Development of Commercial Mitigation Methods for White Polychaete Tubeworm *Pomatoceros taeniata* Fouling in Australian Blue Mussel Offshore Farm

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NON TECHNICAL SUMMARY

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OBJECTIVES:

1. To investigate and evaluate different treatment methods for controlling Serpulid tubeworms fouled on farmed mussel at laboratory scale
2. To trial the best method at large scale offshore mussel farm.
3. To choose and refine the most commercially viable and environmentally sustainable method for controlling tubeworm biofouling in offshore mussel farms
4. To develop a standard SOP for farm treatment application

Non-Technical Summary

Background and Need

In July 2011, SeaBounty mussel Aquaculture Company reported a heavy settlement of the polychaete white tubeworm on its cultured mussels at two main offshore farms in Port Phillip Bay, Victoria. A large proportion of the affected mussels were found to be covered by white calcareous tubes, which were identified as an endemic Serpulid worm called *Spirobranchus taeniata* or formerly named as *Pomatoceros taeniata*. Tubeworm fouling on mussel shells in European countries reported to be highly problematic and costs millions of dollars of losses for the Mussel Aquaculture Industry, as fouled shells are perceived to be an inferior product. Reports also revealed that mussel with more than 7% of the shell surface fouled by tubeworm are not considered Grade A quality, because the product is considered visually unattractive. An urgent action was needed to address this problem in Port Phillip Bay blue mussel farms, prevent further losses and develop an effective and commercially viable treatment method at industrial scale on-farm.

Overall Objective

The overall objective of this project was to develop an effective and economically viable mitigation method, which could be employed at a large scale offshore longline mussel farm, to minimise the impact of the tubeworm fouling.

Life cycle of *Pomatoceros* tubeworm

Pomatoceros taeniata is identified as a primary source of Serpulid tubeworm fouling in Port Phillip Bay's blue mussel offshore farms. Currently, there is no published information available on the biology, ecology or any life aspect of *P. taeniata*. However, the observations made during the course of this project revealed that the first widespread *P. taeniata* recruitment possibly took place in early May 2011 and continued to settle through spring and summer of 2012 at different rate.

Possible cause of tubeworm outbreak

A 10-year record of hydro-biological parameters in different part of Port Phillip Bay reveals a big spike on nutrients loading particularly Nitrogen, and Chlorophyll a as an indicator of phytoplankton, and a record low salinity in last two years in 2010-11 (tubeworm outbreak period). The study indicates a maximum salinity of 38.3 ppt, on January 2004 and a minimum of 29.0 ppt at 3 m depth in Port Phillip Bay on January-February 2011. Therefore, the most likely explanation for the recent mass tubeworms outbreak in Port Phillip Bay offshore shellfish farms could be attributed to a record high spike of nutrient and food and the record drop of seawater salinity in 2010-11.

Mitigation method

In this project, a number of different mitigation methods including: freshwater dipping, air drying, chlorine dioxide solution, saturated saline water, super chilled saturated saline water and heated seawater/thermal treatment were used to kill tubeworm on blue mussel droppers with differing levels of success. Given the scale of operation of minimum 30'000 mussel droppers at SeaBounty offshore farms, one of

the criteria to select the best mitigation method to scale up and utilise at commercial scale was to be able to treat minimum of 500 droppers on board at offshore farms.

In this study, the thermal treatment method is proposed as the best mitigation method for tubeworms on mussel shells based on the extensive laboratory and onshore experimental results as well as offshore trials that were carried out during this project. With the proposed method, the Sepulid tubeworms were effectively eradicated at an average lethal rate of 95% with a minimum mussel crop loss (<5%) within temperature range of 45-50 ºC at an exposure time of only 45-50 seconds. These results successfully met the standard target initially set for a large scale commercial offshore mussel farm. The proposed method enables the farm to treat over 500 mussel droppers per day on board of a farm boat and at a feasible time and cost level with a commercially acceptable mussel crop loss of less than 5%.

In addition, as a direct result of several laboratory and field experiments in this project, a thermal mitigation machinery system called "close circulated seawater heating system" was designed and commercially operated to mechanise the process on board of the farm boats. Several offshore tests proved that the proposed and designed machinery is capable of treating the tubeworm infestations on mussel droppers very effectively and in compliance with the acceptable standards for offshore mussel farming. The operation temperature can be adjusted in this system and can be easily utilised to mitigate other soft and hard shell groups of biofouling on mussel droppers.

OUTCOMES ACHIEVED TO DATE

- The major outcome achieved in this project is the development of a commercially and scientifically effective thermal treatment method for the mitigation of the Australian native tubeworm *Pomatoceros taeniata*. This resulted in design and exploitation of a commercial scale biofouling thermal treatment system. The system, which is called "closed circulated seawater heating system" (CCSHS), is proved to be highly efficient in treating soft shell and hard shell biofouling at offshore longline mussels and oyster farms.

- A final technical report prepared which describes a detail of all the laboratory and commercial scale tubeworm treatment on offshore blue mussel farms.

- A brief Standard Operating Procedure (SOP) and Factsheet for the tubeworm biofouling thermal treatment at commercial scale are prepared which will be available on the SeaBounty website (http://www.seabounty.com.au/) and Ausnik website (http://www.ausnik.com.au/).

- Half day workshop at DPI Queenscliff to present the results of the project.

Acknowledgment

The research was funded by the Fisheries Research and Development Corporation (FRDC)

Within mussel aquaculture industry in Victoria, we wish to acknowledge the financial contribution and full support of SeaBounty particularly Mr. Lance Wiffen and Shane Wiffen during the course of this project

Laboratory and onshore experiments was conducted at the shellfish hatchery facility and chemistry laboratory of Department of Primary Industry, Queenscliff, Victoria

We would also like to extend our appreciation and thanks to:

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Dr. Leanne Gunthrope, Dr. Brett Ingram, Dr. Andy Longmore, John Mercer and Damien Lancaster from DPI Queenscliff, Victoria

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Dr. Alireza Asgari from Deakin University

Dr. Mayumi Allinson and Dr. Graeme Allinson from CAPRIM, Victoria

1 BACKGROUND

1.1 Mussel aquaculture in Port Phillip Bay, Victoria

Commercial cultivation of the Australian blue mussel *Mytilus galloprovincialis* is one of the major marine aquaculture industries in Port Phillip Bay, Victoria. It has been commercially produced for more than 25 years using primarily suspended Longline culture system in offshore. Its production peaked in 2002 when annual mussel production reached to 1600 tonnes using exclusively wild mussel spat. However, mussel production in Victoria continued to decline since then to a minimum of 700 tonnes in 2007 due to shortage of wild seed mussel in Port Phillip Bay.

In 2008, a joint research and development project was initiated and funded by Victorian Department of Primary Industry and Victorian shellfish Association to address the shortage of the mussel seed. Three years of R&D efforts and building a state of the art intensive mussel hatchery production system, help Victorian mussel aquaculture industry to regain its position as a leading mussel producer state in Victoria. Farmers currently receiving approximately around 400 millions seeds annually, which help them to extend the hatchery based mussel production to over 3000 tones premium product in Port Phillip Bay in 2011. Currently, hatchery supply of the mussel spat to Victorian blue mussel farming industry has given an opportunity to maintain its competitive edges over other states rope-grown mussel industry by trading in premium quality mussels which are more uniform in size, available year-round (12 months a year rather than seasonal availability) and grow faster than wild spat (12 months for the hatchery produced seeds compared to average of 18 months for the wild seed). The target is to achieve production level of 10000 tonnes in 2015.

1.2 Outbreak of tubeworm in Port Phillip Bay and project development

In July 2011, SeaBounty mussel Aquaculture Company reported a heavy settlement of the tubeworm on its cultured mussels at two main offshore farms in Port Phillip Bay. In response to this outbreak, representatives of SeaBounty consulted with both industry and government expertise to seek advice and solutions on how best to manage, control and mitigate the economic and allied social consequences that would arise from the product spoilage that occurs as a consequence of this biofouling.

Discussions were held with range of stakeholders including aquaculture managers and researchers from Fisheries Victoria, aquaculture scientists from NSW Fisheries, members of Shellfish Aquaculture industries in Tasmania and South Australia and a representative of FRDC to seek advice and support on reducing the economic impact of tubeworms on Victorian mussel industry. There was wide spread support for this request by both industry, government and FRDC in light of

- The economic impact which could be caused by tubeworm biofouling
- Lack of an effective commercial scale treatment for mussel droppers
- Urgency of the issue – to maintain the economic value of the current infected crop.

As a result, an industrial project application was submitted in October 2011 to FRDC by SeaBounty Pty Ltd to seek fund for an intensive research study on developing a commercial controlling method for tubeworm biofouling in mussel farms. In the first instance, Ausnik Pty Ltd as technical service provider, in collaboration with the Victorian Department of Primary Industries in Queenscliff (DPI Vic) commenced a preliminary research to develop commercial control methods. The FRDC funded component of the research (2011/241) commenced November 2011 with the aim of developing a commercially viable Serpulid tubeworm controlling methods. Thus, a series of laboratory and field treatment methods were examined and studied, as was the settled tubeworms identified for further future biology and life cycle studies.

1.3 Species identification and its biology and life cycle

In November 2011, a series of tubeworms samples were collected from the infected offshore blue mussel farms in Port Phillip Bay, Victoria and sent to Melbourne Museum and Australian National Museum for identification. A world prominent Polychaete biologist Dr. Elena Kupriyanova from Australian National Museum and Mr. Robin from Melbourne Museum both confirmed the tubeworm samples as *Spirobranchus taeniata* or formerly named as *Pomatoceros taeniata*.

A major problem in writing a review on biological and ecological aspects of *P. taeniata*, is a lack of studies and information on the life cycle, general biology, reproduction and settlement of this tubeworm not only in Australia but also worldwide. This report most probably is a first of its kind on the outbreak and heavy fouling of *P. taeniata* in shellfish farms. As the focus of this research project was to develop commercial control methods, time and resources were insufficient to support further study on the biology and seasonal pattern of reproduction of *P. taeniata* in Port Phillip Bay.

In the absence of any information on the biology and life cycle of *P. taeniata*, a brief literature review was carried out on general biology and life cycle of some closely relative biofouling Serpulimorph tubeworms such as *Pomatoceros triqueter* and *Pomatoceros lamarckii* to simulate the possible life cycle scenario for this endemic Australian tubeworm. These two species share some common biological and ecological aspects with *P. taeniata* as all of them belong to genus *Pomatoceros* and Serpulidae family. They are also commonly found in temperate coastal water and of considerable economic importance because of their fouling propensities in shellfish farms.

2 NEED

Blue mussel farming in Victoria is a largest shellfish aquaculture industry producing over 3000 tons annually. This industry is increasingly expanding as the mass production hatchery seed technology enable farmers to have access to mussel spat in large number at year-round basis. However, to date all the mussel farms in Port Philip Bay are suspended longline culture system and are susceptible to fouling since they are continuously submerged in seawater.

In May and June 2011, Serpulid tubeworms settled on mussel crops in great numbers in Port Phillip Bay. This infestation has occurred on hundreds of tonnes of SeaBounty cultured mussel and several other shellfish farms within Port Phillip Bay. This

infestation was heavily affecting over 80% of the stock. Fouling of mussel shells by Serpulid tubeworms can be problematic for the Mussel Aquaculture Industry, as fouled shells are perceived to be an inferior product. Reports also revealed that mussel with more than 7% of the shell surface fouled by tubeworm are not considered Grade A quality, as the product is considered visually unattractive. Therefore, it was assumed that the extensive tubeworm fouling could lead to significant losses in three major SeaBounty aquaculture sites and other shellfish aquaculture industry within Port Phillip Bay. It was expected that the outbreak could reduce the value of the product and cause a negative impact on domestic and export markets, affecting the viability of this industry.

Furthermore, fouling by the Serpulid tubeworm represents a particular threat to industry's viability, productivity and profitability as calcareous tubes >5 mm cannot be removed from the mussel shell degrading product value. Reports also indicated that heavy settlement of tubeworm in Scottish mussel farms resulted in entire mussel stocks being discarded at an estimated cost to the Scottish industry of between £300,000 and £500,000 per annum. Tubeworm biofouling costs millions of dollar of losses in many other shellfish aquaculture industries around the world.

Nevertheless, literature review revealed no commercially viable and effective treatment method for mussel droppers fouled by Serpulid tubeworm at offshore farms. An effective commercial scale treatment must consider the following issues:

1. To kill the worm before the tubes grow >5 mm
2. Not to affect mussel attachment to the ropes
3. To produce mussels that are edible (i.e. comply with health regulations)
4. Ability to be performed at sea on board of commercial mussel boat.

An urgent action was needed to address this problem, prevent further losses and develop an effective and commercially viable treatment method at on-farm scale.

3 OBJECTIVES

1. To investigate and evaluate different treatment methods for controlling Serpulid tubeworms fouled on farmed mussel at laboratory scale
2. To trial the best method at large scale offshore mussel farm.
3. To choose and refine the most commercially viable and environmentally sustainable method for controlling tubeworm biofouling in offshore mussel farms
4. To develop a standard SOP for farm treatment application

4 REVIEW OF LITERATURE

4.1 Taxonomy, distribution and life cycle of tubeworm

According to (Biodiversity Information Explorer 2011) a detail classification of the species *Pomatoceros taeniata* can be presented as:

- Kingdom: ANIMALIA
- Phylum: ANNELIDA
- Class: POLYCHAETA
- Family: SERPULIDAE
- Subfamily: Serpulinae
- Genus: *Pomatoceros*
- Species: *Pomatoceros taeniata*

As indicated before, *Pomatoceros taeniata* belongs to Serpulidae tubeworm family. This family is a sessile, tube-building annelid worm in the class Polychaete.

Generally, the tube-dwelling Serpulid genus *Pomatoceros* has a wide geographical distribution over the Atlantic and Indo-Pacific Oceans and has been reported from low water to abyssal depths (Zibrowius 1971). *Pomatoceros taeniata* is considered as an endemic Australian species which is mainly recorded in South Eastern Australian coastal water, primarily in Victorian (97% of records) and Tasmanian temperate and cold costal water territories (3%) (Figure 1 and Figure 2). A major recent outbreak area of this tubeworm in commercial offshore shellfish farms of Port Phillip Bay, Victoria is shown in Figure 3.

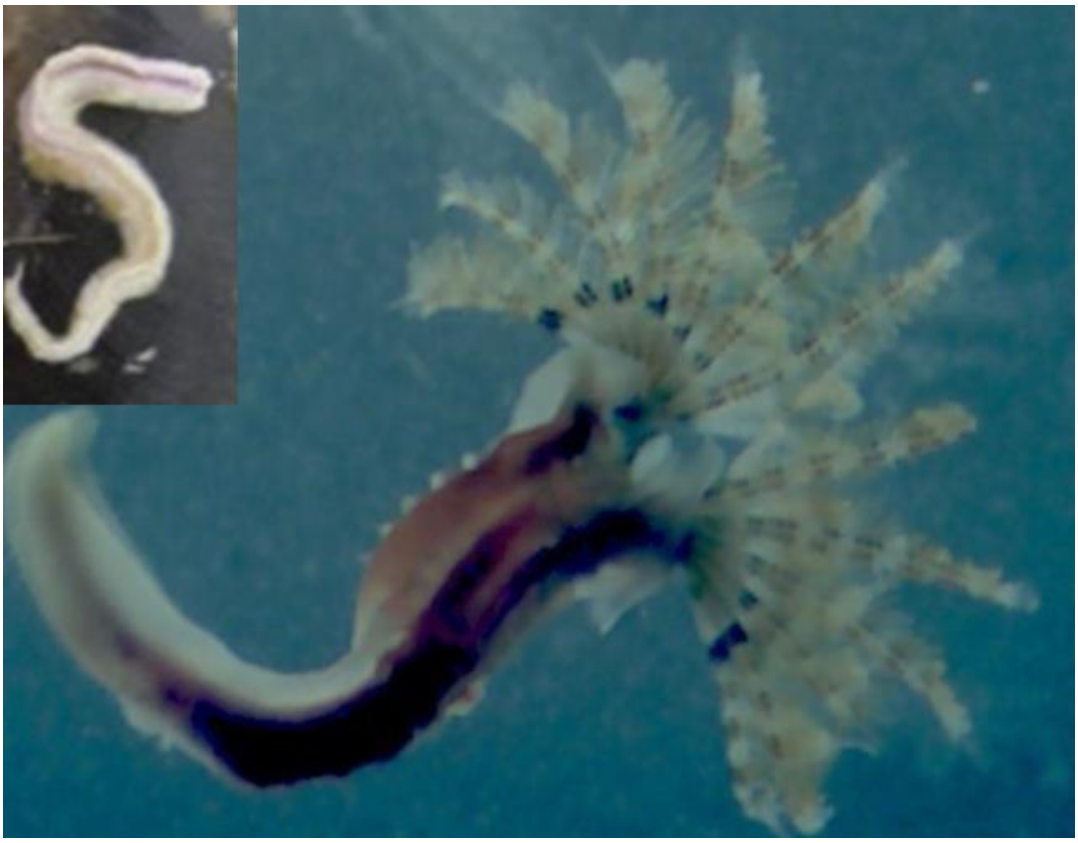

Figure 1: Australian tubeworm *Pomatoceros taeniata* collected from blue mussel farms in Port Phillip Bay

Figure 2: Distribution of *P. taeniata* by state and territory in Australia (highlighted in bold blue area (Biodiversity Information Explorer 2011)

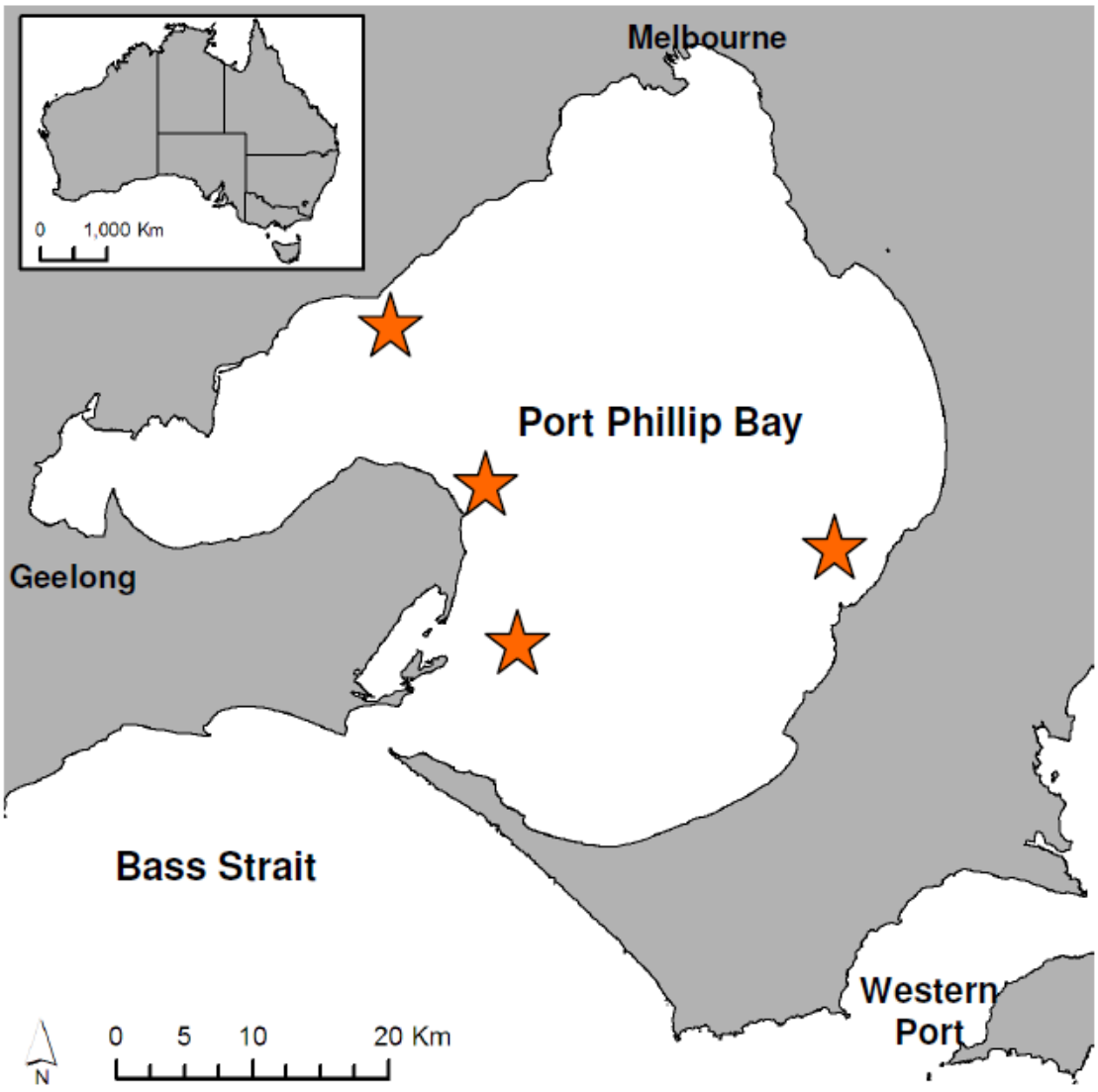

Figure 3: Major tubeworm outbreak area in Port Phillip Bay shellfish farms

Serpulid tubeworms have no specific habitat but live on stones, rocks, shells and occasionally on the hard parts of other animals. Artificial, commercially important structures such as buoys, ship's hulls, docks, offshore oil rigs and aquaculture infrastructures are also colonised by these tubeworms (OECD, 1967), and they are considered to be primary fouling organisms (Crisp, 1965).

4.2 Life History

A typical life cycle of Serpulid tubeworms are shown in Figure 4. These tubeworms generally undergo four main stages of development in their life cycle:

- Embryonic stages
- Larval stages
- Settlement and metamorphosis
- Growth and maturation
- Embryonic stages

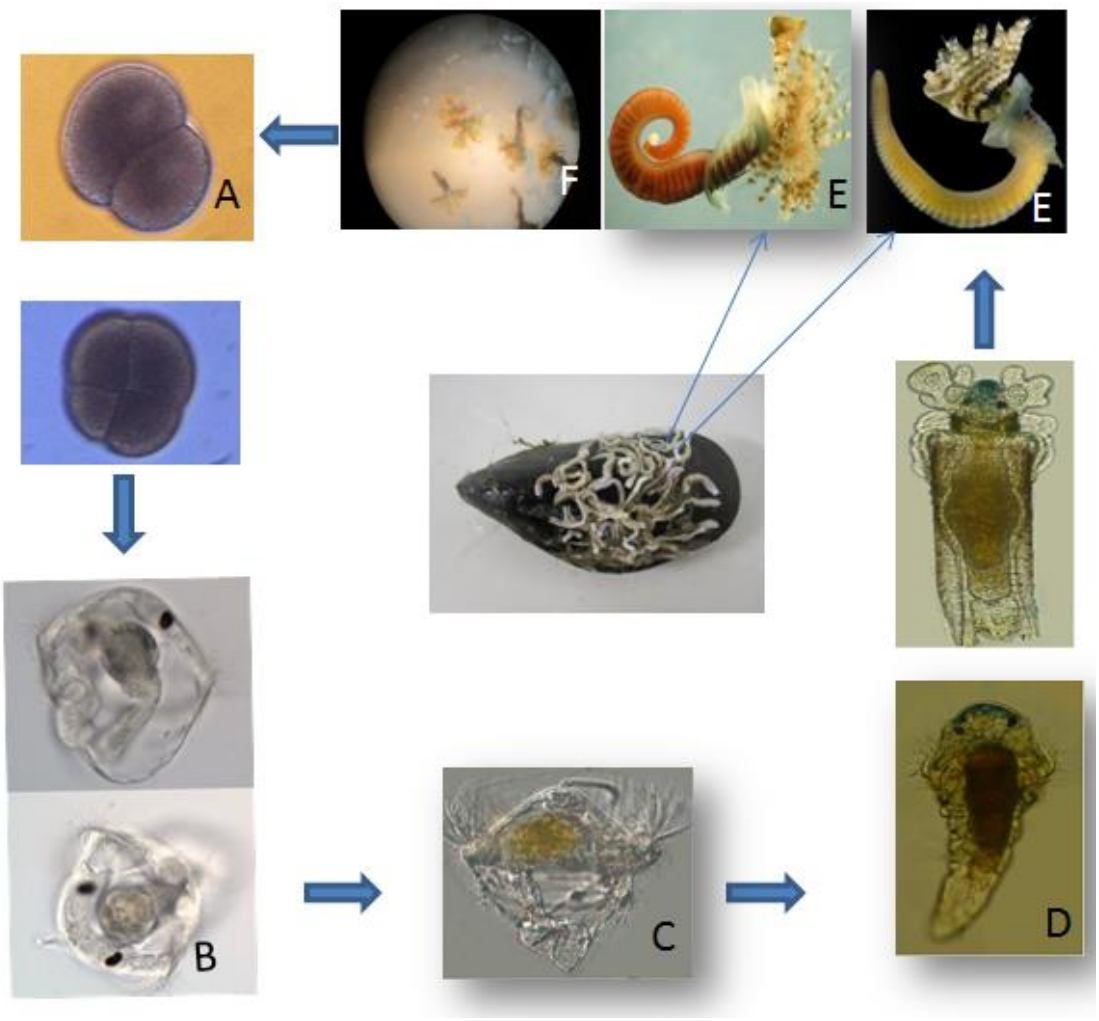

Figure 4: Typical life cycle of *Pomatoceros sp* tubeworm: *A. Embry,* B. Trochophore larvae, C. Metatrochophore, D. Juvenile, E. Adult, F. Spawning adult, F. Embryo

The embryonic development of Serpulids has been studied extensively, especially at the turn of the century. Summarised early embryological information from the literature published mostly prior to 1910 and provided a detailed description of development of *Hydroides dianthus* through the early trochophore stage; see (Rouse 1999) for a (re)definition of the trochophore concept. More recent studies on serpulid embryonic development include the work of (Vuillemin 1965, 1968) on *Ficopomatus enigmaticus* and Groepler (1984, 1985) on *Pomatoceros triqueter*.

The embryonic development of Serpulids has been studied extensively, from the beginning of the 19the century until recently. A detail of these studies and an overview of early development in Serpulids are summarised by (Kupriyanova et al. 2001). Developmental events in genera such as *Ficopomatus*, *Galeolaria*, Hydroides, *Pomatoceros*, Pomatoleios, Serpula and *Spirobranchus*, which have small eggs and planktotrophic larvae, are very similar (Kupriyanova et al. 2001). After fertilisation, the negatively buoyant eggs sink to the bottom, where they undergo cleavage up to the blastula stage. The first cleavage occurs after 1-1.5 h after fertilisation at 20-25°C (Andrews and Anderson 1962; Smith 1984; Wisely 1958) but it takes 2.5 h at 15°C and almost 4 h at 10~11°C (Strathrnann 1987).

Under normal conditions, embryogenesis in Serpulids takes approximately 20 h producing the first feeding larvae. However, the embryonic development period is highly variable among the species, geographical area and different environmental conditions. For example, development time increases with decreasing temperatures, e.g. 7 h at 30°C, and 15 h at 20°C for *Ficopomatus enigmaticus* (Vuillemin 1958)The blastula develops into trochophore larva, a prototroch consisting of a single ring of cilia.

4.3 Larval stages

Larval stages in Serpulids begin with trochophore larvae. Trochophore is a stage in which Serpulid's larvae commence feeding. Development period from embryonic to feeding larvae are found to be different from within 10-14 hours after fertilization in *H. elegans* (Finley 1971)to 48 h in *Protula palliata* (Kupriyanova et al. 2001). The difference probably correlates with egg size. Larval development continues from the Trochophore to the metatrochophore and metamorphose larvae at the final stage. The development time of the larval stage in Serpulids and that of the brooded stage in spirorbids is, like most other reproductive and developmental processes, profoundly affected by temperature (Kupriyanova et al. 2001). For example, (Castric-Fey 1984) reported that the larval development period for *Pomatoceros lamarckii* is three weeks in laboratory conditions at 18°C but could be varied from about 2 months in early spring to 8-15 days in early summer and 20 days in late summer in South Brittany. Similar effect of temperature on larval development are reported for *Serpula columbiana* where the longest larval developmental period of up to 50 days recorded in the laboratory at 12°C (Strathrnann 1987; Young and Chia 1982) and for *Hydroides dianthus* and *H. elegans* where the larval development to metamorphosis takes place for only 5 days at 24-35°C(Carpizo-Ituartc and Hadfield 1998; Strathrnann 1987).The duration and success of larval development to settlement stage also depends on salinity, food availability and external metabolites of other invertebrates (Kupriyanova et al. 2001).

Settlement, metamorphosis and Juvenile Settlement stage is a transition period in which larvae transform from a pelagic swimming to an obligate sessile life style. Metamorphosis is a set of morphogenetic events accompanying this transition and making it possible. The metamorphosis in Serpulids larvae begins with the disappearance of the prototroch and is further characterised by differentiation of the branchial crown in the head region the collar and thoracic membrane in the thoracic region, and the pygidium at the tip of the abdomen (Kupriyanova *et al.* 2001; Marsden and Anderson 1981) describe some initial events of metamorphosis Galeolaria caespitosa, such as collapse of prototroph, development of bronchial buds, pygidial appendages and thoracic membrane rudiments. After the demersal stage, the larvae settle by secreting an abdominal posterior mucous bag. Juveniles originally secrete a mucous tube, covering it later with calcareous matter secreted by the ventral collar surface. Successful attachment and construction of the calcareous tube marks the completion of normal metamorphosis.

During the settlement season, environmental factors such as ambient temperature, salinity, dissolved oxygen and light intensity that generally have measurable effects on larval development, behaviour, and survival also affect the intensity of settlement (Kupriyanova et al. 2001). For example, (Reish 1961) suggested that the settlement of *Hydroides elegans* occurred when the water temperature was above 20°C and more

intensive settlement occurred at stations having a greater concentration of dissolved oxygen.

Settlement in Serpulids and spirorbids living in temperate climates is also highly seasonal and generally the length of the settlement period coincides with the length of reproductive period (Kupriyanova et al. 2001). For instance, larvae of *Pomatoleios kraussi* settle in Kuwait from March to December with the maximum of abundance in August (Mohammad 1975). In Australia and New Zealand, California, Japan and China peaks of *Hydroides elegans* settlement occur in summer and autumn (Kupriyanova et al. 2001), whereas in Hong Kong settlement of this species peaks in early spring to early summer (Qiu and Qian 1997). However, (Castric-Fey 1983) reports all year round settlement for *P. triqueter* and *P. lamarckii*, but with maxima in April, June, August and October in South Brittany, France.

4.4 Growth and maturation

4.4.1 Growth

The rate of post-settlement growth of juvenile Serpulids has been well studied for fouling species. e.g. *Ficopomatus enigmaticus*:(Rullier 1946; Soldatova and Turpaeva 1960; Vuillemin 1965) *F. uschakovi*: (Hill 1967; Straughan 1972a, 1972b); *Hydroides dianthus*: (Grave 1933) *H. elegans*: (Behrens 1968; Dew 1956; Grave 1933; Paul 1937, 1942; Scntz-Braconnot 1968); *H. ezoensis*: (Miura and Kajihara 1984); *Pomatoceros triqueter*: (Foyn and Gjocn 1954; Scntz-Braconnot 1968); *Pseudochitinopoma occidentalis* (Smith and Haderlie 1969). Tubes of juvenile worms grow rapidly but the growth slows down in later life (ten Hove & van der Hurk 1993). Settled *Spirobranchus* juveniles put down at least a body length of tube (0.5-1 mm) per day when first settled (Paul 1937; Smith 1985) reports a growth rate of 14 mm in 9 days for Hydroides elegans. In *H. dianthus* the first three months increases in length by 54 mm but in the next 9 months only 12 mm are added (Grave 1933). *Pomatoleios kraussi* grows 130 µm day-1 for the first 2 months, slowing to 50 µm day-1 in the third month (Crisp 1977).

Under natural conditions the growth of *Spirorbis spirorbis* is much slower in winter (0.17 mm month-1) than in summer (0.66 mm month-1) (De Silva 1967). The same holds true for *Pomatoceros triqueter* (20-30 mm month-1 in spring, 2-10 mm month-1 in winter) and *Hydroide elegans* (12 mm month-1 in spring, 4 mm month-1 in winter) (Scntz-Braconnot 1968). In addition to seasonal changes, the growth rate in Serpulids varies according to temperature, population density, flow speed, salinity, the poIIution and availability of food (Kupriyanova et al. 2001).

4.4.2 Maturation

Serpulids reach sexual maturity after they achieve a certain body size and expose to an optimal environmental conditions (Kupriyanova et al. 2001). It has been demonstrated that tubeworms living under conditions suboptimal for growth reach maturity slowly and some never reach it. For instance, *Ficopomatus uschakovi* juveniles growing in optimum salinity become mature in 4 weeks, while those that exposed to salinities either below 5 or above 30 ppt, grew slowly and never matured (Hill 1967). At salinity >25 and temperature >20°C, the first spawning of *Hydroides elegans* occurred on day 16 after settlement. Both low temperature and low salinity

led to slower growth and subsequently to a longer time to maturation (Qiu and Qian 1998). Furthermore, spawning of *H.elegans* reared in the laboratory was observed on average 40 days after fertilisation at 23°C (Matuso and Ko 1981). However, according to (Paul 1937, 1942) H. *elegans* reaches maturity within 9 days after settlement in Madras, India at 25.5-29.5°C. The first macroscopic signs of sexuality appear 1.5-3 months after settlement in *Pomatoceros triqueter* and *P. lamarckii* (Castric-Fey 1984)

Size at maturation of *Ficopomatus uschakovi* at Lagos, Nigeria started from 6 mm but not all worms of this size were mature (Hill 1967) while in Japan, mature eggs or sperm in F. enigmaticuswere first observed in individuals 6-8 mm long, 3-4 weeks after settlement (Okamoto and Watanabe 1997) whereas in France *F. enigmaticus* becomes mature at 9-10 mm (Fischer-Piette 1937).

4.5 Longevity

The longevity of all organisms, including serpulimorph polychaetes, correlates with body size. The life span of small Serpulidae and Spirorbidae rarely exceeds one year. For example, *N. brasiliensis* live only several months (Rzhavsky and Britayev 1984). However, for the most *Pomatoceros triqueter* has a life span of 4 years according to (Dons 1927) but estimates of the longevity for this species in Northern Europe vary from 1.5 (Foyn and Gjocn 1954) to 2.5 year (Castric-Fey 1983). Larger species, such as *Spirobranchus polycerus* and *Ficopomatus enigmaticus*, can live for 10-12 year (Fox 1963; Marsden 1994). The even larger forms of the *Spirobranchus giganteus* complex can live for 18-35 year (Nishi 1997; Nishi and Nishihira 1996, 1999; Smith 1984).

5 METHODS

5.1 Study area

The outbreak of tubeworms infestation on blue mussel took place in Port Phillip Bay. Port Phillip Bay is a large bay in southern Victoria, Australia. Geographically, the bay covers 1,930 square kilometres (480,000 acres) and the shore stretches roughly 264 km. Although it is extremely shallow for its size, most of the bay is navigable. The deepest portion is only 24 metres, and half the region is shallower than 8 m (26 ft). The volume of the water in the bay is around 25 cubic kilometres (Figure 5). (Wikipedia 2011).

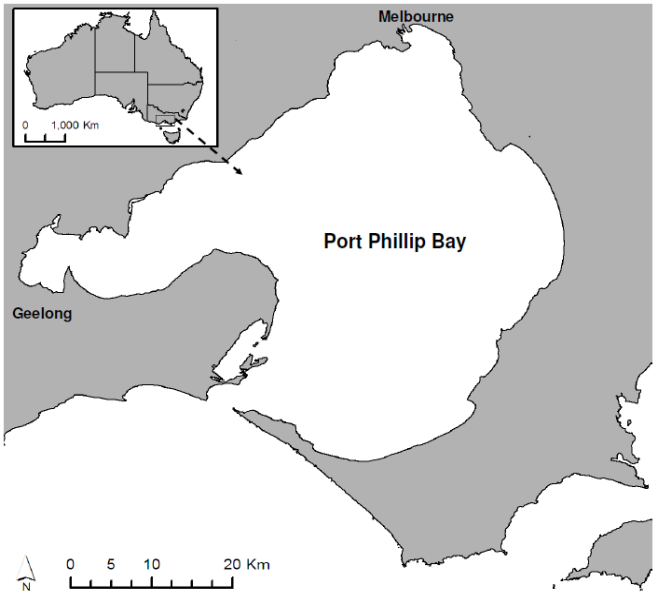

Figure 5: Australia with detailed location map of Port Phillip Bay

5.2 Description of farming operations

Currently, blue mussel cultivations are situated in 5 major offshore aquaculture sites within Port Phillip Bay, where a longline culture of blue mussels is the predominant activity (Figure 6).

Figure 6: Offshore Aquaculture sites in Port Phillip Bay

A long-line is a horizontal rope suspended one meter below the surface by a series of buoys. The longline is anchored at either end with a two tonne block in approximately 10 m of water. Long-lines are typically 250 m in length with only 120 m of the line used for culture. Droppers are the ropes on which mussel spat is attached (socked in industry terms). They are typically 5 m in length and hung from long-lines at intervals of 400 to 800 mm (Figure 7). Droppers with mussel spat attached are handled

approximately 3-4 months post deployment, where 2.5-3 cm spat stripped from the spat ropes and resocked to grow-out droppers at the density of 1200-1600 spat per 5 meter droppers. Grow-out period for blue mussel takes approximately 8-12 months and is highly depend on the source of spat (hatchery/wild), production season and environmental conditions.

At present, SeaBounty Pty Ltd is the largest of the commercial operators in Port Phillip Bay, possessing over 30,000 commercial mussel droppers at Pinnace Channel, Grassy Point, Clifton Spring and Werribee aquaculture sites. In this study, mussels samples infected with Serpulid calcareous tubeworms were obtained from SeaBounty Pty Ltd. long line offshore farms at Pinnace Channel and Grassy Point.

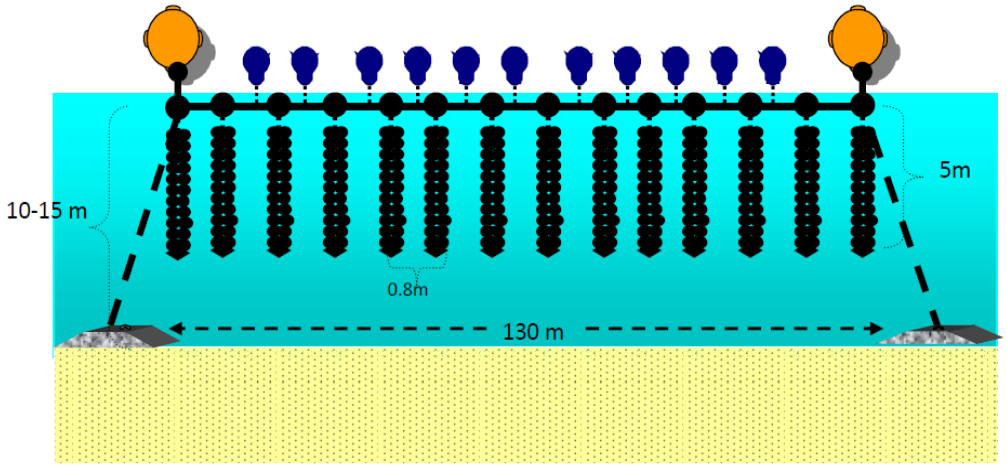

Figure 7: Long-line culture of blue mussels

5.3 Facilities

All the laboratory experiments and treatment trials in this study were conducted at the Aquatic Chemistry Laboratory and DPI Queenscliff Shellfish Hatchery facilities at Marine and Freshwater Resources Institute (MAFRI), Department of Primary Industry, Queenscliff, Victoria. Seawater was obtained from the Institutes' main seawater supply system where the water is sourcing from an oceanic side of Queenscliff and filtered through a 30 µ sand filter.

5.4 Collection and maintenance of tubeworms infested blue mussels

Blue mussels droppers containing infected tubeworm mussels were obtained from SeaBounty offshore farms located at Pinnace Channel and Grassy Point in Port Phillip Bay and transported to DPI Queenscliff facilities using fish bins, covered with wet hessian. On arrival at the laboratories, the mussel droppers were placed in a flow-through 5000 litre ambient seawater tank, acclimatised and used within one week as a source of experimental mussels and tubeworms (Figure 8).

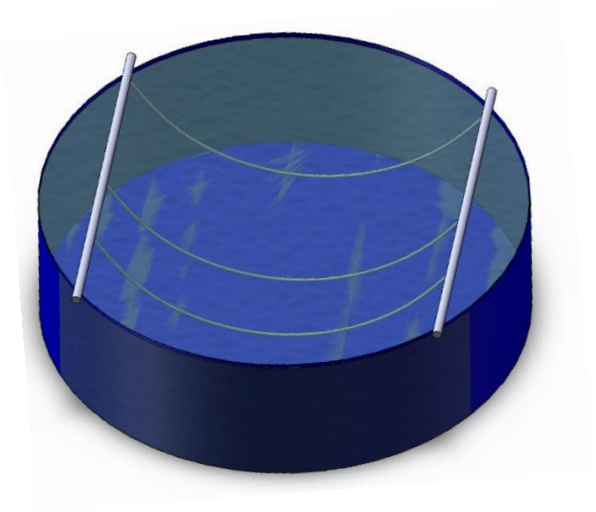

Figure 8: Diagram of 5000 litres ambient seawater tank

Mussels stocks were maintained in flow-through tanks for a maximum period of one week to ensure the quality and fitness of mussels and worms for treatment evaluation and renewed then after. When required, tubeworms infected mussels were then stripped from the droppers and taken into an ambient water aquarium facility in Chemistry lab where they kept for 24 hours and then transferred to a 1 litre take away box to conduct laboratory scale treatment tests.

5.5 General Experimental Procedures

During the course of this project, experimentation was carried out in three phases:

1. A laboratory phase to establish the optimum times required for each treatment to achieve maximum tubeworm mortalities with minimum mussel mortalities using 1 litre take-away plastic box

2. An onshore medium scale trial phase to re-optimise time and intensity for effectual laboratory treatment using commercial size mussel droppers in a 150 litre plastic container.

3. A field-testing phase to scale up the laboratory and onshore medium treatment findings and to determine the efficacy of the treatments on tubeworms and mussels using multiple mussel droppers at offshore commercial farm

A series of mitigation methods were selected based on a literature review including drying, heating, freshwater, chlorine, saturated salt and chilling. Numbers of criteria were considered in selecting the best methods to scale up to onshore and offshore phase, including:

1. Level of intolerance of tubeworms to the physical/chemical conditions of the treatment: Those treatments which resulted to over 90% mortality at post laboratory phase trials were chose for next phase.

2. Impact on mussel crops: Those treatments which resulted to over 90% survival in mussel crops at post laboratory phase trials were chose for next phase.

3. Impact on environment: Those with minimum or no impact on environment were selected for next stage

4. Cost effective at commercial scale

5. Logistical capacity of the farmers to operate at an commercial scale offshore mussel farm

During all laboratory and field experiments, the condition of all treated mussels and tubeworms were determined in a similar manner. Mussels were judged alive if the valves closed and mantle contracted after a mechanical stimulation. Calcareous tubes of polychaete attached to the mussel shells were gently crushed under dissection microscope and individual extracted from the tubes was classified dead when no signs of movement could be detected on examination under a stereo microscope (Figure 9) (Nel *et al.* 1996). The number of tubeworms present on each mussel was determined by counting undamaged tubes.

Figure 9: Extracted tubeworms

The polychaete tubeworms survival were analysed 48 hours post-exposure to each treatment both at laboratory and field trials, while mussels survival were checked 14 days post treatment at laboratory and field stage. In all the trials, a group of non treated infected mussel individuals or droppers was used as control group.

5.6 Air drying

Air drying was performed at laboratory where fully dried tubeworm infested mussels were exposed to a constant temperature of 18 ºC in triplicates. A preliminary onshore air drying trial was also carried out on a board of commercial mussel boats at ambient temperature of 14-16ºC. The experimental schedule and exposure regime is summarised in Table 1.

Table 1: Experimental program for air drying

Treatment scale	Unit	Mussel number per replicate	Exposure time (h)	Number of test conducted
Laboratory	Plastic tray	10	6, 12, 24	1
Onshore	Mussel dropper	1200-1400	12, 24	1
Offshore	Non	Non	Non	0

5.7 Freshwater

Freshwater dipping was performed at laboratory scale where tubeworm infested mussels were exposed to an ambient temperature of 18 ºC in triplicates. The experimental schedule and exposure regime is summarised in Table 2.

Table 2: Experimental program for freshwater treatment

Treatment scale	Unit	Mussel number per replicate	Exposure time (min)	Number of test conducted
Laboratory	Small Onion sacks	10	15, 30	1
Onshore	Non	Non	Non	0
Offshore	Non	Non	Non	0

5.8 Chlorine Dioxide

Chlorine dioxide was obtained from a Queensland supplier and 3 different experimental solutions (volume/volume) were prepared in freshwater and run at open-air laboratory scale in triplicates (Figure 10). The experimental schedule and exposure regime is summarised in Table 3.

Table 3: Experimental program for Chlorine dioxide treatment

Treatment scale	Chlorine dioxide dosage (ppm)	Unit	Mussel number per replicate	Exposure time (h)	Number of test conducted
Laboratory	0,700, 1400, 2800	Plastic box (1 litre)	5	0, 3, 6, 9	1
Onshore	0,100, 150, 200	Non	Non	Non	0
Offshore		Non	Non	Non	0

Figure 10: Chlorine dioxide experiment

5.9 Saturated saline water

A food grade salt was obtained from Cheetham Salt Company, a local supplier in Geelong, Victoria. Saturated saline water (350 ppt) were prepared using magnetic stirrer at laboratory or a commercial stainless steel mixture for 150 litres container at onshore and offshore farm. Salinity level of the saturated saline water was controlled prior and upon completion of each treatment by 10 times dilution of the solution and measured by digital Salinometer to ensure the quality of work. Saturated saline water treatment was performed at laboratory, onshore and offshore scale (Figure 11) where tubeworm infested mussels was exposed in triplicates. The experimental schedule and exposure regime for each treatment phase is summarised in

Table 4.

Table 4: Experimental program for saturated saline water

Treatment scale	Unit	Exposure time (min)	Number of test conducted
Laboratory	Small Onion sacks	10,15, 20, 30	3
Onshore	15 litre container	15,20	3
Offshore	150 litre container	15,20	3

Figure 11: Saturated saline water offshore treatment trail

5.10 Chilled saturated saline water

A cold shock treatment was performed at laboratory scale where tubeworm infested mussels were dipped to -20 ºC chilling saturated brine water in triplicates. The experimental schedule and exposure regime is summarised in Table 5.

Table 5: Experimental program for chilled saturated saline water

Treatment scale	Experimental unit	Exposure time (sec)	Number of test conducted
Laboratory	Small Onion sacks	3, 5,10	1
Onshore	Non	Non	0
Offshore	Non	Non	0

5.11 Saturated saline water plus vinegar

A combined treatment of the saturated saline water and 3 different vinegar dosages was performed both at laboratory and offshore farm on tubeworm infested mussels groups in triplicates. While 10 individual tubeworm's infested mussels kept in a small

onion sack and dipped in saturated saline water at laboratory scale, this numbers increased to 30 individual mussels for each replicate and dipped to mixed solutions at offshore trial. All the treated mussels were transferred to oyster cages and kept at offshore longline mussel farm for monitoring (Figure 12). The experimental schedule and exposure regime for each phase is summarised in Table 6.

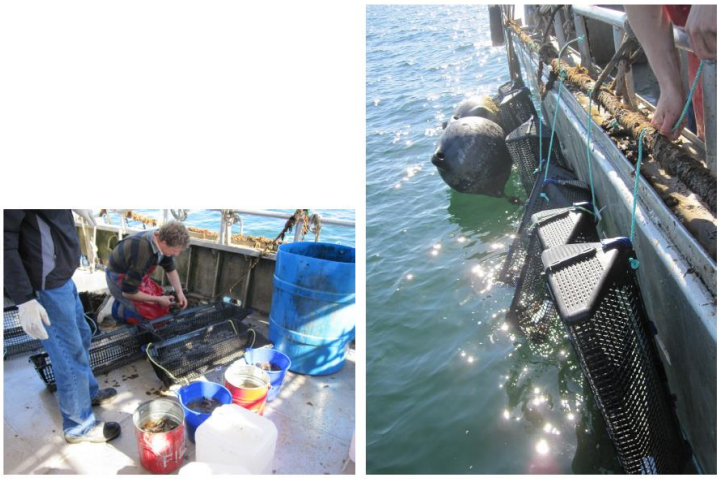

Figure 12: A combined saturated saline water and vinegar treatment at offshore

Table 6: Experimental program combined treatment of saturated salt and vinegar

Treatment scale	Vinegar concentrations (ppt)	Experimental unit	Exposure time (min)	Number of test conducted
Laboratory	0,100, 150, 200	Small Onion sacks	15,20	1
Onshore	Non	Non	Non	0
Offshore	0,100, 150, 200	15 litre container plus oyster cages	15,20	1

5.11.1 Thermal treatment

A series of heat shock treatments was performed at laboratory, onshore and offshore scale where tubeworm infested mussels was dipped to a wide range of exposure temperature and time in triplicates (Figure 13). The experimental schedule and exposure regime for each treatment phase is summarised in Table 7.

Table 7: Experimental program for the thermal treatment

Treatment scale	Experimental unit	Exposure Temperature	Exposure time (Sec)	Number of test conducted
Laboratory	Small Onion sacks	45,48, 51, 53	40, 45,50, 55, 60,65, 70,80	3
Onshore	150 litre	50, 51,52, 55,56, 60, 65	30	2

Offshore	400 litre	45,48, 51	40, 45	1

Figure 13: Onshore thermal treatment of the mussel droppers

5.11.2 Statistical Analyses

Data on tubeworm and mussel mortality rates were analysed using one-way or two-way analysis of variance (ANOVA) where applicable followed by Fisher's Least Significant Difference (LSD) to determine significant difference ($P<0.05$) among the means. All statistical analyses were done using GenStat 13th edition. To ensure a normal distribution, percentage values were arcsine-square root transformed prior to analysis (Zar 1984).

6 RESULTS AND DISCUSSION

6.1 RESULTS

6.1.1 Air drying

Air dying was found as an effective treatment method at laboratory scale where at fully dry conditions, up to 100% of the Serpulid worms were killed within 12 hours exposure with no impact on mussels (Figure 14). Statistical analysis revealed no significant difference in tubeworms mortality among groups exposed for 6 h, 12 h and 18 h.

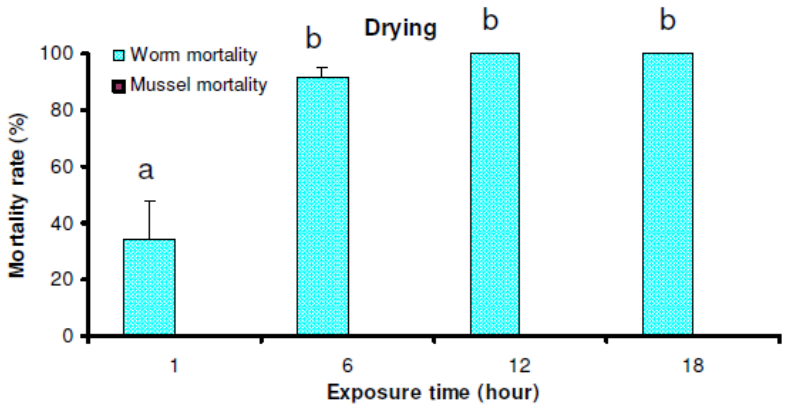

Figure 14: Air drying

When scale up to an onshore commercial scale, air drying was not an effective treatment for commercial size mussel droppers as majority of the Serpulid tubeworms (93%) survived the exposure of 12 to 24 hours period. Most likely effective way to kill the tubeworms was to extend the exposure to 48 hours which neither practically nor economically was feasible to carry out at very big commercial offshore mussel operations such as SeaBounty farms. Therefore, no offshore trial was conducted during the course of this project.

6.1.2 Freshwater

At the start of this treatment, SeaBounty set a 30 minutes time frame as a commercially viable period to treat tubeworms with freshwater at its offshore farms. At laboratory scale, freshwater immersion failed to meet this standard where approximately 50% of the tubeworms killed post exposure (Figure 15). No mussel mortality recorded during the course of this trial. However, given the long exposure time required to treat tubeworms on mussel droppers, and the high cost of logistics, this treatment method was not scaled up to an onshore or offshore trial.

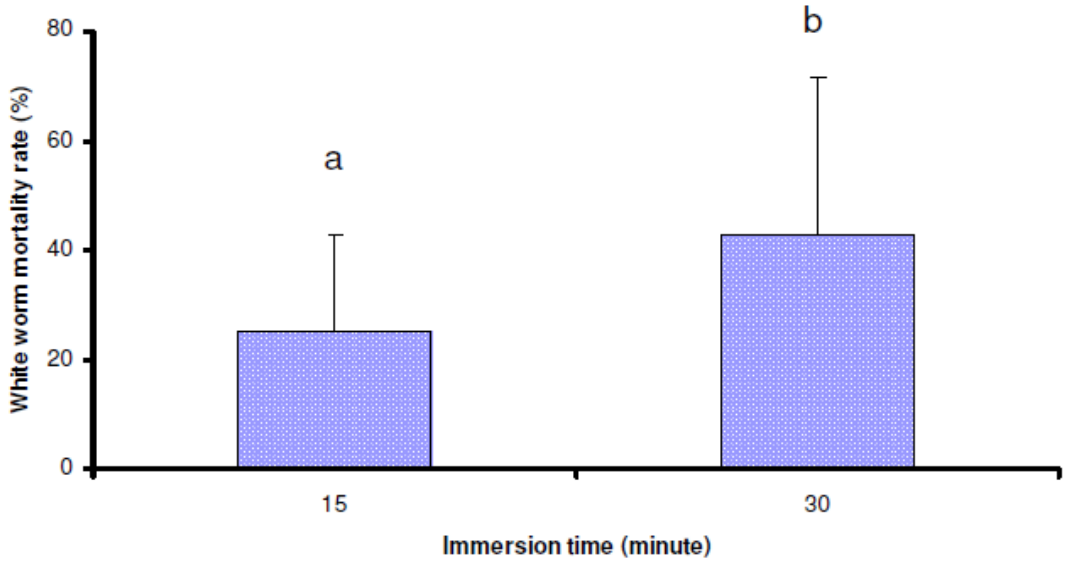

Figure 15: Freshwater treatment: comparison of the Serpulid worm mortality exposed to two different freshwater bathing times

6.1.3 Chlorinated Seawater (Chlorine dioxide)

Chlorine dioxide was not an effective treatment for tubeworm *P.taeniata*. Almost all the Serpulid tubeworms which intentionally exposed to extreme concentrations of Chlorine dioxide (700 to 2800 ppm) for a period of 3-9 minutes at laboratory scale, survived the exposure. The only exception was for 1400 ppm concentration at 9 minute exposure time which only 1% mortality). Similar results were found for mussels with exception of only 3% mortality for those exposed 1400-2800 ppm Chlorine dioxide for 9 minutes (Figure 16and Figure 17)

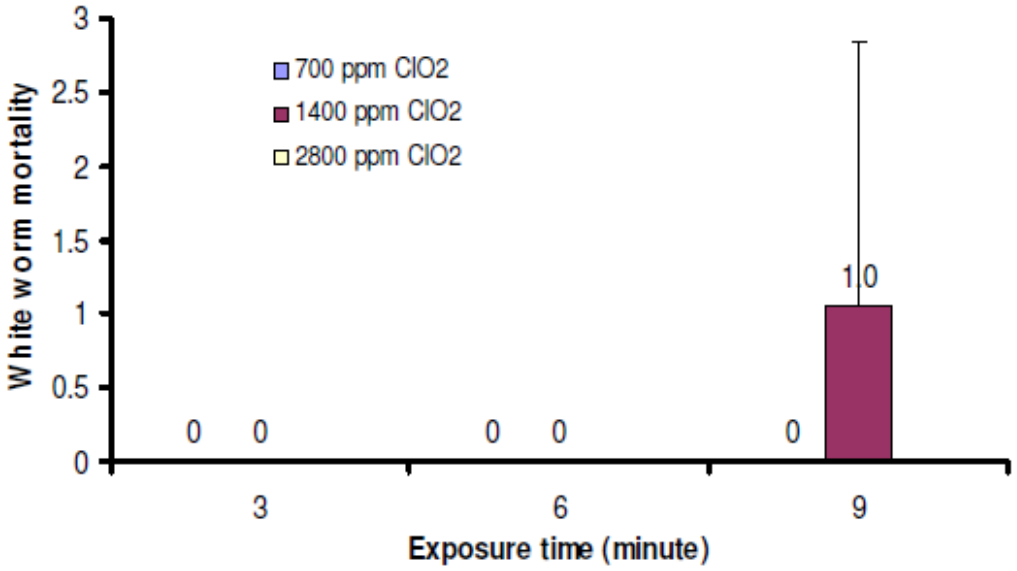

Figure 16: Chlorine dioxide: comparison of the Serpulid tubeworm exposed to Chlorine dioxide at a different time periods

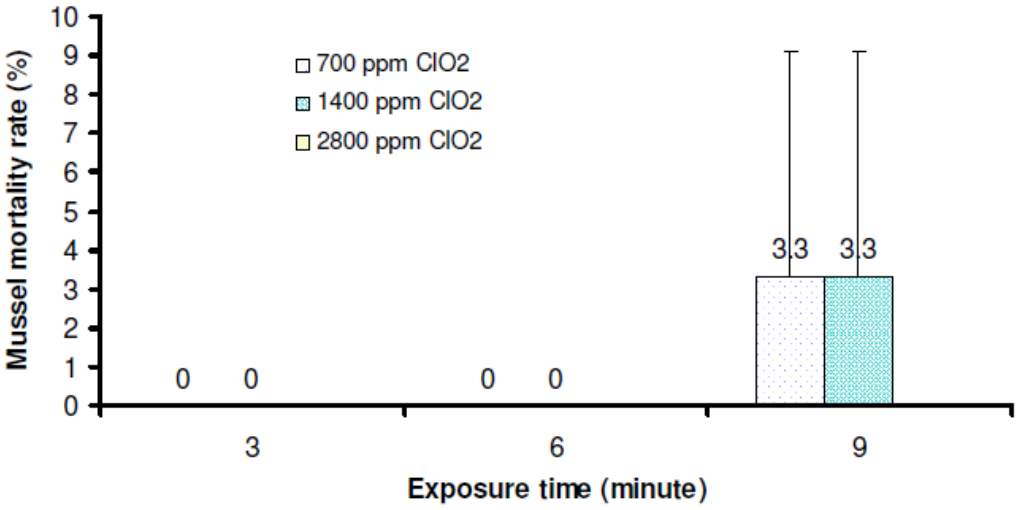

Figure 17: Chlorine dioxide: comparison of blue mussel mortality exposed to Chlorine dioxide at a different time periods

6.1.4 Saturated saline water (350 ppt) (Laboratory scale)

Saturated saline water was found as an effective treatment method at laboratory scale where a mortality rate of 86 to 98% was recorded for the tubeworms at an exposure period of 10-30 minutes (Figure 18). Statistical analysis revealed no significant difference in tubeworms mortality among groups exposed for 10 m, 15 m, 20 m and 30 m. The impact of this treatment method was exceptionally low on mussels with only 3.7% mortality rate at 20-30 minutes exposure time.

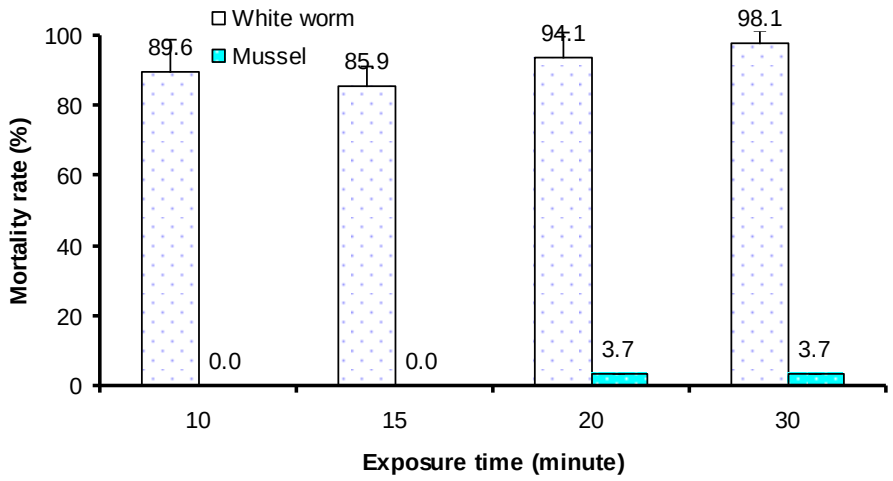

Figure 18: Saturated saline water at laboratory scale: Comparison of the tubeworm and mussel mortality exposed to saturated saline water at different dipping time

6.1.5 Saturated saline water (350 ppt) (Farm scale)

In contrast to laboratory scale trials of saturated saline water treatment, scaling up of this treatment method to an offshore commercial mussel farm, resulted into a much more damage in mussel crops (21-25% mortality). Saturated saline water was also found less effective on tubeworms at commercial scale where between 80-84% of tubeworms killed at an exposure period of 15 and 20 minutes to the saturated saline water. When compare laboratory and commercial scale results, the effectiveness level of the saturated saline water on tubeworm was 5-10% lower at commercial scale treatment than those at laboratory scale. Statistical analysis also shows no significant mortality rate differences (P>0.05) among tubeworms or mussels groups exposed to saturated saline water at 15 minutes or 20 minutes periods (Figure 19).

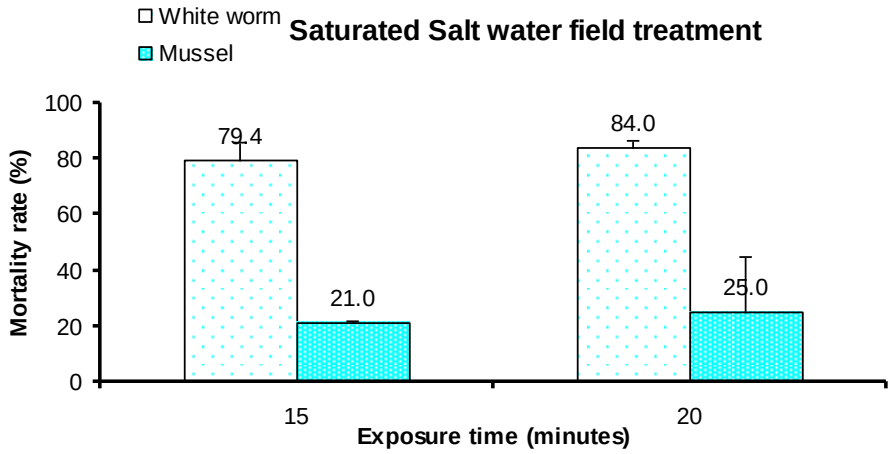

Figure 19: Saturated saline water at offshore farm scale: Comparison of the tubeworm and mussel mortality rate exposed to saturated saline water treatment at 15 and 20 minutes dipping time periods

6.1.6 Saturated saline water plus vinegar (Laboratory scale)

The mortality responses of the tubeworms and blue mussels to a combined treatment of the saturated saline water and three different concentrations of vinegar at laboratory scale and exposure period of 10 minutes is shown in (Figure 20). As mentioned in the methodology, to create a real treatment scenario at offshore farm, none of the experimental mussel groups were subjected to any mechanical stress (i.e. shaking) to induce valve closure in this trial. As expected, the post treatment mussel mortality was higher than a commercially acceptable range (10%) with a record range of 17 to 23% for the exposed groups. Combined treatment of 100 and 150 ppt vinegar caused the highest tubeworms mortality rate (81%) follow by control group (no vinegar) and 50 ppt vinegar concentration. Statistical analysis also shows no significant mortality rate differences (P>0.05) among tubeworms or mussels groups exposed to this combined saturated saline water and different vinegar concentrations.

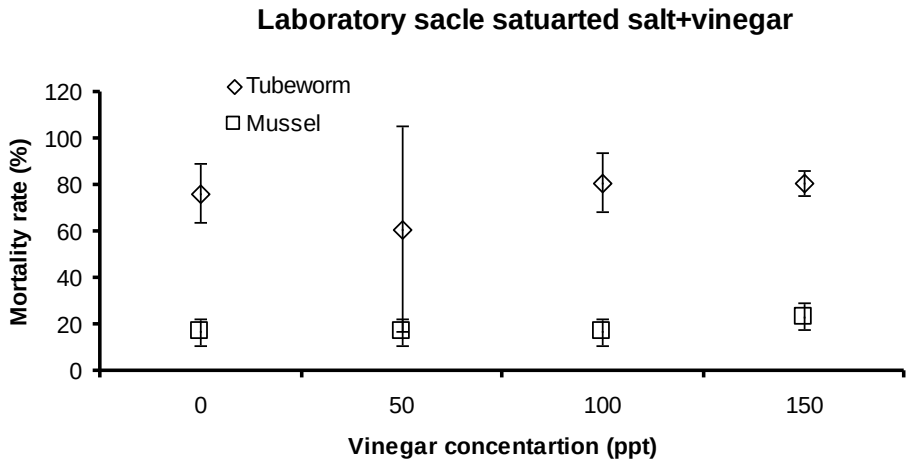

Figure 20: Combined treatment of saturated saline water and vinegar at laboratory scale: Comparison of the tubeworm and mussel mortality exposed to a combined saturated saline water and different 3 different vinegar concentrations at 10 minutes exposure period.

6.1.7 Saturated saline water plus vinegar (Onshore scale)

The mortality responses of the tubeworms and blue mussels to a combined treatment of the saturated saline water and vinegar dosages of 100, 150 and 200 ppt at two different exposure periods of 15 and 20 minutes are shown in (Figure 21 and Figure 22). All the experimental mussel groups were subjected to mechanical stress (i.e. shaking) to induce valve closure in this trial. Generally, tubeworms mortality was considerably high ranging from 85% to 94% for 15 minutes and 92% to 95% for 20 minutes exposure time. Statistically, no significant effect (P>0.05) of vinegar concentration, exposure time or interaction effect of these two factors were detected in this trials (

Table 8). The post treatment mussel mortality was exceptionally low ranging from 3% - 4% for groups exposed to 100 ppt to 200 ppt vinegar concentrations at an exposure period of 15 minutes while the mortality rate slightly increased at 20 minutes exposure time to a range of 3% for the

100 ppt to 9% for 150 ppt followed by 7% for the 200 ppt vinegar concentration. Two ways ANOVA performed in this trial followed by Fisher's LSD reveals a highly significant (P<0.05) effect of the vinegar concentrations, exposure time and interact effects of these two factors on the survival rate of post treated mussels (

Table 8).

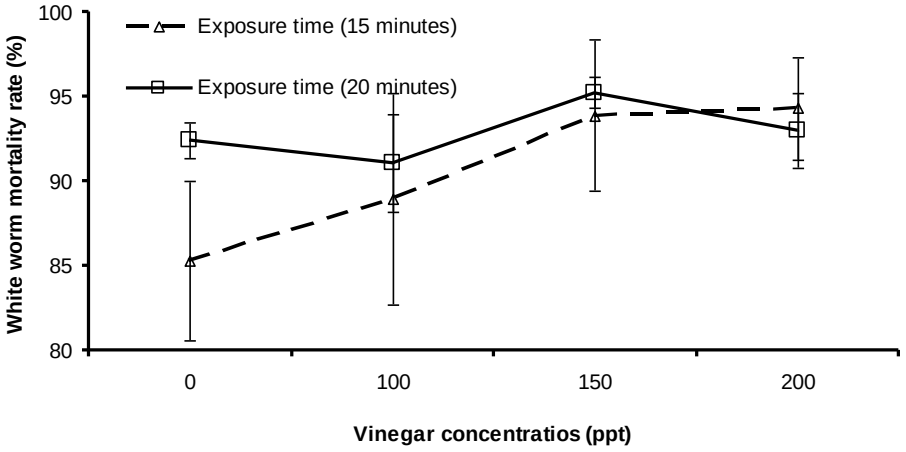

Figure 21: Combined treatment of saturated saline water and vinegar at onshore scale: Tubeworm mortality exposed to different combination of saturated saline water and different vinegar concentrations at 15 and 20 minutes exposure period

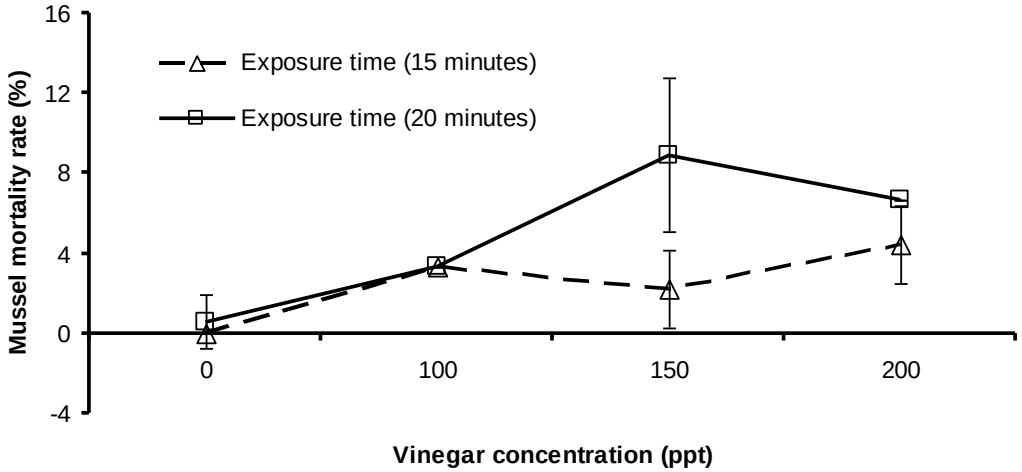

Figure 22: Combined treatment of saturated saline water and vinegar at onshore scale: Mussel mortality exposed to a combinations of saturated saline water and three different vinegar concentrations at 15 and 20 minutes exposure period.

Table 8: Summary of analysis of variance (ANOVA) examining the effect of vinegar concentration and exposure time on mortality of tubeworms and blue mussel

Sources of variation	**SS**	***df***	**MS**	**F-ratio**	***P***
Tube worms					
Vinegar concentration (ppt)	90.25	3	30.08	1.08	0.376
Exposure time (minutes)	25.80	1	25.80	0.93	0.345
Vinegar conc. x time	24.34	3	8.11	0.29	0.345
Mussel					
Vinegar concentration (ppt)	167.963	3	55.988	22.94	<.001
Exposure time (minutes)	30.000	1	30.000	12.29	0.002
Vinegar conc. x time	45.000	3	15.000	6.14	0.003

n=4 ; Significant (*P<0.05)* effects are in bold.

6.1.8 Chilled saturated saline water (-20 ºC, 350 ppt)

Laboratory scale

In the second attempt to test the multiple treatment approach in this study, a combination of osmotic and chilling treatments were applied in which a saturated brine water chilled down to -20 ºC chilling as cold shock treatment were used at laboratory scale. Both tubeworms and blue mussel were extremely highly susceptible to the chilled saturated saline water treatment (Figure 23). The susceptibility (mortality) of the tubeworms to the chilled saturated water was instant, ranging from 93% for 3 seconds exposure time t0 100% for 5-10 seconds. While only 17% mortality was recorded for mussels exposed for 3 seconds to the chilled saturated saline, the mortality was exponentially increased to approximately 90% at 5-10 seconds exposure time. Statistical analysis shows a significant mortality rate differences (P<0.05) among tubeworms or mussels groups exposed to chilled saturated saline water at 3 seconds and those groups exposed for 5 and 10 seconds (Figure 23).

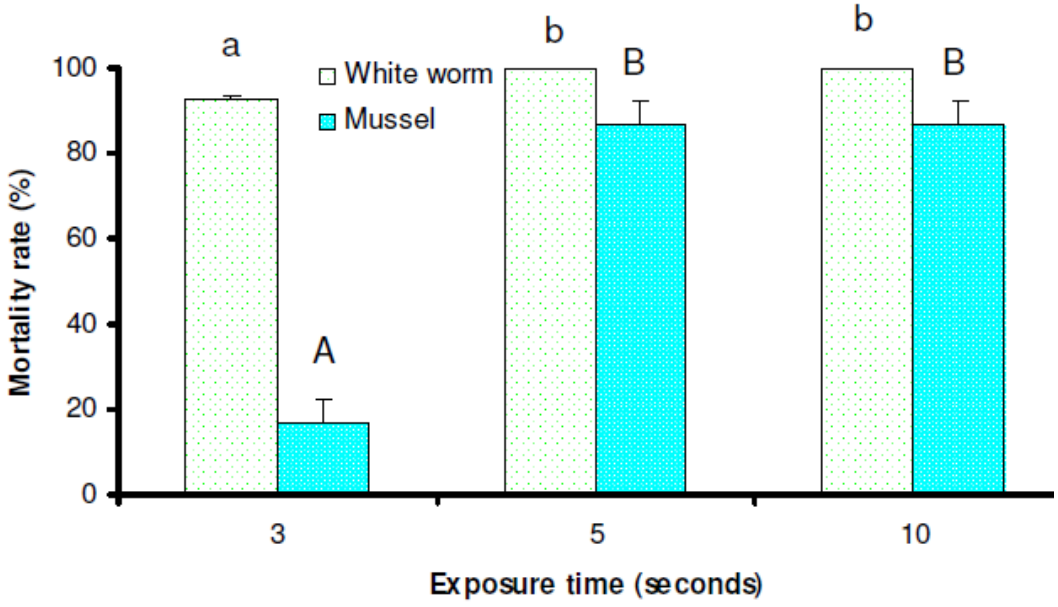

Figure 23: Comparison of the Serpulid worm and blue mussel mortality dipped into a super chilled saturated saline water (-20 ºC, 350 ppt)

6.1.9 Thermal treatment (Laboratory)

In this part, results of several laboratory scale thermal treatments on the tubeworms and mussels are collectively pooled and presented in a separated single graphs (Figure 24 and Figure 27) to avoid confusion and summarised the output. The mortality responses of the tubeworms and blue mussels to a range of temperature (45 to 53 ºC) and exposure time (40-80 seconds) are shown in (Figure 24 and Figure 27).

Highest susceptibility (mortality) for tubeworms was recorded for a group exposed for 60 seconds at 53 ºC (99%), followed by those exposed for 80 seconds at 51 ºC (97%) and lowest was recorded for those exposed to 45 ºC heated water for 40 seconds (Figure 24). Figure 18 showed a significant ($P<0.05$) positive linear relationship between water temperature and tubeworms mortality rate ($R2=0.76$). A similar pattern was also observed between tubeworms mortality rate and exposure time ($R2=0.21$, Figure 26), but was not significantly high ($P>0.05$). Two ways ANOVA performed in this trial followed by Fisher's LSD test reveals a highly significant ($P<0.05$) effect of the water temperature on the survival rate of post treated tubeworms (Table 9), whereas the effect of the exposure time or interaction effects of these two factors was not significantly high ($P>0.05$).

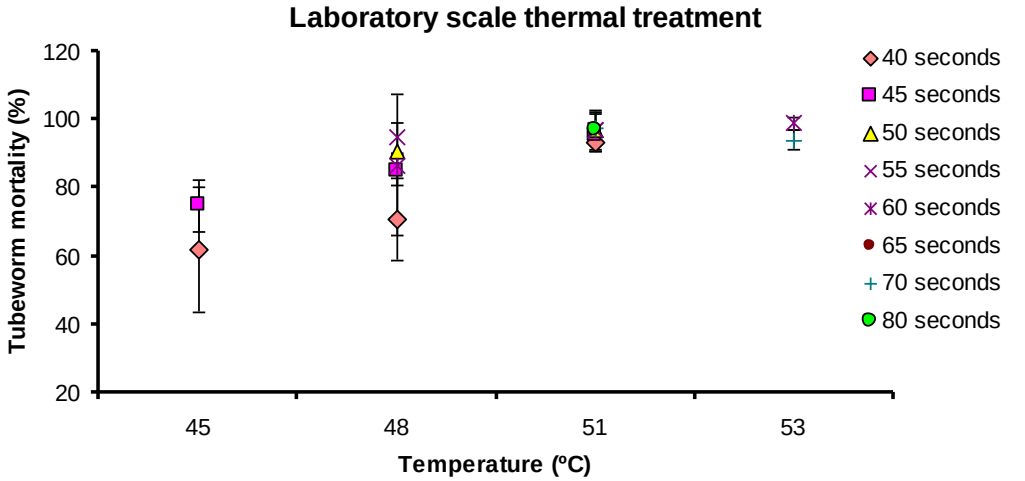

Figure 24: Thermal treatment at laboratory scale: Comparison of the tubeworm mortality exposed to four different temperature ranges at different dipping time.

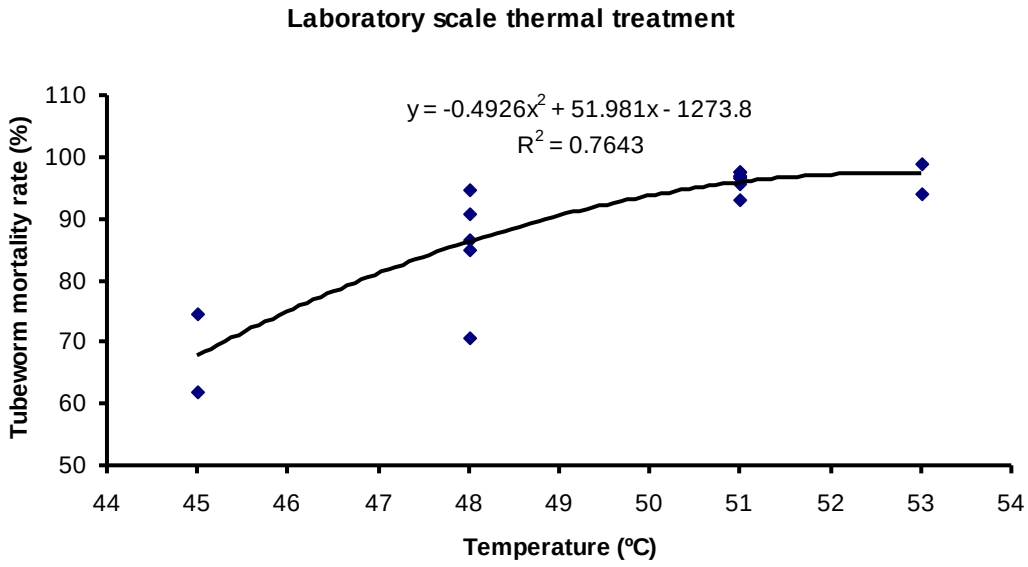

Figure 25: Relationship between water temperature and tubeworm mortality.

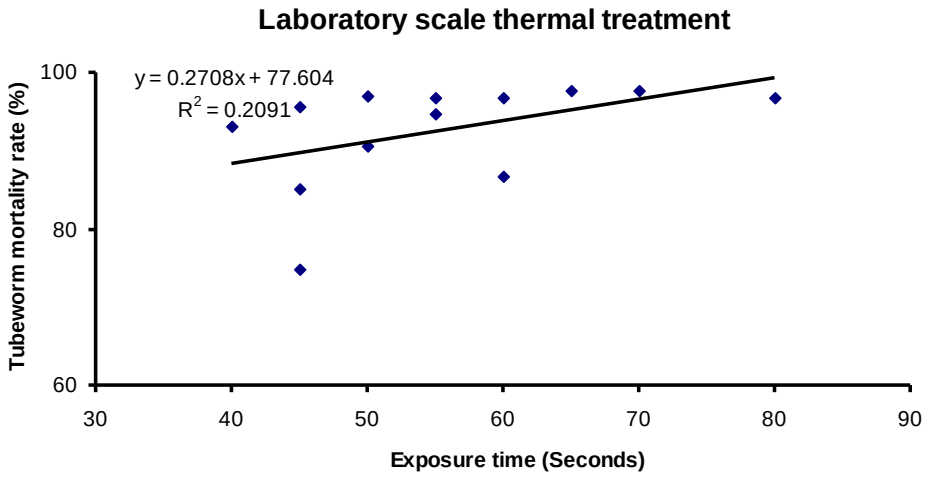

Figure 26: Relationship between exposure time and tubeworm mortality.

All the mussels exposed to 45 and 48 ºC heated water at the laboratory scale at all the exposure times, survived the treatment. However, mussels exposed to 51 ºC for a period of 55 to 65 resulted into 5-7% mortality (Figure 27). Similarly, laboratory exposing of the mussels to 53 ºC for more than 55 or 70 seconds resulted to a mortality range of 7-13%. However, two ways ANOVA followed by a Fisher's LSD test reveals that temperature or exposure time do not have any significant (P>0.05) effect on mussel mortality, individually or interactively (Table 9).

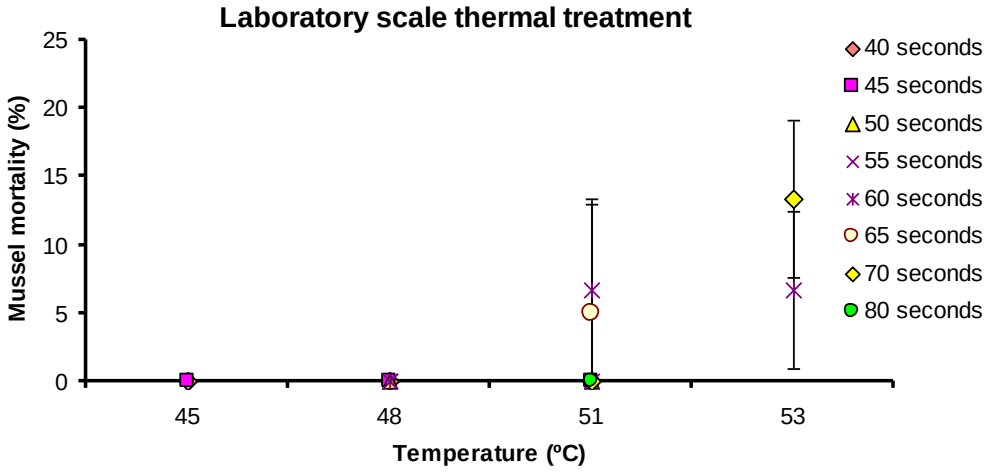

Figure 27: Thermal treatment at laboratory scale: Comparison of the blue mussel mortality exposed to four different temperature ranges at different dipping time.

Table 9: Summary of analysis of variance (ANOVA) examining the effect of temperature and exposure time on mortality of tubeworms and blue mussel at laboratory scale

Sources of variation	SS	*df*	MS	F-ratio	*P*
Tube worms					
Temperature	2246.66	3	748.89	8.98	< 0.001
Exposure time (seconds)	998.51	7	142.64	1.71	0.130
Temperature x time	475.15	6	79.19	0.95	0.469
Mussel					
Temperature	225.35	3	75.12	22.94	0.142
Exposure time (seconds)	133.53	7	19.08	12.29	0.842
Temperature x time	1816.67	6	33.55	6.14	0.539

n=4 ; Significant (P<0.05) effects are in bold.

6.1.10 Thermal treatment (Onshore)

At this stage, the susceptibility of the tubeworms and mussels on real commercial droppers were examined in a series of onshore trials. The mortality responses of the tubeworms and blue mussels exposed to seven ranges of water temperature at 30 seconds exposure period is presented in Figure 28. Generally, tubeworms had shown an extremely high susceptibility (average of 97% mortality) during the course of these trials. The lowest tubeworm mortality at onshore treatment scale was recorded at 51 ºC (94%) and the highest at 56 ºC (98.4%) exposure temperature. However, statistical analysis shows no significant mortality rate differences (P>0.05) among tubeworms exposed to different exposure temperature.

While all the mussels exposed to 50ºC, 51ºC, 52ºC, 55ºC and 56 ºC survived the exposure treatments at rate of 97-100%, those exposed to 60 ºC and 65 ºC suffered a high mortality 54 to 58% (Figure 28).

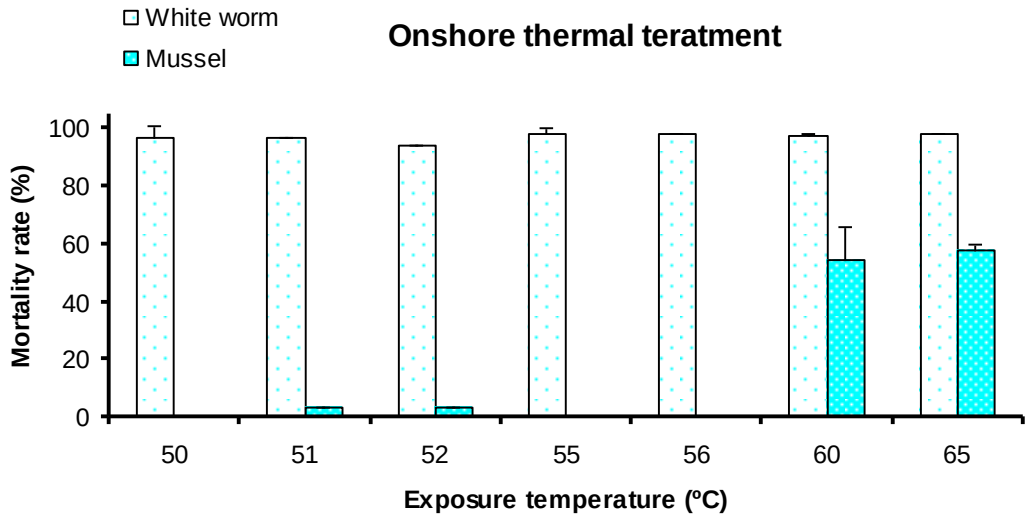

Figure 28: Thermal treatment at onshore scale: Comparison of the tubeworms and blue mussel mortality exposed to seven different temperature ranges at 30 second dipping time.

6.1.11 Thermal treatment using closed circulated seawater heating system (CCSHS) (Offshore)

Following a successful laboratory and onshore trial phases of the thermal treatment methods, "closed circulated seawater heating system (CCSHS)" machinery were tested on a board of the SeaBounty commercial mussel boat at offshore phase to run the thermal experiment (Figure 29).

Figure 29: Offshore thermal treatment of the mussel droppers using "closed circulated seawater heating system (CCSHS)" machinery

Several preliminary tests were carried out to evaluate the performance and to assess the OH&S issues in using CCSHS machinery at offshore environment. Figure 30 and Figure 31 shows the responses of exposed tubeworms and blue mussels treated at 45, 48 and 51ºC exposure temperature at 40 and 45 seconds periods. Mortality rate was highest for the tubeworms exposed to 51ºC for 45 seconds (92%), followed by those exposed to 51ºC for 40 seconds (89%), 51ºC for 40 seconds (78%), 48ºC for 45 seconds (68%), 48ºC for 40 seconds (56%) and 45ºC for 40 seconds (53%). Two ways ANOVA performed in this trial followed by Fisher's LSD test reveals a highly significant ($P<0.05$) effect of the water temperature on the survival rate of post treated tubeworms (Table 10), whereas the effect of the exposure time or interaction effects of these two factors was not significantly high ($P>0.05$).

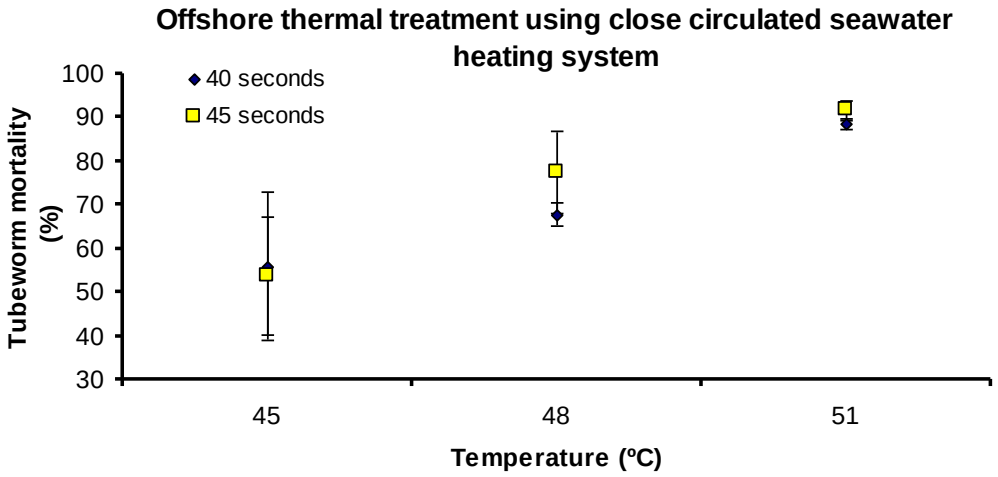

Figure 30: Thermal treatment at an offshore scale: Comparison of the tubeworm mortality exposed to three different temperature ranges at two different exposure times.

Offshore thermal treatment using (CCSHS) machinery proved to be a safe and economically viable treatment system where the average casualty of the treated mussels was 3% for all the experimental temperature and exposure times of 40-45 seconds (Figure 31). The post treatment mortality of the exposed mussels (average 3%) in this trial is well below the norm accepted for commercial scale biofouling treatment (10%). Two ways ANOVA followed by a Fisher's LSD test reveals that temperature or exposure time do not have any significant (P>0.05) effect on mussel mortality, individually or interactively (Table 10).

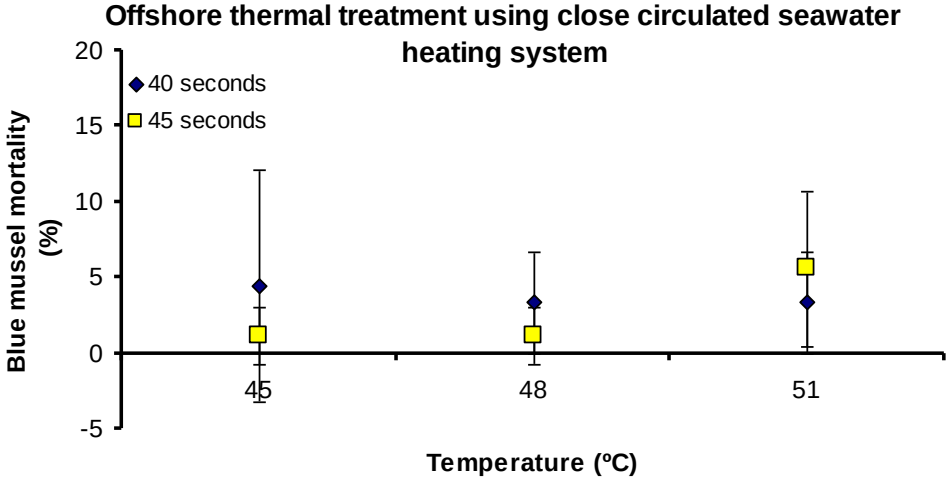

Figure 31: Thermal treatment at an offshore scale: Comparison of the blue mussel mortality exposed to three different temperature ranges at two different exposure times.

Table 10: Summary of analysis of variance (ANOVA) examining the effect of temperature and exposure time on mortality of tubeworms and blue mussel at offshore farm scale

Sources of variation	SS	*df*	MS	F-ratio	*P*
Tube worms					
Temperature	3744.38	2	1872.19	19.82	<.001
Exposure time (seconds)	55.99	1	55.99	0.59	0.456
Temperature x time	108.05	2	54.03	0.57	0.579
Mussel					
Temperature	16.05	2	8.02	0.42	0.667
Exposure time (seconds)	5.56	1	5.56	0.29	0.600
Temperature x time	25.93	2	12.96	0.68	0.526

n=3 ; Significant (P<0.05) effects are in bold

6.2 Discussion

To date, intensive outbreak of Serpulid calcareous tubeworm continues to infect shellfish aquaculture operations and marine infrastructures within Port Phillip Bay. According to the shellfish farmers and senior marine biologists in Marine and Freshwater Research Institute (MAFRI), DPI Victoria, recent extensive Serpulid tubeworm outbreak on commercial shellfish farms in Port Phillip Bay is a historic phenomenon. It was assumed that the first outbreak was started in May 2011 where the newly installed hydrographical devices of MAFRI were heavily fouled by juvenile Serpulid tubeworms in four different underwater locations of Port Phillip Bay (Longmore and Nicholson 2011). It remains as a matter of concern for aquaculture operations as it is degraded the market value of products. This concern intensified in June 2011, as several numbers of shellfish farmers reported a heavy Serpulid tubeworms biofouling on their offshore longline farms within Port Phillip Bay.

Fouling of mussel shells by Serpulid calcareous white worm can be problematic for the Mussel Aquaculture Industry, as fouled shells are perceived to be an inferior product. Generally, mussel with more than 7% of the shell surface fouled is not considered Grade A quality, as the product is considered visually unattractive. The mussel industry in Port Phillip Bay maintains its competitive edge in Australian domestic and export markets by trading in premium quality mussels only. Indeed, fouling by the Serpulid tubeworm represents a particular threat to industry viability, productivity and profitability as calcareous tubes >5 mm cannot be removed from the mussel shell degrading product value.

6.2.1 Possible cause for tubeworm outbreak in Port Phillip Bay, Victoria

The most likely explanation for the mass tubeworms outbreak in offshore shellfish farms 2011 could be the unprecedented ecological and biological changes in Port Phillip Bay water which provided the optimal conditions for mass breeding of this biofouling organism. Recent report shows that 1998–2009 period was the driest on record for the Melbourne region as a main freshwater catchment and nutrient contributors to the Port Phillip Bay, whereas rainfall in 2010/11 was the fifth highest since records began in 1855, and the highest since 1954/55 (Longmore and Nicholson

2011) report is the most recent extensive review on 10 years hydro-biological monitoring in different part of Port Phillip Bay. This report reveals a big spike on nutrients and primary productivity and a record low salinity in last two years in 2010-11 (Figure 34 and Figure 35). This report indicates that the salinity varied by more than nine part per thousand (PPT) over the reporting period (2002-2011), from a maximum of 38.3 ppt at 3 m depth in Long Reef, Port Phillip Bay on 8 January 2004 to a minimum of 29.0 ppt at the same depth in Hobsons Bay, Port Phillip Bay on 17 January and 18 February 2011 (Figure 34 and Figure 35).

(Longmore and Nicholson 2011) emphasised that at all monitored sites within Port Phillip Bay, salinity in 2011 was consistently lower than in other years. The report also shows that heavy rains and high river flow in October 2004–March 2005 and from August 2010–June 2011 have been the main contributor factor in reducing surface salinity by 2–5 at Hobsons Bay (Figure 35) by July 2011, salinity of 34–35 was 2–3 lower at each site than it had been in 2002–09.).

Several recent reports indicate that the changes in salinity may lead to subtle changes in water circulation in the Port Phillip Bay (EPA 2010; Lee *et al.* 2011) and (Longmore and Nicholson 2011) which in turn cause some ecological and biological implications. A widespread outbreak of Serpulid tubeworms in June-July in 2011 within Port Phillip Bay at a time of lowest salinity in record (34-35) in 2011 may well demonstrate the ecological impact of lower water salinity.

This finding is also consistent with several other reports where increased nutrients and lower salinity triggered the outbreak of Serpulid tubeworms (Scotland, New Zealand, and Canada)

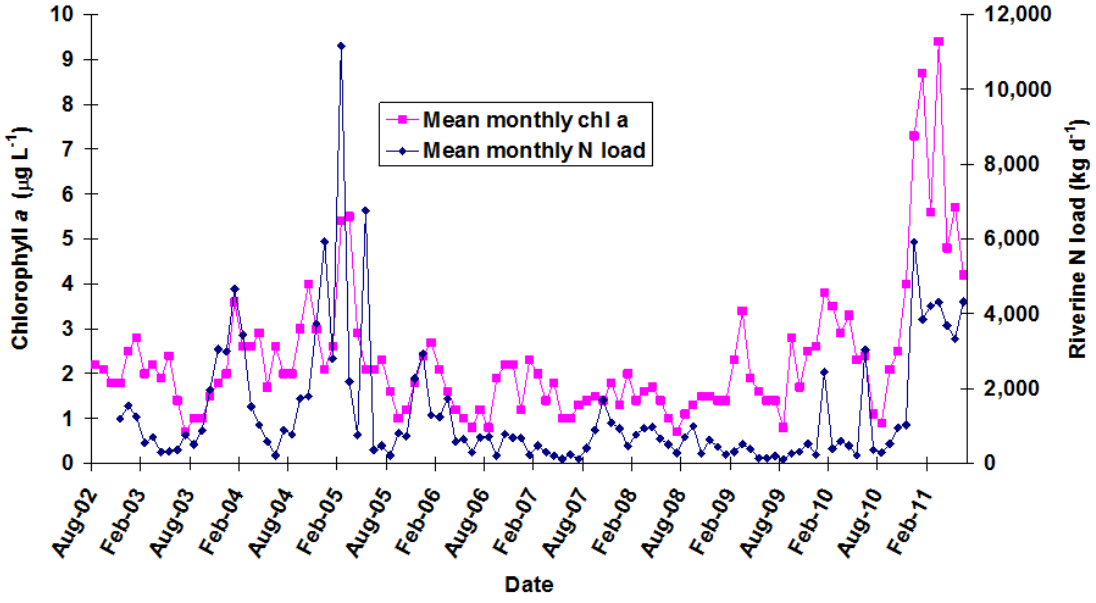

Figure 32: Mean monthly chlorophyll response at (upper) PPB to riverine total N load and (lower) Long Reef to Western Treatment Plant total N load.

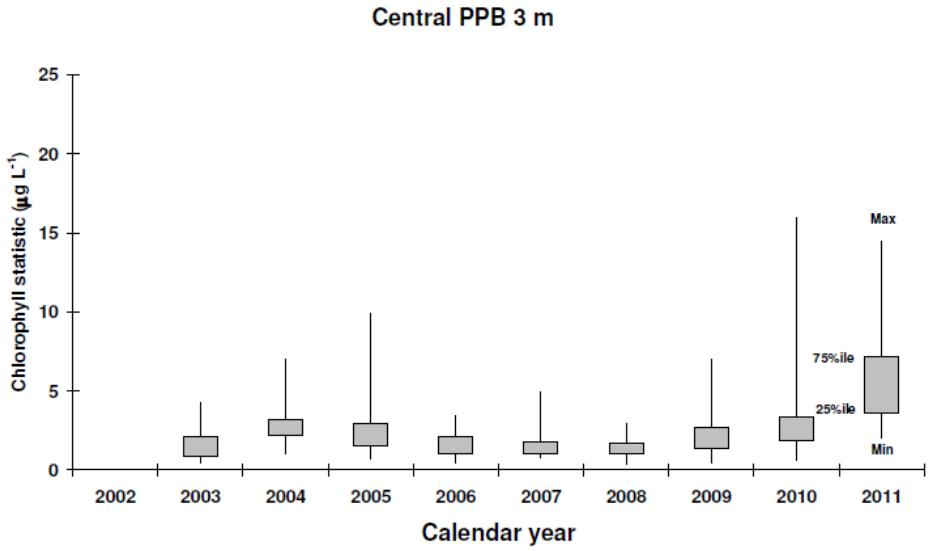

Figure 33: Summary statistics (min, max, 25 percentile, 75 percentile) for chlorophyll at Central PPB 3 m and 18 m sites, by calendar year

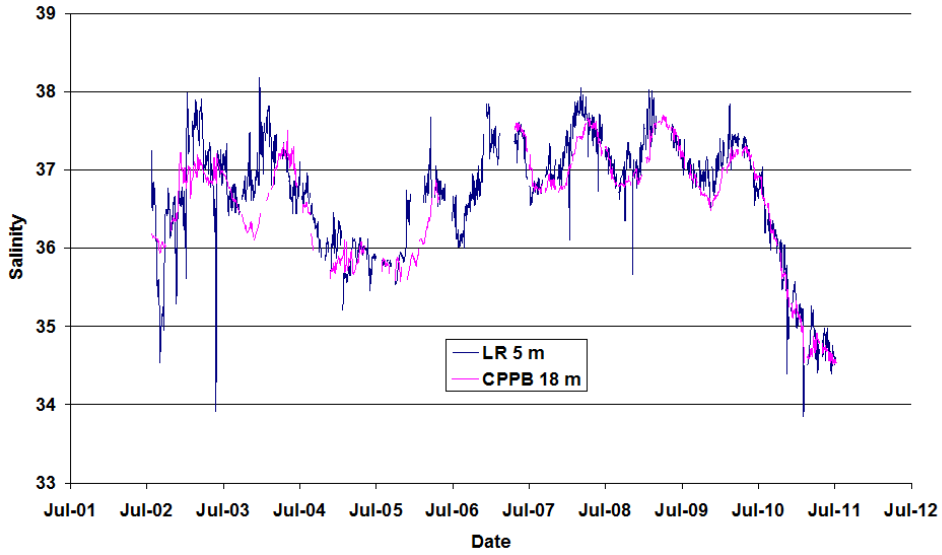

Figure 34: In situ salinity at Central PPB from July 2001 to June 2011.

NB Gaps indicate servicing periods

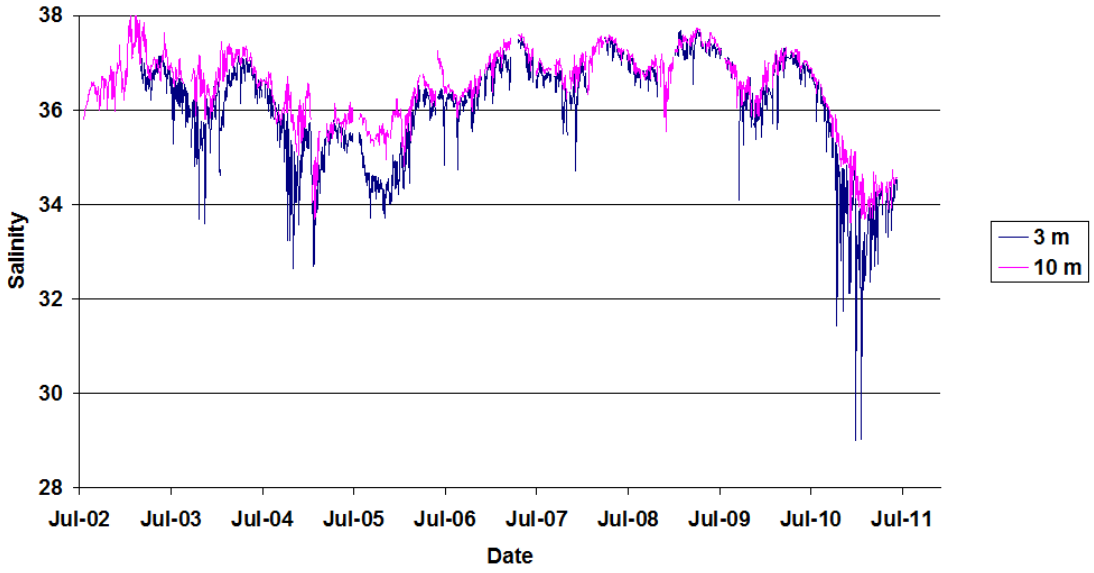

Figure 35: In situ salinity concentration at Central PPB from July 2002 to June 2011

6.2.2 Challenges in developing commercial control method

Biofouling is a complex and recurring problem in all sectors of the European aquaculture industry. Considering the low cost margins, current priorities and operating environments, it is vital that low cost, practical methods are found and introduced to control biofouling.

In this study, there were two main challenges to be overcome in a short period of time to minimise the economic impact of Serpulid infestation in Port Phillip Bay mussel aquaculture farms. The first challenge was the lack of information on the biology, ecology or any life aspect of *Pomatoceros taeniata* which currently identified and exist as a primary source of Serpulid tubeworm fouling in Port Phillip Bay shellfish farms. Having appropriate knowledge on the life cycle, biology and behaviour of any biofouling would facilitate the design of eradication strategy From the few studies addressing infestation of commercial bivalves by Serpulid (Forrest *et al.* 2007), none of them have identified *Pomatoceros taeniata* as biofouling. In majority of these studies H. elegans (Forrest *et al.* 2007; Kupriyanova 2000), P. *lamarckii* have been reported as most common tubeworm species in shellfish farms in New Zealand and Europe.

Secondly, tolerance of tubeworms as hard shell biofouling organisms is generally high to conventional biofouling control measures as compared to other groups of fouling organisms. A number of control methods such as exposure to air, hyper or hypo-saline water, heat and various chemical treatments have been examined and or proposed to treat these common Serpulid tubeworms in shellfish aquaculture industry mostly in Europe . Some other suggests a combination approach include exposing mussel ropes to air, freshwater, lime, or saturated brine dips (90 parts per thousands) followed by air (Shearer and MacKenzie 1997). However, neither of them specifically examined at large scale long line mussel farm nor specified the optimum effective range at similar circumstances. Therefore, at the first step in this study, several common treatments were examined at laboratory scale on both *Pomatoceros taeniata* and blue mussel to

optimise the most effective level/concentrations of treatments and exposure time period.

As a commercially large scale operation of offshore longline farm, SeaBounty owns over 30,000 blue mussel droppers at its culture sites, each dropper contains an average of 1200 mussels and weight between 70-100 kg of biomass. Under these circumstances, a treatment system has to have a capacity to effectively treat over 500 mussel droppers per day to be considered as a commercially viable and timely manageable method (Lance Wiffen, Pers. Com.). Therefore, the second challenge in this project was to develop a method to mitigate tubeworms fouling on mussel droppers at highest rate (>90%) and at lowest possible crop loss (<10%) on a commercially extensive scale offshore longline farm. Indeed, to date to our knowledge no commercially viable control treatment developed, in which could be used at substantial scale longline shellfish farm.

6.2.3 Air drying

Several studies suggest this method as an effective and environmentally friendly control method for tubeworm biofouling control In this study, air drying treatment found to be 100% lethal for *P. taeniata* tubeworms at 12 hours exposure time at laboratory scale. However, for a number of reasons, application of this treatment method on mussel droppers at commercial scale was proved to be practically not viable. Firstly, *P. taeniata* tubeworms appear to be able to tolerate a long period of exposure to air on mussel droppers. More than 70% of tubeworms survived on mussel droppers when exposed to air for over 24 hours. The high survival of tubeworms may be largely assisted by a high level of water and moister exerted by mussels and other biofouling organisms attached to the droppers which prevent droppers microenvironment to be dried. The water retained within *P. taeniata* protective tube may also help enhance the survival of the worms on mussel droppers. A similar observation reported for exotic tubeworm *Sabella spallanzanii*, when blue mussel droppers exposed for air drying in Port Phillip Bay for a period of 24 hours(Gunthorpe 2001) and Galeolaria as an intertidal species which is well adapted to periodical dry periods and their thick tubes and tightly fitted opercula reported to prevent desiccation in this species (Elena Kupriyanova and Jon Havenhand, 2000).

Secondly, for the air drying to be an effective control method for tubeworms fouling, mussel droppers would have to be exposed for periods greater than 48 hours. While mussels are capable of tolerating long periods of time out of seawater, periods greater than 72 hours out of water may jeopardise crop viability (Gunthorpe 2001).

Thirdly, a large scale mobilisation, high logistic costs and a wide space are needed to mobilise over 20’000 to 30’000 mussel’s droppers out of water to carry out this treatment. In addition, as a result of long air exposure many of the mussels start dropping from the mussel droppers upon redeployment to the farms (Lance Wiffen, per. com.) Therefore, all the mussel droppers must be resocked prior to redeployment to avoid losing mussels which is costly, time consuming and very hard at big scale farm operation. In practice, all these requirements make the air drying treatment technique neither feasible nor economic to be carried out by mussel farmers in Port Phillip Bay using long line offshore culture system.

6.2.4 Dipping in chemical solution

A number of different chemical treatments (acetic acid, hydrated lime, saturated brine or hypochlorite solution) and nonchemical (exposure to air and heat) have been used to kill fouling species on shellfish with differing levels of success (Bailey-Brock and Ringwood 1982; Caceres-Martinez *et al.* 1998; Leighton 1998; Lleonart *et al.* 2003; Nel *et al.* 1996; Oakes and Fields 1996). Chemical treatments are reported as being more effective against soft bodied fouling species. From the chemical treatment methods common used in biofouling control studies, freshwater, Chlorine dioxide and saturated hyper saline water were evaluated in this project.

6.2.5 Freshwater treatment

Dipping in freshwater is a very common and relatively low cost method to control biofouling in shellfish industry (Gunthorpe 2001). It is particularly considered as a safe treatment method for shellfish as majority of commercial species can survive along exposure without harm. For example, it has been shown that mussels can survive well at a two day soak in fresh water. Although, this method is potentially a very powerful technique in killing the majority of fouling organisms, work carried out in this project has revealed a number of potential bottlenecks as practical method to control tubeworm biofouling. Firstly, approximately 60% of tubeworms immersed for 30 minutes in freshwater bath survived the treatment. This indicates that *P. taeniata* tubeworms resist to osmotic stress and a longer immersion time required to completely kill this tubeworm on mussel droppers at offshore farm. Resistance to osmotic stress (i.e. freshwater immersion) in Serpulid tubeworms has also reported by (Kupriyanova 2000), where she indicated that 3 days immersion time required to kill Galeolaria. (Kupriyanova 2000), also pointed out that resistance to osmotic stress increases as the worms grow, so longer treatment should be required for older worms. (Gunthorpe 2001) applied a combine treatment method approach for example a mixture of freshwater and detergent follow by an overnight drying to increase the lethal effect of freshwater treatment on tubeworm biofouling on mussel droppers. In this project, within the context of large mussel industry operations (e.i. SeaBounty), in which tens of tonnes of mussel droppers has to be routinely treated each day , the relatively long dipping time required to fully eliminate tubeworm fouling would often make the use of freshwater dipping impractical.

Secondly, previous work has shown that the osmotic efficacy of freshwater on biofouling organisms could be depleted very quickly at a large scale dipping process, as it turn to brakishwater within few hours (CRAB 2006); Barry et al., 2006; Jahangard, unpublished data). Such findings are supported by work conducted as part of the present study, in which we observed that a 5000 litter freshwater bath tank turned to brakishwater following a dipping of only 3 commercial size mussel droppers in DPI Queenscliff facility. Clearly, while freshwater bath is not an appropriate field-based treatment method in the commercial scale mussel aquaculture operations in Port Phillip Bay, its efficacy in eliminating a large group of soft body biofouling suggests that it may nevertheless be useful in reducing the occurrence of other types of fouling. In conclusion, although freshwater treatment may be considered feasible in the case of small farms or low-level soft body biofouling infestations, these options is not logistically and practically viable for tubeworm biofouling particularly the case of large scale operations.

6.2.6 Chlorine treatment

While chlorine serves as an excellent biocide for controlling biofouling organisms in cooling water systems (Rajagopal et al. 2003)and exotic marine organism in shipping blast water (De Silva 1967).its use was ineffective in this study. Although concentrations as high as 2800 ppm were used in this experiment, both mussel and Serpulid worms were able to protect themselves against chlorine by closing their shell valves or operculum and survive for long periods. These data also further proved the findings of other that both organisms have the capacity to seal off their body parts against toxic environment. (Day 1967)and (Forrest *et al.* 2007) suggested that the special morphology of the polychaete worms specially its calcareous tube and operculum would enable it to prevent or reduce its exposure to chemical treatment such i.e. Chlorine and acetic acid. Therefore, the ineffectiveness of Chlorine treatment method most likely is due to the protection conferred by *P. taeniata* tubeworm during its exposure to Chlorine.

(Mattice and Zittel 1976; Rajagopal *et al.* 2002)reported that the efficacy of chlorine as an antifoulant depends on various parameters, most importantly residual levels of chlorine and exposure time. For example, many reports suggesting the chlorine concentration of up to 3000 ppm and exposure time of several days to effectively kill the shell protected biofouling organisms (Lewis 1985; Rajagopal *et al.* 2002). Therefore, extending the exposure time to few days may required to kill the tubeworms on mussel droppers. However, it is practically and economically unfeasible to extend exposure time to a long period for large scale mussel farms. Consequently, in this study, commercial scale Chlorine treatment experiment was rolled out at this stage.

While Chlorine dioxide treatment method was ineffective for tubeworms treatment in this study, it is likely suitable to use at lower concentration for sterilising of mussel droppers at farm scale. Nevertheless, its application must be carefully considered for safety of work force, along with containment or neutralisation procedures to avoid environmental contamination.

6.2.7 Saturated saline water

Generally, the responses of both mussel and tubeworms to a saturated saline water was or combination of saturated saline water and vinegar exposure were highly inconsistent and were very much subjected to the level of handling and inducement of mechanical stress to the stock prior to the dipping. Previous work has shown that the saturated saline water is an effective method to control polychaete tubeworms or mudworm infestations in oyster and other commercial bivalves. Such findings are supported by laboratory scale trials in current study, where immersing tubeworm's infested blue mussel to saturated saline water resulted into 90-98% of tubeworms mortality at a time period of 10-30 minutes. In contrast, mussel tolerance to a long exposure period (20-30 minutes) to saturated salt at laboratory scale was exceptionally high where 96%-100% of exposed crops survived.

However, scaling up of this treatment method to commercial mussel farm resulted into much more damage in mussel crops (21-25% mortality) and relatively lower mortality rate in tubeworms (80-85%), than those in laboratory scale trials. Increasing mortality of mussels at commercial scale treatment was highly likely attributed to an

instantaneous immersion of mussel droppers to saturated saline solution and sudden exposure of the internal soft body parts of the gapping mussels to a very extreme hyper saline osmotic solution of the saturated saline water. Further field trails in this projects reveal that the reaction of the gapping mussels is very slow to the quick immersion process and to avoid this problem, mussels valve closure have to be induced (e.g. by shaking). Several other studies also reported a considerable post treatment mortality of mussels using chemical treatment immersion without inducing valve closure (e.g., by shaking) prior to immersion stage (Campbell and Kelly 2002; Cox 2010) . Whilst mussel valve closure was induced (e.g., by shaking) in laboratory mussel stock to avoid contact with hyper osmotic solution of saturated saltwater, application of this technique at commercial scale was practically not feasible. In this study, several farm based pre-treatment attempts to induce valve closure were ended up with a big chunk loosing of mussel crops on mussel droppers shortly after treatment.

Although, saturated saline water treatment proved to be a very effective, efficient, cheap and environmentally friendly method at laboratory scale, its application on mussel droppers at offshore farm requires a supportive socking to prevent mussel crop loss which often happened during the course of inducing valve closer by shaking. Therefore, from the commercial point of view, factors such as cost of protective socking on mussel droppers and time requires covering the ropes and inducing valve closer could be considered as limited factors in this method.

Toxicologically, it has been known that the application of a combination of stressors either in parallel or sequentially have a synergistic/additive effect (Landis and Yu 1995). The effectiveness of this approach have been very well proved in previous works where a combination treatment of freshwater bath followed by an overnight air drying (Gunthorpe 2001) or hyper saline solutions and cold shock (Cox 2010)were used to control tubeworms or mud worm infestations in bivalves. In this study, further step to optimise the application of saturated saline water treatment in tubeworm control on mussel farms was to use combination treatments approach. In the first attempt, a combination of saturated saline water and different level of vinegar as acetic acid source were used. Results of this trial suggest that a combination of saturated saline water and 200 ppt vinegar is almost 100% lethal for *P. taeniata* tubeworm. However, this combined treatment method should be applied with a great caution as over 30% of mussel crops could be destroyed as a result of instantaneous exposure of mussel droppers without valve closure induction. Caution in using acetic acid products as biofouling control method also recommended by others. For example in a field treatment in New Zealand (Cox 2010) suggested that within an exposure period required for acetic acid solution (5% concentration) to kill hard bodied foulers, over 50% mussel stock could be destroyed.

In the second attempt to test the multiple treatment approach in this study, a combination of osmotic and chilling treatments were applied in which a saturated brine water chilled down to -20 ºC chilling as cold shock treatment were used at laboratory scale. Results suggest a range of 93-100% lethal rate for tubeworms population exposed within 3-5 seconds period to this combined treatment method. While only 17% of exposed mussel was destroyed at 3 seconds exposure time, the mortality was exponentially increased where to approximately 90% at 5-10 seconds exposure time. In practice, it takes between 7-12 seconds to dip a 5 m mussel dropper

(~ 50-70 kg biomass) at offshore farm. Given the longline farming as main mussel farming technique at Port Phillip Bay, a very heavy mussel mortality within a very short period of exposure time (more than 2-3 seconds) and cost of building a massive super chilled treatment system on board of the mussel boat, no commercial scale trials was conducted at offshore field.

7 BENEFITS AND ADOPTION

As acknowledged in the original application, the offshore mussel farmers in Victoria and other Australian states are the major beneficiary of the research. As indicated earlier the extend of the tubeworms infestation was very extensive and occurred on hundreds of tonnes of SeaBounty cultured mussel and several other shellfish farms within Port Phillip Bay. The widespread infection created a market havoc and cause anxiety and stress among the shellfish farmers in Port Phillip Bay.

This extensive fouling could lead to significant losses in three major SeaBounty aquaculture sites and other shellfish aquaculture industry area in Port Phillip Bay. It could reduce the value of the product and cause a negative impact on domestic and export markets, affecting the viability of this industry. The loss of confidence associated with the early tubeworm infestation at SeaBounty farms has at least been partly eased as a result of this project. SeaBounty as a biggest blue mussel operation in Victoria has begun to extend further its operation and planning for export market and regaining its domestic market. A new and relatively large sea based farm has been established since the project began and consulted with project staff on mud worm risk assessment during the development phase.

The mitigation methods and the commercial treatment system developed during the course of this project are also applicable for other commercial bivalve farming system such as edible and pearl oyster industry and have a potential to adopt for offshore abalone farming too. Other beneficiary sector can be the marine pest control and interstate marine translocation authority.

In general, the outcomes of this project could be adopted and used by other mussel farming industry in neighbouring states. It can also be used by other offshore or inshore shellfish industries such as the oyster and scallop farming

8 FURTHER DEVELOPMENT

There are two main areas where further developments could make substantial improvements in reducing the impacts of tubeworm infestation on blue mussel farms Port Phillip Bay Victoria and in Australia in general.

There is still an absolute lack of information on the breeding season and life cycle of *P. taeniata* in Port Phillip Bay. The main area of development following the project would be in undertaking life cycle studies to better understand the major spawning and recruitment season of this endemic tubeworm in Port Phillip Bay thought the year. These studies will enable farmers to prepare a better management strategy to reduce the impact of the heavy tubeworm settlement on the offshore shellfish farms at their earlier life cycle stages. The outcomes of these studies will benefit all other stakeholders such as offshore abalone and oysters framers and help ensure impacts of tubeworm settlement are minimised.

Thermal treatment method proved to be a best effective method on controlling the adult size tubeworms on blue mussels. The second area of development would be to assess the impact of thermal treatment on earlier stages of this tube worm. It is anticipated that t thermal treatment method will be effective at lower temperature and probably shorter exposure time, but an offshore trial is required to optimise the exposure and the temperature level.

9 PLANNED OUTCOMES

- An effective, environmentally friendly and efficient treatment method for tube worm infestation at commercial blue mussel farm scale: In this project, thermal mitigation treatment was identified as an environmentally friendly, biologically effective and commercially viable method for tubeworm fouling in mussel farms. It has scaled up to a commercial scale biofouling thermal treatment system called "closed circulated seawater heating system" (CCSHS) machinery and successfully tested for its competence to kill the tubeworms with minimum impact on the mussel crops at offshore shellfish farms.

- A Standard Operating Procedure (SOP) for tube worm biofouling treatment at commercial scale: Current report and the data presented in this repost can be simply used as guideline for shellfish farming industry

- An estimate of the effect of the treatment method on the other major biofouling groups: Due to the limited time and urgency of exploring the mitigation methods for the tubeworm biofouling, less attention was paid to check the effect of tested methods on other biofouling through the course of this project. However, two to three weeks post treatment observation of the tagged mussel droppers by authors and Mr. Lance Wiffen and his crew reveals highly clean mussels on the tagged droppers which are highly suggesting that the thermal treatment has a very high potential to mitigate other major biofouling organisms particularly soft body organisms.

- A final technical report: Draft prepared and submitted.

- Results from these trials will provide baseline information for development of a larger industry-support project for other shellfish species: The progress made in this project is highly applicable for controlling the tubeworm outbreak and infestation in other commercial shellfish species such as oyster

10 CONCLUSION

In response to the heavy settlement of tubeworms on cultured mussel in Port Phillip Bay, this research project was developed and supported by FRDC. The main objectives of the project were to evaluate different treatment methods for controlling Serpulid tubeworm fouling on mussels, trials and scale up the best and most effective method to a commercial scale and finally help develop a practical offshore mitigation method with maximum impact on tubeworms and minimum impact on mussel.

It was found that the unprecedented environmental changes in Port Phillip Bay including a historical spike in nutrients, very high level of Chlorophyll a and lowest

record salinity may have play a part in widespread outbreak of the tubeworms at offshore shellfish farms in mid 2011 to early 2012.

In this project, a number of different mitigation methods including: freshwater, air drying, chlorine dioxide, saturated saline water, super chilled saturated saline water and heated seawater/thermal treatment were used to kill tubeworm on blue mussel droppers.

Air dying was found as an effective treatment method at laboratory scale, but was not considered for offshore trials as more than 70% of the worms were survived an exposure time of 12-24 hours on mussel droppers. Freshwater treatment failed to meet the standard requirement as a viable method at laboratory scale, and therefore, was not considered for commercial scale treatment. Chlorine dioxide as third option was also not considered as an effective treatment for tubeworm *S. taeniata* at any scale as majority of this tubeworm survives at exposure to extreme concentrations of Chlorine dioxide (700 to 2800 ppm). None of those three treatment methods met the criteria to scale up to commercial scale.

Saturated saline water treatment proved to be a very delicate and practically sensitive and risky treatment method. While it was found as an effective and highly efficient method at laboratory scale (90-98% tubeworms mortality within 10-30 minutes exposure), it caused a significant mussel crop mortality (20-25%) on droppers as a result of gapping mussel impulsive exposure to saturated saline water. It could be also diluted every 30 minutes during offshore treatment and required to a regular salinity monitoring. A regular salt re-mixing is required throughout the course of this treatment to keep it effective. To avoid this problem, an induce valve closure (.i.e. shaking), must be applied which may itself cause further losing of the crops on the mussel droppers.

Cold shock using chilled saturated salt (350 ppt, -20 ºC) was extremely effective on tubeworm (over 90% mortality within 3 seconds) but highly risky as the farmers may loos the entire mussel crops within 5-10 seconds exposure time.

Based on the extensive laboratory and onshore experimental results as well as offshore trials that were carried out during this project, the thermal treatment method is proposed as the best mitigation method for tubeworms attached to mussel shells. With the proposed method, the Sepulid tubeworms were effectively eradicated at an average lethal rate of 95% with a minimum mussel crop loss (<5%) within temperature range of 45-50 ºC at an exposure time of only 45-50 seconds. The effective lethal range achieved in this method for the tubeworms and exposure times are far better than those achieved with other methods examined in this study. These results successfully met the standard target initially set for a large scale commercial offshore mussel farm. The proposed method enables the farm to treat over 500 mussel droppers per day on board of a farm boat and at a feasible time and cost level with a commercially acceptable mussel crop loss of less than 5%.

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12 APPENDICES

12.1 Appendix 1: Intellectual property

All components of this research are in the public domain.

12.2 Appendix 2: Staff

Ladan Asgari, researcher from Ausnik Pty Ltd

Sam Jahangard, researcher from Ausnik Pty Ltd

Lance Wiffen, SeaBounty Pty Ltd

Shane Wiffen, SeaBounty Pty Ltd

12.3 Appendix 3: Raw data table

A	B	C	D	E	F	G	H	I	J
Deployemnt Date	Date sampled	Mussel source1	Mussel source 2	Treatment	Temperature start	Temparature finished	Experimental scale (Lab/field)	Replicates	Density
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	1	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	2	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	3	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	1	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	2	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	3	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	1	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	2	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Chill seawater	-17	-12	Lab	3	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Control	-17	-12	Lab	4	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Control	-17	-12	Lab	5	10
28/08/2011	28/08/2011	Pinnace Channel	Lance Wiffen	Control	-17	-12	Lab	6	10

K	L	M	N	O	P	Q	R	S	T	
Average length (mm)	Exposure time (secon	Total worm no.	Worm survive(	Worm mortality No.	Worm Mortality (%)	Total mussel no	Mussel survived	Mussel Survival (%	Mussel mortality(%	Commen
76	10	30	0	30	100.0	10	1	10	90	Extr
76	10	28	0	28	100.0	10	1	10	90	Extr
76	10	20	0	20	100.0	10	2	20	80	Extr
76	5	38	0	38	100.0	10	1	10	90	Extr
76	5	27	0	27	100.0	10	2	20	80	Extr
76	5	31	0	31	100.0	10	1	10	90	Extr
76	3	26	2	24	92.3	10	8	80	20	Extr
76	3	31	2	29	93.5	10	9	90	10	Extr
76	3	25	2	23	92.0	10	8	80	20	Extr
76	10	29	29	0	0.0	10	10	100	0	
76	10	23	23	0	0.0	10	10	100	0	
76	10	34	34	0	0.0	10	10	100	0	

Figure 36: Raw data for the Chilled water laboratory scale experiment