DIFFERENT HOLDING CONDITIONS DURING TRANSPORT AND THEIR EFFECT ON TEMPERATURE

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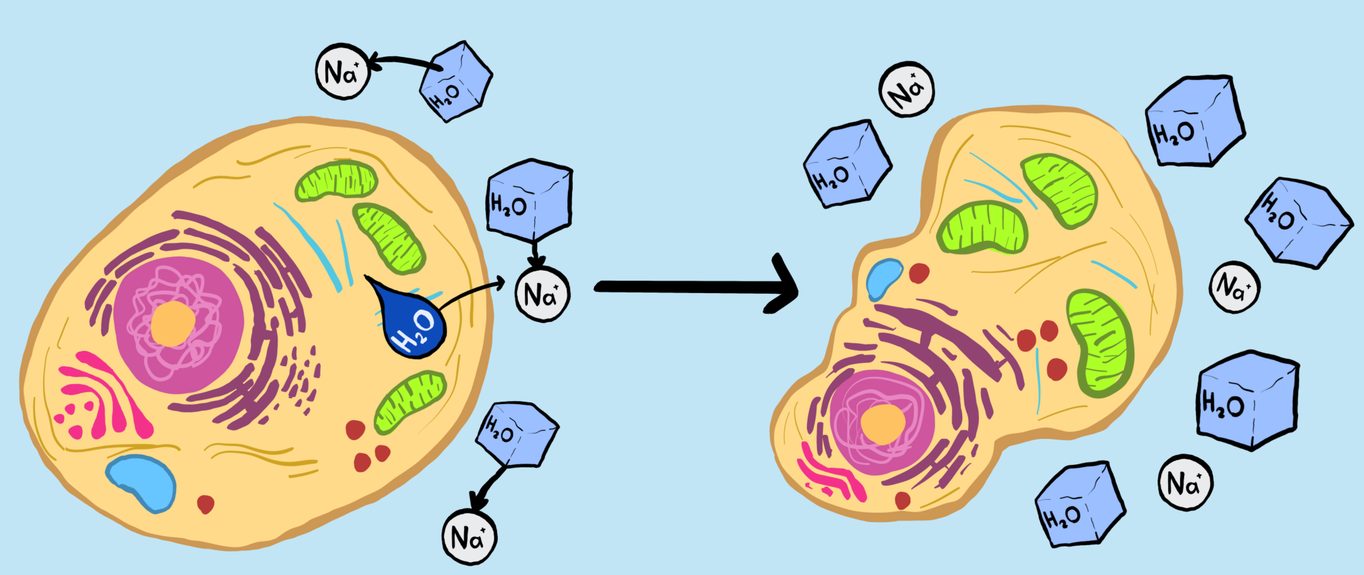
Abstract

Routine surveillance of farm operations is crucial to ensure health of animals and minimize risk to public health. The shipping of tissue samples collected from farms and facilities is an essential part of providing an accurate assessment of the animal's health status. There can be a challenge preserving the integrity of the tissue during long transport times. Biological samples are often frozen to prevent normal decay. Freezing can compromise the sample through the formation of ice crystals and the damaging of cell membranes and internal structures. Depending on the analysis being performed, this could potentially impact results. The integrity of kidney samples in different holding conditions during transport of Atlantic salmon (*Salmo salar*) kidney samples were evaluated. Temperature logging data determined that plastic coolers with frozen foam ice packs are not able to insulate contents from the external environment. Foam ice packs frozen at −80 (°C) cannot maintain the internal environment for longer than 33 hours. Foam ice packs held at both −20 (°C) and 4 (°C) cannot maintain an internal environment for longer than 2 hours. Pre-chilling the cooler was effective in maintaining temperature for a longer period. Changing the transport container of samples in the future or finding ways to pre-chill the container might be effective solutions in maintaining sample integrity through the transportation process. The results of this study will inform transport methods.

Introduction

How does freezing impact the physiology of a cell?



Dehydration! As water freezes in the extracellular space, solutes are pushed out of the ice lattice causing the remaining liquid have a higher solute concentration. Water is attracted to solute, so water will leave the cell to enter the more concentrated extracellular fluid, causing dehydration. Dehydration prevents ice crystals from forming which protects the delicate RNA and DNA structures that might be inside the cell from damage from ice crystals. This is why maintaining a consistent and specific temperature is important in preserving genetic material upon storage and transport.

Results

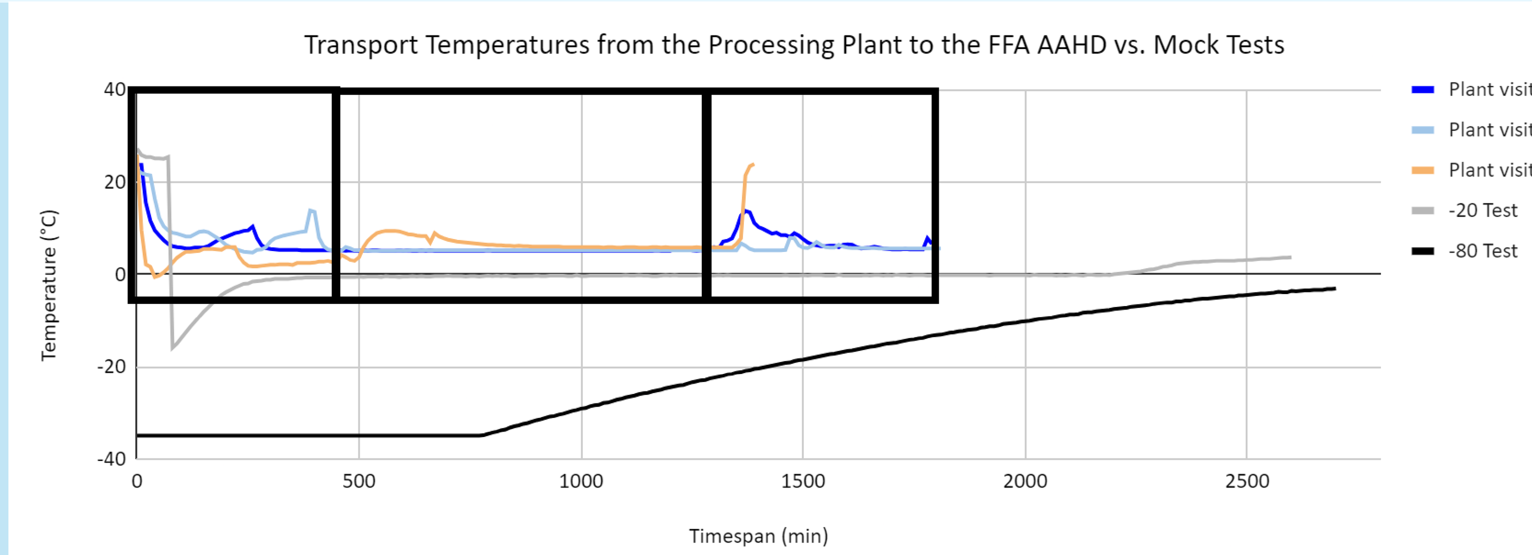


Figure 1: Temperature (°C) of delivery time from the processing plant before sample collection to the delivery of the samples back to the lab (top three) compared to test runs conducted in a stable lab environment of two different temperatures of ice packs (bottom two)

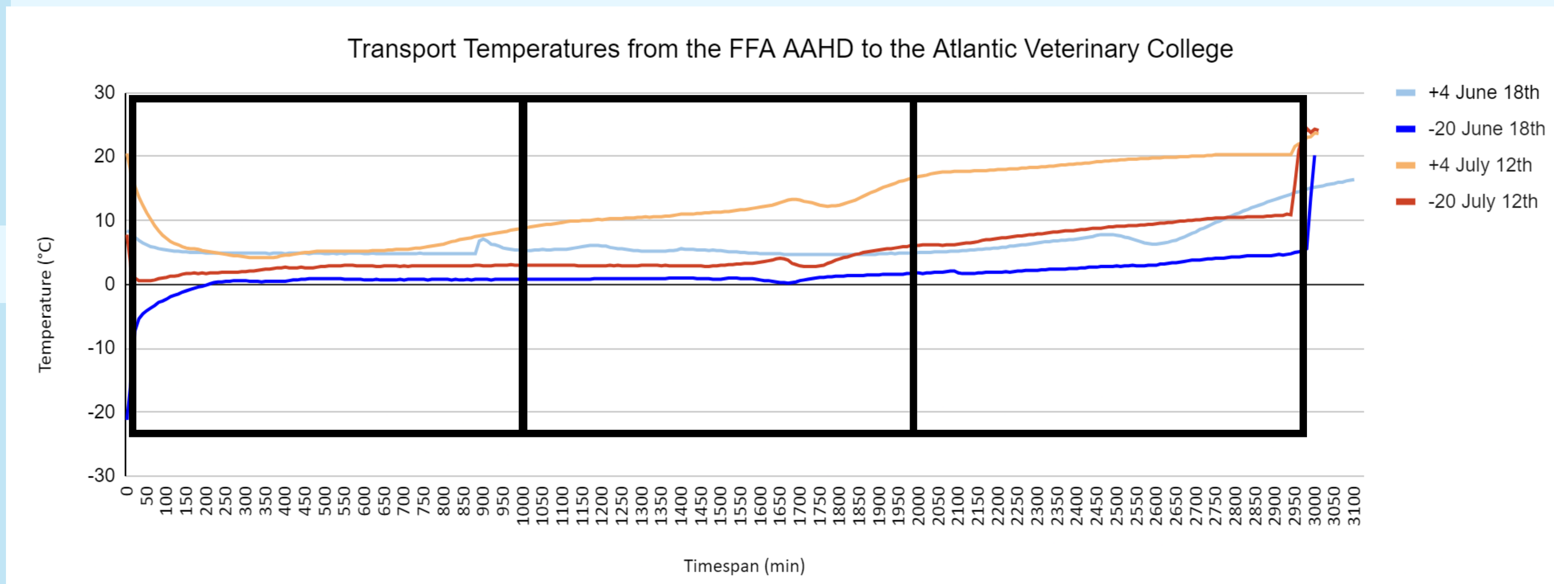


Figure 2: Temperature (°C) of delivery time of the samples in *figure 1* during plant visits 1 and 2 after being shipped from the laboratory to the Atlantic Veterinary College after being kept at +4 (°C) or −20 (°C)

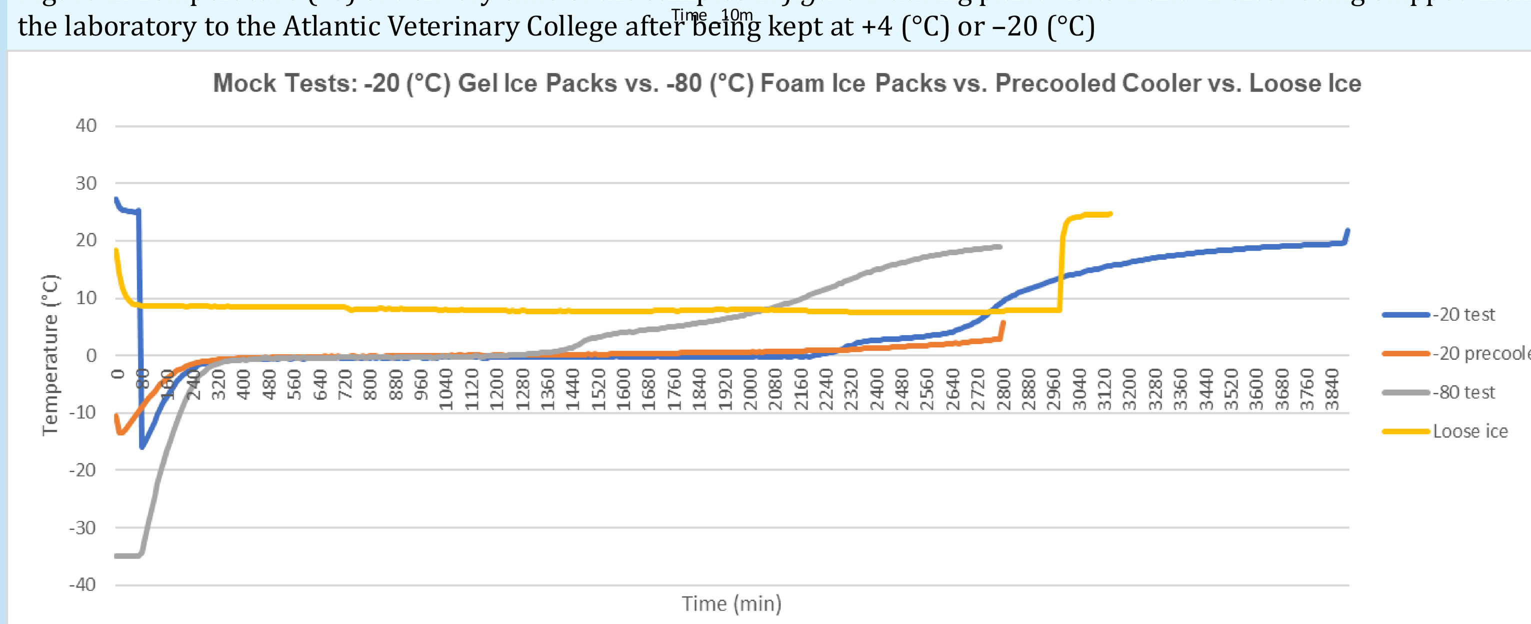


Figure 3: Temperature (°C) of experimental situations of using foam ice packs at −20 (°C) (blue), foam ice packs at −80 (°C) (grey), using a cooler pre-chilled at −20 (°C), and using loose ice in response to previous results in *figures 1* and *2*

Discussion

Plant Visits vs. Mock Tests

- The target temperatures of the tests in all scenarios were never reached
- The temperature logger was started at the plant and put into the cooler with -20 (°C) ice packs (initial dip in temperature)
- Within 3-4 hours, the temperature rose 5-6 (°C)
- Temperature fluctuates every time the cooler is opened during the sampling process at the plant
- Environmental temperature of the transport time impacted the data outcome. There was a 1 (°C) difference between plant visits 1 and 2 during transit
- The −20 (°C) test was conducted in a lab environment in which the cooler was never opened—comparing the actual transit temperature to this baseline shows the plastic coolers do not insulate the samples from their external environment
- The −80 (°C) test was compared to the −20 (°C) test and it was found that even in this scenario, the coldest the cooler was able to keep the samples was −35 (°C)
- The −80 (°C) test had a more gradual heat dissipation and took 11.5 hours before the temperature began to rise compared to the −20 (°C) test

Transport Temperatures

This graph is a chronological continuation after figure 1, showing the transit time of 36-48 hours after post-processing in coolers with −20 (°C) ice packs to the Atlantic Veterinary College

- Intended temperature during transport was never maintained
- Samples kept at +4 (°C) had a higher average temperature during shipment
- Second transport on July 12th, 2024, had a higher average temperature than the shipment on June 18th, 2024—this could be due to July having a higher average daily temperature than June

Ice Packs vs. Loose Ice

After the results found in figures 1 and 2, a series of experimental tests were performed to test the stability of thermal cooling elements: foam ice packs, thermal gel ice packs in plastic casing, loose ice, and a cooler precooled with foam ice packs

- Foam ice packs kept at −20 (°C) and −80 (°C) never reached intended temperatures as seen in *figure 1*
- In the −80 (°C) test, heat began to deplete after 33 hours
- In the −20 (°C) test, heat began to deplete after 2 hours
- Precooling was achieved by placing the entire cooler in a -20(°C) fridge overnight
- In the −20 (°C) with precooling, heat began to deplete after 47 hours until the temperatures reached 0 (°C) where it became stable at that temperature for 3 to 4 days
- Loose ice had the most stable results, but the temperature remained at around 7 (°C)

Materials and Methods

Method used for kidney samples shipped to Atlantic Veterinary College:
Ice packs kept at −20 (°C)
- 1 rep kept at 4 (°C); Shipped with a few ice packs
- 1 rep kept at −20 (°C); Shipped with many ice packs

Experimental Test: 6 foam ice packs frozen at −20 (°C) measured with datalogger

Experimental Test: Loose ice cubes measured with datalogger

Experimental Test: 6 foam ice packs frozen at −80 (°C) measured with thermocouple

Experimental Test: 3 gel ice packs frozen at −20 (°C) measured with datalogger

Sampling Procedure

- Atlantic Salmon (*Salmo salar*) were sampled from a processing plant in Newfoundland from 25 randomly selected animals off the processing line on both June 10th and July 8th—50 fish were sampled in total
- The animals were necropsied using a scalpel from in between the ventral pectoral fins to in between the ventral pelvic fins and the organs were moved aside to reveal the kidneys where a sample was taken using a sterile technique
- The samples were stored in coolers where temperature dataloggers were started as sampling begun at the plant
- The samples were taken back to the FFA AAHD and stored at 4 (°C) to be processed the next day where they were homogenized
- The day after processing, the samples were sent to the Atlantic Veterinary College

Fish 1-25 were collected June 10th and processed on June 11th
- One rep was stored at 4 (°C) and shipped on June 12th
- One rep was frozen at -20 (°C) and shipped on June 12th

Fish 26-50 were collected on July 8th and processed on July 9th
- One rep was stored at 4 (°C) and shipped on July 10th
- One rep was frozen at -20 (°C) and shipped on July 10th

Conclusion

Temperature logging data from initial plant visits determined that plastic coolers with foam ice packs are not able to insulate contents from the external environment. Additional tests in a laboratory study also implied that the density of contents inside the coolers pose a challenge in ensuring everything is cooled equally. Foam ice packs cannot be used as a cooling method at −80 (°C) for longer than 33 hours before heat begins to wane and they cannot be used as a cooling method at −20 (°C) and 4 (°C) for longer than 2 hours before heat begins to wane. Gel ice packs were more effective than foam ice packs, but not enough to compensate for the loss of heat through the cooler. Loose ice was effective but did not keep the samples at the desired temperature, and pre-chilling the cooler was effective in maintaining temperature for a longer period. Changing the transport container of samples in the future or finding ways to pre-chill the container might be effective solutions in maintaining sample integrity through the transportation process.

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