

**Feeding pressure of *Mytilus edulis* and *Styela clava*  
on phytoplankton and zooplankton, including lobster larvae  
(stages I and IV)**

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## ABSTRACT

Sonier, R., Filgueira, R., Daoud, D. and Comeau, L.A. (2018). Feeding pressure of *Mytilus edulis* and *Styela clava* on phytoplankton and zooplankton, including lobster larvae (stages I and IV). Can. Tech. Rep. Fish. Aquat. Sci. 3263: vi +19 p.

This report provides insight on feeding pressures of cultivated blue mussels *Mytilus edulis* and the invasive solitary tunicate *Styela clava* on phytoplankton and zooplankton, including American lobster (*Homarus americanus*) larvae (stages I and IV). In 2015-2016, experiments were conducted in a portable aquatic laboratory on Prince Edward Island (PEI) using natural sea water. Average standardized (for 1g of dried tissue weight) clearance rates (CR) were of  $12.5 \pm 1.12 \text{ Lh}^{-1}\text{g}^{-1}$  and  $6.7 \pm 0.7 \text{ Lh}^{-1}\text{g}^{-1}$  for mussels and tunicates respectively. Scaling up these results to a bay scale model that integrates mussels and tunicates feeding pressures on phytoplankton in Malpeque Bay (PEI) showed the importance of mitigation measures and treatment regimes on tunicate populations. A retention of zooplankton was detected at approximately  $35 \pm 9.4\%$  and  $28 \pm 9.0\%$  by mussels and tunicates respectively with a slight preference for copepods. As for predation on lobster larvae (both stages I and IV), *M. edulis* and *S. clava* demonstrated no retention or capture of lobster larvae, even at densities which surpass significantly natural lobster larvae abundance in the natural environment. It is anticipated that the information presented in this report will serve in future modelling and management analysis.

## RÉSUMÉ

Sonier, R., Filgueira, R., Daoud, D. and Comeau, L.A. (2018). Feeding pressure of *Mytilus edulis* and *Styela clava* on phytoplankton and zooplankton, including lobster larvae (stages I and IV). Can. Tech. Rep. Fish. Aquat. Sci. 3263: vi +19 p.

Ce rapport fournit un aperçu de la pression de filtration exercée par les moules d'élevage *Mytilus edulis* ainsi que le tunicier solitaire *Styela clava* sur le phytoplancton et le zooplancton, incluant les larves (stades I et IV) de homard (*Homarus americanus*). En 2015-2016, des expériences en milieux contrôlés ont eu lieu dans un laboratoire mobile à l'Île-du-Prince-Édouard (I.-P.-É.) utilisant de l'eau mer naturelle. La capacité de filtration standardisée (individu de 1g, chair sèche) des moules et tuniciers furent de  $12,5 \pm 1,12 \text{ Lh}^{-1}\text{g}^{-1}$  et  $6,7 \pm 0,7 \text{ Lh}^{-1}\text{g}^{-1}$  respectivement. L'exercice de modélisation numérique intégrant les la capacité de filtrations de ces deux espèces dans la baie de Malpeque (I.-P.-É.) démontre clairement l'importance des méthodes de contrôles des tuniciers par l'industrie aquacole. Une rétention du zooplancton fut détectée entre  $35 \pm 9,4\%$  et  $28 \pm 9,0\%$  par les moules et les tuniciers respectivement, avec une préférence apparente pour les copépodes. À ce qui a trait à la prédation sur les larves de homard (stades I et IV), aucune filtration ou capture ne fut descellée par les deux espèces de filtreurs, et ce même en utilisant des densités surpassant nettement l'abondance que l'on peut retrouver dans l'environnement. Il est attendu que les informations présentées dans ce rapport serviront aux exercices de modélisation et de gestion futures.



## INTRODUCTION

Ascidian tunicates are often recognized as important pests in shellfish aquaculture settings, with the potential to cause considerable economic losses (Comeau et al. 2015) and have therefore gained considerable research interest as fouling organisms on shellfish culture gear (Carver et al. 2003). As for mussels, ascidian species are efficient filter-feeders that are capable of depleting important concentrations of suspended particles in the water column (Thompson and MacNair, 2004) and sometimes further modify phytoplankton assemblages in the marine environment. The notorious invasion of *Styela clava* (clubbed tunicate) in shellfish aquaculture farms represents a good example that merits research attention. This species is ubiquitous in coastal hard-substrate habitats worldwide. In 1997, it established and proliferated in mussel (*Mytilus edulis*) farms in Prince Edward Island (PEI, Atlantic Canada), which provides excellent substrate for settlement (LeBlanc et al. 2007). Those mussel farms create suitable habitat for tunicates with plenty of substrate for recruitment. The abundance of *S. clava* now ranges between 573 to 3136 individuals per mussel sleeve, depending on whether mitigation measures, such as lime treatments, are being applied (Ramsay et al., 2014). Comeau et al. (2015) recently showed that the phytoplankton demand in mussel leases infested with *S. clava* increases by 47% (despite treatment) compared to non-infested scenarios. This estimate relates to median-size ( $47.8 \pm 1.3$  mm) *S. clava* and therefore represents one snapshot in time through the infestation cycle. This increased filtration pressure due to tunicates infestations can exert potential impacts on phytoplankton biomass and communities, which is relevant to ecosystem functioning and commonly studied in carrying capacity (here forth CC) research.

In bivalve aquaculture, the concept of CC has traditionally been considered in the context of maximizing the level (biomass) of aquaculture production in a farm. The latter is also referred as production CC. The CC concept and its level of complexity have changed over the past decade. Important consideration is now given to ecological CC, which is defined as the magnitude of aquaculture activity that can be supported without leading to unacceptable changes in ecological processes. Recent CC models have focused on the dynamics of phytoplankton (biomass and communities) and their interactions with cultured bivalves, given that phytoplankton constitutes the primary level in plankton-based marine food webs. Such numerical models are now capable of predicting to what extent bivalves utilize resource-limited phytoplankton and seston (Filgueira et al. 2013, 2014; Guyondet et al. 2013, 2015). Models can integrate fouling communities, however the information on the physiology, life cycle or biomass of these invasive organisms are sometimes scarce or incomplete. A more detailed understanding of how *S. clava* impacts CC is not possible from the existing scientific literature, which is rather dated (Holmes, 1973) or focussed on the actual cultivation of the tunicate in Korea and China (Jiang et al., 2008; Kang et al., 2011). For that reason, information on real biomass data as well as filtration capacity of tunicates from PEI is lacking and needed to incorporate in current modelling exercises.

Estuaries on PEI are often shared by multiple users targeting different species of economic importance for the region. In the present case, Malpeque Bay is 19,640 ha of which 1,400 ha (~7%) are currently leased for mussel and oyster culture (Filgueira et al. 2015b), although mussel leases expansion proposals (increase to 10% usage) are being evaluated. Expansion scenarios encompass areas in the northern part of this embayment, which is also fishing grounds for lobster (*Homarus americanus*). The lobster fishery community presented some concerns on the potential interactions between the presence of lobster larvae in the system and the increasing biomass of filter-feeders (mussels and tunicates). In terms of lobster larvae dynamics, Hudon et al. (1986) published a report on lobster larvae vertical and horizontal distribution in the Magdalen Islands and presented important vertical distribution in the water column mostly dependent on light intensity (especially for Stage I larvae) where the abundance is greater in surface waters (0–0.8m). To our knowledge only one secondary paper (Leblanc, A.R. et al. 2007) addressed zooplankton ingestion by *S. clava*. However, the latter research was conducted in a controlled environment using artificial sea water, and presented no allometric relationships. This particular knowledge gap requires attention to pursue dynamic modelling and higher-trophic level modelling (e.g., Ecopath) and improve our understanding of the linkages between mussel farming and species of commercial, recreational and aboriginal importance (CRA species).

Accordingly, this project proposes four objectives: (1) determine *S. clava* clearance rates on natural phytoplankton communities, (2) measure *S. clava* and *M. edulis* predation on zooplankton communities, (3) evaluate *S. clava* and *M. edulis* capacity to retain lobster larvae (stages I and IV), (4) update an existing carrying capacity numerical model for Malpeque Bay with these new measurements to estimate the impact of *S. clava* on phytoplankton dynamics.

## **MATERIAL AND METHODS**

### **Portable aquatic lab (PAL)**

All retention efficiency and clearance rate trials were conducted in a Portable Aquatic Laboratory (PAL), which can be deployed on wharfs to be supplied with natural sea water from the studied area. The PAL can be connected to the electrical grid, provides lab space holding 5 large tanks (250 L), 24 medium sizes acrylic tanks and a workbench (Figure 1 and 2). The inflow consists of unfiltered sea water pumped from close proximity (< 0.25km), 2m below surface, at a rate of approximately 1500 L h<sup>-1</sup> (Tsurumi Titanium 50TM2.4S submersible pump, semi-vortex/propeller). A stainless steel cage covers the pump to prevent macro algae and organisms being uptaken into the laboratory installations.

## Clearance rate (*Styela clava* and *Mytilus edulis*)

Clearance rate (CR) is defined as the volume of water cleared of suspended particles per unit time ( $\text{L h}^{-1}$ ) (Comeau et al. 2015). Clearance rates experiments were conducted in summer 2015 and fall 2016, over the course of three consecutive days. A total of 6 experimental trials were carried using  $n = 12$  feeding chambers each trial. Clubbed tunicates of variable sizes (mean full length  $73.5 \pm 17.6\text{mm}$ , max =  $118.8\text{mm}$ , min =  $42.4\text{mm}$ ,  $n=250$ ) were collected from Malpeque Bay (PEI) and transferred to the portable aquatic laboratory. Individuals were held in two large tanks (250 L) supplied continuously with natural seawater ( $12.5 \pm 1.4^\circ\text{C}$ ,  $30.8 \pm 0.6\text{ppt}$ ). The high flow rate provided by the pump ensures that mussels and tunicates do not significantly cause food depletion during the acclimatization phase. After a 2-week acclimatization period, acrylic feeding chambers (Figure 3) (1.9 L,  $n=12$ ) were installed and supplied with the same seawater as the holding tanks. Using those feeding chambers, trials were conducted using only one mussel or tunicate per chamber as single individual trials.

Serving as controls to account for gravitational settling of particles, each trial dedicated one chamber for a dead tunicate previously treated in vinegar (acetic acid), a mitigation measure used by the mussel industry against *S. clava* to control their fouling biomass (Forrest et al. 2007). The same control procedure was used for mussel where shells were placed in the control chamber. Particle (food) mixing was promoted by fine aeration (bubbling), introduced to homogenize the entire water volume while minimizing feces resuspension. Actively feeding individuals were confirmed through feces production. Individual clearance rate was measured as follows. Following a 1-h adaptation period, each trial started when the water flow in the chambers was halted and the decline of particles over time was monitored by taking water samples (10ml) in each chamber at a 10-min interval over an hour incubation for particle counts (Beckman Coulter Counter Z2TM fitted with a  $100\text{ }\mu\text{m}$  aperture). Aliquots ( $100\text{ }\mu\text{l}$ ) were processed in the instrument within the  $5\text{--}19\text{ }\mu\text{m}$  size range which is known to be efficiently retained by mussels (Ward et al. 2003) and *S. clava* (Petersen 2007). Only individuals that had their valves or siphons open and chambers that showed continuous decrease in particle counts were included in the final analysis. This approach provides the maximum individual clearance rates (CR) estimates. For the CR calculations only the 10-min interval exhibiting the greatest depletion of particles (greatest slope) for each chamber ( $\text{CR}_{\text{ch}}$ ) was used following this equation (based on F from Petersen et al. 2003 and  $\text{CR}_{\text{ind}}$  from Comeau et al. 2015). This method represents the maximum CR estimates for each chamber:

$$\text{CR}_{\text{ch}} = V / t \times \ln (C_0 / C_t)$$

Where  $V$  is the volume of the chamber,  $t$  is time elapsed between measurements (10 min), and  $C_0$  and  $C_t$  are particle concentrations at times 0 and  $t$  respectively. An exponential curve was fitted to the decline in particles concentrations with time, and only experiments with  $r^2 > 0.90$  were considered. This triage enable the exclusion of trials that presented artifacts in particle depletion such as particle expulsion by the filter feeder (particle squirting) or inability to filter (i.e. valve or siphon lasting closure).

## **Retention of zooplankton assemblages by *Mytilus edulis* and *Styela clava***

The assessment of predation on zooplankton by *S. clava* and *M. edulis* was conducted in the fall 2016 in the PAL over the course of three days using the above-mentioned acrylic feeding chambers. A total of 5 experimental trials were conducted. Five chambers for mussels, five for tunicates, and two controls (dead individuals) were used in each trial. The same acclimatization protocol was used during the CR trials followed this time by 1-h static incubation. Following the incubation period, 600 ml of water from each homogenized chamber was collected and sieved through a 63 µm sieve to only retain zooplankton size above that threshold. All samples were rinsed and preserved in 20ml vials containing a formaldehyde dilution for taxonomic analysis carried out at the Atlantic Reference Centre (St. Andrews, New Brunswick). All animals easily identifiable were identified to the species level while others were identified to the class taxonomic level. Retention efficiencies of zooplankton by *M. edulis* and *S. clava* were estimated by comparing identifiable zooplankton counts from incubations with active filter-feeders and control chambers (dead individuals).

## **Predation on lobster larvae by *Mytilus edulis* and *Styela clava***

Lobster (*Homarus americanus*) larvae were produced by Homarus Inc. in Shippagan (northeast New Brunswick) at the Coastal Zones Research. Homarus Inc is a non-profit research and development organization created by the Maritime Fishermen's Union (MFU) whose mission is to develop tools to ensure the sustainability of the lobster resource and its fishery. Among other projects, they produce and release into the wild about 500,000 lobster larvae yearly.

Stage I (~8 mm) (Factor 1995) and IV (~15 mm) lobster larvae were transported to the PAL on PEI for our feeding trials in cold aerated water (Figure 4) while feeding them regularly with *Artemia sp.* to limit die-off and intraspecific cannibalism during transport. Acclimatization and rearing of lobster larvae were performed in custom made upwellers (Figure 5) with strong aeration (bubbles) to minimize mortality due to cannibalism. A supply of frozen *Artemia sp.* was also provided for regular feedings. All upwellers were assembled with ample running *in-situ* sea water with no interruption mainly to preserve a constant temperature and reduce the ammonia accumulation created by excretion and, in some instances, degrading surplus food.

Predation trials were done in acrylic feeding chambers with various densities of lobster larvae in proximity of mussels or tunicates. Experimental combinations of lobster larvae, mussels and tunicates, as well as the chosen sample analysis method used for each trial are presented in Table 1. All predation trials on lobster larvae were of one-hour incubations and solely chambers with actively filtering and feeding mussels and tunicates were accounted for in the results. Feeding activity was monitored using the clearance rate protocol depicted earlier (particles counts). Feces and pseudofeces production was also looked at as secondary proof of ingestion activity. Two different approaches were used to acquire predation data: 1) trials in 2015 were done in a "count-recount" fashion where a known number of lobster larvae was introduced in each

chamber and then recollected (counted) after the incubation, 2) in 2016, a known numbers of lobster larvae was added to each chamber and all experimental mussels and tunicates were then flash frozen after each incubation and later dissected in the laboratory (DFO, Gulf Region) for gut content analysis and to evaluate the potential presence or entrapment of lobster larvae in mussel and tunicates internal cavities.

### **Bay scale modelling (predation on phytoplankton)**

The novel measures of *S. clava* clearance rate were used to update an existing hydrodynamic biogeochemical spatially explicit model for Malpeque Bay (Filgueira et al. 2014, 2015). Filgueira et al. (2014) provide a detailed explanation of the model equations, coupling, parameterization, forcing, calibration and validation. The fully-coupled model was run from 24 May 2012 to 7 October 2012 (137 days), based on data available to force the model. In the original configuration, the feeding pressure of tunicates was assumed to increase the filtering capacity of cultured mussel operations by 15% (Filgueira et al. 2014, 2015a). According to the average pumping rate measured in this current study,  $1.6 \text{ L h}^{-1} \text{ ind}^{-1}$  for a  $90.3 \pm 1.4 \text{ mm}$  tunicate, the filtration pressure of the original model corresponds to approximately 225 *S. clava* individuals ( $90.3 \pm 1.4 \text{ mm}$ ) per sleeve.

Recently, Comeau et al. (2015) has shown that the increase in filtration capacity of tunicates could range from 30–47% to 150–171% for sleeves that did or did not receive antifouling treatment, respectively. Accordingly, three scenarios were run to test the effect of different fouling scenarios: 0%, 47% and 171%, representing an absence of invasive tunicates, treated, and untreated sleeves, respectively. These scenarios correspond approximately with 0, 700 and 2500 *S. clava* individuals ( $90.3 \pm 1.4 \text{ mm}$ ) per sleeve, respectively. This report assumes that no occlusion is occurring between mussels and tunicates in terms of filtration capabilities. Thus, presenting the worst case scenarios in terms of feeding pressures.

For comparative purposes, the impact of these infestation scenarios has been compared to the baseline scenario presented in Filgueira et al. (2014, 2015b). The comparison has been carried out in terms of net impact on phytoplankton populations, using chlorophyll concentration ( $\mu\text{g chl L}^{-1}$ ) as a proxy for phytoplankton biomass.

## RESULTS

### Clearance rate

A total of 59 *S. clava* were used for particle depletion analysis in feeding trials. The mean total length ( $\pm$  SE, including the peduncle) of tested individuals was  $90.3 \pm 1.4$  mm with maximum and minimum lengths of 111 mm and 63 mm, respectively. Individual total dried tissue weight averaged  $0.27 \pm 0.009$  g (mean  $\pm$  SE). Clearance rates (mean  $\pm$  SE) for tunicates averaged  $1.6 \pm 0.1$  L h<sup>-1</sup> ind<sup>-1</sup> or  $6.7 \pm 0.7$  L h<sup>-1</sup>g<sup>-1</sup> of dried tissue weight. A total of 24 mussels were used for particle depletion analysis in feeding trials. The mean ( $\pm$  SE) shell length of mussels was  $51.2 \pm 1.1$  mm with maximum and minimum shell length of 60.6 mm and 41.1 mm, respectively. Individual total dried tissue weight was  $0.384 \pm 0.024$  g. Mean clearance rates for mussels ranged from  $4.3 \pm 0.3$  L h<sup>-1</sup> ind<sup>-1</sup> to  $12.5 \pm 1.12$  L h<sup>-1</sup>g<sup>-1</sup> of dried tissue weight. No significant allometric relationship between CR and individual size (length or dried tissue weight) was detected for tunicates or mussels.

### Predation on zooplankton assemblages

Trial assessments of mussel and tunicate predation on zooplankton showed that overall zooplankton abundances were fairly low (Figure 6) with crustaceans being the dominant taxonomic group. Comparisons between control and experimental chambers using all zooplankton classes combined showed that retention efficiency (mean  $\pm$  SE, n= 25) on zooplankton by *M. edulis* and *S. clava* was approximately  $35 \pm 9.4\%$  and  $28 \pm 9.0\%$ , respectively (Tables 2 and 3). Both species displayed a superior retention of copepods, which was the most abundant group. Copepods included numerous individuals from the order Harpacticoida, which are considered as benthic copepods. Bivalve larvae were detected in all ten trials and other ascidians (colonial tunicate species) were collected in one experimental trial (Tables 2 and 3).

### Predation on lobster larvae (stage I and IV)

Predation on stage I and IV lobster larvae by *M. edulis* and *S. clava* was not demonstrated (i.e. no lobster larvae were captured, filtered or ingested by either test species). However, some stage I lobster larvae were particularly hard to find following incubation, mainly due to the accumulation of feces and pseudofeces on the bottom of the feeding chamber and fragments of *Artemia* sp. (lobster larvae conditioning food source). The following year we attempted a different approach where trials were performed with stage I lobster larvae (n = 25 repetition) and relied on dissections of each filter feeder to determine the potential for entrapment, filtration, or ingestion of lobster larvae. In all cases, no lobster larvae were found in the gut, gills, palps, mucus net region, or cavity of the test animals.

Using the lobster larvae (stage I and IV) remaining after the above mentioned feeding trials, some quick ad-hoc trials were done on an exploratory basis. Instead of integrating a single mussel or tunicate in each feeding chamber, we opted to add 6 individual to maximize filter-feeders densities. Up to 50 lobster larvae were added to trial chambers to maximize predator-prey contact. Results consistently demonstrated no retention of lobster larvae (stage I and IV) by *M. edulis* and *S. clava*.

### **Bay scale modelling (predation on phytoplankton)**

Three different infestation scenarios of *S. clava* were explored and compared to the baseline aquaculture scenario (Filgueira et al. 2014, 2015b), which assumes an increase in filtration pressure of 15% caused by tunicates. The reduction in fouling filtration capacity from 15% to 0%, that is, the absence of invasive tunicates, caused an increase in phytoplankton concentration in the bay (Figure 7a), with a maximum increase of  $0.39 \mu\text{g chl-a L}^{-1}$  in Darnley Basin. As expected, the most significant effects from changing fouling filtration capacity were observed in aquaculture areas where tunicates are located. The increase of filtration capacity to 47%, which corresponds with some degree of fouling treatment, caused a reduction of chlorophyll in the system, with a maximum reduction of  $0.72 \mu\text{g chl-a L}^{-1}$  in Darnley Basin (Figure 7b), where current chlorophyll concentration is  $\sim 3.83 \mu\text{g chl-a L}^{-1}$ . The total absence of fouling treatment, which increases filtration capacity up to 171%, causes a maximum reduction of  $1.72 \mu\text{g chl-a L}^{-1}$  (Figure 7c) in Darnley Basin, a reduction of  $\sim 45\%$  compared to the current chlorophyll levels in that area ( $\sim 3.83 \mu\text{g chl-a L}^{-1}$ ).

## **DISCUSSION**

### **Predation on phytoplankton**

Solitary tunicates, such as the clubbed tunicate *Styela clava*, are recognized as nuisance species in many aquaculture settings, since they may cause considerable economic losses (Carver et al. 2003; Ramsay 2014). They reproduce seasonally (Bourque et al. 2007), producing free-swimming larvae that settle on mussel socks, where they grow rapidly (Boothroyd et al. 2002). Solitary tunicates are also efficient filter-feeders that are capable of depleting suspended particles or even controlling phytoplankton communities in the water column (Thompson and MacNair, 2004). For instance, solitary tunicates in longline mussel farms may increase filtration rates (per unit lease area) by 150–171% in farms not using any mitigation measures against tunicates (Comeau et al. 2015). Clearance rates data for *S. clava* presented in this report is consistent with Comeau et al. (2015) who reported  $0.6 \text{ L h}^{-1} \text{ ind}^{-1}$  for relatively small individuals ( $< 40 \text{ mm}$  body length). For comparisons purposes, the smallest individual tunicate used in our trials (66 mm body length) reported a clearance rate of  $0.9 \text{ L h}^{-1} \text{ ind}^{-1}$  whereas our mean individual (90 mm body length) showed a mean clearance rate of  $1.6 \text{ L h}^{-1} \text{ ind}^{-1}$ . However, it is important to highlight the high CR variability, which resulted in non-significant allometric relationships with regards to total length as well as dried tissue weight. This is not surprising since commercial size

mussels were targeted in this study, and that tunicate tunic length (including the foot) is very variable between individuals.

### **Predation on zooplankton, including lobster larvae (stage I and IV)**

Both *M. edulis* and *S. clava* have been shown to feed on their own larvae and larvae of other species such as gastropods, barnacles, polychaetes and echinoderms (André and Rosenberg 1991, Osman and Whitlatch 1995, Davenport *et al.* 2000, Lehane and Davenport 2002, 2004). Perturbations at the zooplanktonic level can alter the energy transfer to higher trophic levels since zooplankton is preyed on by small fishes, especially fish larvae (Gibbs 2007). Filtered but non-ingested zooplankton (i.e. crustaceans) may become mucus-bound and expelled in pseudofaecal particles. They are usually dead or moribund (Davenport *et al.* 2000). Predation on zooplankton by *M. edulis* and *S. clava* was also observed in this research; however, since zooplankton concentrations were low in the feeding chambers, the uncertainty on zooplankton retention results is high. This artefact is mainly due to the size of the feeding chambers which contain a small volume of water (1.9L) for work on zooplankton. Despite this uncertainty, copepods were the group that was most commonly taken up in relation to its relative abundance in our trials. Copepods are very common crustaceans and are one of the most numerous metazoan groups in aquatic communities (Turner, 2014) and mussels have been shown to feed them (either whole or in parts) in the literature (Robinson *et al.* 2002, Maar *et al.* 2008).

Our results on lobster larvae predation by mussel are in agreement with Gendron *et al.* (2003) who also did not record any ingestion of lobster larvae in 4L beakers with the exception of two occasions (<1%) where a larva was seen entering a filtering mussel and subsequently did not come back out. These authors were cautious with their observations since those isolated situations occurred both at the end of trials when lobster larvae were less active. In our case, even with unrealistic densities of lobster larvae incubation in a confined space with filter-feeders (mussels or tunicates), we observed no loss of larvae (stage I or IV).

### **Bay scale modelling**

Bay scale modelling is a crucial tool to represent present or future scenarios of tunicate infestations in mussel aquaculture areas. The output presented in this work demonstrates the important contribution of treatments and mitigation measures on the overall filtration pressure attributed to tunicates on PEI. Low densities of *S. clava* presently reported in Malpeque Bay (Comeau *et al.* 2017) are a direct reflection of the industry's intense mitigation activities conducted in mussel farms to control this invasive species. Their efforts result in lower cumulative feeding pressure on phytoplankton populations at the bay-scale level by the commercial species (*M. edulis*) and its invader (*S. clava*). Furthermore, Comeau *et al.* (2017) conducted an extensive biomass survey of mussels and tunicates in several mussel producing estuaries on PEI. They concluded that a low proportion (< 11%) of samples collected contained invasive tunicates, showing that mitigation practices in place are working well. Having a good



understanding of tunicate counts and size is key to understand the impact of fouling on ecosystem functioning via ecosystem modelling. For example, the average size of *S. clava* individuals collected during this survey was  $32.1 \pm 3.9$  mm, which is approximately one third of the average size of tunicates ( $90.3 \pm 1.4$  mm) we used in our research. This mismatch increases uncertainties in modelling predictions. Accordingly, large scale biomass surveys such as Comeau et al. (2017), paired with robust CR measurements, are key information to conduct meaningful numerical model outputs for aquaculture management purposes.

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Figure 1 – Fisheries and Oceans Canada, Gulf Region, Portable aquatic lab (PAL).

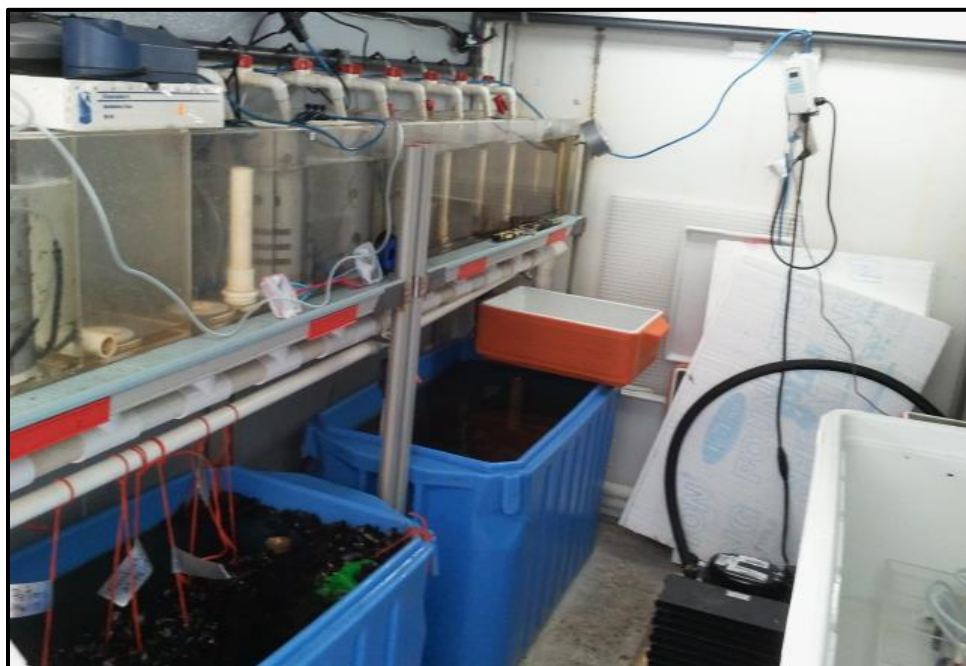


Figure 2 – Tank sizes available inside the PAL.

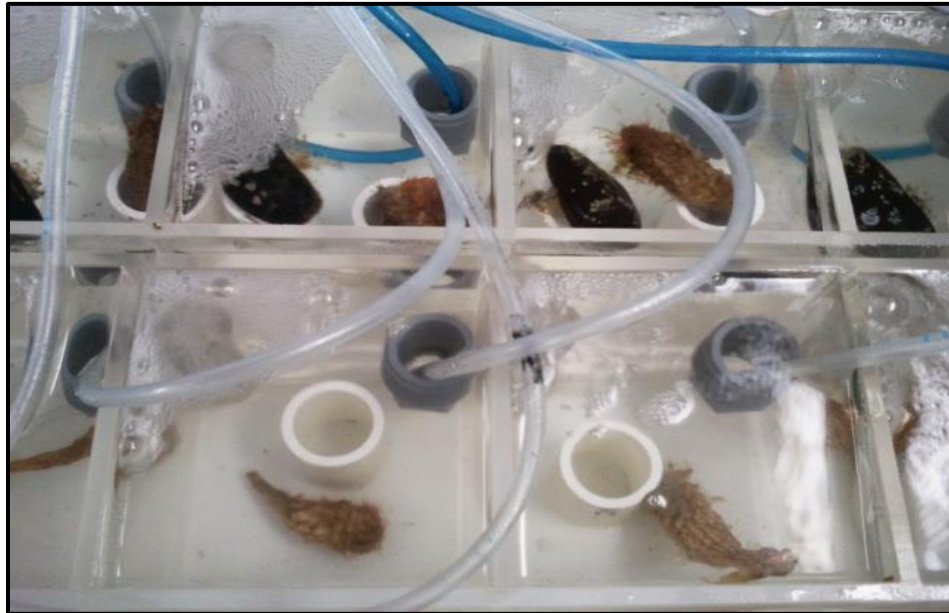


Figure 3 – Acrylic feeding chambers (volume of 1.9L each).



Figure 4 – Cooler with aerators containing thousands of lobster larvae.

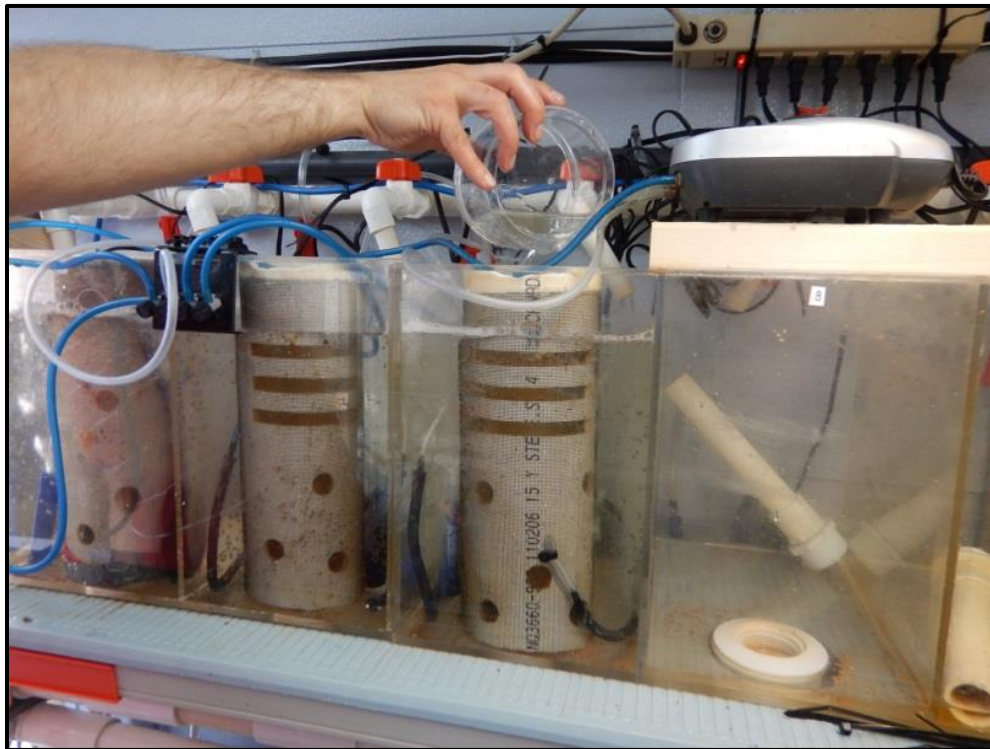


Figure 5 – Upwellers for rearing larvae in the mobile laboratory facilities.

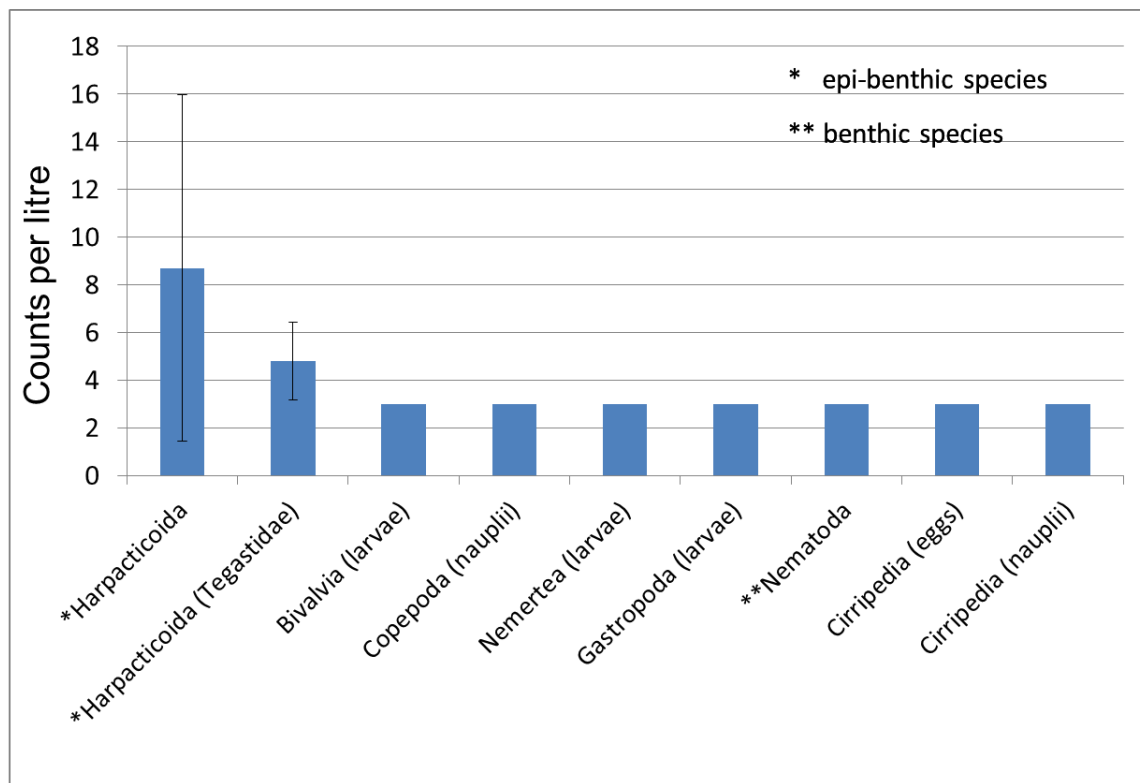


Figure 6 – Zooplankton counts (mean  $\pm$  SE) for the retention efficiency trials by *Mytilus edulis* and *Styela clava*.



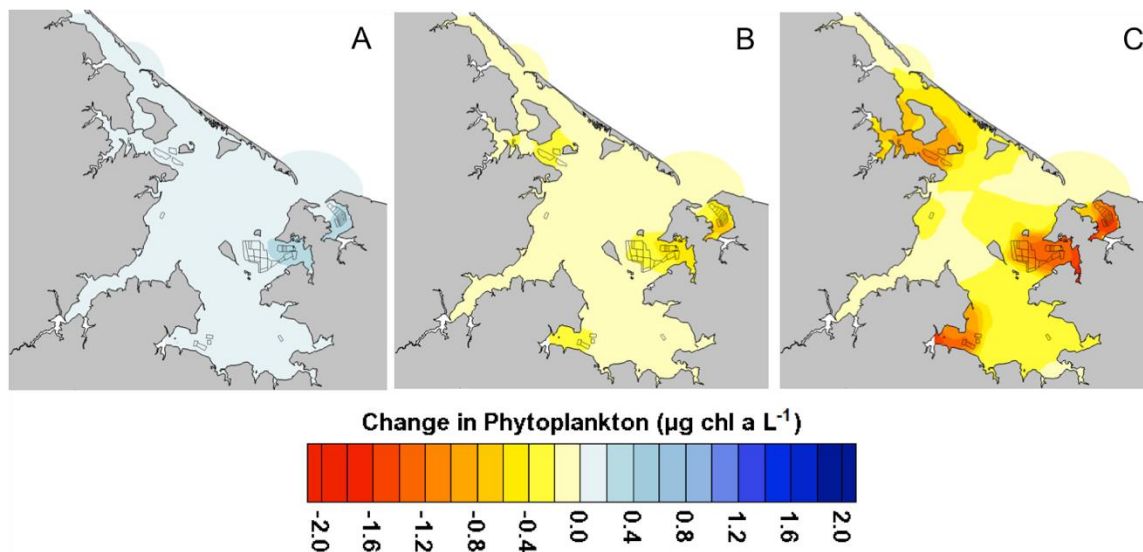


Figure 7 – Model results for the net change in phytoplankton concentration in a scenario without fouling (A), with an increase in filtration capacity of 47% due to the presence of treated fouling (B) and with an increase in filtration capacity of 171% due to untreated fouling (C), compared to the Filgueira et al. (2014, 2015) scenario, which assumes an increase of filtration activity of 15% due to fouling.



Table 1 – Experimental trials information for predation on lobster larvae (stages I and IV) by *Mytilus edulis* and *Styela clava*.

<b>Year</b>	<b>Trials #</b>	<b>Trials repetitions</b>	<b><i>M. edulis</i> / chamber (n =replicates)</b>	<b><i>S. clava</i> / chamber (n = replicates)</b>	<b># Lobster larvae</b>	<b>Larval stage</b>	<b>Larvae Analysis</b>
<b>2015</b>	1	1	1 (n=5)	1 (n=5)	10	I	<b>Recounts</b>
<b>2015</b>	2	1	3 (n=5)	5,6,7,9 (n=5)	20	I	<b>Recounts</b>
<b>2015</b>	3	1	3 (n=5)	6 (n=5)	25	IV	<b>Recounts</b>
<b>2016</b>	4	5	1 (n=25)	1 (n=25)	10	I	<b>Dissections</b>

Table 2. – *Mytilus edulis* zooplankton feeding trials results: counts and taxonomic information. Results from post-incubation control (red) and experimental (black) trials are presented.

	Trial 1						Trial 2					Trial 3					Trial 4					Trial 5								
	Control	Mussel 1	Mussel 2	Mussel 3	Mussel 4	Mussel 5	Control	Mussel 1	Mussel 2	Mussel 3	Mussel 4	Mussel 5	Control	Mussel 1	Mussel 2	Mussel 3	Mussel 4	Mussel 5	Control	Mussel 1	Mussel 2	Mussel 3	Mussel 4	Mussel 5	Control	Mussel 1	Mussel 2	Mussel 3	Mussel 4	Mussel 5
Polychaeta (larvae)			3																											
Ostracoda																						3	3							
Nemertea (larvae)	3								3	3			3		3				3								3			
Gastropoda (larvae)	3		3	3							3							3				3					3			
Copepoda (eggs)								3		3	3														3					
Copepoda (nauplii)	3		3	3		3	3	3					3	3					3										3	
Cirripedia (cypris)													3																	
Cirripedia (nauplii)																		3												
Bivalvia (larvae)	3		3	3	3	3	3	3	3				3	3	3				3		3				3	3			3	
*Harpacticoida	6		6	6	3	6	9	6	6		6	6	6	6	6	3			6	3	3		3	3					3	
*Harpacticoida (Tegastidae)	6	3	9	3	3	3	3	6					6			3			3			3				6	3	3		
**Nematoda				3					3																					
**Hiatella arctica (juvenile)					3																									
**Gastropoda (juvenile)											3																			
**Corophiinae (dam)															3															
**Ascidacea (colonial)												3																		

\* indicates epi-benthic species

\*\* indicates benthic species

Table 3. – *Styela clava* zooplankton feeding trials results: counts and taxonomic information. Results from post-incubation control (red) and experimental (black) trials are presented.

	Trial 1					Trial 2					Trial 3					Trial 4					Trial 5				
	Control	Tunicate 1	Tunicate 2	Tunicate 3	Tunicate 4	Tunicate 5	Control	Tunicate 1	Tunicate 2	Tunicate 3	Tunicate 4	Tunicate 5	Control	Tunicate 1	Tunicate 2	Tunicate 3	Tunicate 4	Tunicate 5	Control	Tunicate 1	Tunicate 2	Tunicate 3	Tunicate 4	Tunicate 5	
Polychaeta (trochophore)		3																							
Ostracoda																								3	
Nemertea (larvae)	3													3					3						
Gastropoda (larvae)	3	3			3									3				3	3					3	
Evadne nordmanni													3												
Copepoda (eggs)																							3		
Copepoda (nauplii)	3	3		3		3	3		3					3	3	3							3	3	
Cirripedia (cypris)											3														
Cirripedia (nauplii)														3											
Bivalvia (larvae)	3	3		3		3	3	3						3	3				3				3		
*Cyclopoida								3																	
*Harpacticoida	27	15	6	6	12	3	9	9	6		3	6	9	3	3	3	3	3	3	6		3	3	3	
*Harpacticoida (Tegastidae)	6	3		3						6					3			3				3			
**Nematoda					3			3					3								3				
**Monocorophium acherusicum												3													
**Caprellidae (dam)											3														

\* indicates epi-benthic species

\*\* indicates benthic species