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Experimental tests of *Littorina* Herbivory on the Basin Head Form of Irish Moss (*Chondrus crispus*)

Essais expérimentaux avec le mollusque herbivore *Littorina* sur la forme de de la mousse d'Irlande (*Chondrus crispus*) présente à Basin Head

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ABSTRACT

Basin Head is a small lagoon on the eastern shore of Prince Edward Island. The Irish Moss (*Chondrus crispus* Stackhouse) in the lagoon has not been faring well over the past few years. Various hypotheses have been brought forward to explain the recent loss in biomass from this red alga. Field and laboratory experiments were performed to test herbivory pressure on the Irish Moss. *Littorina* is the primary herbivore in the area of the Irish Moss bed in the lagoon. The snail was placed in close contact with the alga in field cages and laboratory tanks, with no significant detrimental effect on the *Chondrus*. The secondary effects of eutrophication in the lagoon (deposition of flocculent organic ‘sludge’, blooms of *Ulva*, low oxygen events) are suggested as more likely causes for *Chondrus* decline.

RÉSUMÉ

Basin Head est une petite lagune de la côte est de l’Île-du-Prince-Édouard. La mousse d’Irlande (*Chondrus crispus*, Stackhouse) présent dans la lagune ne se comporte pas bien depuis quelques années. Diverses hypothèses ont été avancées pour expliquer la perte récente de biomasse de cette algue rouge. On a mené des expériences sur le terrain et en laboratoire pour tester l’incidence des organismes herbivores sur cette algue. *Littorina* est le principal mollusque herbivore présent dans le secteur occupé par la mousse d’Irlande dans la lagune. Le mollusque a été mis en contact étroit avec l’algue dans des cages sur le terrain et dans des réservoirs en laboratoire, et aucun effet négatif important n’a été observé chez *Chondrus*. Les effets secondaires de l’eutrophisation de la lagune (dépôt d’une boue organique floculante, poussées d’*Ulva* et épisodes de faibles concentrations d’oxygène) seraient les causes les plus probables du déclin observé chez *Chondrus.*

INTRODUCTION

The Basin Head lagoon on the eastern shore of Prince Edward Island is known for a unique form of the red alga Irish Moss (*Chondrus crispus* Stackhouse). The seaweeds are much broader and more deeply coloured than the form most often seen in nature on exposed shores near the low tide mark. The Basin Head Irish Moss is also interesting in its association with mussel clumps embedded in the muddy bottom in the central portion of the northeast arm of the lagoon. The mussels hold onto dense clumps of Irish Moss via byssal threads (Sharp et al. 2003). It appears that drifting clumps of detached *Chondrus* (the plants normally attach to hard substrata via holdfasts[1]) were 'captured' by these mussels and glued in place, purpose unknown[2]. Perhaps the mussels benefit by some mechanical protection from predation (e.g. crabs), or camouflage. The *Chondrus* benefits from this association because the mussels hold the plants in place, preventing them from drifting onto shore as wrack or into deeper waters. The mussel clumps also occur in higher current areas that are rarely exposed at low tides – this habit protects the plants from desiccation and aids in gas exchange and nutrient uptake, enhancing growth.

The *Chondrus* population in the lagoon has been decimated in recent years. There are several hypotheses for this loss in biomass[3]:

- Loss of available substrate (i.e. the mussel patches)
- Mussels detaching from *Chondrus*
- Illegal harvesting of *Chondrus*
- Eutrophication effects
- Alterations in hydrodynamics
- Herbivores attacking *Chondrus*

There is not much evidence for the first 3 hypotheses. Recent surveys at Basin Head (2007 and 2008, performed by the author and members of his field crew) indicate that the mussel patches are still present and viable (although recruitment of new mussels may be an issue). If there had been wholesale detachment of Irish Moss, someone would have noticed the resulting tonnes of *Chondrus* rolling loosely about the lagoon[4]. No Irish Moss harvesting has been observed (this would have been a large scale activity, considering the tonnes of plant material that has been lost).

Eutrophication effects are suspect, as nitrogen loading to this lagoon is high (Crane pers. comm.). There are large blooms of *Ulva* (and associated crashes and water column anoxia) just to the north and east of the main *Chondrus* bed. Many *Ulva* plants drift loosely around this area and over the main *Chondrus* bed, often covering mussel clumps and even the *Chondrus* itself. The water around the main *Chondrus* bed is often murky and full of brown particulate matter (that material is flocculent enough to suspect an organic origin). The *Ulva*, particulates, and low oxygen events are all potentially detrimental to *Chondrus* growth and the associated mussels.

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[1] A few plants were discovered in the summer of 2008 attached via holdfasts to some rope tied to a buoy in the main part of the lagoon. This is the first observation of more normal attachment.
[2] The author has also seen young mussel spat attach to fragments of *Chondrus* in tumble culture tanks in the laboratory; this behaviour appears to be common.
[3] Suggested by a meeting of technical experts in the fall of 2008.
[4] Although chronic low level loss of the *Chondrus* may be an important, but undetected, factor – see conclusion section of this report.

The only connection between Basin Head and the open sea is an artificial channel. The channel has been damaged by recent storms and rebuilt during approximately the same time frame that the *Chondrus* started to disappear. The hydrodynamics of the lagoon are now being studied.

The final hypothesis in the list, herbivory, is the subject of this report. The hypothesis was tested by 2 experiments, field and lab. The dominant herbivore in Basin Head is the grazing snail, *Littorina*; most likely *L. littorea* L. The snail is very abundant in the *Chondrus* bed in Basin Head, dominating over any other mid-sized invertebrate. The author has not seen sea urchins anywhere in the area of the main *Chondrus* bed in Basin Head, and other potential herbivores (e.g. limpets) appear to be absent as well. *Littorina littorea* does well in nutrient enriched waters (Karez et al. 2004).

Littorina is known to have an appetite for macrophytic seaweeds (Scott and Marsham 2006), especially filamentous forms (Harley 2006). *Littorina obtusata* L. is a species found in Atlantic Canada which can feed on the relatively robust and 'tough' rockweed, *Ascophyllum nodosum* L. (Toth and Pavia 2006). Carlson et al. (2006) note that *Littorina littorea* grazes preferentially on macroalgal germlings, diatoms, and ephemeral green algae such as *Ulva*, *Enteromorpha*, and *Cladophora*.

MATERIALS & METHODS

Field Herbivory Experiment

Concrete patio slabs (76x61 cm, 45 kg each) were used as bases for herbivore exclusion cages. Three large eye bolts were drilled in each slab to act as cage anchor points (Figure 1). Stainless steel flour sifters 26.5 cm in diameter with a 10 cm deep bowl shape were turned into herbivore exclusion cages. 'Fine mesh' sifters had a 1.5 mm screen and 'coarse mesh' sifters had a 4 mm screen. Sifters were attached to 31 cm diameter white plastic pail lids via 2 hook bolts. Foam pipe insulation 1 cm thick was used as a gasket to seal the sifter to the lid (Figure 1).

The cages were transported to the field where approximately 80 g fresh weight of Irish Moss was placed in each cage. 'Fresh weight' was measured after spinning the plants in a mesh SCUBA goodie bag to remove excess moisture. Half of the cages were provided with 8 snails each while the other cages received no snails. The snail shell size was 16 – 21mm. The snail number to *Chondrus* biomass ratio was similar to that seen in the main *Chondrus* bed in this area. That ratio was determined by haphazardly collecting clumps of *Chondrus*, removing and counting all snails, and then taking fresh weights as described above.

The field experiment began on May 17, 2007. Two cages (one coarse mesh, one fine) were attached to each patio slab via tie wraps through the eye bolts (Figure 1). The cages were assembled on the east shore of the northeast arm, right next to the main Irish Moss bed and then carried directly into the water and placed in a line on the bottom about 2 m apart (Figure 2). This location is on the west side of the channel which drains the central portion of the northeast arm of the Basin Head lagoon (Figure 2). Just to the west and south of this area is a cottage development with a gazebo and steps leading to the shore. The bottom in this general area has supported a consistent bed of Irish Moss (attached to mussel clusters via byssal threads) for over a decade. At this site, approximately 30 cm of water would cover the cages on a low tide, and 2 m of water on a high tide.

Laboratory Herbivory Experiment

The results of the field-based herbivore exclusion experiment were confounded by the deposition of flocculent particulate matter and potential oxygen problems. A laboratory experiment was conducted to control for the 'sludge' effect observed in the field-based experiment.

In July 2007, an aquarium array was set up at the Bedford Institute of Oceanography Fish Lab (Dartmouth, NS). This consisted of a series of 8 glass aquaria (5.5 gal each) filled to about 10 cm depth (6 L) with 20 ppt filtered salt water from the Fish Lab supply (Figure 3). Water was not exchanged for the duration of the experiment (i.e. batch culture). Water temperature stayed at 19 to 22ºC for the duration of the experiment, with continuous air stone aeration. The salinity and temperature were chosen to match Basin Head waters at that time of year.

Four banks of paired fluorescent lights were suspended above the tanks on a 15:9 light:dark cycle (on 6 AM, off 9 PM), to match a summer light cycle. The lights provided 80 to 160 µEinsteins • m^{-2} • s^{-1} at the water surface, a low light level but enough to keep the plants healthy[5].

On July 26, 2007, approximately 1 kg wet weight of Irish Moss was collected from Basin Head in the area of the main bed by the gazebo steps, along with about 100 *Littorina* snails. The water was notably murky, and the plants were dusted with flocculent particulate material.

That same night, the tanks were each stocked with about 80 g wet weight of this *Chondrus*, with four tanks receiving 8 snails each. This is a similar *Chondrus* / snail ratio to the field experiment and in the wild as well. The experiment ran from July 26 to August 9 (15 days).

RESULTS

Field Experiment

The field experiment was conducted from May 17 to June 12, 2007. When the cages were opened on June 12, 2007, they were in good condition and not overgrown by epiphytes (Figure 4). All plants were washed to remove debris and invertebrates prior to weighing. Although all 'snail' cages retained their snails in good condition (just one mortality in coarse mesh cage #1) and the 'no snail' cages excluded new snails from entering (with one exception, a single snail in coarse mesh cage #7), many small amphipods (and some scale worms) were able to enter the cages irrespective of mesh size (Figure 4). Many of the snails in the cages had attained a shell size of about 23 mm, indicating some growth over the experimental period.

The results of the field experiment are shown in Table 1. The Irish Moss lost weight over the 27 day experiment in all cages. In general, the plants in the coarse mesh cages appeared to be quite healthy and in good color with little fragmentation. However, the plants in the fine mesh cages were often fragmented and discoloured (greenish) at the margins.

[5] No data to substantiate this claim. However, these same lights were used for a tumble culture system developed from these aquaria in the fall of 2007. The Basin Head *Chondrus* has been growing happily in this tumble culture since December 2007.

All of the data in Table 1 were subjected to a Two-Factor ANOVA with replication (i.e. snails as main factor, mesh size as second factor). The average weight loss of plants exposed to snails versus those protected from snails was not significantly different ($P>0.05$). However, the average weight loss in all coarse mesh cages was significantly less than the weight loss in the fine mesh cages ($P<0.05$).

Weight loss seems to be driven by the use of fine mesh, rather than the presence of snails. Even the presence of amphipods (detrital scavengers) did not seem to affect the plants – they were actually more abundant in the coarse mesh cages (25 – 200 individuals per cage) than the fine mesh cages (10 – 100 individuals).

Laboratory Experiment

By the end of the experiment, some evaporation had occurred and the water in the tanks had risen from 20 to 25 ppt. This salinity change is considered insignificant to the physiology of the *Chondrus* or the snails.

Snails occasionally climbed out of the water, but were placed back in as they were discovered. All snails were alive at the end of the experiment. Organic debris appeared to build up in the tanks as the experiment progressed, but this may have been particulate material that fell off the plants and settled to the bottom over time (Figure 5).

The *Chondrus* in all the tanks appeared to be in good condition at the end of the experiment with very little fragmentation or green discoloration (Figure 5). Snail ‘chew’ marks (round edge holes, pits) were not observed. Snails appeared to actively graze flocculent particulate material and epiphytes on the *Chondrus*, while not harming the Irish Moss itself (Figure 3). This observation is consistent with the growth observed in the tanks. In some tanks the Irish Moss lost weight, and in some weight was gained (Table 2). However, any observed weight loss was less than that seen in the field experiment. There was no significant difference in weight change associated with the presence of snails ($P>0.1$, Table 2).

CONCLUSIONS

The presence of herbivores (snails) or detritivores (amphipods) did not significantly influence Irish Moss biomass in cage exclusion experiments. However, the experimental design may have inadvertently tested for another factor influencing *Chondrus* growth in Basin Head.

The lagoon is eutrophic and flocculent material (probably of organic origin) covers all surfaces (the clay bottom substrate, mussels, *Chondrus* and *Ulva*, stray debris, etc.). Our cages appear to have amplified this deposition effect. All cages collected this particulate material, especially the fine mesh cages. Field notes from June 12, 2007, indicate ‘moderate’ or ‘light’ particulate build-up in the coarse mesh cages, and ‘heavy’ particulate build up in all of the fine mesh cages. It is reasonable to expect that the fine mesh cages would trap more flocculent material. Boundary layer currents are less likely to enter and ‘clean out’ a fine mesh cage versus a coarse mesh cage.

Notably, one concrete slab at the end of the line of slabs appears to have been in a location that supported higher flocculent material deposition rates than the others. The fine mesh cage (#12) on that slab contained more organic particulate material than any other cage in the experiment; it resembled a thin sludge that gave off a hydrogen sulphide odour when disturbed.

The fine mesh cages were associated with heavy accumulations of sludge and potentially anoxic conditions as a result (the H_2S noted in cage #12). The fine mesh cages also had significantly higher weight loss of Irish Moss than the coarse mesh cages.

Although the cages were placed in an area of strong tidal flow, the fine mesh cages attracted flocculent material. It is reasonable to assume that the secondary effects of eutrophication (i.e. organic particulate build-up with associated reduced oxygen exchange) are detrimental to *Chondrus* survival in Basin Head. This effect may be very site specific, occurring most strongly in small patches of low current flow related to depressions or obstructions on the bottom.

Findings of the laboratory experiment are consistent with those of the field experiment. In both instances the presence of herbivores at densities similar to field conditions does not significantly affect *Chondrus* growth.

The poor growth rate of *Chondrus* in the lab was probably due to sub optimal conditions. The tanks were batch culture and so did not receive a consistent salt water inflow to provide a carbon supply and nutrients, and the plants were not agitated to enhance growth[6].

The presence of herbivores (snails) and detrital scavengers (amphipods) does not seem to affect Irish Moss (*Chondrus*) growth in Basin Head. *Chondrus* is a sturdy seaweed and the presence of snails and amphipods probably serves to keep the plants clean of epiphytes more than anything else. Indeed, Lubchenco (1978) described *Chondrus* as a less preferred food item for *Littorina*. The density of snails used in the field and laboratory experiments was similar to that in the field, if artificially high densities had been used the snails may have had more of an effect on the *Chondrus* tissue.

The ‘sludge effect’ noted in the field experiment may be important to the maintenance of the *Chondrus* population in Basin Head. It is potentially driven by the very high nitrogen loading that the lagoon receives from local agricultural practice. The particulate material may shade or interfere with gas exchange on the Irish Moss thalli, or indicate microscale anoxic conditions – all of which could reduce plant growth and lead to fragmentation. It is even possible that mussels may ‘toss off’ attached *Chondrus* (by breaking byssal thread attachment points) as a result of anoxic or low oxygen events in order to improve water circulation through their bodies.

The fragmentation or direct loss of *Chondrus* plants by the mechanisms described above need testing in the laboratory and field. Unfortunately, these may be subtle and chronic effects.

ACKNOWLEDGEMENTS

Bob Semple remains a stalwart field hand for Basin Head. This study would not have been possible without his help. The 2007 Basin Head summer field staff, Megan MacIsaac and Crystal Larocque, were invaluable for the field study. Glyn Sharp, Megan Wilson, and Sarah Nebel provided field observations, advice and support. This study was supported by Department of Fisheries and Oceans (DFO) offices in Moncton (NB) and Charlottetown (PEI).

[6] The establishment of a tumble culture system (mentioned previously) corrected these problems. However, tumble culture is too vigorous for snails to hold onto the plants, and an herbivory experiment could not be performed under those conditions.

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Table 1. Results of the field caging experiment of herbivore effects on growth of Chondrus crispus in Basin Head lagoon, 2007.

Factor - herbivore	Slab number	Cage number	Factor mesh size	Start weight (g wet)	End Weight (g wet)	Weight difference (end – start)
With snails	1	1	Coarse	80.15	66.20	-13.95
		2	Fine	79.73	40.00	-39.73
	2	3	Coarse	79.36	56.35	-23.01
		4	Fine	81.18	40.70	-40.48
	3	5	Coarse	79.71	68.00	-11.71
		6	Fine	78.90	38.85	-40.05
Without snails	4	7	Coarse	80.23	70.75	-9.48
		8	Fine	79.90	55.90	-24.00
	5	9	Coarse	80.71	64.18	-16.53
		10	Fine	80.17	51.80	-28.37
	6	11	Coarse	79.40	57.15	-22.25
		12	Fine	78.61	46.50	-32.11

Factor herbivore	Factor mesh type	Mean weight loss	Factor herbivore	Factor mesh type	Mean weight loss	F-value
With snails	Coarse	-16.22	Without snails	Coarse	-16.09	
	Fine	-40.09		Fine	-28.16	
	Overall	-28.16		Overall	-22.12	F = 4.68 (P > 0.05)
Overall	Coarse mesh	-16.16	Overall	Fine mesh	-34.12	F = 41.5 (P < 0.05)

Table 2. Results of the laboratory aquarium experiment of herbivore effects on growth of Chondrus crispus, 2007. The 2-tailed t statistic was 0.710 and was not significant (P > 0.1).

Factor herbivore	Aquarium number	% change in weight (end – start)
With snails	1	-4
	2	5
	3	1
	4	-10
	Overall mean	-2
Without snail	5	-7
	6	12.5
	7	0
	8	-5
	Overall mean	0.125

Figure 1. Field structures used in the experiment on herbivory effects on Chondrus crispus in Basin Head lagoon, 2007. Photos show concrete patio slabs with eye bolts (left image), stainless steel flour sifters turned into herbivore exclusion cages (upper right image), and attachments of paired cages containing Irish Moss on each patio slab (one coarse mesh, one fine mesh; bottom right image).

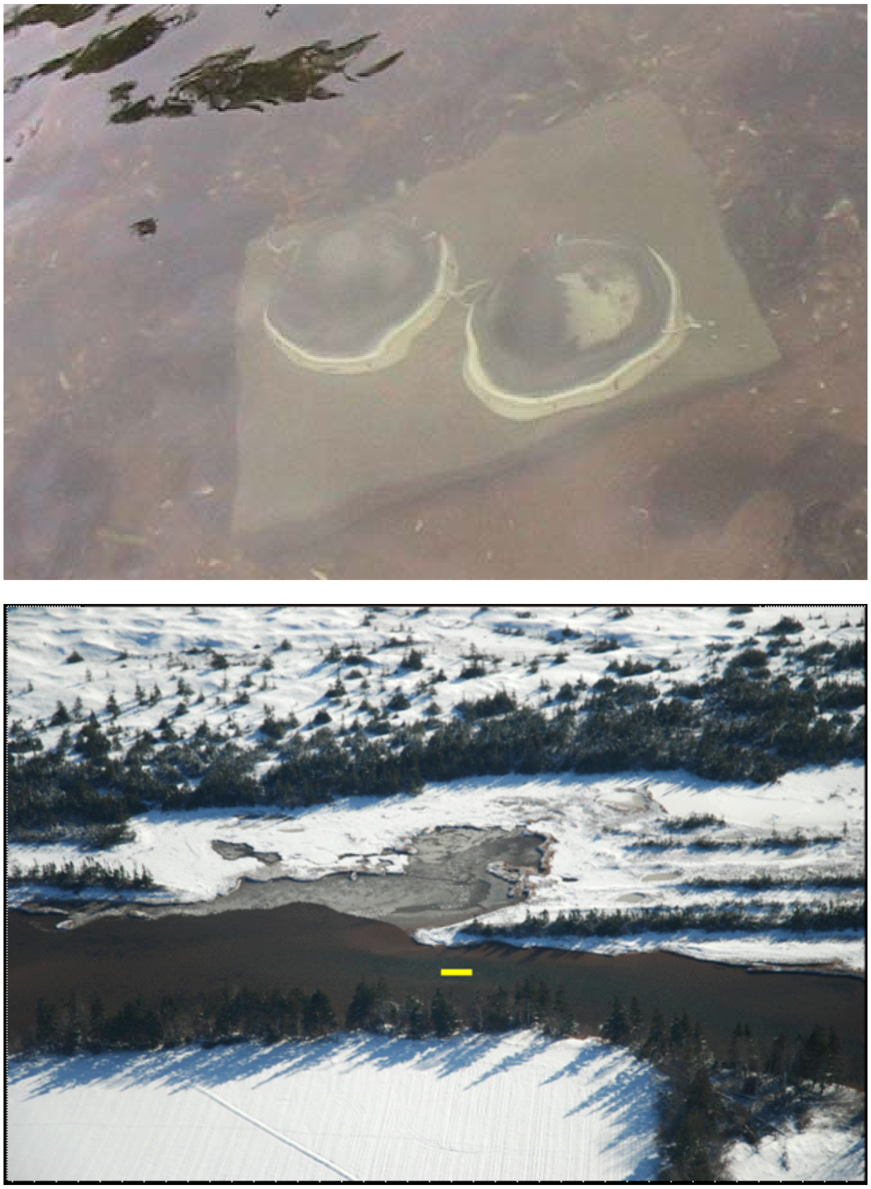

Figure 2. Placement of slabs (upper image) in a line on the bottom on the west side of the channel which drains the central portion of the northern arm of the Basin Head lagoon, and a winter air photo (bottom image) looking east over the central portion of the northeast arm of Basin Head. The exclusion cages were located in the area of the yellow rectangle. The main Chondrus bed occurs in this area and off to the right outside of this image.

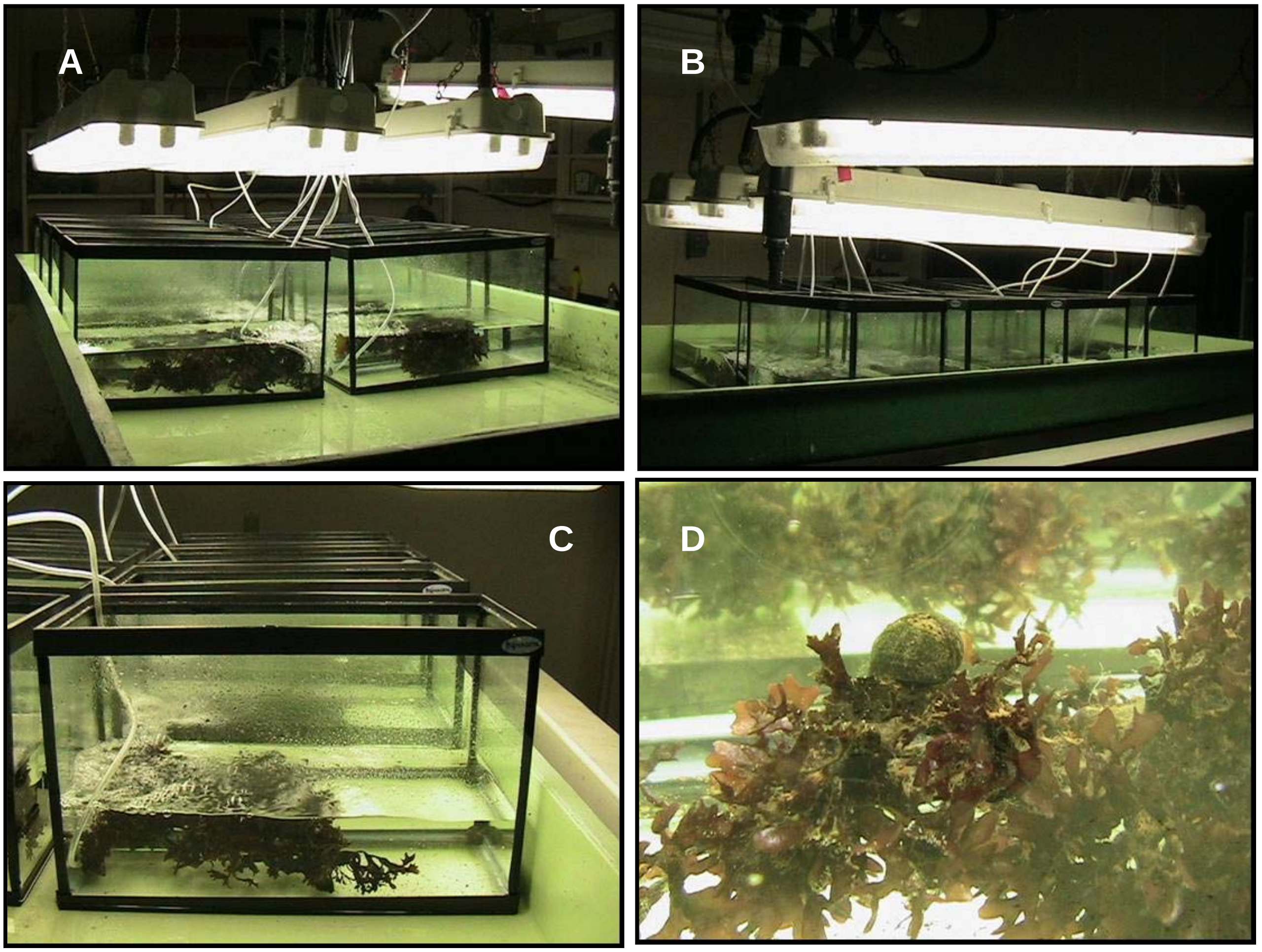

Figure 3. Aquarium set up at the Bedford Institute of Oceanography (Dartmouth, NS) used to test for effects of herbivory on Chondrus crispus growth. A) end view, B) side view, C) aquarium with air stone, Irish Moss, and note snail in back right corner of the tank, and D) snail actively grazing epiphytes on Irish Moss. All images are from July 27, 2007.

Figure 4. Retrieval, sorting and sampling of Irish Moss and cage contents on June 12, 2007. Snails were counted and measured. Bottom image is of numerous small amphipods which were able to enter the cages irrespective of mesh size. The amphipods in this image average about 3 to 4 mm long.

Figure 5. Aquaria at the end of the experiment (August 12, 2007) showing brown organic debris on bottom of the tanks, and the errant snail up the aquarium wall and out of the water (upper image) and Irish Moss in good condition at the end of the experiment (bottom image).