

Feasibility Investigation of De-fouling Tunicates from Mussel Socks with Cavitating and Pulsed Waterjets

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ABSTRACT

The objective of this project was to investigate whether reverse flow cavitating waterjet (RFCWJ) and the forced pulsed waterjet (FPWJ) technologies are capable of removing or, mortally wounding *Ciona Intestinalis* and *Styela Clava* in an underwater (subsea) environment and, *Styela Clava* in an air environment (proof of principle) from a mussel sock. The results would be considered as acceptable if the cultivated mussel survives the waterjet treatment and remains affixed to the sock for future harvesting.

1.0 BACKGROUND ON TUNICATES

Tunicates are among the most common marine invertebrates with around 3,000 species. Details of tunicates, relevant to this investigation, are given by Gill, et al. (1) and Davidson (2). According to Gill, in recent years the mussel culture industry in PEI has been plagued by the invasion of tunicate species which have fouled socks in which mussels are grown (Fig. 1), equipment, decreased the production, and increased the operating costs. The four tunicate species that are of primary concern to PEI industry are the clubbed tunicate (*Styela clava*; Fig. 2A), the vase tunicate (*Ciona intestinalis*; Fig 2B), the golden star tunicate (*Botryllus schlosseri*) and the violet tunicate (*Botrylloides violaceus*). As depicted in Fig. 2, the first two of these grow as



Figure 1. A general view the mussel socks (to be submerged in the sea for growth) infested with tunicates.

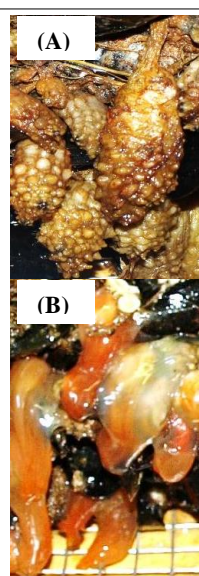


Figure 2. Close-up views. (A) Styela Clava (B) Ciona Intestinalis

large individuals, while the others grow in colonies of very small individuals. Of these four species, the Club tunicate and Vase tunicate have caused the most harm and therefore have been the focus on treating and controlling these species of the tunicate. Government, industry and the University of PEI have undertaken a multi-faceted program to understand the nature of this invasive species and devise effective methods of combating it. Thorough literature review, on topics such as original morphological and taxonomic descriptions, physiology, morphology, preferred habitat, and ecological requirements of the tunicates has been undertaken. Several other biological factors, such as physiology and composition of the skin, are currently being investigated by the industry. There are many biological factors (for example, strength of adherence vs. age) that have not been investigated as yet, all of which could impact the effectiveness of treatment systems.

Any treatment method to remove or kill tunicates, or prevent them from settling on mussel socks must be based on the differences between the characteristics of Mussels and tunicates. Some of the known differences between tunicates and mussels are: the outer covering, (shell vs. “Tunic”), unique chemistry of tunicate epidermis (it is unlike many other animals), tunicate blood is hypertonic to seawater (that is, more salty) and tunicates are less tolerant of water turbulence. When any differences in these parameters are known they can be translated into treatment options that can fit in to one of the following three categories: biological, chemical and physical. In the case of mussel farms, where the culture is conducted extensively in the natural environment, the introduction of biological agents such as predators or diseases is problematic, for obvious reasons. Similarly options for chemical treatment are limited to agents that degrade quickly with negligible effect on other organisms. Following this logic, physical treatment options are the most attractive, and the most obvious difference is that tunicates have a soft skin, while mussels have shells. Thus options that cause trauma to the tunicates are the most common concepts.

Ciona Intestinalis and *Styela Clava* are two particular tunicate species of interest being investigated in this project as they have invaded the mussel farming estuaries of Prince Edward Island, Canada (the problem also exists in many other countries, for example, South Africa). These types of tunicates rapidly occupy and overrun cultivated mussels. Current methods of tunicate mitigation include traditional *continuous* pressure wash systems. Although somewhat effective on *Ciona Intestinalis*, *Styela Clava* is a hardier species that is impervious to this type of treatment. A chemical-based lime treatment has been developed in order to preserve crops infested with the *Styela Clava* tunicate (Fig. 3). Both regiments have major disadvantages. Mussel socks must physically be suspended above water in order to undergo chemical or, pressure wash de-fouling. Implementing a system that is capable of treating both species of tunicate either submerged or, highly effective in air (suspended above water) was the ultimate goal of the project. The collaborative project with Atlantic Veterinary College University of Prince Edward Island was initiated assuming that RFCWJ under submerged or, open air environment and, FPWJ in open air environment would provide the required solution.

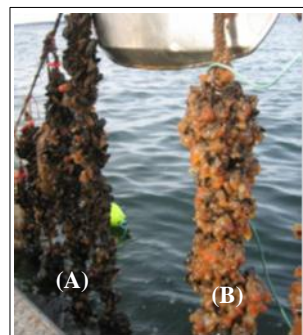


Figure 3. (A) Mussel socks treated with high concentration lime, (B) untreated sock (2).

2.0 TECHNICAL BACKGROUND – RFCWJ AND FPWJ

2.1 RFCWJ

Although the destructive effect of cavitation bubble has been known for more than a century, the method of harnessing that power for enhancing the cutting/cleaning ability of continuous waterjet emerged around 1970 (3, 4, 5, 6). In principle, any submerged waterjet generates gaseous and vaporous cavitation bubbles in the mixing zone of the jet (4). Conn and his collaborators have given an elegant and lucid description of the fluid mechanics of cavitating waterjets (7, 8). Vijay and his associates conducted extensive series of tests and corroborated the erosion results with remarkable photographs of the bubbles in the vicinity of the submerged jet issuing from a variety of nozzles (9). With the exception of a few new applications (10, 11), the widespread

commercial applications has not been possible because of the limitation of submergence. Having realized this, Vijay and his associates developed a novel nozzle, called reverseflow cavitation nozzle, for both submerged and open (*in air*) applications (12, 13, 14). While its principle of operation was reported in Ref. 12, its application for the removal of coating (also, deburring and peening which have not been reported due to commercial confidentiality) was reported in Ref. 13.

In the reverseflow cavitation nozzle, as disclosed in Ref. 14, the mixing zone is highly turbulent due to the adverse shear gradient generated by the interaction of the central continuous jet (CJ) with the annular reverse jet (RJ). The thickness of the mixing zone, indicated by δ in Fig. 4, is quite important. The magnitude of δ depends on the flow rate of reverseflow, which is controlled by the number of turns of the nut of the nozzle (the flow paths inside the nozzle are quite complex). Turn = 0 implies the nut is closed tightly and the reverseflow is shut off. In this case, only central jet (CJ) emerges (regular blasting). When the nut is turned by 1/8th of a turn, thickness of the mixing zone increases. However, it may not be enough to generate cavitation bubbles in the mixing layers. The reason for better performance (compared to continuous waterjet) is due to the angle β of the reverseflow. For generation of cavitation, the central and the reverse streams must be parallel (that is, $\beta = 0$). If there are slight defects in the fabrication of items, which generate and control the reverseflow rate, then β will not be zero. In this case, RJ may interrupt the CJ. The interruptions may be periodic due to circulation in the mixing chamber, with vortex shedding. This may generate both cavitation bubbles and pulses of water. When the nut is loosened further, indicated by 1/4 and 1/2 turns, the thickness δ will increase further, which may be better for generating cavitation bubbles. In summary, it is probably a combination of both cavitation bubbles and pulses, which contributes to better performance compared to a continuous waterjet. Results reported by

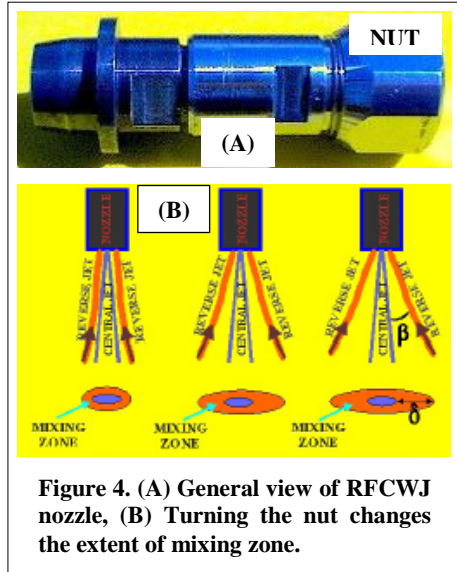


Figure 4. (A) General view of RFCWJ nozzle, (B) Turning the nut changes the extent of mixing zone.

Vijay et al. (13) confirm that the performance of the nozzle under submerged and in open atmosphere environments are significantly better than the continuous waterjet (CJ).

2.2 FPWJ

As operating principles of FPWJ have been reported in several publications (15), only a brief description is given here. High-frequency FPWJ is produced by placing small probe inside the nozzle energized by ultrasonic power. When the ultrasonic power input is matched (resonance) with the operating parameters, fully developed pulses issue from the nozzle as illustrated in Fig. 5.

3.0 EXPERIMENTAL PROGRAM

3.1 Overview

All tests were conducted at VLN's waterjet laboratory. *Ciona Intestinalis* and *Styela Clava* infested mussel socks were transported from PEI. In order to ensure that the socks will remain in satisfactory condition for the purpose of testing, adequate onsite life support was provided to the socks (controlled conditions). This was supervised by Dr. Davidson, a veterinary doctor.

As stated in **Section 1**, it was quite important to make sure that the mussels were not damaged (hurt) while de-fouling or mortally wounding the tunicates. This required conducting trial runs in the laboratory on a material that was similar to the skins of both types of tunicate. Although FARD (Fisheries, Aquaculture and Rural Development) suggested using leather, due to the uncertainty of getting the appropriate type of leather, soft vinyl samples were employed for selecting optimum set of operating parameters. This procedure was also important as there was no time to conduct systematic tests on the tunicates (as their condition could deteriorate while testing, yielding erroneous results). These preliminary trials are described in the **Appendix**.

3.2 Sample Collection

Styela Clava – club tunicate (Figure 2A): Sections of mussel socks fouled by the club tunicate were collected by members of the Atlantic Veterinary College (AVC), Shellfish Health Research Group (SHRG) from Marchwater Bay, PEI. These sections contained market size mussels and were double socked. They were transported in coolers to Georgetown where they were held in a flow through system with water from Georgetown Harbor (information provided by AVC-SHRG). This ensured their health until shipping.

Ciona Intestinalis – vase tunicate (Figure 2B): Sections of mussel socks fouled by the vase tunicate were collected by members of the AVC-SHRG team from the Montague River (information provided by AVC-SHRG). Three socks were immediately packed for transportation and three were held in the flow through system on the Georgetown Harbor for shipment the following day. These sections also contained market size mussels.

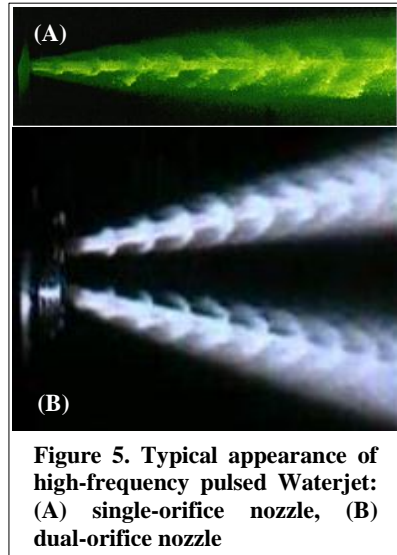


Figure 5. Typical appearance of high-frequency pulsed Waterjet: (A) single-orifice nozzle, (B) dual-orifice nozzle

3.3 Transportation of Samples

1.2-m sections of mussel socks fouled with vase tunicate and with club tunicate were packed into two coolers with ice packs and paper towels (information provided by AVC-SHRG). In order to make sure that the biological characteristics of the fouled socks do not deteriorate, the shipments were transported by a direct flight from Charlottetown to Ottawa. AVC-SHRG personnel accompanied the shipments to ensure sample preservation. Upon arrival in Ottawa, the samples were immediately delivered to VLN laboratory and were transferred into a holding tank filled with salt water (28 ppt @ 18.1°C). The tank water was treated with Instant Ocean and Nutrafin tap water conditioner along with an aeration system. The water chemistry was checked and maintained by AVC-SHRG personnel to ensure specimen mortality was not a result of inadequate life support.

3.4 EXPERIMENTAL SETUP AND PROCEDURE

3.4.1 Experimental Setup

For testing with both RFCWJ and FPWJ, the following equipment and nozzles were employed:

- Pratisolli triplex plunger pump rated to deliver 50-litre/min of water at the rated pressure of 103.5-MPa;
- RFM 2020 (Retrofit Module), pulsed waterjet generator (illustrated in Fig. 6);
- RFCWJ nozzle assembly with $d = 1.54\text{-mm}$ (Fig. 7);
- $d = 0.76, 1.01, 1.37$ and 1.90-mm for the FPWJ nozzle.

For RFCWJ nozzle, a special jig was fabricated to hold the mussel socks in place in the tank while they underwent waterjet treatment (Fig. 7). It was implemented to accurately monitor the effects of pressure (P), turn of the nut (T), standoff distance (S_d) and traverse speed (V_{tr}). Since the socks were soft, special care was taken to secure them in order to obtain reliable data. A heavy gauge wire mesh was fastened to the backside of the mussel sock for proper orientation to the impinging waterjet. As pointed out earlier, based on the prior tests conducted on vinyl samples (**Appendix**), multiple runs were conducted with appropriate variations in the operating parameters.

The tests with the RFCWJ were conducted by articulating the robotic arm of the 6-axis Kawasaki robot (Model ZZX 165U). Tests with the FPWJ were conducted on the X-Y-Z gantry. Performance indicator was basically visual observation of the socks before and



Fig. 6. A general view of pulsed waterjet generator (RFM).



Figure 7. Salt water tank showing the RFCWJ nozzle positioned over the infested mussel sock.

after exposure to the jets. Evaluation of the state of the tunicates (mortally wounded, etc.) was performed by Dr. Davidson and his associates.

4.0 RESULTS

4.1 RFCWJ

Results obtained with the RFCWJ are summarized in Table 1. The table includes operating parameters, comments (observations based on the visual examination of the socks) and corresponding photographs of interest.

4.2 FPWJ

The results obtained with the FPWJ are summarized in Table 2. The table includes operating parameters, remarks (observations based on the visual examination of the socks) and corresponding photographs of interest.

5.0 DISCUSSION

The primary objective of this project was to determine whether or not the RFCWJ/FPWJ would be able to effectively remove or mortally wound *Ciona Intestinalis* and *Styela Clava* tunicate without adversely affecting the health of the cultivated mussel (proof of principle). A brief description of the results is presented herein although the final evaluation of the efficacy of the process for removing tunicates was in the hands of the technical teams of AVC-SHRG and FARD. It must also be emphasized that evaluation (that is, 'yes' or 'no', etc) while conducting the tests was essentially subjective as it was based on simple visual observations of the specimens before and after exposure to the jets.

5.1 RFCWJ

A few initial trials were conducted at $P = 6.9\text{-MPa}$, $V_{tr} = 2.54\text{-m/min}$ with 0-Turn of the nut. As illustrated in Fig. 8, visual observation appeared to indicate that the RFCWJ at this low pressure was somewhat effective in removing the *Ciona Intestinalis* tunicate. However, no further tests were conducted at this pressure as the traverse was considered to be quite slow. Therefore, in order to match the V_{tr} of the nozzle to that of the existing equipment in the field, further tests were conducted at higher traverse speeds and consequently, at higher pressures. It was also important to keep in mind that the maximum pressure would be limited by: (i) ease and safe operation of the RFCWJ in a submerged (subsea) environment and (ii) without causing significant mussel loss while at



Figure 8. Appearance of the sock after testing at 6.9-MPa.

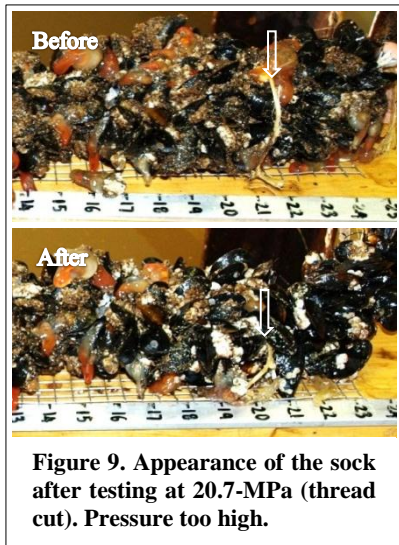


Figure 9. Appearance of the sock after testing at 20.7-MPa (thread cut). Pressure too high.

the same time preserving the delicate mussel byssal thread. Although not obvious from Fig. 9, Dr. Davidson noticed significant loss of mussel at 20.7-MPa, and stated that it would be unacceptable to the sea farmers. Figure 10, on the other hand, shows $P \approx 17.2$ -MPa was satisfactory. Although this test run was conducted on *Styela Clava* mussel sock, it was believed to be applicable to *Ciona Intestinalis* as the mechanical properties of the mussel byssal are assumed to be similar.

Figure 11 shows the sock from which the *Ciona Intestinalis* was removed. The fact that it was achieved for almost zero turn of the nut at a low pressure (17.2-MPa), and very high traverse rate of 19.8-m/min was considered to be significant. At these

conditions, loss of mussel was considered to be negligible. The traverse speed of 19.8-m/min was selected based on the following information (provided by Mussel Growers Association):

1. The time taken by the nozzle to travel within the existing wash treatment system;
2. Up and down movement of the nozzle;
3. The speed of the boat travelling down the mussel sock line (see Fig. 1).

Other relevant remarks are listed in Table 1.

6.0 FPWJ

A very limited number of runs were conducted with the FPWJ on *Styela Clava* tunicate sock. The robot was programmed to cover a 76.2-mm wide swath using an index of 12.7-mm per pass, which allowed a more realistic assessment of the FPWJ's performance. The operating conditions, to mimic the actual service condition, were: $d = 0.076$ -mm, $P = 27.5$ -MPa, $V_{tr} = 8.12$ -m/min and $S_d = 127$ -mm. Figure 12 shows that FPWJ was able to remove some of the *Styela Clava* without significant mussel loss. As testing was incomplete due to lack of socks, further work needs to be conducted to determine the potential of FPWJ for removing both types of tunicates.

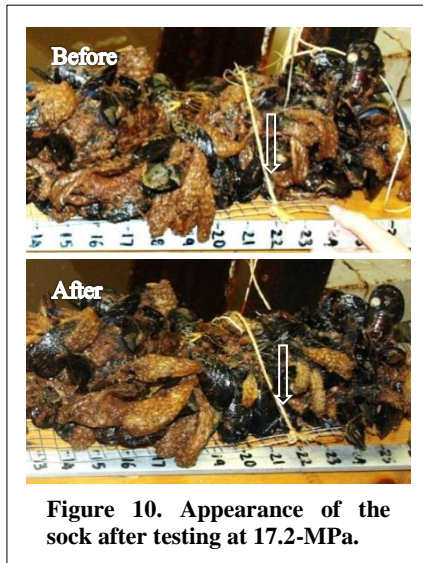


Figure 10. Appearance of the sock after testing at 17.2-MPa.

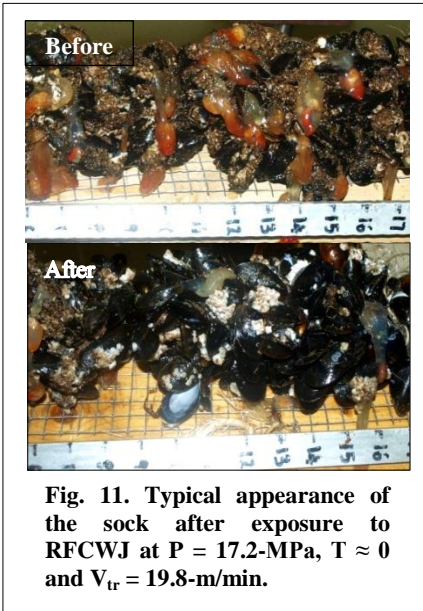
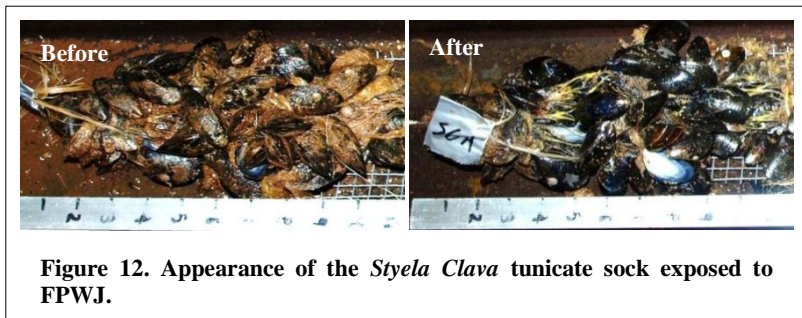


Fig. 11. Typical appearance of the sock after exposure to RFCWJ at $P = 17.2$ -MPa, $T \approx 0$ and $V_{tr} = 19.8$ -m/min.



7.0 CONCLUSIONS

The conclusions, from the limited tests conducted on de-fouling or mortally wounding *Ciona Intestinalis* and *Styela Clava*, are:

- The RFCWJ appears to be quite effective for de-fouling *Ciona Intestinalis* tunicate from a mussel sock in a submerged (subsea) environment;
- Operating parameters that appeared to be effective were: $d = 1.54\text{-mm}$, $P = 17.2\text{-MPa}$, $S_d = 127\text{-mm}$, $T \approx 0$ for RFCWJ and $V_{tr} = 18.3\text{-m/min}$;
- Further work is required to optimize (that is, maximize rate of treatment) the operating parameters (for example, testing at $T = 1/8$ for the RFCWJ);
- The RFCWJ does not appear to be effective for de-fouling *Styela Clava* tunicate from a mussel sock in a submerged (subsea) environment, suggesting further work;
- With regard to the FPWJ, further work is required to establish if it could effectively de-foul *Styela Clava* tunicate from mussel socks in-air environment.

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9.0 NOMENCLATURE

a = Position of the microtip in the FPWJ nozzle.

d = Orifice diameter, mm

L = Location where the run was conducted as indicated on the photographs, in

P = Pump pressure, MPa

S_d = Standoff distance, mm

T = Turn of the nut on the nozzle (for RFCWJ). For FPWJ, indicates the position of the microtip 'a' in the nozzle.

V_{tr} = Traverse speed of the nozzle over the sock, m/min

10.0 ACKNOWLEDGEMENTS

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FARD (Fisheries, Aquaculture and Rural Development), PEI Aquaculture Alliance and, Atlantic Innovation Fund with ACOA (Atlantic Canada Opportunity Agency).

11.0 Table 1. Brief summary of results obtained with the RFCWJ

Species (S_p): $C = Ciona Intestinalis$, $S = Styela Clava$ $S_d = 127$, $d = 1.54$, $V_{tr} = 8.12$ (unless otherwise stated within the comments column).					
Run	S_p	P	T	Comments:	L
1	C	6.9	0	No mussel loss, cleaned mussels over 51-mm swath; some tunicates remained. Required second trial.	20
2	C	6.9	0	Fig 8: No mussel loss, cleaned mussels over 2 inch swath; some tunicates remained.	22
3	C	6.9	¼	No effect on tunicates or mussels	16
4	C	6.9	0	Accidental run, similar results as run #1 and 2	12
5	C	13.8	0	Sample flipped. No loss of mussels - clean (tunicates removed).	9
6	C	13.8	¼	No loss of mussels. Clean, 63.5 mm – 76.2 mm swath path (2.5 in – 3 in).	6
7	C	20.7	0	Fig 9: Significant mussel loss, 101.6 mm swath path (4 in).	21
8	C	20.7	¼	Significant mussel loss, 101.6 mm - 127 mm swath path (4 in – 5 in).	16
9	C	20.7	½	Minimal mussel loss, 76.2 mm swath path (3 in).	11
10	C	20.7	¾	Significant mussel loss (76.2 mm swath)	7
11	C	20.7	1	Significant mussel loss 76.2-mm swath)	3
12	C	17.2	0	$S_d = 25.4$. Significant mussel loss. Test was conducted to observe effect of S_d .	18
13	C	17.2	0	Fig. 10 ($V_{tr} = 19.8$). The remaining tunicates eviscerated. Kinematics (63.5-mm; index = X 3).	8-16
14	S	17.2	0	Fig 11. Some tunicate removed with minimal mussel loss (some tunicates perforated).	22
15	S	17.2	¼	Cleaned mussels. Some tunicates removed with no mussel loss.	17
16	S	17.2	½	Removed several tunicates with no mussel loss.	13
17	S	17.2	¾	Cleaned mussels. No tunicates removed & no mussel loss.	7
18	S	17.2	0	Cleaned mussels. No tunicates removed & no mussel loss.	22
19	S	17.2	¼	Cleaned mussels. Few tunicates removed with no mussel loss.	16
20	S	17.2	½	Cleaned mussels. No tunicates removed & no mussel loss.	10
21	S	20.7	0	Tunicate and mussel loss.	5

12.0 Table 2. Brief summary of results obtained with the FPWJ

Species (S_p): $C = Ciona Intestinalis$, $S = Styela Clava$ $S_d = 127$, $d = 1.90$, $V_{tr} = 8.12$ (unless otherwise stated within the comments column).					
Run	S_p	P	T	Comments:	L
22	S	20.7	3	Wounded a few tunicates & mussel loss.	22
23	S	20.7	3	Complete removal of tunicates and mussels. 5 broken shells with meat inside. 25% mussel shell damage. Stripped tunicates off by the holdfast. Kinematics: 12.7-mm, index = X 6 (12.7-mm).	20-17
24	S	13.8	3	Removed mussels and mesh visible but not as drastically as 20.7-MPa. Perforated several tunicates in lower 1/3 of body. No broken shells. Kinematics: 12.7 mm, index = X 6.	11-8
25	S	13.8	4	$d = 1.37$. Lost mussels that were not double socked. No cracks in shells. Kinematics 12.7 mm, index = X 6.	19-16
26	S	13.8	4	$d = 1.37$. Tunicates removed. Mussel valves damaged (cracked/broken). Kinematics: 12.7-mm, index = X 6.	11-14
27	S	13.8	4	$d = 1.37$ mm. Cleaned mussels slightly. No tunicates removed; No mussel damage. Kinematics: 12.7 mm, index = X 6.	4-1
28	S	20.7	4	$d = 1.01$. Cleaned mussels. Removed dead tunicates. No mussels removed. Kinematics: 12.7-mm, index = X 6.	20-17
29	S	24.1	4	$d = 1.01$. Mussel loss, removed dead tunicates. Kinematics: 12.7-mm, index = X 6.	16-13
30	S	24.1	4	$d = 1.01$. Loss of few mussels and tunicates. Kinematics: 12.7-mm, index = X 6.	29-26
31	C	24.1	5	$d = 1.01$. Mussel and tunicate loss. Kinematics: 12.7-mm, index = X 6.	23-20
32	C	20.7	5	$d = 1.01$. Removed a few tunicates with no mussel loss. Kinematics: 12.7-mm, index = X 6.	18-15
33	C	27.6	5	$d = 0.76$ mm. Removed a few tunicates with no mussel loss. Kinematics: 12.7-mm, index = X 6.	10-7
34	C	27.6	5	Fig 12. $d = 0.76$. Removed a few tunicates. Mussel loss but uncertain about condition of Byssal threads prior to waterjet treatment. Kinematics: 12.7-mm, index = X 6.	6-4

Appendix

General Remarks

Preliminary tests were conducted using both RFCWJ and FPWJ to determine the best possible protocol for tunicate laboratory trials. Parameters set forth by VLN in the formal proposal as well as those outlined in the FUNDY technical report (Ref. 2) were taken into consideration. The goal was to remove the tunicates without damaging the mussels.

Procedure of RFCWJ

Visualization and performance testing of the submerged RFCWJ were used to determine a starting point for the tunicate test protocol. Discussions with FARD indicated that leather would be an appropriate substitute to simulate clubbed tunicate. However, due to the uncertainty of getting the right type of leather, vinyl samples were employed for obtaining the results.

Procedure of FPWJ

As the statement of work (SOW) in the proposal required lower operating pressures (≤ 34.5 -MPa), it was decided to try a different approach for achieving the desired results from FPWJ technique (15). A variety of large diameter nozzles together with different microtips at various ultrasonic power settings were investigated to obtain the most effective operating parameters.

Visualization of RFCWJ

Multiple tests were conducted to visualize the structure of submerged RFCWJ at various operating parameters. The experimental arrangements for visualization and testing the vinyl samples are illustrated in Figs. A1 and A2. To illustrate the effects of pressure, photographs were taken with the RFCWJ operating at the same flow but different nozzle turns. Typical appearance of the jets is shown in Fig. A3.



Figure A1. A general view of the experimental set-up using the robot for manipulation of the nozzle.

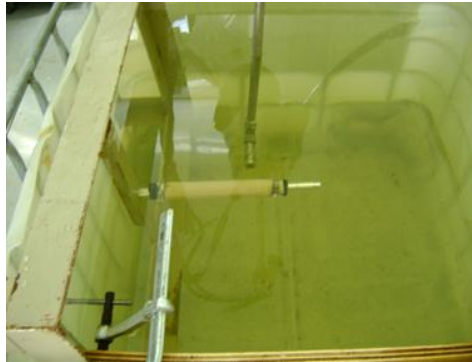


Figure A2. A close-up view of the set-up showing the vinyl sample used to simulate the skin of tunicate.

Performance of RFCWJ

Tests were conducted utilizing a six axes robot with the RFCWJ submerged in a modified container as illustrated in Fig. A2.

With the information provided by FARD, the vinyl sample was prepared as depicted in Fig. A4. While the closed micro cell foam was used to simulate the fleshy portion of the club tunicate's body, the vinyl was used to simulate its tough outer skin. It should be pointed out, however, that in practice removing the tunicates from the socks is more relevant. Cutting the vinyl sets the limit on operating parameters (exceeding the limit could damage the mussels).

All RFCWJ performance tests were conducted at a standoff distance of 127-mm and at two traverse speeds of 1.27 and 2.54-m/min. Pressure and nozzle turns were adjusted until the vinyl skin was cut. Typical data are indicated in Fig. A4. From these data, it appears that the RFCWJ issuing from an orifice diameter of 1.55-mm would cut the vinyl at pressures of the order of 27.6-MPa and a traverse speed of 2.54-m/min. This information was quite useful in conducting the tests on mussel socks, as the time (duration) of testing was critical (to make sure that the tunicates were alive).

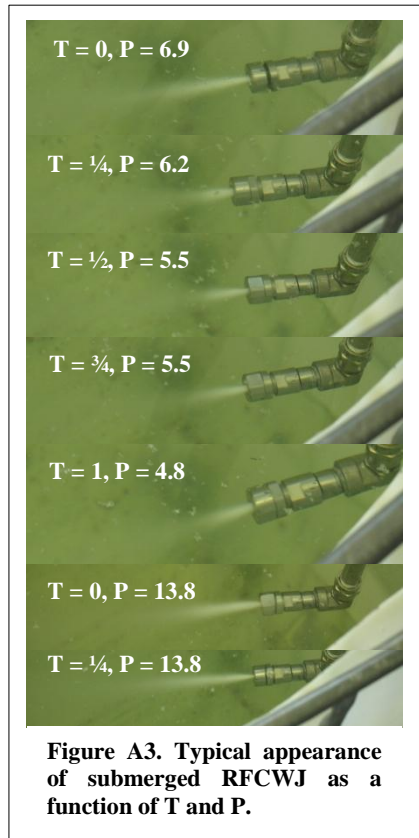


Figure A3. Typical appearance of submerged RFCWJ as a function of T and P.

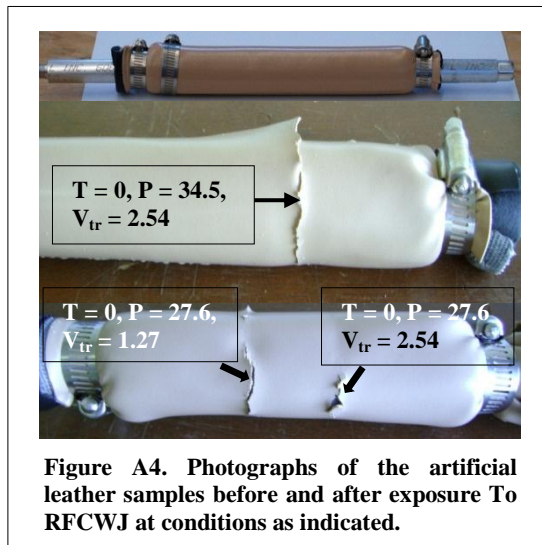


Figure A4. Photographs of the artificial leather samples before and after exposure to RFCWJ at conditions as indicated.