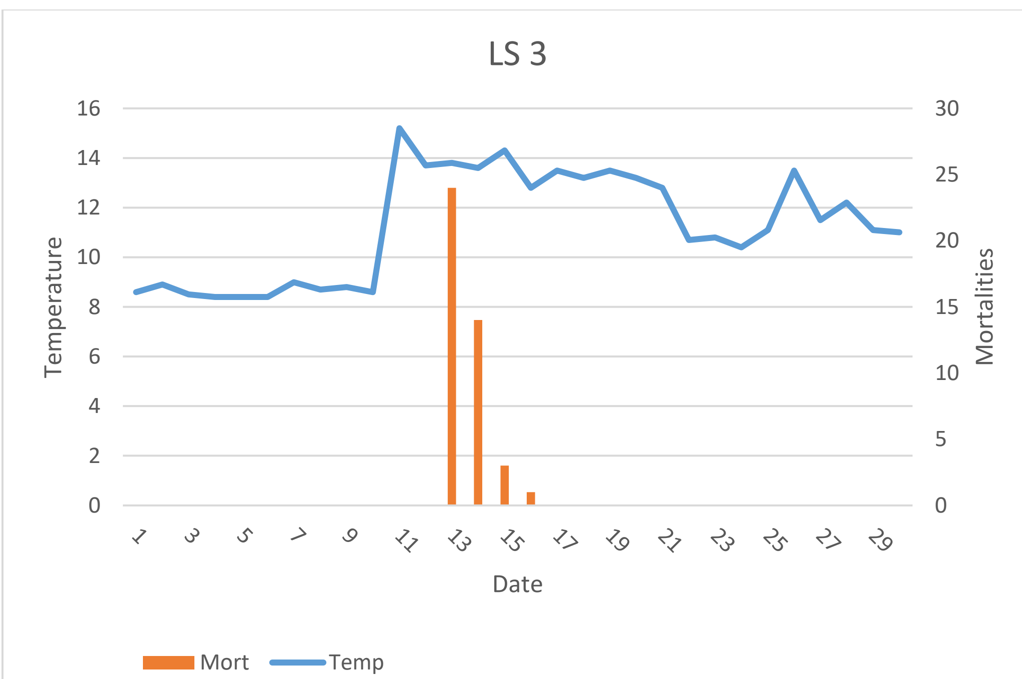


Figure 27: Tank temperature throughout the month with mortalities caused by temperature spike in LS 3.

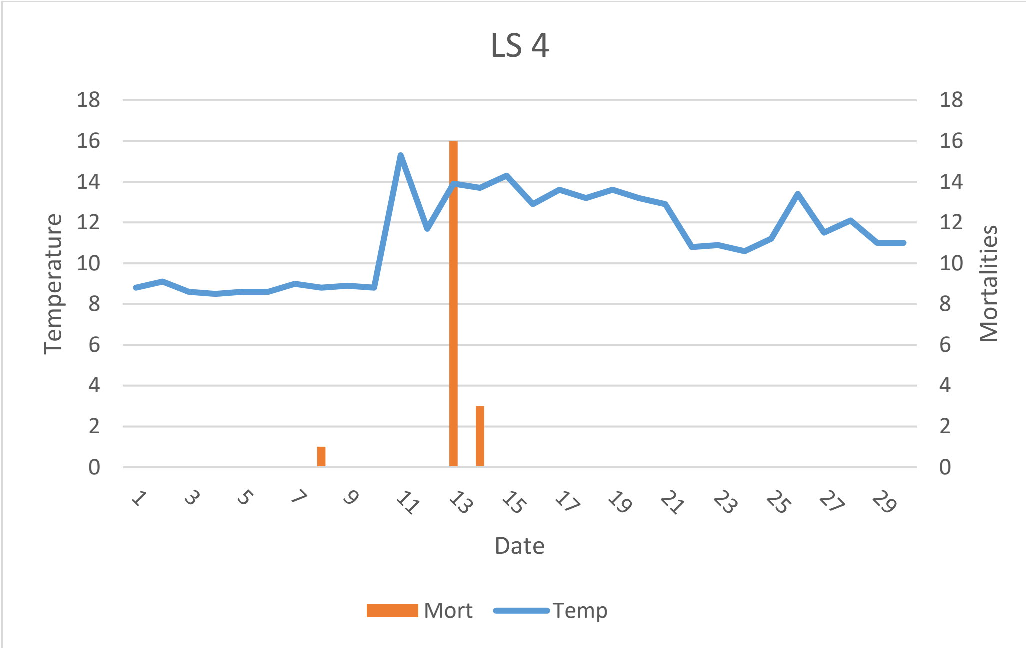


Figure 28: Tank temperature throughout the month with mortalities caused by temperature spike in LS 4.

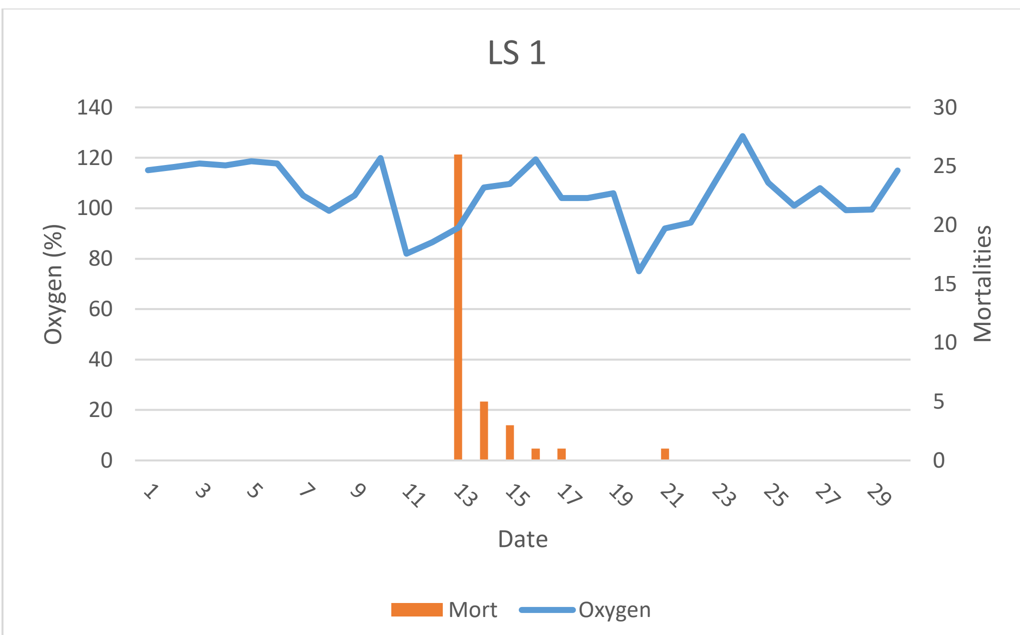


Figure 29: Tank oxygen levels throughout the month with mortalities caused by temperature spike in LS 1.

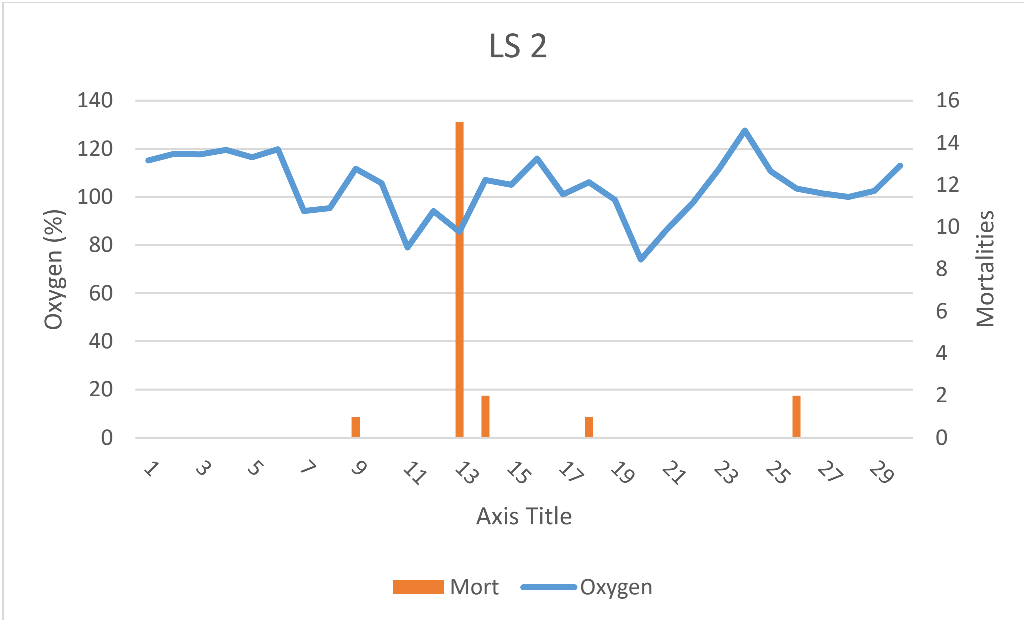


Figure 30: Tank oxygen levels throughout the month with mortalities caused by temperature spike in LS 2.

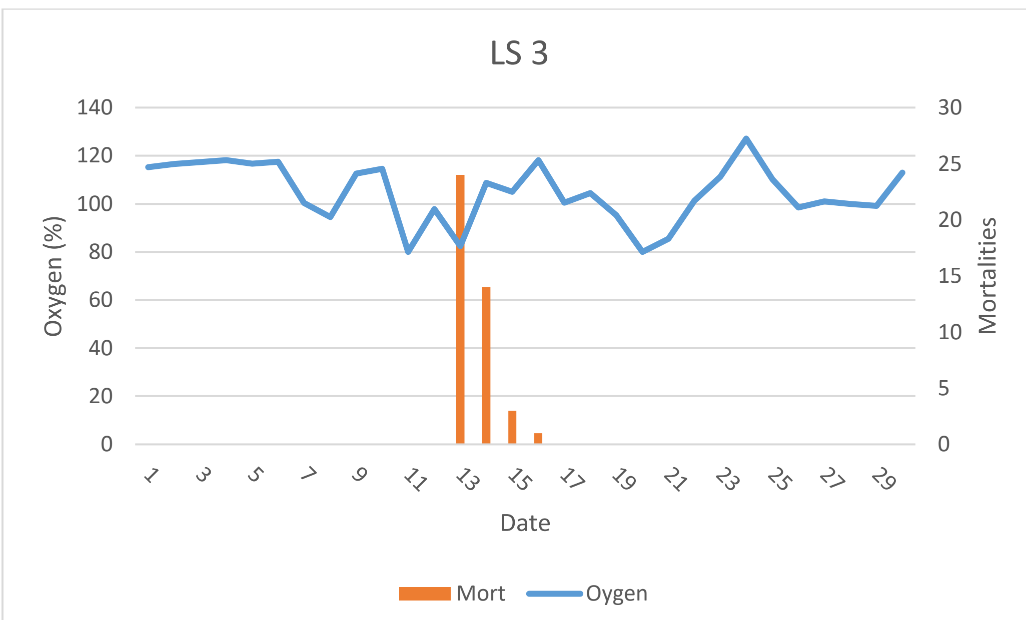


Figure 31: Tank oxygen levels throughout the month with mortalities caused by temperature spike in LS 3.

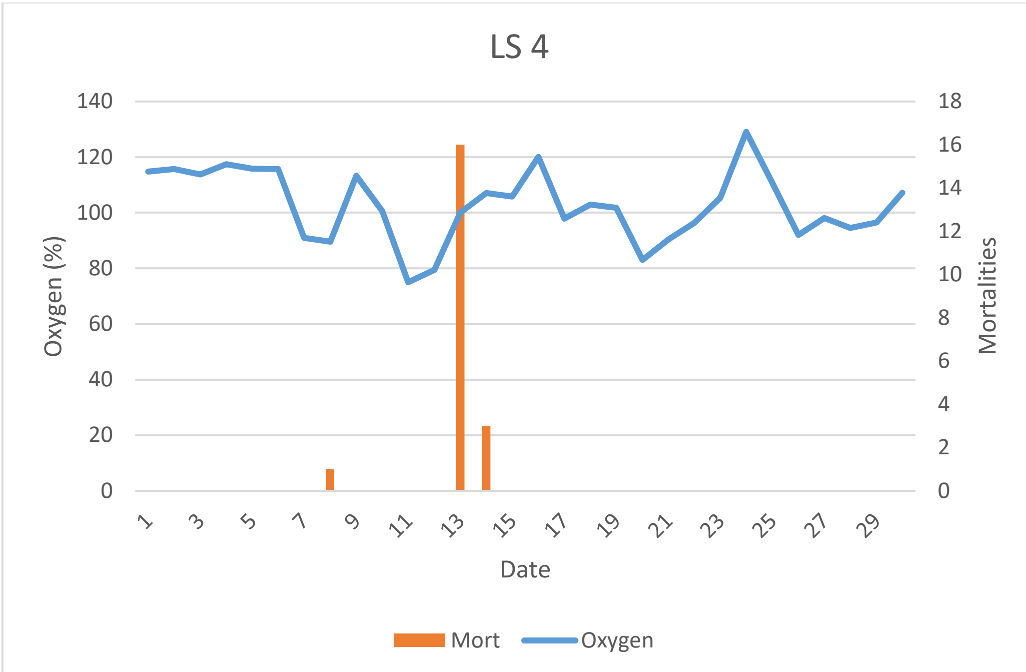


Figure 32: Tank oxygen levels throughout the month of September with mortalities caused by temperature spike in LS 4.

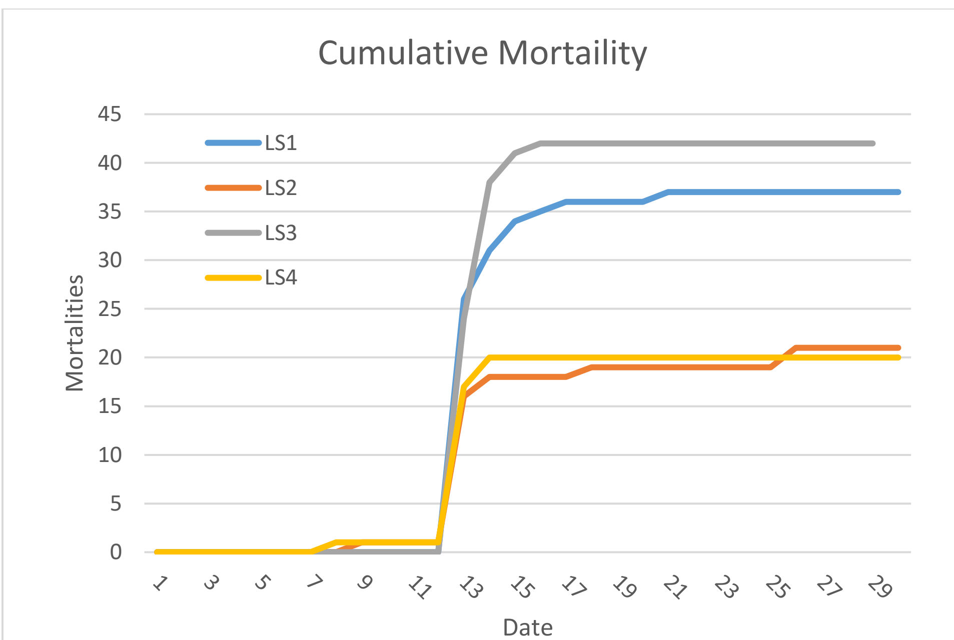


Figure 33: Cumulative mortality from tanks LS 1-4 due to temperature spike

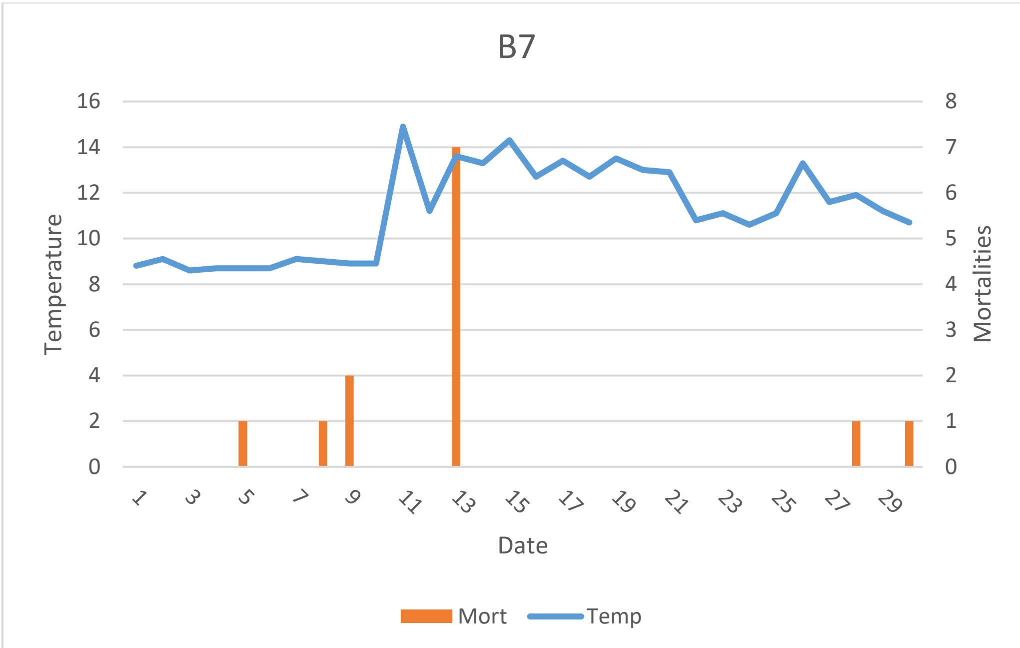


Figure 34: Tank temperature throughout the month with mortalities caused by temperature spike in BS 7.

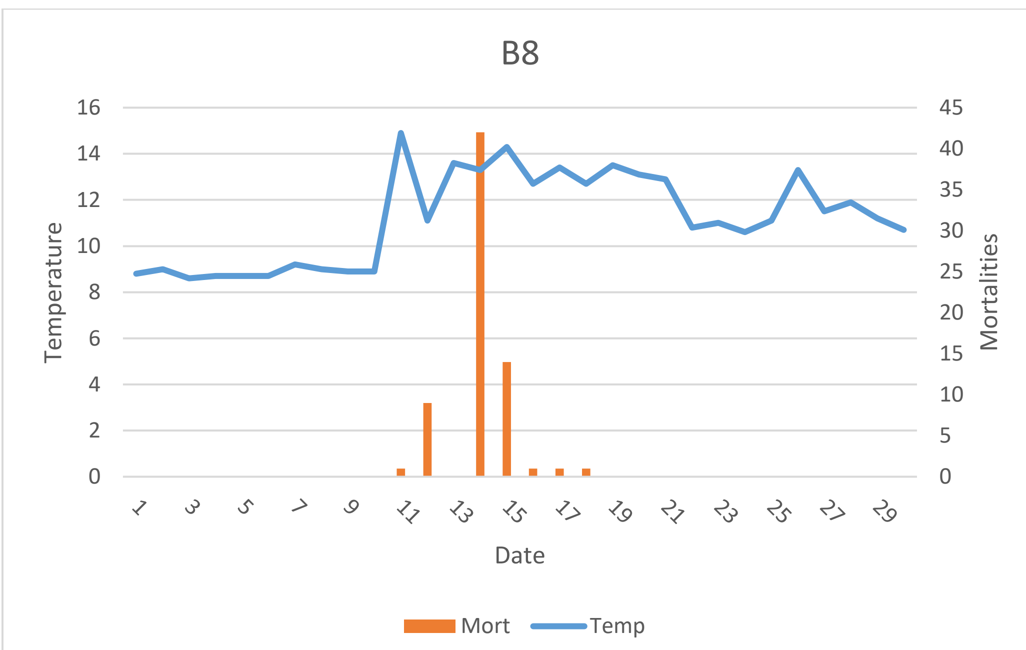


Figure 35: Tank temperature throughout the month with mortalities caused by temperature spike in BS 8.

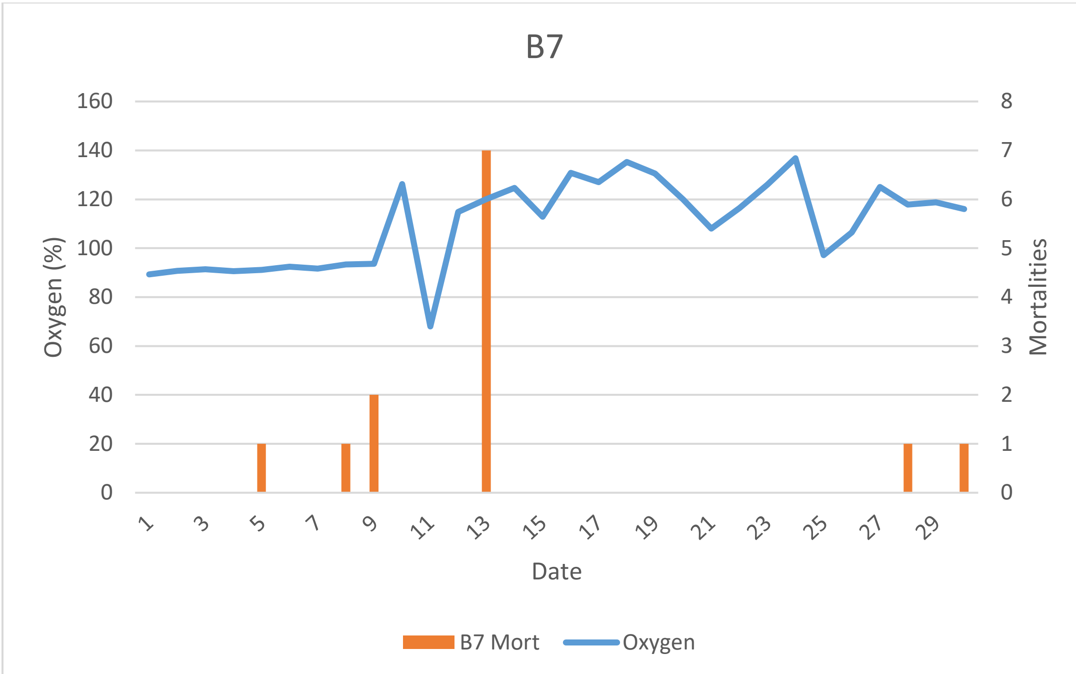


Figure 36: Tank oxygen levels throughout the month with mortalities caused by temperature spike in B7.

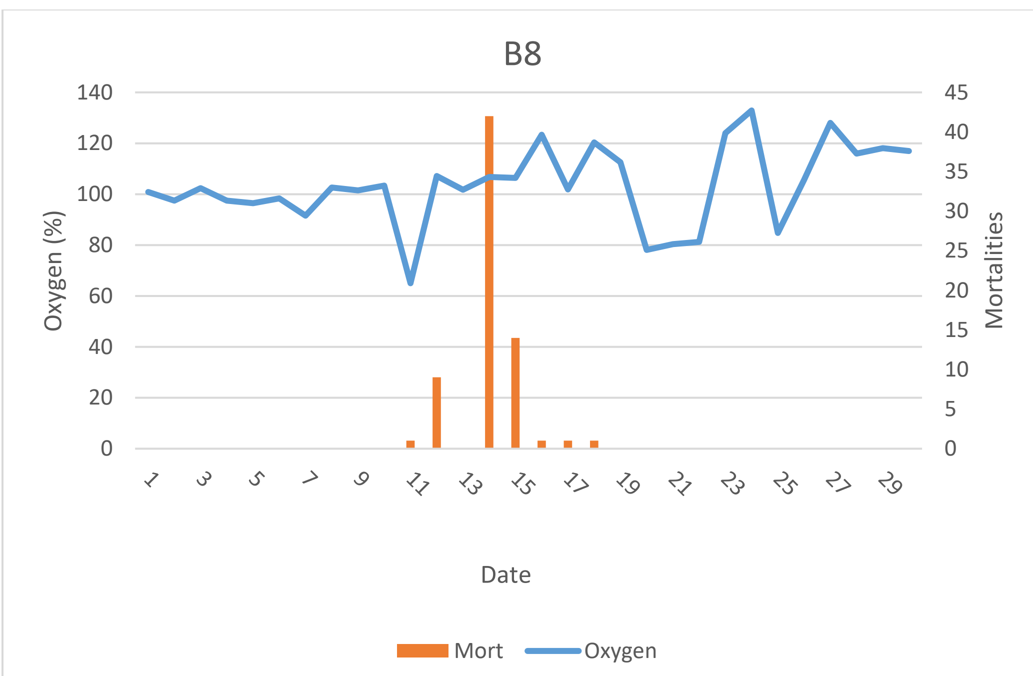


Figure 37: Tank oxygen levels throughout the month with mortalities caused by temperature spike in B8.

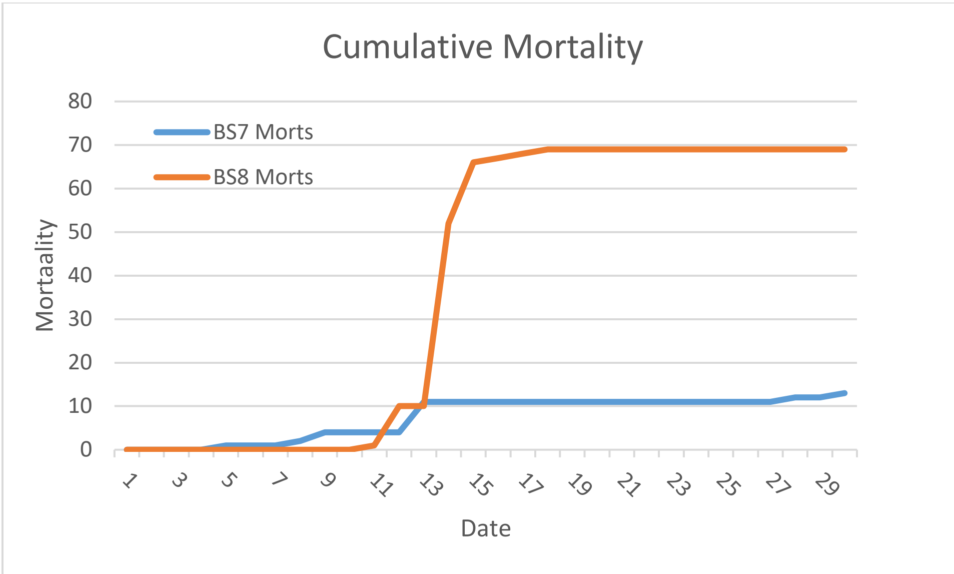


Figure 38: Cumulative mortality from tanks B7-8 during high temperature spike

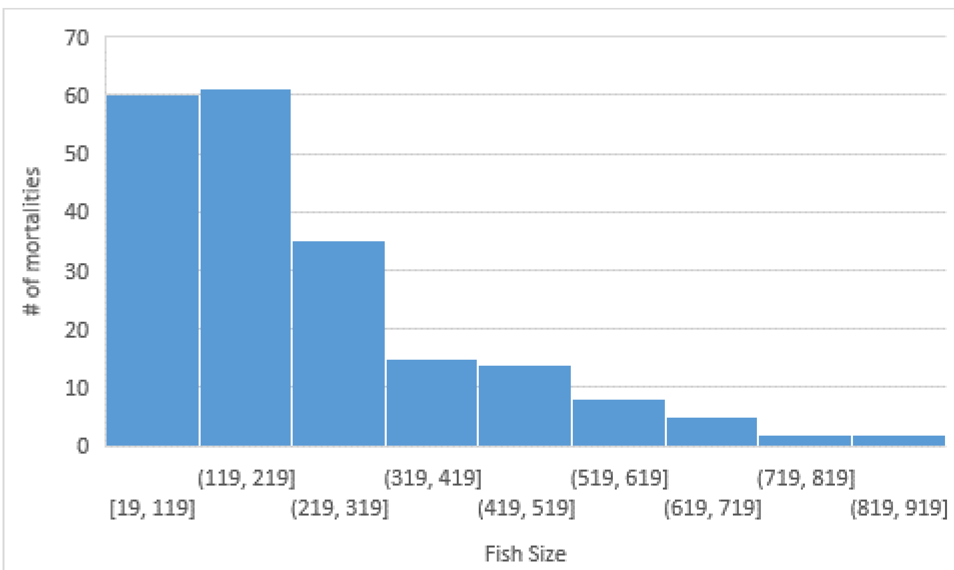


Figure 39: Frequency distribution of the mortalities from all tanks.

Table 7: Summary of the fish mortalities from a high temperature event

Tank		# fish September 1	# fish September 30	Mortalities	Average Weight September 1	% mortality	Stocking density kg/m^2
LS 1		524	485	37	216	7.1	53.9
LS 2		515	494	21	190	4.1	46.6
LS 3		530	488	42	225	7.9	56.8
LS 4		583	563	20	179	3.4	49.7
BS 7		1827	1814	14	372	0.77	60.5
BS 8		1886	1818	69	401	3.7	67.6
Total Fish		5865	5662	203			

3.7 M2 – A7 Camera Feeding System

3.7.1 Artificial Intelligence (AI) Feeding System.

Timeline: A7- April 1, 2022 - December 31, 2023

We requested a change from Artificial Intelligence (AI) due to timelines and equipment purchase issues with global supply chain.

To remove the Artificial Intelligence portion and continue with the making of feeds with Hobart Feeding machine, purchase as planned the automatic feeders and testing of moist versus dry feeds in tanks. No change to budget.

Feed Testing:

Soft versus hard pellets. The fish seem to really enjoy the softer diets. They are performing very well to date. There are no issues with water quality in our flow through system.

Below are a few figures of pellets, making feeds, feed storage and handling (Figures 40-43).

Figure 40: Left- Soft Pellet Right- Hard Pellet

Figure 41: Hobart Feed mixing and extruder.

Figure 42: Soft feeds 6 mm & 10 mm.

Figure 43: Soft feeds storage in JBARB -20°C Freezer 6, 10 & 15, 22 mm in bags within styro boxes.

3.8 M2 – A8 Fish Transport

Wolffish Transport Tank

We have successfully completed multip ttransport trips to date with zero mortality. A few pictures below (Figures 44-48) and technical details (Table 8-9).

Figure 44: OSC Fish Transport

Figure 45: Offloading Wolffish that arrived from out of province to start project.

Figure 46: Preparing to load fish in Quebec. Transfer to PEI.

Figure 47: Preparing site management plan and trailer for the loading of wolffish to begin transport.

Figure 48: Preparing for off-loading of wolffish during night time.

We use the above trailers for wolffish transports (Figures 44-48).

5 Transport boxes per trailer with top open hatch. Oxygen capabilities on board.
Inside tank dimensions.

- Width (across trailer) 85 inches or 2159 mm
- Length (front to back on trailer) 88 inches or 2235.2 mm
- Height - 52 inches or 1320.8 mm

~6000 L of water/ 4.4 m^2 of bottom surface area.
Trip Duration – 12-36 hours depending on route.
Water Temperatures – 4-12 degrees depending on time of year and location.
Transport stocking densities: 20-80 kg/ m^2 depending on size of fish, transport route.
Starvation period - 2 days for juveniles, 3 days for larger broodstock.
Oxygen Levels,
Mortality rates

Table 8: Transport Technical

Transport #1- December 19, 2022
Tanks- 4.4 m2 surface area
Aim- 40 kg/m2

Wolffish		Number	Size	Move to PEI				
MLI		73	2-3 kg	73				
MLI		33	10 kg	33				
		106						
Location	**Re-locate**	**Fish #**	**Fish Size (gms)**	**Biomass (kg)**	**Density (kg/m2)**	**Tank #**	**Load #**	**Truck**
MLI	PEI	36	2500	90	20.45	1	1	Cooke Smolt Tank
MLI	PEI	37	2500	92.5	21.02	2	1	Cooke Smolt Tank
MLI	PEI	11	10000	110	25.00	3	1	Cooke Smolt Tank
MLI	PEI	11	10000	110	25.00	4	1	Cooke Smolt Tank
MLI	PEI	11	10000	110	25.00	5	1	Cooke Smolt Tank
Total		106						

Warm Water Resistent Clothing
Boots
Gloves (bite proof)/balaclava
Large Nets/ baskets?
O2 meter and batteries
Transport Permit
Bags Salt for
truck
Pencil / clip board
Fish Feed
Head Lamp/ Flash Lights

Completed - all okay and 0
mortality.

Table 9: Transport Technical

Transport #2- January 12, 2023
Merinov to PEI

Wolffish		Fish Number	Size	Move to PEI
Broodstock		134	6500	134
Immature Broodstock		107	4000	107
Total Fish				134

Location	Re-locate	Fish #	Fish Size (gms)	Biomass (kg)	Density (kg/m2)	m2	# tanks required	Load #	Tank Size
Merinov Broodstock	PEI	134	6500	871	65.98	13.2	3.0	1	4.4 m2
Merinov Broodstock	PEI	107	4000	428	48.64	8.80	2.0	2	4.4 m2
Total		241		1299					

Warm Water Resistant Clothing
Boots
Gloves (bite proof)
Large Nets/ baskets?

O2 meter and batteries
Transport Permit
Bags Salt for truck

Pencil / clip board

Completed - all okay and 0 mortality.

10 hr from Grande Riviere - to Victori PEI
Cold but clear -
8°C
really cold air temp on arrival -20°C

Need carts, more people and offloading- add multiple fish per tub and get cart etc.
Oxygen stays high even with 1/16 on flow meter with 50 PSI- need 0-1 lpm oxygen flow meters

Parameters Measured

At each time point a visual check was performed on the fish and both Oxygen and temperature were obtained and recorded using an Oxyguard Handy Polaris 2 or YSI Pro 20 portable dissolved oxygen Meter (Table 9B, Figure 49).

Amar Seafoods shipped 97 Broodstock (~ 20 per tank) to the Ocean Sciences Center, Logy Bay 2023 at a stocking density of approximately 35-40kg/m^2. No mortalities during transport.

The broodstock were handled by netting them from the tank and walking the net to the truck. The journey duration was approximately 31 hours and the average time between visual checks and sampling was 2.79 hours.

Table 9B: Dissolved oxygen and temperature readings Wolffish Transport

Spotted Wolffish Broodstock Shipping Log 2023											
Details	Approximately 20 Fish per tank, total 97 fish per transport, approximately 35-40kg/m^2										
Date	Time point	Transport Tank 1		Transport Tank 2		Transport Tank 3		Transport Tank 4		Transport Tank 5	
		Temp	DO %	Temp	DO %	Temp	DO %	Temp	DO %	Temp	DO %
Day 1	1	6.8	85	6.7	85	6.7	85	6.7	87	6.7	89
	2	7	127	7	127	6.9	135	6.9	141	7	131
	3	7.1	132	7.1	135	7.1	135	7	148	7.2	138
	4	7.3	126	7.3	123	7.3	124	7.2	136	7.4	123
	5	7.7	122	7.8	117	7.6	117	7.5	130	7.8	122
	6	8	111	8.2	129	7.9	118	7.8	129	8.1	124
	7	8.2	114	8.4	131	8.1	116	7.9	124	8.3	122
Day 2	8	8.3	118	8.7	112	8.2	113	8.1	118	8.3	122
	9	8.3	116	8.7	116	8.2	107	8.1	121	8.3	122
	10	8.6	118	9.1	133	8.5	113	8.3	125	8.6	118
	11	8.8	118	9.5	116	8.8	115	8.5	128	8.9	121
	12	9.2	124	9.9	112	9.1	119	8.8	122	9.3	121
	13	9.4	120	10	105	9.2	116	9	113	9.4	117

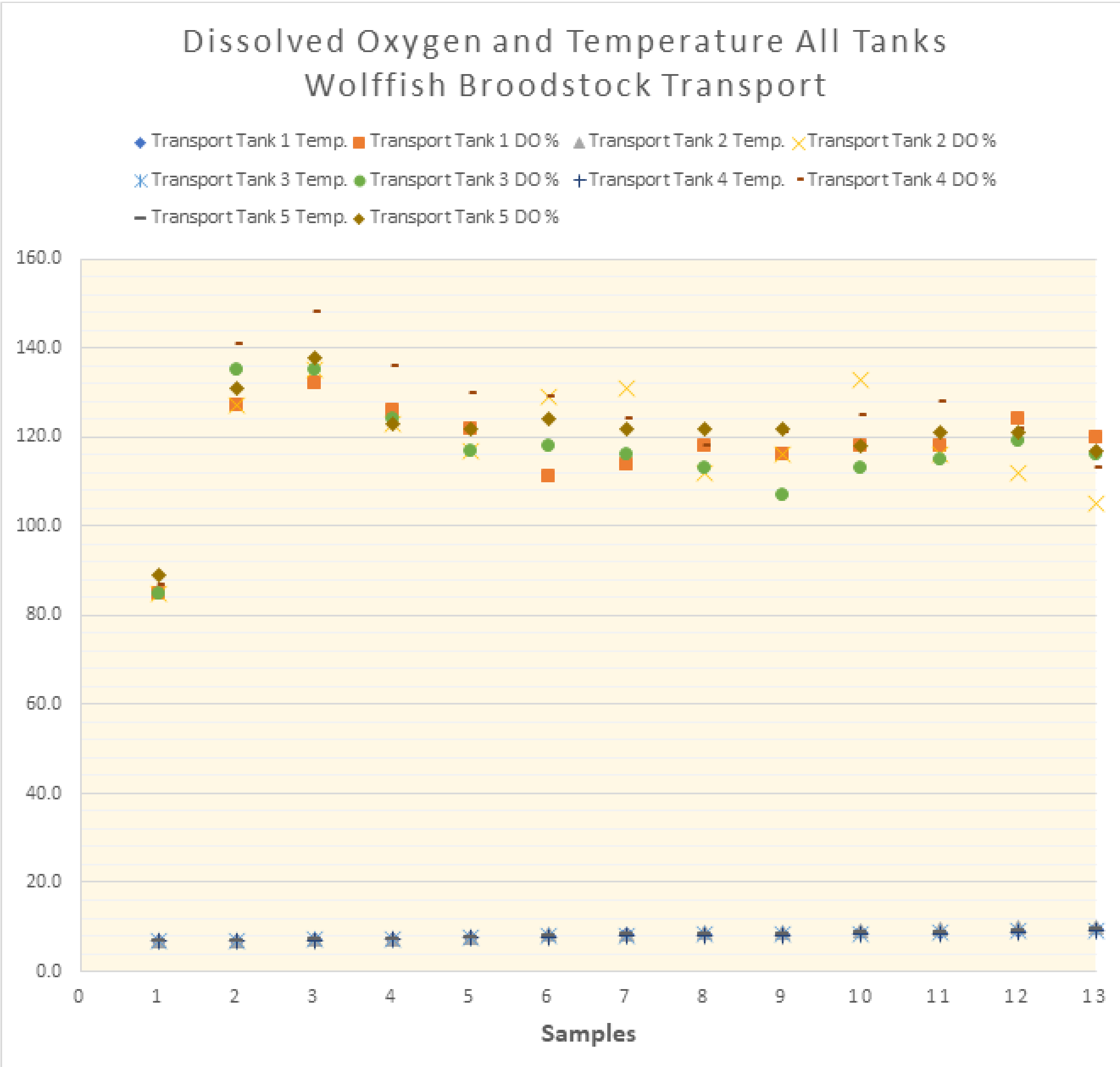


Figure 49: Wolffish broodstock transport temperature and dissolved oxygen readings for all tanks.

3.9 M3 – A9 Aquatic Animal Health Surveillance Program

Wolffish samples have been taken by provincial authorities multiple times during the past year as part of our ongoing active surveillance program and all results have been negative to ate with no pathogens noted. Fish are in good health (Figure 50).


Figure 50: Juvenile wolffish.

3.9.1 – Spotted wolffish Genomics
3.9.2 – Transcriptomics and Temperature Changes
3.9.3 – Microbiota Profile and Pathogen Surveillance

A 3.9.1 – Spotted wolffish Genomics- completed & ongoing. High molecular weight (HMW) DNA was extracted from blood and fin clip from male and female wolffish (~1.5 Kg). The wolffish were maintained in 5,000 L tanks with flowthrough filtered seawater at Prince Edward Island aquaculture facility (Figure 51A). Wolffish blood was taken from the caudal vein and immediately fixed in ethanol 100% (Figure 51B and C). For this, we used the MagAttract HMW DNA kit (QIAGEN, Hilden, Germany). Quality of the extracted DNA was evaluated by electrophoresis using 1% agarose gel (Figure 51D). The concentration of extracted DNA was between 20-57 µg/ml for fin clips and 160-199 µg/ml for blood samples (Table 10).

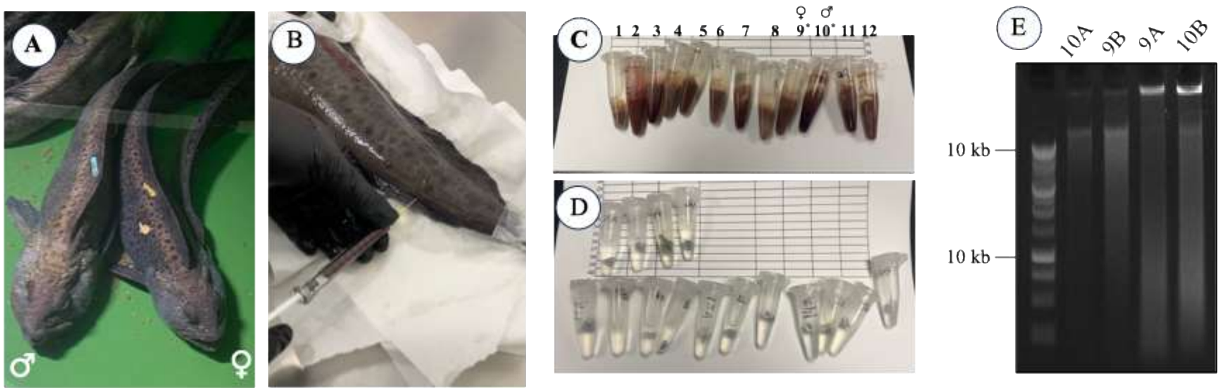


Figure 51. Wolffish and DNA samples. A. Male and Female wolffish from PEI facility; **B.** Blood sampling; **C.** Blood samples fixed in 100% EtOH; **D.** Fin clips fixed in 100% EtOH; **E.** Electroforesis of HMW DNA purification in a 0.8% agarose gel. Female #9 and Male #10 blood and fin clip samples were used.

HMW DNA extracted from blood showed the highes quality (Fig. 1D) and is currently under sequecing using Oxford Nanopore technology, Ultra-Long DNA Sequencing Kit and MinION™ and MinION Mk1C flow cell (R10.4.).

Table 10. DNA concentration and quality purified from wolffish blood and fin clip samples.

Sample	DNA µg/mL	DNA ng/mL	260/280	260/230
Female-Blood-9B	199.31	19,931	1.883	2.214
Male-Blood-10B	160.44	16,044	1.833	1.193
Female-FIN-9A	56.159	5,615	1.778	1.945
Male-FIN-10A	20.004	2,000	1.608	1.114

3.9.2 – Transcriptomics and Temperature Changes- ongoing. For transcriptomics analysis a total of 100 fish (~600 g) were exposed to temperatures changes mimicking deep ocean, and ocean rising temperatures. Three temperatures were selected ≤3 °C, 8 °C and 14 °C. Before proceeding with the temperature setup, fish were acclimated at 10°C during a period of 3 weeks, followed by increasing or decreasing the temperature in a range of 0.1 °C- 0.4 °C per day during a 3-week period. Once, the desired temperatures were reached, fish were kept for 6-week period until sampling (Figure 52). A total of six fish liver were flash frozen for each temperature. Total RNA samples were extracted using mirVana RNA kit according to manufacture´s instructions. RNA sequencing was performed commercially at Genome Quebec, QC, Canada. All samples passed the quality controls from Genome Quebec and successfully sequenced (Figure 53).

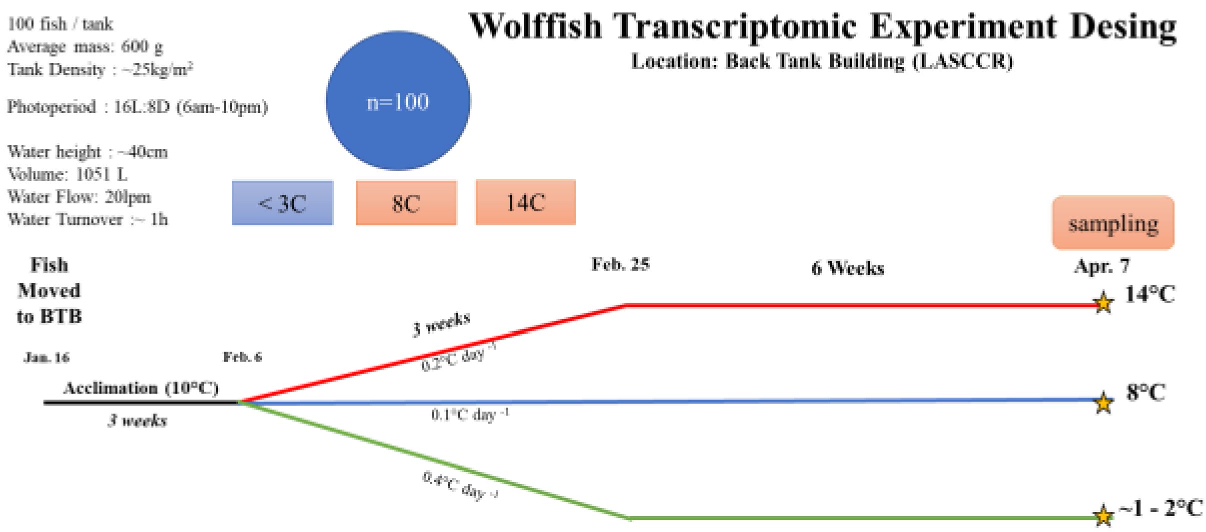
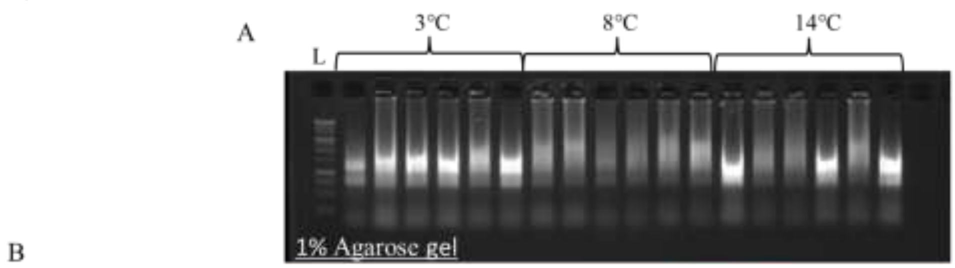


Figure 52. Experimental design for temperature changes.



Samples								Bioanalysis (RNA)							
	Name	Alias	Barcode	Reception Date	Library Type	Starting Amount (ng)	Starting Vol. (ul)	Image	28s/18s	RIN	Qual. QC Status	Vol. (ul)	Conc.	Total RNA (ng)	Quant. QC Status
1	L_3C_F1	L_3C_F1	MPS174451	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.968123900302215	8.5	✓	24	697 ng/ul	16728	✓
2	L_3C_F2	L_3C_F2	MPS174452	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.731468093228888	7.8	✓	24	692 ng/ul	16608	✓
3	L_3C_F3	L_3C_F3	MPS174453	2023-10-05	polyA-enriched RNA (stranded)	250	55		0.859705686945692	8.5	✓	24	419 ng/ul	10056	✓
4	L_3C_F4	L_3C_F4	MPS174454	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.852183688084318	8.4	✓	24	483.5 ng/ul	11604	✓
5	L_3C_F5	L_3C_F5	MPS174455	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.757714585404513	8.4	✓	24	299 ng/ul	7176	✓
6	L_3C_F6	L_3C_F6	MPS174456	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.739096333552584	8.6	✓	24	391.5 ng/ul	9396	✓
7	L_8C_F1	L_8C_F1	MPS174457	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.73832112974119	8	✓	24	355.5 ng/ul	8532	✓
8	L_8C_F2	L_8C_F2	MPS174458	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.561287281375971	7.8	✓	24	312 ng/ul	7488	✓
9	L_8C_F3	L_8C_F3	MPS174459	2023-10-05	polyA-enriched RNA (stranded)	250	55		0.785698438889153	8.6	✓	24	273 ng/ul	6552	✓
10	L_8C_F4	L_8C_F4	MPS174460	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.897747238717916	8.9	✓	24	222 ng/ul	5328	✓
11	L_8C_F5	L_8C_F5	MPS174461	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.730648874888073	8.8	✓	24	186 ng/ul	4464	✓
12	L_8C_F6	L_8C_F6	MPS174462	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.738428529993119	8.8	✓	24	211 ng/ul	5064	✓
13	L_14C_F1	L_14C_F1	MPS174463	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.935325258894209	8.8	✓	24	606.5 ng/ul	14556	✓
14	L_14C_F2	L_14C_F2	MPS174464	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.849804882881332	8.6	✓	24	180.5 ng/ul	4332	✓
15	L_14C_F3	L_14C_F3	MPS174465	2023-10-05	polyA-enriched RNA (stranded)	250	55		0.821267828373479	8.6	✓	24	149.5 ng/ul	3588	✓
16	L_14C_F4	L_14C_F4	MPS174466	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.797890164739488	8.7	✓	24	392 ng/ul	9408	✓
17	L_14C_F5	L_14C_F5	MPS174467	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.793753891145387	8.1	✓	24	353 ng/ul	8472	✓
18	L_14C_F6	L_14C_F6	MPS174468	2023-10-05	polyA-enriched RNA (stranded)	250	52		0.84913793877282	9	✓	24	235 ng/ul	5640	✓

Figure 53. RNA samples quality control reports. A. Inner laboratory quality control of RNA samples extracted run in a 1% agarose gel. **B.** Genome Quebec quality controls report of sent RNA samples.

3.9.3 – Microbiota Profile and Pathogen Surveillance-ongoing. For metagenomic analysis we utilized 10 fish hind gut and their content as represented in Figure 54. Samples were flash frozen in liquid nitrogen and kept at -80°C. DNA extraction was performed using the QIAamp

PowerFecal Pro DNA kit (QIAGEN, Hilden, Germany) according to manufacture´s instructions. DNA concentrations obtained range from 124 – 4000 µg total DNA (Table 11).

Metagenomics analysis was performed using Oxford Nanopore sequencing technology, by sequencing the bacterial 16S rDNA, using the 16S Barcoding Kit 1-24 (SQK-16S024) manufacture instructions and MinION flow cell R9.4.1 (FLO-MIN106) from Oxford Nanopore. The sequencing report showed a range of 1.25 – 11.76 GB of data produced with a success of 99.93-100% reads called with an average size of 1.5 Kb read length, sequencing was performed in a period of 72 h (Figure 55).

Table 11. Gut-Hind gut DNA extraction concentrations for metagenomic analysis.

8C			14C			3C		
Samples	[µg/ml]	Total DNA [µg]	Samples	[µg/ml]	Total DNA [µg]	Samples	[µg/ml]	Total DNA [µg]
F1	2.874	143.7	F1	5.221	261.05	F1	3.515	175.75
F2	8.034	401.7	F2	19.373	968.65	F2	2.859	142.95
F3	3.471	173.55	F3	8.153	407.65	F3	10.641	532.05
F4	3.064	153.2	F4	9.48	474	F4	3.483	174.15
F5	7.183	359.15	F5	98.286	4914.3	F5	3.917	195.85
F6	4.315	215.75	F6	8.644	432.2	F6	4.281	214.05
F7	4.694	234.7	F7	2.956	147.8	F7	56.398	2819.9
F8	4.371	218.55	F8	8.212	410.6	F8	3.446	172.3
F9	12.963	648.15	F9	3.398	169.9	F9	2.489	124.45
F10	11.051	552.55	F10	2.94	147	F10	2.804	140.2

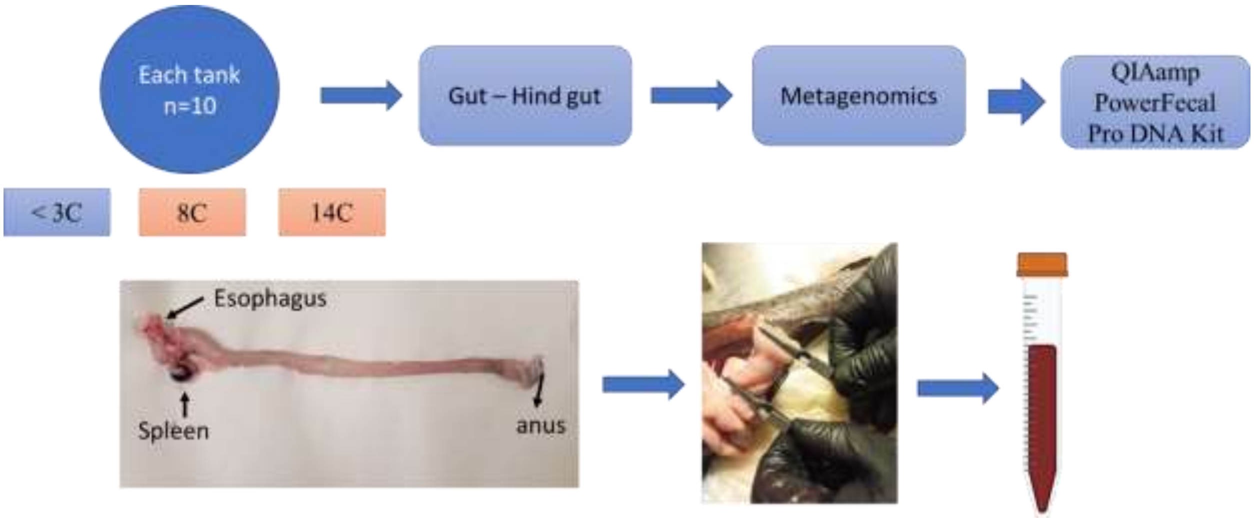


Figure 54. Experimental design for metagenomics analysis, representation of wolffish gut-hind gut and content obtention.

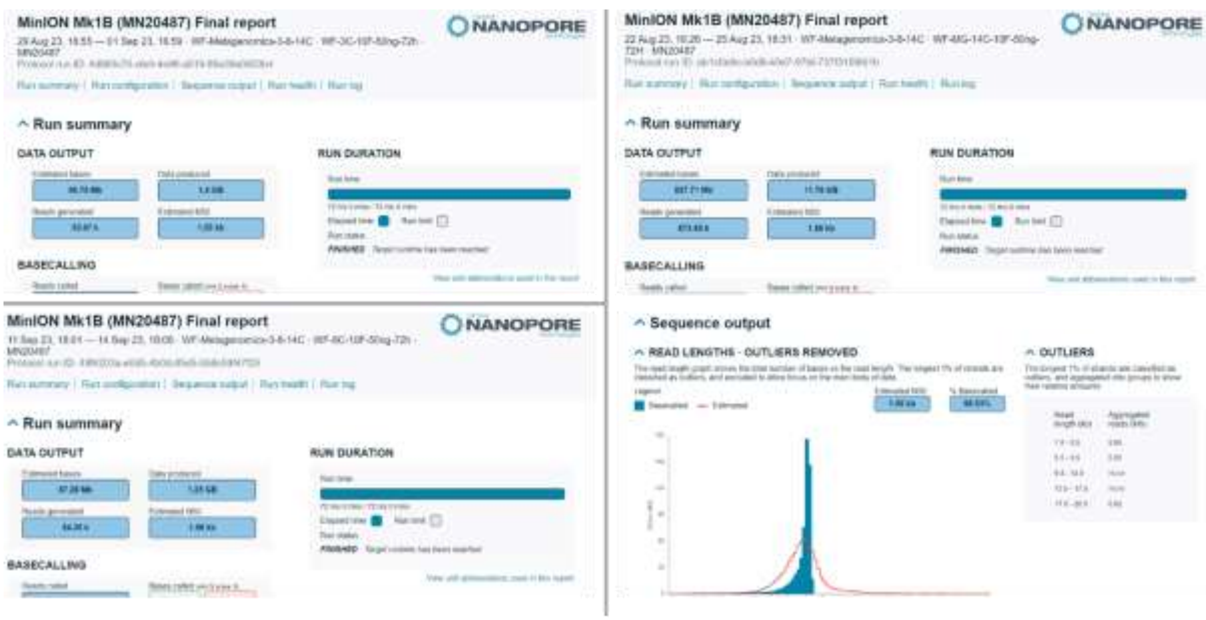


Figure 55. Nanopore 16S-Barcoding sequencing report representation.

Bioinformatic analysis of the data obtained was performed using the fastq files from passed sequenced reads by using the EPI2ME software from Nanopore Technologies, through the pipeline analysis of Fastq-16S, using defaults parameters for the Blast Component analysis as follows: BLAST E-value filter = 0.01; Minimum coverage, % = 30; Minimum identity, % = 77; Max target sequences = 3. Analysis of the genus and species matches for each temperature was addressed by GraphPad Prims 9.0. Results obtained were represented as Top 20 genus (Figure 56); and Top 20 species (Figure 57) per temperature group considering the counts numbers as operational taxonomic units (OTUs). Preliminary results suggest that there are changes in the wolffish microbiota when ocean temperature rises, for instance we identified that the most predominant genus at 3°C are *Malacoplasma*, *Mycoplasma*, *Photobacterium* and *Romboutsia*, whereas at 8°C and 14°C changes in the microbiota composition were noticed (Figure 56). At 8°C we identified *Aliivibrio* as de most predominant genus, followed by *Romboutsia*, *Photobacterium*, *Malacoplasma*, *Clostridium* and *Mycoplasma* (Figure 56). Similarly, at 14°C *Aliivibrio* is significantly the most predominant genus, followed by *Shewanella*, *Fusobacterium*, *Ilyobacter* and *Vibrio* (Figure 56).

In the other hand, identified most predominant species at 3°C are *Malacoplasma muris*, *Mesomycoplasma moatsii*, *Photobacterium carnosum*, *Romboutsia timonensis* (**Figure 57**). While at 8°C are *Aliivibrio wodanis*, *Photobacterium carnosum*, *Malacoplasma muris*, *Romboutsia timonensis*, *Aliivibrio logei*, *Mesomycoplasma moatsii*, *Romboutsia ilealis* (**Figure 57**). In contrast, we observed at 14°C that most of the species identified tend to be or possible be commensal pathogens, such as *Aliivibrio wodanis*, *Shewanella halifaxensis* HAW-EB4, *Aliivibrio logei*, *Shewanella pealeana*, *Shewanella pneumatophore*, *Aliivibrio sifiae*, and *Ilyobacter polytropus* (Figure 57). These results suggest that at increasing temperature the normal microbiota of wolffish tends to change reducing beneficial intestinal flora towards increasing presence of commensal pathogens such as *Aliivibrio wodanis*, causative agent of ulcer diseases (https://doi.org/10.3389/fmicb.2021.626759).

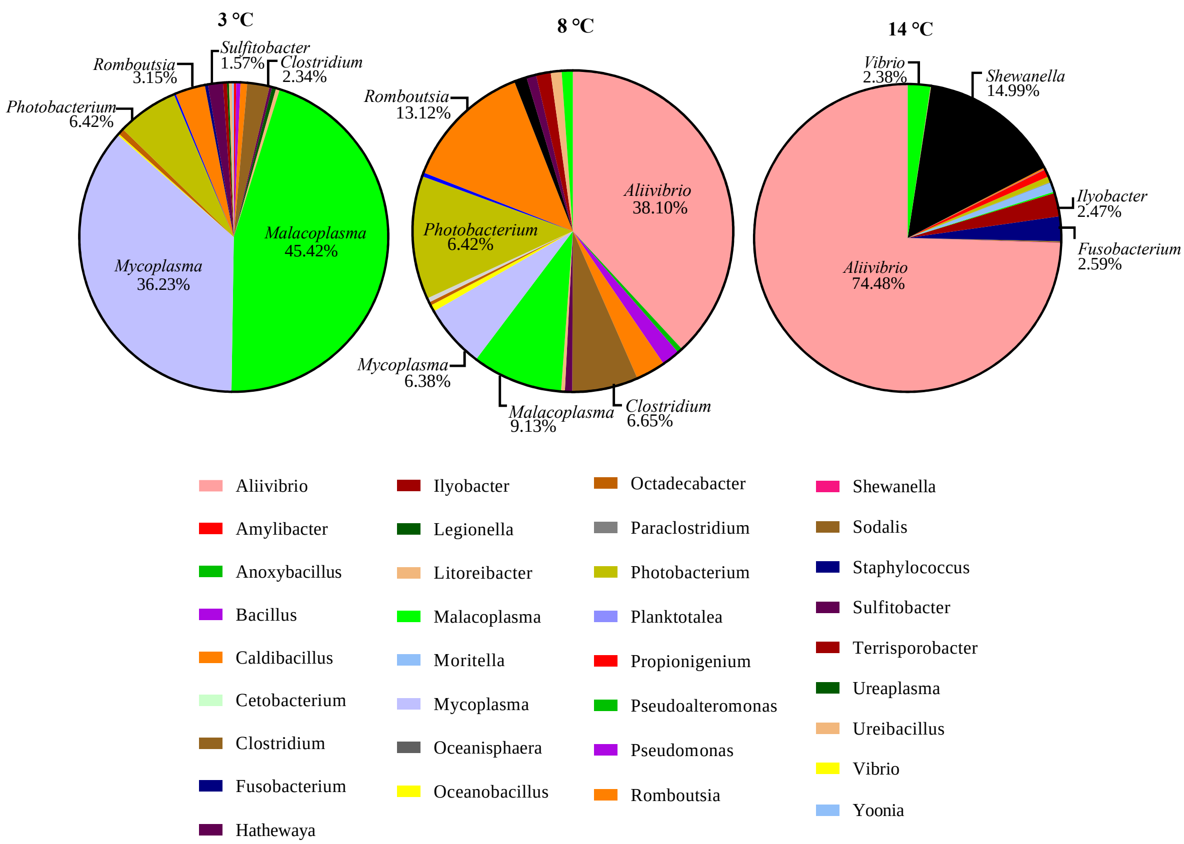


Figure 56. Identified predominant bacterial genus in wolffish exposed to temperature changes.

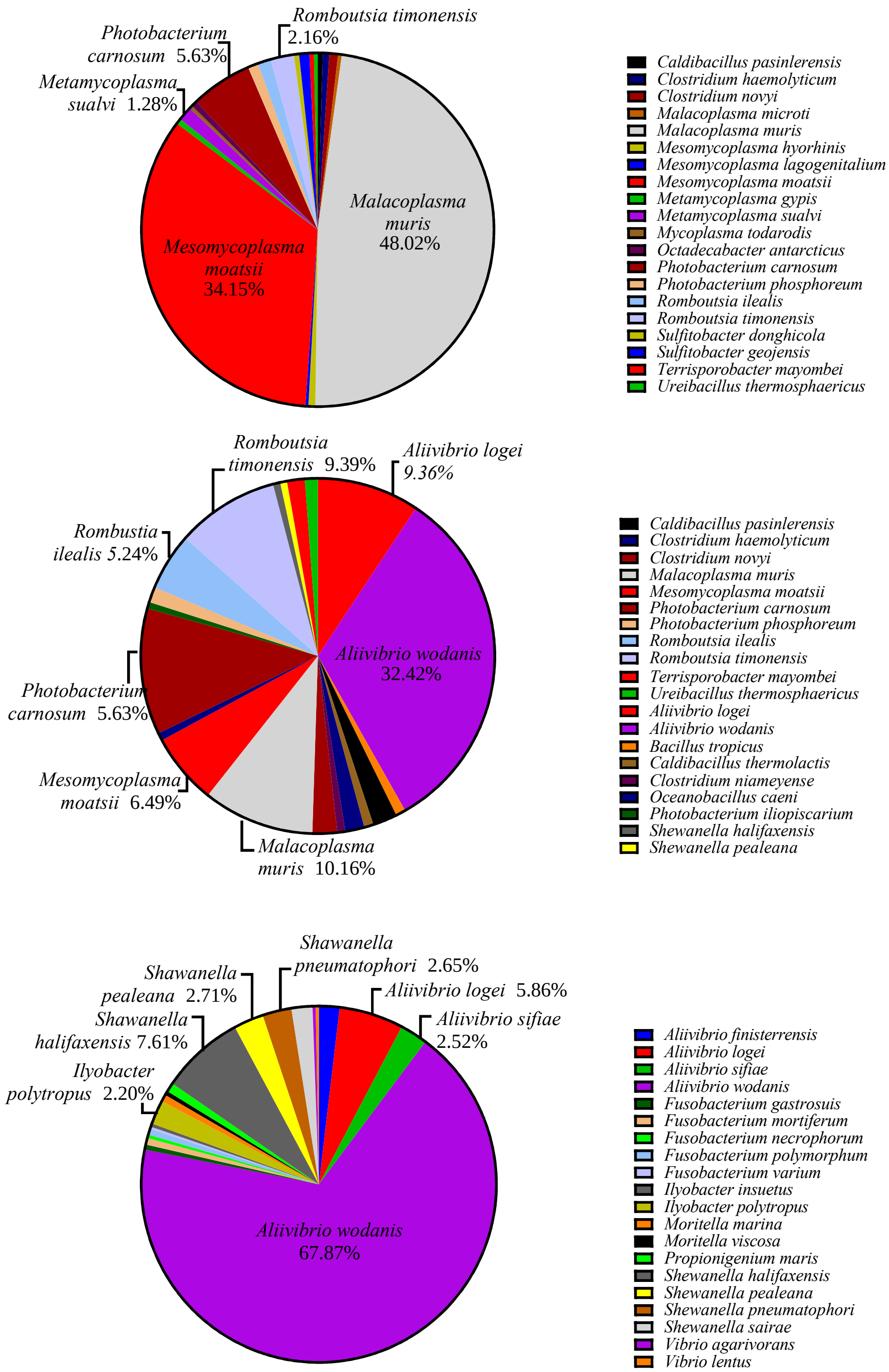


Figure 57. Identified predominant bacterial species in wolffish exposed to temperature changes.

Figure 58: Broodstock wolffish.