Fevers Aren't *Cool*: But This Possible New Oyster Disease Therapy Is

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Introduction

- *Perkinsus marinus* is a protozoan parasite that causes dermo disease which can lead to widespread mortality in Eastern oysters
- No therapy exists to prevent or treat infections
- When people get sick, their immune system typically raises their body temperature to fight the pathogen causing the illness
- Oysters do not have this capacity, but it may be possible to raise their temperature inducing a "fever" to help them fight dermo
- Previous studies have shown that *P. marinus* cannot survive temperatures above 50°C, while oysters living intertidally in the Southeast typically survive temperatures up to 55°C
- This may provide a mechanism to treat farmed oysters infected with dermo in the Northeast and a mechanism for oysters to adapt to climate change

Objectives

Exp.1) Demonstrate that 50°C will kill *in vitro* cultured *P. marinus*

Exp.2) Test the impact of repeated exposure of naturally infected oysters to 50°C on oyster survival and dermo infections

Exp.3) Test methods to elevate temperature in a field setting and evaluate the effect on oyster survival and dermo infections

Methods

Exp.1 Three replicate *in vitro P. marinus* cultures (Fig 1) were placed in a drying oven at 50°C, 1 hour per day for 2 weeks while controls remained at room temperature. Cultures were allowed to recover for 1 week then compared.

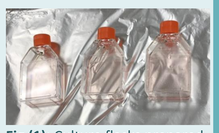


Fig (1): Culture flasks prepared for the drying oven.

Exp.2 Sixty naturally infected Delaware Bay oysters were held in bags off the Haskin Lab dock. Half were baked in a drying oven at 50°C for approximately 2 hours per day for one week while the rest remained in the water as controls. Dermo disease (Fig 2) was compared pre- and post-thermal exposure.

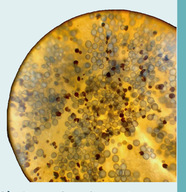


Fig (2): *P. marinus* in oyster tissue after incubation in Ray's Fluid Thioglycolate Medium (RFTM). 100x magnification.

Exp.3 Oysters from three beds spanning the salinity gradient in Delaware Bay (Fig 3) were hung from the dock (2 bags per bed, 37 oysters per bag). All bags were exposed to the sun on the dock for 1 hour daily. One bag from each bed was enclosed in clear marine vinyl to elevate temperature which was monitored with a bluetooth logger (Fig 4). Bags were vented when temperature approached 55°C to target a mean temperature spike of 50°C. Mortality and dermo intensity was measured after 14 exposures.

Fig (3): Collection Sites in Delaware Bay. Adapted NASA satellite image

Fig (4): Oysters in bags with logger

Results

Experiment 1

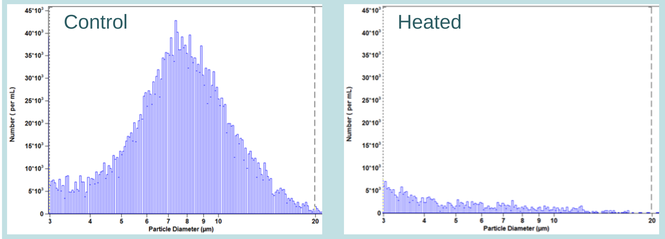


Fig (5): Size distribution shows heated cultures don't contain live *P. marinus*

P. marinus cells range in size from 3-20 µm. The heated cultures contained few particles within that range (Fig 5), and what was observed appeared to be dead cells and debris (Fig 6) that were an order of magnitude less abundant (Fig 7).

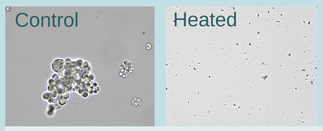


Fig (6): Control and heated cultures at 40x magnification

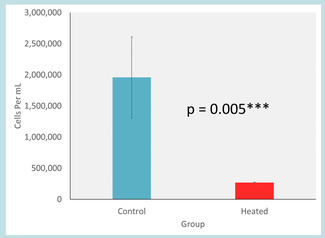


Fig (7): Control cultures were ten times as dense as the heated cultures

Conclusive:
Cultured cells were clearly killed *in vitro* when exposed to 50°C.

Experiment 2

Fig (8) Results of oven experiment with infected oysters

Infections were rare (7% for control and 13% for heated) and light in both groups. A two tailed t-test revealed no significance between the groups (Fig 8).

Inconclusive:
T_0 sample showed oysters were largely uninfected, and project timeline was too short to allow the oysters more time to pick up infections.

Experiment 3

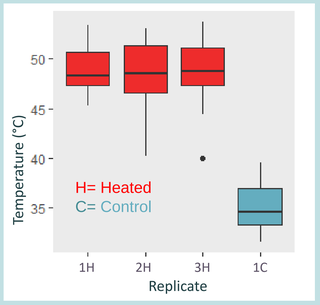


Fig (9): Average Temperature During Dock Exposure:

Clear marine vinyl covering successfully increased temperature as heated bags averaged 49°C with similar levels of variation whereas control bags averaged 34°C (Fig 9).

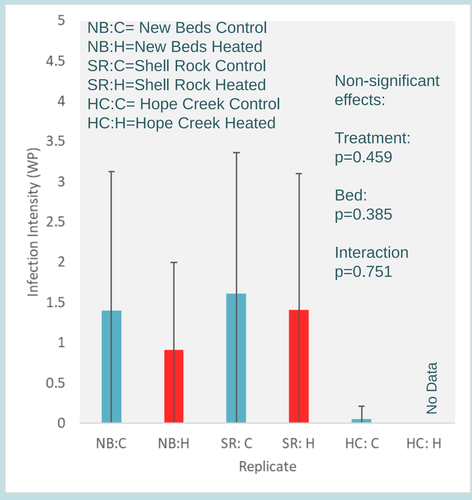


Fig (10): *Field Treated Oysters vs Controls show a non-significant trend towards lower dermo infection in heated groups*

The Weighted Prevalence (WP) of the RFTM ratings for each oyster bag was lower in the heated bags (Fig 10). All HC-H oysters died during experiment. Two-way ANOVA (Hope Creek omitted) indicated no interaction between bed and treatment, and no effect of bed or treatment on infection intensity.

Fig (11): Oysters being prepared for dermo analysis

Informative:
Low salinity oysters did not tolerate heat exposure. Other groups suffered 20-30% mortality regardless of heat exposure. A trend towards lower dermo infection in heated groups was not significant.

Conclusions

Exp.1) Conclusive

In vitro P. marinus cultured cells were killed by heating to 50°C.

Exp.2) Inconclusive

Oysters selected for oven experiment did not have adequate infections to demonstrate effect. Experiment should be repeated using oysters with higher, more prevalent infections.

Exp.3) Informative

Oysters from low salinity were less tolerant of heat. Although not significant, the trend towards lower dermo infection in heated groups indicated that a higher sample size and longer duration may demonstrate an effect.

Discussion

Application:

- There are no known therapies to prevent or treat dermo disease.
- Induced fevers remain a possible therapeutic for oyster farmers to protect their crops

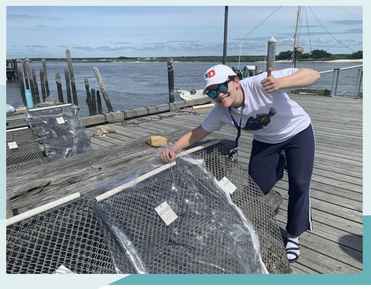
Fundamental implications:

- This study observed the effect of rising temperatures and varying salinities on oyster survival and dermo infection.
- As sea level and global temperatures rise in Delaware Bay and elsewhere, oysters may respond by moving into the intertidal zone like populations in the Southeast.

Next Steps

Experiments two and three should be repeated due to their inconclusive results. When repeated:

- Sample size and duration should be increased
- Experiments should be performed in Fall, when *P. marinus* infections are at their seasonal peak
- Alternative thermal conductors should be explored



Acknowledgements

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