FINAL REPORT

AWARD RECIPIENT: James Wynne

ADDRESS: CSIRO Marine Laboratories, Castray Esplanade, Hobart, TAS 7004

HOST ORGANISATION: CSIRO

DATE: 12/3/19

ACTIVITY UNDERTAKEN

A joint project between CSIRO and Tassal was conducted to increase our knowledge and research capacity concerning hydroid biofouing by funding a visiting expert (Dr Nina Bloecher) to conduct a one-month educational visit to Tasmania. The purpose of visit was to 1) assist CSIRO/Tassal to taxonomically identify hydroid species of relevance to the Tasmanian Atlantic salmon industry, 2) establish a hydroid culture system at CSIRO for evaluating various inactivation methods, and 3) provide educational seminars and a workshop to the broader research and aquaculture industry within Tasmania.

OUTCOMES ACHIEVED TO DATE

1. Hydroids species derived from Atlantic salmon net pens were taxonomically identified.
2. Hydroids were cultured at CSIRO, and electric current inactivation methods were assessed.
3. An industry wide seminar was held at CSIRO (Hobart) where Dr Bloecher presented her research on Atlantic salmon biofouling.
4. One-on-one training between key Tassal staff and Dr Bloecher on site at Tassal was conducted.

Acknowledgments

Tassal and CSIRO (Agriculture and Food) co-invested with the FRDC in this activity, 1/3 each.

Background

Marine biofouling poses a major problem to many aquaculture operations, including Atlantic salmon farming in Tasmania. The accumulation of organisms on submerged surfaces, such as salmon cage nets, can significantly reduce water flow and can severely affect fish health during in situ net cleaning operations. In recent years in southern Tasmania the most common and problematic biofouling organisms associated with salmon farming have been hydrozoans, and particularly the hydroid life stage. Hydrozoans have a complex life cycle, including both polypoid and medusoid life stages. In Tasmania, hydroids settle on salmon cage nets and increases in abundance to form large monocultural communities which may cover whole nets. Significant hydroid growth can cause deformation and structural fatigue of cages, restrict water exchange across the net, reduce water quality, and thus ultimately decrease fish health. Furthermore, nomadcystes released and dispersed from hydroids during in situ net washing causes severe gill injury, particularly in larger fish. Currently no methods have been developed to reduce the impact of hydroid biofouling within Tasmanian Atlantic salmon farms. A lack fundamental knowledge concerning hydroid biofouling dynamics within Tasmanian Atlantic salmon farms has hampered progress.

Need

In recent years an increase in hydroid biofouling and related gill injuries have been observed within major salmon farming regions of Tasmania. In 2016, over 96,000 hydroid related mortalities were observed within Tassal's D'Entrecasteaux Channel sites in southern Tasmania. This accounted for 6% of the production and contributed to an estimated economic loss of 4.8M. The abolishment of copper based antifoulants in combination with long-term in situ net cleaning practices has increased incidence of hydroid biofouling within Tasmania. Current net cleaning practices/rotations have failed to reduce hydroid biofouling within Tasmanian salmon farming operations. Consequently, there is an urgent need to developed new strategies to reduce the burden of hydroid biofouling within salmon farming operations. The issue of hydroid biofouling is not unique to the Tasmania. Other major salmon producing nations such as Norway and Scotland are also severely affected, particularly within the summer period.

To reduce the negative impact of hydroid biofouling new research is required. Particularly in the area of species identification, seasonal dynamics, and net cleaning/inactivation methods. Currently within the Tasmanian industry and research communities we lack the scientific expertise to undertake this research. Therefore, a clear need exists to import this expertise in the form of a visiting scientist. Dr Nina Bloecher is a Norwegian based scientist with significant expertise in hydroid biofouling research, particularly related to salmonid aquaculture.

Objectives

1) Taxonomically identify hydroid species of relevance to the Tasmanian Atlantic salmon industry.

2) Establish a hydroid culture system at CSIRO for evaluating various inactivation methods.

3) Evaluate if electric current can trigger the release of nematocysts in Tasmanian hydroid species

4) Provide educational seminars and a workshop to the broader research and aquaculture industry within Tasmania.

Methods

Objective 1, 2 and 3.

Dr Nina Bloecher (Fig 1) conducted all experiments with technical assistance from both CSIRO and Tassal staff. The hydroids for the experiments were collected from nets and other structures (e.g., ropes or walkways) at various Tassal farm sites in Tasmania between 12. – 23. February 2018. Samples were kept in holding tanks (100 L) with aerated sea seawater at 16 ºC and a salinity of 31 to 32 ppm.

Fig 1. Dr Nina Bloecher on site at Tassal marine farm.

Experimental setup

Electric current experiments were conducted in the Hobart CSIRO laboratory using metal rod electrodes of 20 cm length and 0.8 cm diameter. Two setups were used:

Petri dish setup: The electrodes were fixed vertically in the Petri dish with a water depth of 0.8 cm, without touching the bottom (Fig. 2a). The distance between the electrodes was varied during the experiment.

Tank setup: The electrodes were fixed horizontally onto a plastic sheet in opposing directions (see Fig. 2b, c). The sheet was submerged in a plastic box (60 x 38 x 20 cm LxWxD) filled with water. A porcelain funnel was used to hold the sheet under water. A water depth of 10 cm was considered a realistic approximation to field conditions, resembling the distance between a net cleaner and the net. Various electrode distances were tested.

The electrodes were connected to one of two optional power sources:

(i) a battery connected to a converter which allowed the regulation of the frequency and the duty cycle, delivering a maximum voltage of 25 V to the electrodes (Fig 1a). The experiments were carried out in an AC setting, with 25 V at 50 Hz and with a 100% duty cycle.

(ii) a 240vAC Variac combined with a 240vAC – 48vAC step-down transformer to deliver 48 V at 50 Hz.

Two digital multimetres (Tenma, Australia) allowed the reading of the current between the electrodes (not carried out for every setup) as well as the chosen frequency and duty cycle. Experiments were conducted with individual hydroid polyps that were placed between the electrodes before a current was allowed to flow for the duration of approximately 1 second (unless indicated otherwise). In addition, the reaction of 3 colonies, consisting of 13, 10, and 9 polyps, was assessed for a single setting (50 V, 3.2 cm electrode distance). Higher replication was not possible due to a lack of sample material. Prior to the start of the electric current experiments, several individuals of each hydroid species examined were exposed to acetic acid (5 %), a proven trigger for nematocyst release, to validate that the species was in principle capable of the release of nematocysts.

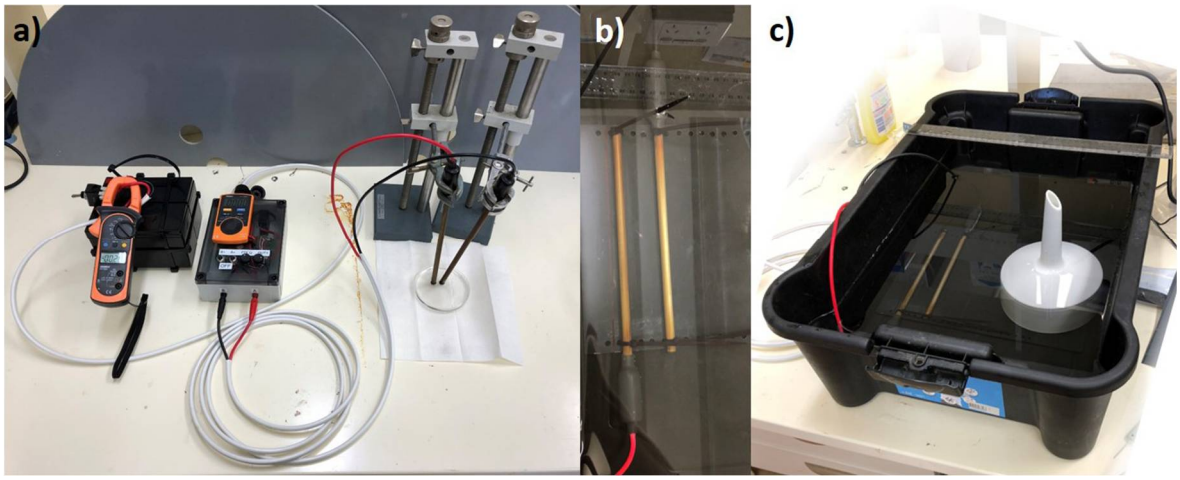

Figure 2: Test setup with electrodes (a) vertically in a Petri dish or (b and c) fixed horizontally to a plastic board in a plastic box.

Assessment of the hydroids:

The general condition of the hydroid polyps was assessed under a dissecting microscope where intact body shape and tentacle movement were able to be discerned. Before exposure to electric currents, each individual polyp was further investigated using a stereo microscope. Polyps were placed on a concave microscope slide, and the tentacle surface was searched for the presence of fired nematocysts. Typically, some (1 to 30) fired nematocysts were present at the base of the

tentacles (Fig. 3), but rarely at the tentacles. If nematocysts were present at the tentacles, the polyp was excluded from the trial (<10 instances).

After exposure to electric current, the hydroid was again investigated under the microscope and the presence of additional fired nematocysts was noted individually for the tentacle base and the tentacles. The presence was ranked into 4 release rate categories:

0: None - no additional nematocysts fired

1: Low - some fired nematocysts present (not necessarily on all tentacles)

2: Medium - fired nematocysts present on all tentacles / base

3: High - large amounts of fired nematocysts present

When entire colonies of hydroids were exposed to electric current, nematocyst condition could not be assessed beforehand to prevent the destruction of the hydroid colony structure. The ranking could therefore not be corrected for the nematocysts that may have been present before exposure.

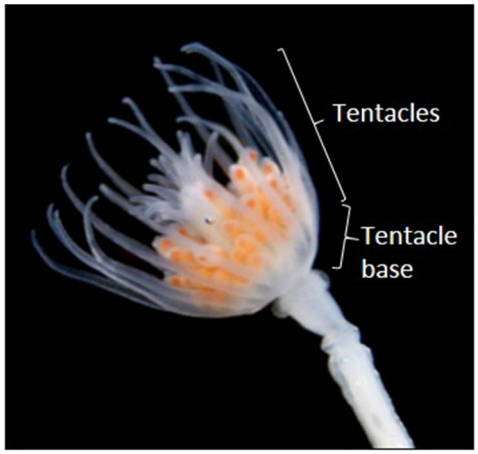

Figure 3: Tentacles and tentacle base of a hydroid

Objective 4.

A seminar by Dr Nina Bloecher was advertised to all three Atlantic salmon farms, including Tassal, Huon Aquaculture and Petuna. It was also advertised to other research organisations including the University of Tasmania.

Results/Discussion

Hydroids on Tassal salmon farms

The collection of hydroid material was more difficult than anticipated due to low abundances at previously deployed collector panels. The most abundant species found on net samples collected during the first week was of the family *Corynidae* (e.g., *Sarsia* sp.; Fig. 4a). This species did not release nematocysts after exposure to acetic acid and was therefore not included in further experiments. In addition, some polyps of the genus Obelia were found (Fig. 4c). They did react to acetic acid (80 % of n = 10), although the assessment was difficult due to the diminutive size of the polyps.

During the second week of this research, some colonies of *Ectopleura crocea* were found in two instances (Fig. 4d). These polyps did react to acetic acid (100 % of n = 10) and were used for the main experiments. In addition, individuals of another, unidentified *Ectopleura* species were found. They, too, had a nematocyst reaction to acetic acid and electric current, but there were too few individuals for replicated experiments.

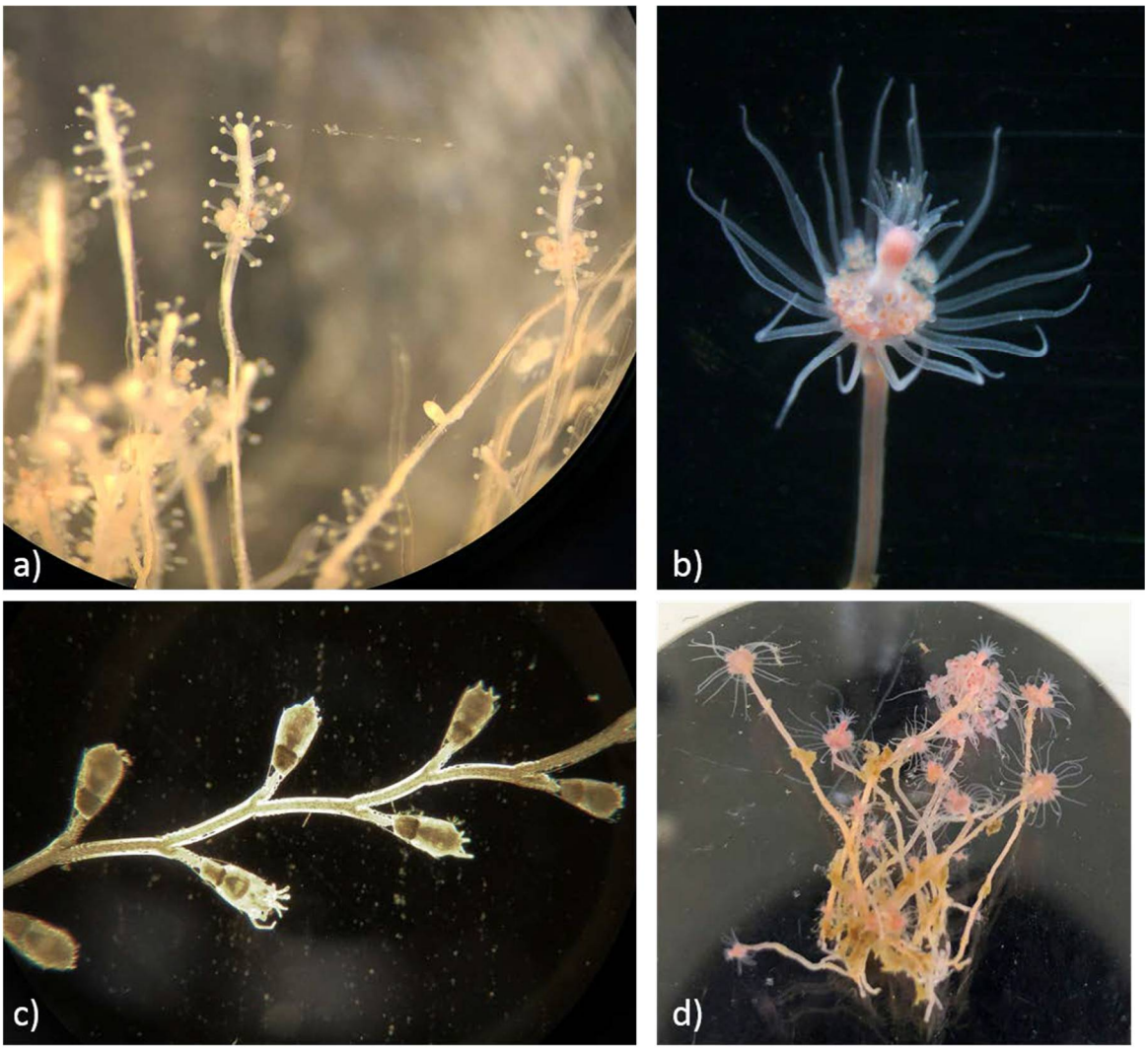

Figure 4: a) unidentified hydroid, potentially genus Corynidae; b) polyp of *Ectopleura crocea*; c) colony of an unidentified hydroid, potentially *Obelia* sp.; d) *E. crocea* colony before exposure to electric current

Exposure of hydroids to electric currents

Experiments with Obelia sp.

Obelia sp. was exposed to electric currents at various frequencies and exposure times (Table 1). The polyps did not react to the electric current and were still able to release nematocysts when exposed to acetic acid following electricity exposure. Therefore, experiments with Obelia sp. were not continued.

Table 1: Settings tested for *Obelia* sp.; N = number of colonies tested.

Setup	Power source	Water depth (cm)	Electrode distance (cm)	Frequency (Hz)	Current (A)	Exposure duration	N (colony*)
Tank	25 V	4	2	50	5 sec	5	
Petri dish	25 V	0.8	0.5	25	2.3	5 sec	5
Petri dish	25 V	0.8	0.5	25	2.3	10 sec	1
Petri dish	25 V	0.8	0.5	100	5 sec	1	
Petri dish	25 V	0.8	0.5	25	2.3	5 sec	1
Petri dish	50 V	0.8	0.5	50	4	2 sec	1
Petri dish	50 V	0.8	0.5	50	4	3 sec	1
Petri dish	50 V	0.8	0.5	50	4	10 sec	1

* a tested colony consisted of approx. 8-12 polyps

Experiments with *Ectopleura crocea*

Individual *Ectopleura crocea* polyps were exposed to an electric current at various settings. In general, the release of nematocysts was highest at the tentacle base, but was also present at the tentacles. However, the intensity of nematocyst release depended on the amount of electricity delivered to the hydroids, determined by the factors of power used, depth of the water in the sample container, and distance between the electrodes.

At the low power setting (25 V) it was not possible to increase water depth beyond 1.4 cm or increase electrode distance significantly without losing efficacy. At the high power setting (50 V), the highest and most consistent nematocysts release rates at the targeted water depth of 10 cm were obtained at an electrode distance of 3.2 cm (Average incidence of nematocyst release 90 % for both tentacle base (TB) and tentacles (T); average release rate: TB 2.2 and tentacles: 1.1; Fig. 5). When the electrode distance was increased to 5.2 or 6.5 cm, the incidence of nematocyst release dropped and release rates were considerably lower.

None of the treatments caused instant death of the hydroid polyps. All tested polyps were able to move their tentacles after treatment.

Colonies of *E. crocea* were exposed to electric current only at the previously identified optimal setting of 50 V, 3.2 cm electrode distance and 10 cm water depth (Fig. 4d). The reaction to electric current was very low with only 58% of the polyps showing released nematocysts at the tentacle base, and only 15 % with released nematocysts visible at the tentacles. All treated polyps in the colonies were alive and moving after exposure to electric current.

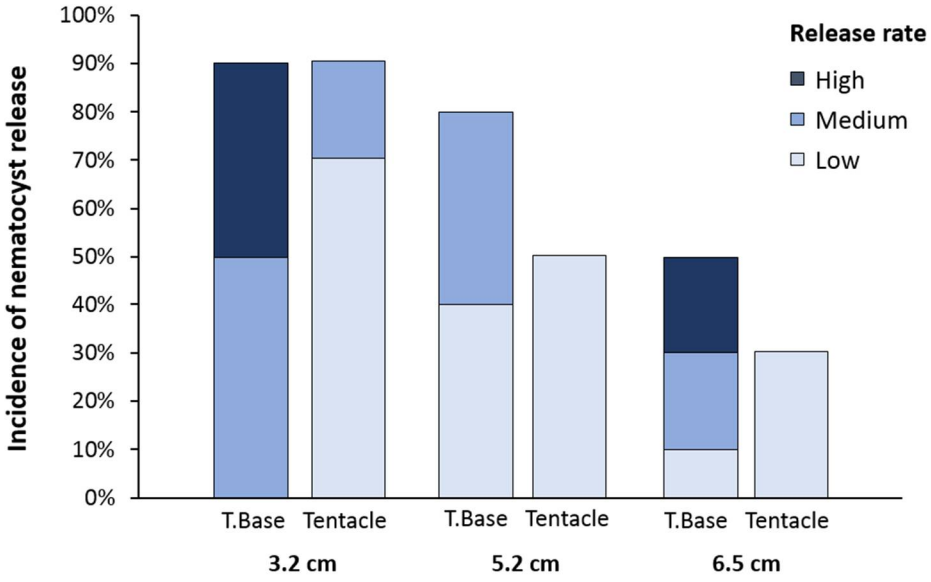

Figure 5: Average incidence of nematocyst release at the tentacle base (T.base) or the tentacles of *E. crocea* hydroids at different electrode distances.

The experiments show that individual polyps of Ectopleura crocea tested in 10 cm deep water reacted best when 50 V and an electrode distance of 3.2 am were used. Under these circumstances, 90 % of the individuals released nematocysts. This setting also achieved the highest incidence of high release rates, making it the most promising for further testing (achieving a high proportion of released nematocysts would conceivably result in a high reduction of risk of physical damage caused to salmon tissues).

During the tests with hydroid colonies, only very few nematocysts were released. This could indicate that the test setup is not effective when the current encounters several hydroids at once. However, low replication (n = 3) may not have achieved representative results and treatment of colonies should be examined in more detail.

Benefits and Adoption

The activities undertaken in this training scheme has provided significant benefits to both Tassal and CSIRO. Specifically, a number of key Tassal and CSIRO staff have now been up-skilled in both the identification and culture of hydroids that are of importance to the Tasmanian Atlantic salmon industry. Tassal are now in a much better position to undertake future R&D on hydroids as a consequence of this training activity. While our trials using electric current to deactivate hydroid colonies was only moderately successful, we built new capacity at both CSIRO and Tassal that will be used in the future to assess additional methods the can either kill or deactivate hydroids.

Further Development

On a general scale, it would be beneficial for Tassal to increase the company's knowledge on biofouling organisms on the nets and their impact on the fish. By correlating the occurrence of specific cnidarian species throughout the year with the gill health of the fish, species that may impact fish health stronger than others could be identified. This would also confirm if *Ectopleura* spp. are the correct species to target for nematocyst deactivation, or if there are others with

stronger impacts on fish health. Identification could be conducted through analysis of net samples, or potentially also through genetic analysis of collected cleaning waste.

An alternative way of identifying cnidarian species with the potential to harm the fish could be the analysis of gill swabs for hydroid DNA to identify species that indeed reach the gills. This data, too, could be correlated with gill health data.

References

N/A

Intellectual property

No immediate IP was generated.