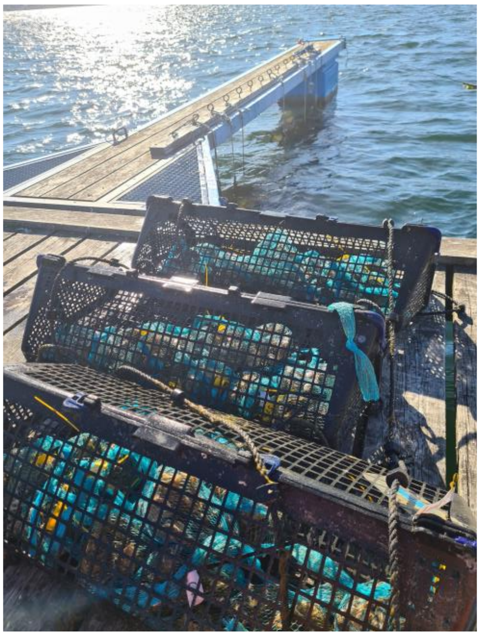
DEPARTMENT OF BIOLOGICAL AND
ENVIRONMENTAL SCIENCES

EVALUATING HEAT TREATMENT AS A METHOD TO REDUCE BIOFOULING IN OYSTER AQUACULTURE



Heat tolerance in Swedish grown *Magallana gigas* and *Ostrea edulis*

Niklas Agblad

Degree project for Bachelor of Science with a major in Biology
BIO603, Bachelor's degree project 30 hec
First cycle
Semester/year: Spring/Summer 2022
Supervisor: Anna-Lisa Wrange, IVL Swedish Enviromental Research Institute & Åsa Strand, IVL Swedish Environmental Research Institute/ Department of Marine Sciences, University of Gothenburg
Examiner: Sam Dupont, Department of Biological and Environmental Sciences

Photo by: Agblad, 2022

Table of Contents

Abstract

Aquaculture is a growing business in various parts of the world, Sweden included. As the commercial use of oysters increases, so does its demand. Farmers will need to produce a high quality product that is appealing to the customer and cost-effective for the farmer. One major issue for farmers is biofouling, as unwanted organisms settle and grow on the oysters, reducing their value and quality. This thesis explored a method for reducing the problems caused by a group of biofouling organisms, the calcifying tubeworms. The method included using a heated sea water treatment, in which the oysters were dipped into heated water for a short time period. Included in the experiment was the Swedish native oyster *Ostrea edulis* and the invasive species *Magallana gigas*. Due to its invasive status, *M. gigas* is prohibited to culture in Swedish water. The demand from the market has farmers wanting a legislation change, therefore its inclusion in this thesis was relevant. Using a range of temperatures based on previous successful studies on blue mussels, tests were conducted to see if the method could be applied to oysters too and if so, if the two different species had any differences in tolerance to stress from the heat treatment. Tubeworm mortality was also tested separately. The results indicate that *M. gigas* had a higher tolerance towards the stress from heat treatment while the mortality of *O. edulis* significantly increased with temperature. Alongside a surprisingly low threshold for high mortality in tubeworms, a conclusion was made that the method was suggested to be applicable to Swedish aquaculture, but the method must be optimized further to suit our native species to yield an effective result with low oyster mortality.

Sammanfattning

Vattenbruk är en växande industri i flera regioner av världen, inkluderat Sverige. Då konsumtion av produkterna ökar, gör även efterfrågan. Odlare måste kunna producera en produkt av hög kvalité som är attraktiv för kunden och kostnadseffektiv för odlaren. Ett av de stora hindren för detta är påväxt, då oönskade organismer fastnar och växer på ostronen, vilken resulterar i en produkt av lägre kvalité med minskat värde. I denna uppsats utvärderas en metod för att reducera påväxt och stoppa tillväxt av en av de mest problematiska påväxtorganismerna kalkmaskar. Metoden inkluderar att sänka ner ostron i upphettat vatten under en kort tid, med syfte att döda av kalkmaskarna utan att påverka ostronen. I försöken används två arter av ostron, den inhemska arten *Ostrea edulis* (Europeiskt ostron) och den invasiva arten *Magallana gigas* (Stillahavsostron). På grund av sin klassificering som invasiv är det förbjudet att odla *M. gigas* i Sverige, men då arten importeras för konsumtion finns det aktörer som vill se en lagändring. Att inkludera *M. gigas* i experimenten var därför relevant. Baserat på tidigare lyckade försök på blåmusslor valdes ett spann med olika temperaturer och exponeringstid ut, för att se om metoden var applicerbar på ostron. Experimentet var också utformat på ett sätt så att man skulle kunna se skillnader på de två olika arterna av ostron, om någon av arterna var mer tolerant mot stressen från värmebehandlingen. Dödlighet hos kalkmaskarna under värmebehandling testades i ett separat experiment. Resultaten indikerar att *M. gigas* hade en hög tolerans mot värmebehandling, medan dödligheten hos *O. edulis* ökade signifikant i högre temperaturer. Då dödligheten hos kalkmaskarna var förvånansvärt högt vid relativt låg temperatur drogs slutsatsen att värmebehandling är en metod som är applicerbar i svenskt vattenbruk. För att säkra en låg dödlighet behöver metoden optimeras för vår inhemska art *O. edulis*.

List of figures

List of tables

Abbreviations

MG	*Magallana gigas*, Pacific oyster.
OE	*Ostrea Edulis*, European flat oyster.

1. Introduction

1.1 Aquaculture of bivalves

Aquaculture of bivalves is a growing market all over the world. In Sweden, which has a relatively small number of farmers, around 17 tons of oysters were produced 2020 (SCB, 2020). This includes both cultured and oysters harvested in the wild. The number of oysters produced nationally does not meet the demand, in the same year 600 tons of oysters were imported (SCB, 2020). The two most common species of oyster both imported and produced nationally are *Magallana gigas* and *Ostrea edulis* (SCB). The Pacific oyster, *Magallana gigas* (MG), formerly *Crassostrea gigas* (figure 1, left) originates from Japan and south-east Asia. MG has been introduced to most continents for its aquaculture value (Padilla, 2010). A increase in demand over the last half of the century from costumers have led to overexploitation of wild populations (Smyth et al., 2016). MG itself offers a solution to this problem, with a fast growing rate, resilience towards diseases and the ability to survive in a wide range of temperatures (Kerckhof et al., 2007). The European flat oyster, *O. edulis* (OE) is native to the Swedish west coast (figure 1, right). Due to the species susceptibility to diseases and parasites which is further amplified by exotic pests brought with invasive species such as MG (Laing et al., 2006), Swedish farmers may have a vital role in keeping the population healthy by providing the species with communities in which to grow.

Figure 1. Left: Magallana gigas. Right: Ostrea edulis. (Agblad, 2022)

There are a vast number of methods used for oyster aquaculture today including extensive methods such as oysters relayed on the bottoms for grow-out (on-bottom culture) to more intensive methods such as floating tracks or rafts (Strand et al., 2022). Off-bottom systems refers to methods where the oysters are elevated from the sea bottom but still below surface level, which could be for example cages hung down in the ocean. The term biofouling refers to attachment of unwanted organisms on surfaces such as bivalves, cages and ships. Production of oysters include many suitable surfaces for biofouling organisms to grow on. Compared to on-bottom based systems, surface-based systems often have lower exposure to sedimentation and predators which is beneficial to biofouling organisms (Adams et al., 2011), and consequently, these systems are particularly exposed to biofouling. Biofouling lowers the market value of oysters while also damaging equipment, thereby increasing the production cost. Expenses due to biofouling is mostly related the extra weight and the toll it has on gear (Lacoste & Gaertner-Mazouni, 2015). Approximately 20% of the final market price for an oyster (Adams et al., 2011) is used to combat biofouling, but that number is thought to be an underestimation as a limited amount of resources has been used to research marine biofouling in aquaculture (Fitridge, et al., 2012). As the aquaculture take place in open systems in the ocean, combat with biofouling is continuously a problem for farmers. Shown by studies on blue mussels, when the growth period spans several seasons biofouling can have devastating economic effects which are hard to recuperate from (Asgari & Jahangard, 2012).

Biofouling can be divided into two main groups, the soft bodied fouling which includes macroalgae and tunicates. The other group is hard bodied fouling such as barnacles and tubeworms. While smaller and carrying less weight, the hard bodied fouling is harder to remove compared to soft bodies fouling. *Spirobranchus triqueter* and *Hydroides norvegica* are two biofouling organisms found on aquaculture structures. They are both calcifying tubeworms, therefore hard to remove from the oysters and equipment once settled (Figure 2).

Figure 2. Oyster with a high density of tubeworms (Agblad, 2022).

Historically several methods to remove biofouling have been evaluated, such as chemical removal (Paul & Davies, 1986) which had the problems of too high of an oyster mortality and of course, the use of chemicals. More recently methods using a lime solution has been tested to remove the biofouling organism *Didemnum vexillum* successfully without disrupting the oysters (MG) (Rolheiser et al., 2012). This method does, however, require sophisticated gear to keep the solution at correct concentration to not put the oysters at risk. Methods to remove biofouling by scraping existing organisms off the oyster can be costly and ineffective, which is why heat treatment to remove biofouling organisms has been experimented on previously and to great success on mussels (Asgari & Jahangard, 2012). Using 45-50 seconds of exposure to 45-50 °C water resulted in a 95% mortality rate for tubeworms, while more than 95% of the mussels survived (Asgari & Jahangard, 2012). Follow-up studies in Sweden have shown similar results on blue mussels *Mytilus edulis* (Svedberg, K., unpublished data). Asgari and Jahangard (2012) also note that heat treatment might be applicable to oysters as well, and temperatures as high as 80-85 °C but with shorter exposure time using *Crassostrea virginica* as test subject has been evaluated (Mayrand et al., 2015), with a high survival rate in oysters.

A factor to consider during selection of temperature and exposure times is the size and age of the oysters, as studies shown that larger individuals are more tolerant to stress (Sukhotin et al., 2003). It has also been shown that sensitivity to different temperatures may differ between species (Laing, 1998).

1.2 Aims of the experiments

The aim of this thesis was to expand upon previous work on blue mussels and test if the heat treatment method is applicable to oysters cultured in Sweden. The goal was to evaluate the effects of different temperatures and how these affected the two oyster species found in Sweden and if size was a factor that affected the outcome. This thesis strived towards finding a temperature where a low mortality rate in bivalves (<10%) was observed while being effective on removing biofouling (>90% mortality of fouling). It was hypothesized that there would be a difference in mortality between MG and OE. Furthermore, it was also hypothesized that larger oyster of both species would be more tolerant than smaller individuals. Hypotheses going into the experiment were as follows:
H_0: Warmer temperatures during the treatment within the range of 48-57 °C will not affect mortality rate of MG and OE.
H_1: Warmer temperatures during the treatment within the range of 48-57 °C will increase mortality rate of MG and OE.

H_0: Tolerance to heat treatment will be in the same in MG and OE.
H_1: MG will have a higher tolerance to heat treatment with a lower mortality rate than that of OE.

H_0: A larger size does not affect survival of MG and OE in heat treatment.
H_1: A larger size in MG and OE does increase the chance of survival in heat treatment.

2. Methodology

2.1 Collection of materials and preparation for the experiment.
The experiment was conducted at Kristineberg Marine Research Station. All the oysters used in the experiment were obtained from the Långgap oyster rig close to the research station (58°15'00.7"N 11°26'40.7"E), where the oysters had been cultured in cages for approximately 2-4 years (different size classes included). For the first experiment, oysters were separated by species (OE and MG), then divided into rough size groups depending on their length (measured using digital calipers). The final range of length used was 3 centimeters to 11 centimeters for both species. This was done to help the process of dividing all the oysters into plastic nets with a weight close to 500 grams (measured using a MyWeighWP6K scale), approximately 17 oysters of different sizes of in each replicate. The similar weight per replicate was used to reduce variation in temperature change occurring when the oysters were submerged in the water. Heat trials were conducted separately for OE and MG. The total number of individuals used were 510 for MG and 473 for OE. This resulted in 6 replicates for each treatment in MG, and 5 replicates in each treatment for OE. This meant a total of 30 MG nets and 25 OE nets.

Additionally, 60 MG with a lot of visible calcifying tubeworms as biofouling were chosen for the second experiment, related to calcifying tubeworm mortality.

2.2. Experiment design

2.2.1. Oyster survival

Temperatures used in the experiment was selected based on previous experiments (Asgari & Jahangard, 2012). A temperature range between 48 to 57 C was selected, as well as ambient seawater at 12 °C (control), resulting in five treatments in total (Table 1). Surface sea water was heated to desired temperature using a hot plate and large pot. The heated sea water was transferred to an insulated box (H=13 cm, W=9 cm, L=12 cm), to keep a steady temperature during the treatment. The water volume was 13 liters for every treatment. The temperature was monitored using a mounted Thermometer logger (COMETU0111), which updated the temperature every 10 seconds. Data was collected on temperature at the start of the treatment of each replicate, and after 45 seconds when the replicate was taken out of the water again. After the treatment the nets with oysters were transferred to a box with running sea water to cool down. The control treatment used to same procedure, only the sea water was not heated. When all the treatments were finished, the nets were placed in cages which were hung by the jetty (at 1-3 meters) at Kristineberg Marine Research Station for 14 days. After 14 days the oysters were collected, measured by shell length, width, depth, as well as wet weight of each oyster and classified as dead (shell gaping with no response when touching the shell) or alive (shell firmly closed). The same procedure was performed for both experiments 7 days apart, with MG being the first species in the experiment (3rd of May) and OE trials being conducted at a later date (10th of May) because of logistic reasons.

Table 1. Overview of the heated sea water treatment design. All treatments were run for 45 seconds. Magallana gigas experiment was conducted 3rd of May while the Ostrea edulis was conducted 10th of May.

Species	Replicates (number of nets)	Temperature
Magallana Gigas	6	48°
Magallana Gigas	6	51°
Magallana Gigas	6	54°
Magallana Gigas	6	57°
Magallana Gigas	6	Control 12°
Ostrea Edulis	5	48°
Ostrea Edulis	5	51°
Ostrea Edulis	5	54°
Ostrea Edulis	5	57°
Ostrea Edulis	5	Control 12°

2.2.2 Tubeworm survival

The 60 MG with high density of calcifying worms underwent similar treatment as the oysters in the first experiment, but with other temperatures and exposure times. The new settings were chosen to test a method which would require the least amount of energy to heat water, while still being effective to kill off biofouling. Based on previous trials on tubeworm mortality on blue mussels showing sufficient mortality of worms at 45 °C for 45 seconds (Svedberg, K. 2022), a treatment of 45 C was selected (as well as a control at 12 °C) but with two different exposure times (15 and 45 seconds), see table 2. The oysters were divided into two nets for each treatment. The range size of oysters were 6 to 11 centimeters, as these contained the most amount of biofouling tubes. After the treatment, the oysters were put in cages and put in the ocean for 48 hours. When collected, survival of calcifying tubeworms was examined. This was done using a tweezer, carefully destroying the worm's tube, starting at the narrow part of the tube. The worms were then examined to determine survival. An alive worm would move try to escape through the other end of the tube.

A dead worm would be immobile or partly disintegrated. Empty tubes were to be excluded from the experiment, but no examined tube was empty. 1-3 tubes were checked on one individual oyster. The tubes were selected by giving all tubes on an individual oyster a number and then using a random number generator to select the tubes.

Table 2. Overhaul of the treatments used during the tubeworm survival experiment.

Species	Total number of tubes examined	Temperature	Treatment (seconds)
Magallana Gigas	30	45°	45
Magallana Gigas	30	45°	15
Magallana Gigas	30	Control 12°	45
Magallana Gigas	30	Control 12°	15

2.3 Statistical analysis

To analyze oyster mortality, logistic regression was performed. This was chosen as the dependent variable was binary (dead/alive). To account for the different shapes of the two oyster species, the formula $height * length * depth/3$ (equation 1) was used to estimate volume. This was done as a volume equation was deemed to be the best fit to estimate a size for both species. Control temperature was set to 12°C. Independent variables was size (equation 1), treatment temperature and oyster species. To evaluate if this model could explain the data, a goodness-of-fit test was performed.

For analysis of heat loss during treatment (variable: temperature loss during 45 the seconds, fixed factors: species, starting temperature) two-way anova was used, controlled for homogeneity of variances, outliers and normality.

Statistical analysis was conducted using the program IBM SPSS statistics 27.

3. Results

3.1 Heat-treatment temperature loss (to evaluate method)

During the 45 seconds the oysters were exposed to heated seawater, a significant difference in heat loss between the temperatures were found, however these were only between treatments and the control. (F=24.7 df= 4, p=<0.001). There was no significant difference between the species (F=2, df=1, p=0.159). Biggest loss of temperature for OE was found in the 57 °C treatment with a mean loss of 0.5 °C for the 5 replicates (figure 3).

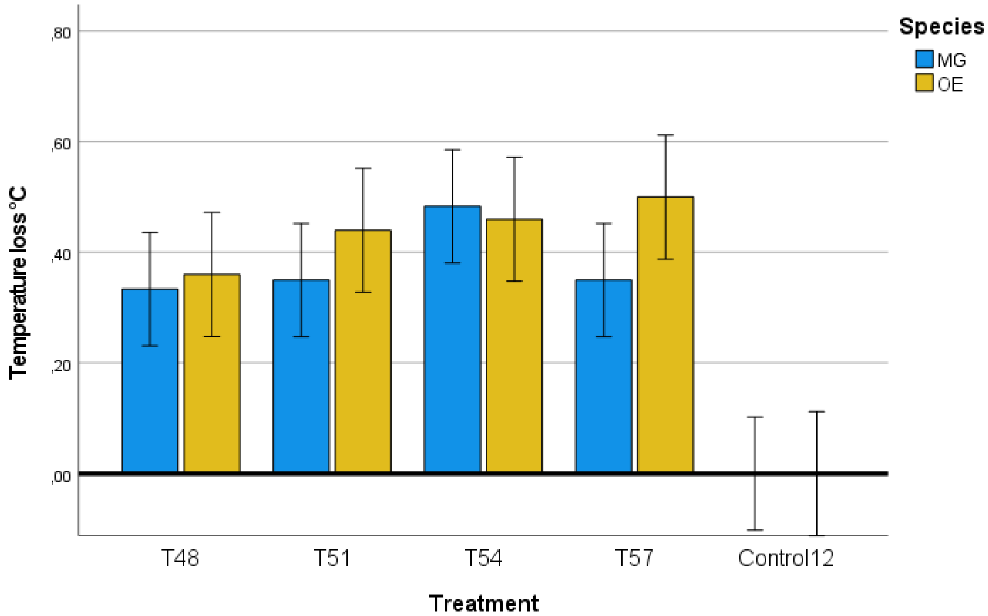


Figure 3. Showing the mean temperature loss during the 45 seconds of treatment. T= temperature, following numbers represents temperature in C. Each MG treatment had 6 replicates while each OE treatment had 5 replicates. Error bars show the standard error.

3.2 Oyster mortality experiment

This experiment was conducted with the method shown in *table 1*. The only group outside of controls with a survival rate >90% was MG treated in 48 °C. For higher temperatures the mortality rate of OE increases more than that of MG. At a temperature of 57 °C, only 4 out of 94 OE survived. In comparison, at the same temperature 76 out of 102 MG survived (*figure 4*).

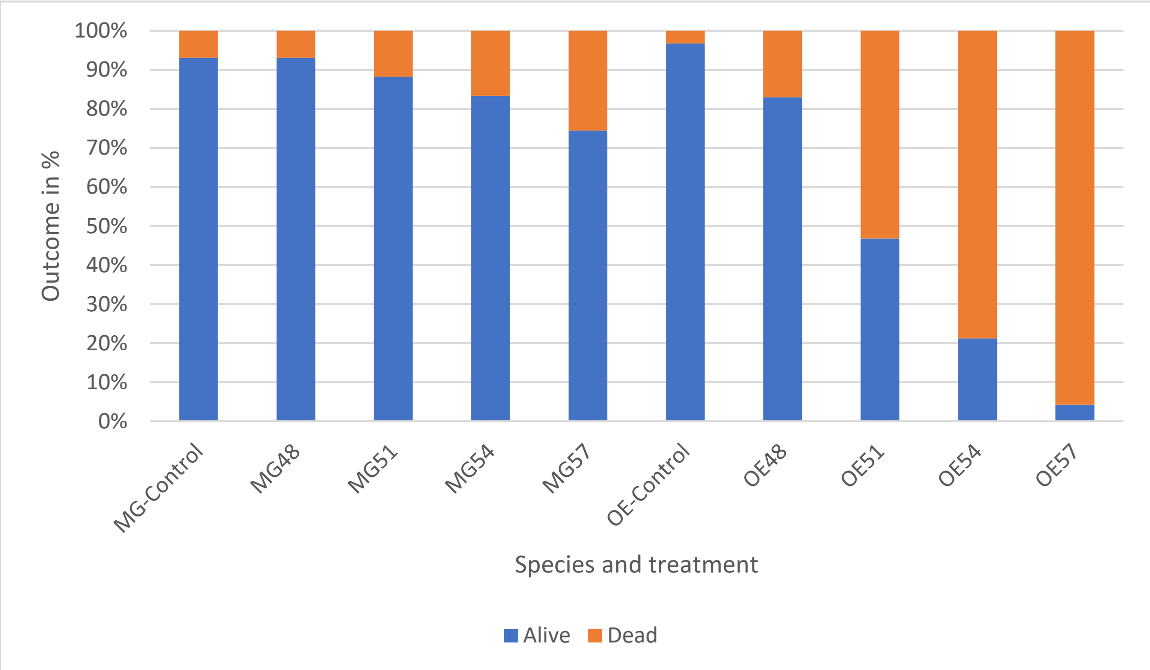


Figure 4. Survival and mortality from each group of treatments. MG and OE are abbreviations of species, the following number represents the temperature for the treatment. A total number of 510 MG were used (102 in each treatment). The total number of OE was 470 (94 in each treatment) MG bars show the mean of 6 replicates each. OE bars show the mean of 5 replicates each.

Logistic regression was used to develop a model to predict the outcome of a treatment. The model explained the data well (Omnibus: df=3, p=<0.001, Hosmer and Lemeshow: df=8, p=<0.001). The model made the correct assumption in 81.5% of the cases and all variables included in the model significantly contributed to predict oyster mortality (table 3). Exp(B) were (B) is the estimate and is called odds ratio, which means that for every unit the variable is increased the Exp(B) should be multiplied with the odds of the outcome. For example, if the temperature was to increase one °C, the existing risk of mortality should be multiplied with 1.121 (table 3). For size, the existing risk of mortality should be multiplied with 0.961 for each unit increase in oyster size, therefore decreasing the risk of mortality as the oyster grows larger.

Table 3. Results from logistical regression. Df= degrees of freedom. Exp(B)= odds ratio. All variables were significant.

Variable	df	p-value	Exp(B)
Species	1	<0.001	15.240
Temperature	1	<0.001	1.121
Size	1	<0.001	0.961

3.3 Worm mortality

The effectiveness of heat treatment to kill tubeworms and possible optimization strategies (through shortening the exposure time) of the heat treatment to save energy, showed that reducing the time from 45 seconds to 15 seconds resulted in similar results as at 45 seconds with 97% respectively 100% mortality in tubeworms (Figure 5). Both treatments met the criteria of >90% mortality in tubeworms. 45 °C for 45 seconds had 100% mortality rate. 45 °C for 15 seconds had 97% mortality rate. In the control, there was a 100% survival for tubeworms in both treatments. The control had unheated sea water at the temperature of 12 °C.

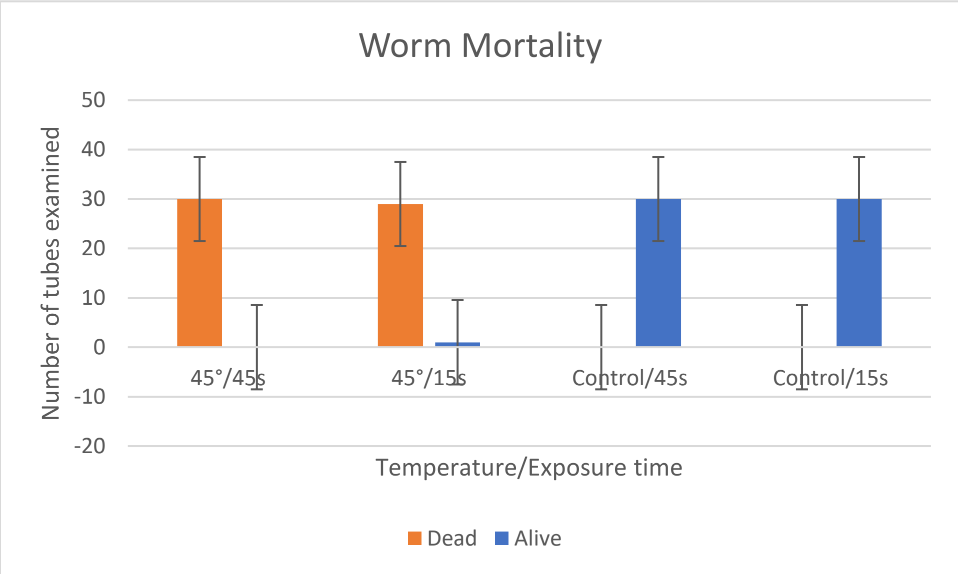


Figure 5. Worm mortality from experiment explained in section 2.3.2. Control temperature is 12 degrees C. Both treatments met the criteria of >90% mortality in tubeworms.

4. Discussion

These experiments show that heated sea water treatment could be a possible method for Swedish bivalve farmers to reduce the problem with calcifying tubeworms, although temperature and exposure time differs between species. The study indicated that MG were more tolerant to high temperatures, while OE were more sensitive and the treatment needs to be optimized fully for the treatment to be effective, as in keeping the OE oyster mortality <10% while having a high mortality (>90%) of biofouling. For OE, no treatment met the criteria of >90% survival rate, however as worm mortality met its criteria (<10%) at 45 °C with an exposure time of 15 seconds further research could find a method applicable to Swedish grown OE. The tubeworm mortality and its effectiveness of >90% mortality is similar to results of an experiment conducted on the same species of tubeworms in February (Svedberg, K., unpublished data). However, if differs from the results from the same study conducted in October in which higher temperatures (50 °C) was needed to reach the criteria. The reason for this discrepancy was not investigated is so far unknown.

Previous studies showed a 100% survival rate in MG exposed to heated water of 48, 50 and 52 °C (Svedberg, K., unpublished data). The experiment in this thesis had two treatments within that span (48, 51 °C) in which only the 48 °C treatment of MG met the >90% survival rate criteria set up beforehand. As the method was adapted from Svedberg and was working correctly without any detrimental loss in temperature during treatment, possible reasons for the difference in results could be found in other factors. In this experiment a wider range of size for the oysters were used, than that of Svedberg. Therefore, a larger number of small oysters could have been included in this thesis which could explain the increase the mortality rate, since size was shown to affect mortality (section 3.2). This is backed up by previous studies showing a lesser stress tolerance in smaller oysters (Salewski & Proffitt, 2016).

MG has previously been shown to have a higher tolerance to the stress from heat treatment than mussels such as *Mytilus edulis* (Rajagopal et al., 2005). This study showed that MG also has higher tolerance than OE. MG could, for an extended period of time survive in water temperatures of 40 °C (Strand & Lindegarth, 2014), while OE is believed to only manage just above 30 °C (Strand et al., 2019). The information from these studies therefore strengthens the conclusion that MG are more tolerant to heat than OE.

This experiment was conducted during the spring, due to time limitations this thesis was not able to repeat the experiment during any other season. The experiment was designed to be performed before the oyster spawning season which takes place in June and July (Gosselin & Sewell, 2013; Lango-Reynoso et al., 2006). Therefore, a limit in the thesis is not being able to examine how seasonal changes depict outcomes of the treatment and how the spawning season could interact with the mortality rate. As shown previously MG has a lower tolerance towards stress during their spawning season (Wendling & Wegner, 2013) due to spawning being an energy sink and therefore reducing the oysters ability to handle stress from other factors. Higher mortality during the reproductive period has also been found in blue mussels (Svedberg, K., unpublished data).

Good methods to remove or reduce biofouling should also be applicable to the gear used in aquaculture as well. The temperatures used in this study are relatively low and could be used without damaging equipment. The treatment do not remove the manual labor in scraping off the biofouling, therefore cheaper methods such as air drying (Hillock & Costello, 2013) may be more cost effective due to not investing in energy to heat up water. However, it is a longer process and could have logistical problems (Carver et al., 2003). Methods that directly removes biofouling from the equipment (Sala & Lucchetti, 2008) are also an alternative to heat treatment but does not have the benefit of being applicable to equipment as well as the cultivated bivalve.

Combining different fouling treatment methods may cause them to function in synergy with each other and be more effective (Shin et al., 2006). This has been tested using freshwater with the addition of airdrying (Gunthorpe, 2001). Using methods in combination seems to be an unexplored area and future research combing heat treatment with other methods could be of interest.

From the perspective of aquaculture, the criteria of more than 90% mortality in tubeworms may be satisfying, however as the tubeworms are also biofouling in infrastructure related to other industries in the ocean, selecting for heat tolerant worms have to be considered. To avoid genetic selection for heat tolerance, which has been shown in other animals (Carabaño et al., 2019) a 100% mortality rate should be considered to have the least impact on the ecosystem. Selecting for tubeworms with a different tolerance to temperatures may have consequences for seasonal behaviors such as settling.

In conclusion, this thesis showed that heat treatment can be used for Swedish oyster aquaculture. Further research to optimize the method for Swedish native species are required. The results in this thesis showed potential for an efficient treatment with relatively low temperatures. This implies a low investment of energy which could provide the farmer with a cheap method to reduce the impact of biofouling. In addition, with the data from this experiment it can be concluded that sensitivity differs between species of oysters and therefore specifics in heat treatment must be tailored to specific regions and species.

Acknowledgements

A warm thank you to my supervisors Anna-Lisa Wrange (IVL Swedish Environmental Research Institute) and Åsa Strand (IVL/University of Gothenburg) for their patience with my many questions and inviting me into the world of bivalve aquaculture.

Also a big appreciative thank you to Kristina Svedberg (Bohus Havsbruk) for the insight on experiment design and the long drive just to help me set up the laboratorium.

Thanks to Hannes Waldetoft (IVL Swedish Environmental Research Institute) for helping me out with the statistical analysis.

References

Adams, C. M., Shumway, S. E., Whitlatch, R. B., & Getchis, T. (2011). Biofouling in Marine Molluscan Shellfish Aquaculture: A Survey Assessing the Business and Economic Implications of Mitigation. *Journal of the World Aquaculture Society*, *42*(2), 242-252. https://doi.org/10.1111/j.1749-7345.2011.00460.x

Asgari, L., & Jahangard, S. (2012). Development of Commercial Mitigation Methods for White Polychaete Tubeworm Pomatoceros taeniata Fouling in Australian Blue Mussel Offshore Farm.

Carabaño, M. J., Ramón, M., Menéndez-Buxadera, A., Molina, A., & Díaz, C. (2019). Selecting for heat tolerance. *Animal Frontiers*, *9*(1), 62-68. https://doi.org/10.1093/af/vfy033

Carver, C., Chisholm, A., & Mallet, A. (2003). Strategies to mitigate the impact of Ciona intestinalis (L.) biofouling on shellfish production. *Journal of Shellfish Research*, *22*(3), 621-632.

Gosselin, L. A., & Sewell, M. A. (2013). Reproduction, larval development and settlement of the intertidal serpulid polychaete Spirobranchus cariniferus. *Journal of the Marine Biological Association of the United Kingdom*, *93*(5), 1249-1256. https://doi.org/10.1017/s0025315412001701

Gunthorpe, L. (2001). *Best practices for the sterilisation of aquaculture farming equipment: a case study for mussel ropes*. Marine and Freshwater Resources Institute.

Hillock, K. A., & Costello, M. J. (2013). Tolerance of the invasive tunicate Styela clava to air exposure. *Biofouling*, *29*(10), 1181-1187.

Kerckhof, F., Haelters, J., & Gollasch, S. (2007). Alien species in the marine and brackish ecosystem: the situation in Belgian waters. *Aquatic Invasions*, *2*(3), 243-257. https://doi.org/10.3391/ai.2007.2.3.9

Lacoste, E., & Gaertner-Mazouni, N. (2015). Biofouling impact on production and ecosystem functioning: a review for bivalve aquaculture. *Reviews in Aquaculture*, *7*(3), 187-196. https://doi.org/10.1111/raq.12063

Laing, I. (1998). Comparative low temperature tolerance of small juvenile European, Ostrea edulis L., and Pacific oysters, Crassostrea gigas Thunberg. *Aquaculture Research*, *29*(2), 103-113. https://eurekamag.com/research/003/072/003072822.php

Laing, I., Walker, P., & Areal, F. (2006). Return of the native - is European oyster (Ostrea edulis) stock restoration in the UK feasible? *Aquatic Living Resources*, *19*(3), 283-287. https://doi.org/10.1051/alr:2006029

Lango-Reynoso, F., Chavez-Villaba, J., & Le Pennec, M. (2006). Reproductive patterns of the Pacific oyster Crassostrea gigas in France. *Invertebrate Reproduction & Development*, *49*(1-2), 41-50. https://doi.org/10.1080/07924259.2006.9652192

Mayrand, E., Sonier, T., & Comeau, L. A. (2015). Hot water immersion lowers survival, shell growth rate and lysosomal membrane stability of oysters Crassostrea virginica (Gmelin). *Aquaculture Research*, *46*(8), 1974-1987. https://doi.org/https://doi.org/10.1111/are.12364

Padilla, D. K. (2010). Context-dependent impacts of a non-native ecosystem engineer, the Pacific oyster Crassostrea gigas. *Integr Comp Biol*, *50*(2), 213-225. https://doi.org/10.1093/icb/icq080

Paul, J. D., & Davies, I. M. (1986). Effects of copper- and tin-based anti-fouling compounds on the growth of scallops (Pecten maximus) and oysters (Crassostrea gigas). *Aquaculture*, *54*(3), 191-203. https://doi.org/https://doi.org/10.1016/0044-8486(86)90328-5

Rajagopal, S., van der Velde, G., Jansen, J., van der Gaag, M., Atsma, G., Janssen-Mommen, J. P. M., Polman, H., & Jenner, H. A. (2005). Thermal tolerance of the invasive oyster Crassostrea gigas: Feasibility of heat treatment as an antifouling option. *Water Research*, *39*(18), 4335-4342. https://doi.org/10.1016/j.watres.2005.08.021

Rolheiser, K. C., Dunham, A., Switzer, S. E., Pearce, C. M., & Therriault, T. W. (2012). Assessment of chemical treatments for controlling Didemnum vexillum, other biofouling, and predatory sea

stars in Pacific oyster aquaculture. *Aquaculture*, *364*, 53-60. https://doi.org/10.1016/j.aquaculture.2012.07.038
Sala, A., & Lucchetti, A. (2008). Low-cost tool to reduce biofouling in oyster longline culture. *Aquacultural Engineering*, *39*(1), 53-58. https://doi.org/https://doi.org/10.1016/j.aquaeng.2008.06.001
Salewski, E. A., & Proffitt, C. E. (2016). Separate and Combined Effects of Estuarine Stress Gradients and Disturbance on Oyster Population Development on Restored Reefs. *Estuaries and Coasts*, *39*(2), 510-528. https://doi.org/10.1007/s12237-015-0007-7
Shin, J.-H., Lee, S.-Y., Dougherty, R. H., Rasco, B., & Kang, D.-H. (2006). Combined effect of mild heat and acetic acid treatment for inactivating Escherichia coli O157:H7, Listeria monocytogenes and Salmonella typhimurium in an asparagus puree. *Journal of Applied Microbiology*, *101*(5), 1140-1151. https://doi.org/https://doi.org/10.1111/j.1365-2672.2006.02996.x
Smyth, D., Kregting, L., Elsasser, B., Kennedy, R., & Roberts, D. (2016). Using particle dispersal models to assist in the conservation and recovery of the overexploited native oyster (Ostrea edulis) in an enclosed sea lough. *Journal of Sea Research*, *108*, 50-59. https://doi.org/10.1016/j.seares.2015.12.009
Strand, Å., & Lindegarth, S. (2014). *Japanska ostron i svenska vatten, Främmande art som är här för att stanna*. https://doi.org/10.13140/2.1.2897.8562
Strand, Å., Wrange, A.-L., Hogström, P., Nielsen, J., Persson, P., & Persson, K. (2019). *Produktion av ostronyngel (Ostrea edulis) i havsbaserade tankar Biologisk och teknisk förstudie*. https://doi.org/10.13140/RG.2.2.10789.14565
Strand, Å. B., James., Rydstedt, A., James, P., Legat, J., & Sühnel, S. (2022). Overview of culture systems for low trophic species.
Sukhotin, A. A., Lajus, D. L., & Lesin, P. A. (2003). Influence of age and size on pumping activity and stress resistance in the marine bivalve Mytilus edulis L. *Journal of Experimental Marine Biology and Ecology*, *284*(1-2), 129-144, Article Pii s0022-0981(02)00497-5. https://doi.org/10.1016/s0022-0981(02)00497-5
Wendling, C. C., & Wegner, K. M. (2013). Relative contribution of reproductive investment, thermal stress and Vibrio infection to summer mortality phenomena in Pacific oysters. *Aquaculture*, *412*, 88-96. https://doi.org/10.1016/j.aquaculture.2013.07.009