Preliminary study of the diel variation of ascidian larvae concentrations in Prince Edward Island.

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Abstract

The arrival of the clubbed tunicate, *Styela clava*, in Prince Edward Island (PEI) coastal waters has caused a great deal of problems for the mussel aquaculture industry. High abundances of this invasive ascidian have created the need for effective mitigation strategies. To determine the most efficient mitigation strategies and monitoring capabilities a basic knowledge of *S. clava* reproduction is necessary. The aim of this study was to document diel variations of *S. clava* larvae concentration in order to establish effective larvae sampling procedures. Preliminary results have shown that diel larval concentration variations are significant. *S. clava* larvae concentrations peaked at noon and declined gradually thereafter. A second ascidian species, *Molgula sp.*, showed a larval concentration peak at mid afternoon. Temperature and salinity were not found to be associated with diel variations of concentration. These results clearly demonstrate the effect of sampling time on the variation of larvae concentration estimates, however, generalization of results may not be possible due to varying environmental parameters that may affect the reproductive cycle of ascidians at different geographic locations.

Résumé

L'arrivée de l'ascidie plissée, *Styela clava*, dans les eaux côtières de l'Île-du-Prince-Edward (IPE) a causé de graves problèmes à la mytiliculture commerciale. Les grandes abondances de cette espèce invasive ont créé un besoin de trouver des stratégies efficaces d'atténuation des impacts. Une connaissance fondamentale de la reproduction de *S. clava* est nécessaire afin d'obtenir des stratégies efficaces et d'assurer un meilleur suivi. Le but de cette étude était de documenter les variations nycthémérales de concentration de larves de *S. clava*, afin d'établir des procédures d'échantillonnage efficaces pour les larves d'ascidies. Nos résultats préliminaires démontrent que les variations nycthémérales de concentration de larves sont significatives. La concentration de larves de *S. clava* a atteint un maximum vers midi et diminue graduellement par la suite. Une deuxième espèce d'ascidie, *Molgula sp.*, a montré un maximum de concentration larvaire vers 15h00. Les variations nycthémérales de la concentration larvaire n'ont pas pu être reliées à la température et la salinité. Ces résultats démontrent clairement l'effet du temps d'échantillonnage sur la variation de la concentration de larves mesuré. Une interprétation fine de nos résultats ne peut-être possible en raison de la forte variabilité des paramètres écologiques par rapport aux sites de culture pouvant affecter le cycle reproducteur des ascidies.

Introduction

The solitary ascidian *Styela clava* (Herdman) is indigenous to Asia; however it has been introduced and has established in many parts of the world (Lützen, 1999). *S. clava* was first identified in Prince Edward Island (PEI) coastal waters in 1998 (MacNair pers. comm.) and since then populations have established. This invasive species has caused a great deal of problems for the aquaculture industry, especially with mussel culture (Thompson and MacNair, 2004). *S. clava* attaches itself to hard substrates, such as culture gear (e.g. buoys, anchors, rope, etc.) as well as shells of mussels (Lützen, 1999). Husbandry has become labor intensive and could become cost prohibitive. Additionally, *S. clava* are filter feeders that could potentially compete directly with mussels for food resources. The high abundances of *S. clava* in culture areas have created the need for effective mitigation strategies. To obtain the most efficient mitigation strategies, reliable recruitment monitoring capabilities are needed. There is a need for reliable information of the reproductive cycle of ascidians in PEI costal waters since it is virtually unknown. Larvae concentrations in the water column may be a good indicator of ascidian recruitment on settlement structures in aquaculture sites (Bourque et al. Unpublished).

Ascidian larvae generally spend less than 24 hours in the planktonic community before settlement (Svane 1984). Because of their short lived larval stage, knowledge of diel variations in larvae concentrations is critical to acquire accurate abundance measurements for monitoring recruitment potential. Standardized larval sampling procedures are needed to acquire reliable larvae concentration data.

Many factors are responsible for variations in larval concentrations, such as spawning events, period of embryonic development and duration of larval period. Spawning generally occurs in response to a period of light following a period of darkness (Svane and Young, 1989). In fact, Lambert et al. (1981) defines the latency period as the time duration of light prior to spawning events. Studies have shown that not all ascidian species spawn at the same time of day, and that latency period can vary greatly between species (Svane and Young, 1989). Time of spawning can also vary for a given species in relation to different geographic locations. For example, *Styela plicata* (Lesueur) has been found to spawn in the evening in Japan (Yamaguchi, 1975) while in California, spawning takes place in the afternoon (West and Lambert, 1976).

In solitary ascidians, fertilization and embryogenesis are external events (Svane and Young, 1989). Both species found in the current study use this reproductive strategy. The duration of this embryonic (pre-hatching) period can also have an effect on the concentration of larvae found in the water column. Duration of the embryonic period is partly attributed to egg size and water temperature (Svane and Young, 1989). Therefore, the embryonic period could not only vary between species but also spatially and temporally.

Ascidian larval period is short lived. However, they can postpone metamorphosis for several days if no suitable substrate is available (Svane 1984). Duration of larval stage could be associated with several variables that are not fully understood, such as habitat selection (Young, 1982), presence of conspecifics (Svane et al. 1987) and light conditions (Crisp and Ghobashy, 1971).

The aim of the present study was to acquire preliminary information on diel variations of ascidian larvae concentrations and relate this to sampling procedures. The best time for larvae sampling will be determined. The period of highest larvae concentration was chosen as the best sampling time, because it corresponds to the highest recruitment potential. Tidal cycle, temperature and salinity were also evaluated as environmental factors that may influence larvae concentrations.

Materials and methods

The first sampling day was conducted on 27 August 2003 in Murray River, Prince Edward Island, Canada. This site was chosen because it had the highest abundance of *S. clava* in PEI (pers. observation). Hourly larvae sampling were carried out between 4:00 and 18:00. Sampling was conducted from a boat anchored on the outside of a mussel aquaculture growing lease located next to the channel. Temperature and salinity were measured at a depth of 1 m throughout the sampling period with a Sea-Bird Electronics CTD profiler.

Larvae samples were acquired with the aid of a Rule 2000 bilge pump. A weight was attached to the pump so it could be lowered vertically into the water column. For each sample the pump was lowered and raised approximately every 10 seconds through the first meter of water. Water was pumped through a 64 μm sieve for 3 minutes to collect larvae. Total volume of water per sample varied from 160-200 L due to the slow decrease in power from the battery. Water flow was measured hourly and taken into account when larvae concentrations were calculated. Larvae samples were done in triplicate. The sieve was rinsed with a wash bottle containing filtered ambient seawater

to transfer the sample to a storage container where they were fixed with approximately 2 ml of formalin. On a later date the samples were analysed by concentrating them to 10 ml and placing them into a Ward Counting Wheel. All tunicate larvae were then identified and counted under a stereoscope.

A second larvae sampling day was conducted on 22 September 2003 to compare results with those observed on 27 August. Procedures were similar to those described above. The date was selected in relation to tidal charts in order to sample during a reverse tidal cycle. The sampling period was shortened since results of the first sampling indicated very few larvae where found before 10:00 and after 16:00. Hourly larvae sampling was conducted between 10:30 and 16:30. Due to logistic problems, salinity was not measured during this sampling period.

Statistical significance was set at a probability level of 0.05. Statistical tests were conducted using SPSS 10.0©. Tunicate larvae concentrations for each sample time were analyzed using a one-way ANOVA. Fisher's Least-Significant-Difference Test (LSD) was applied to significant results in order to identify which groups differed. Linear regression models were applied to determine whether variation in larvae concentrations can be explained by the tidal height.

Results

Two species of ascidian larvae were observed in the water samples: 1) *S. clava* and 2) *Molgula sp*. One-way ANOVA found a significant peak in larvae concentrations for both species on 27 August (Table 1). Fisher's Least-Significant-Difference Test revealed several significant differences in larvae concentrations at different times of the

day for both species (Table 1). A significant peak in *S. clava* larvae concentrations was observed at 12:00. Larvae concentrations gradually declined thereafter (Figure 1A). *Molgula sp.* larvae concentrations started to increase at 14:00, peaked at 15:00 and declined afterwards (Figure 1A).

One-way ANOVA also found a significant peak in larvae concentrations for both species on 22 September (Table 2). Fisher's Least-Significant-Difference Test (LSD) revealed several significant differences in *S. clava* larvae concentrations at different times of the day (Table 2). Fisher's Least-Significant-Difference Test also revealed several significant differences in larvae concentrations at different times of the day for *Molgula sp.* (Table 2). *S. clava* larvae concentrations increased from 10:30 to 12:30 (Figure 1B). Larvae concentrations were significantly higher at 11:30 and 12:30. A significant peak in *Molgula sp.* larvae concentrations was observed at 13:30 (Figure 1B).

Linear regression analysis showed no relationship between *S. clava* larvae concentration variations and tidal height (Figure 1A and 1B) on 27 August (r^2=0.050; P=0.146) and 22 September (r^2=0.018; P=0.567). Measured temperature and salinity were nearly constant during the sampling period and did not explain variation in larvae concentrations. Mean temperature and salinity on 27 August were 19.0ºC and 31.6 ppt respectively and varied no more than 0.67 ºC and 0.64 ppt throughout the sampling period. Mean temperature on 22 September was 19.3ºC and varied no more than 0.2ºC during the sampling period.

Discussion

Larvae of both species of ascidians found during our sampling showed evidence of diel variations of concentration. The species found earliest in the day was *S. clava*, it was found throughout the sampling periods. Larval concentration peaked around noon on both sampling dates, and decreased steadily afterwards to pre-peak concentrations. *Molgula sp.* concentration peaked in mid-afternoon and no larvae could be found during the period of darkness (from 4:00 to 6:00).

The best sampling periods of the day for *S. clava* and *Molgula sp.* seems to be around noon and mid-afternoon, respectively. That being said, it is important to note that this may only apply to Murray River and for the sampling procedure used in this study. A possible explanation for the diel variation in larvae concentration is the vertical migration of larvae. Diel vertical migration by various zooplanktons is a common occurrence in all the world's oceans (Hays, 2003). Phototaxis has an important influence on the vertical distribution and settlement of ascidian larvae (McHenry and Strother, 2003). Some ascidians switch from positive to negative phototaxis before settling (McHenry and Strother, 2003). Larvae seem to accumulate in regions of low light intensity just before settling (Crisp and Ghobashy, 1971). Variation in larvae concentration could therefore be attributed to sampling procedures that do not take into account possible vertical migration of larvae. It is possible that actual larvae abundances earlier in the day were similar to those found during the peak concentration indicated by the sampling, but were not found in the first meter of water. Further investigations on

vertical migration of larvae are needed to develop effective sampling procedure for monitoring purposes.

Horizontal movement of larvae could also affect the distribution of larvae. Horizontal movement, at least in colonial ascidian species, depends mainly on water current, not swimming (Swane and Young, 1989). Since tidal cycles have an important influence on water current, samples were collected over two different tidal cycles to investigate it as a possible factor in the variation of larval concentration. Tidal cycle, however, did not explain the peak in larval concentration of *S. clava*. High adult abundances and larval concentrations throughout the river system could account for the lack of tidal influence on *S. clava* larvae concentration. Nonetheless, tidal effect cannot be ruled out in systems where adult abundance is reduced and distribution is patchy. The effect of tidal cycles on the larval dispersal of solitary ascidians has not been studied as extensively as for colonial species because of their smaller size. Further studies are needed to better understand the effect of tidal cycles. Larval concentrations should be compared over a complete tidal cycle and throughout the water column to confirm results found in this study.

Other possible reasons for the diel variation in larvae concentrations are environmental conditions such as light intensity and temperature that may vary spatially and temporally. Light has been identified as an important spawning trigger for many ascidian species (Svane and Young, 1989). The latency period in *S. plicata* has been shown to be influenced by the length of the dark adaptation period prior to spawning (West and Lambert, 1976). Increasing the length of the dark adaptation period resulted in a decrease of the length of the light period needed to induce spawning. If *S. clava* reacts

similarly, photoperiodism may be an important factor to take into account when conducting larvae sampling. No obvious differences were noted in the timing of the peak larvae concentration of *S. clava* between both sampling dates. However, other factors such as temperature may have had an effect on early developmental stages (further details below). Another study also showed that light intensity can control the duration of the latency period (Forward et al. 2000). Variations in light intensity could therefore regulate spawning events and also be of great importance for larvae sampling procedures. Further studies are needed to document variations of light intensity in mussel culture sites and their effect on ascidian spawning events.

It is believed that water temperature and salinity did not vary enough through the sampling period to contribute to any diel variations in larvae concentration. These parameters did not vary more than 0.67 ºC and 0.64 ppt. The slight changes in salinity were associated with the tidal cycle in the estuary. Although it did not seem to have influenced diel variations of larvae concentration, temperature may play an important role in long term monitoring programs. Studies have shown that temperature influences the duration of the embryonic period (Svane and Young, 1989). The time of the day where peak concentration occurs may change as a function of temperature. Therefore, seasonality may play an important role in selecting the appropriate time for sampling. This study did not take this into consideration therefore further investigations are needed to provide standardized sampling procedures to be used for monitoring purposes.

Conclusion

Results of this study clearly demonstrate the effect of sampling time on the variation of larvae concentration estimates, however, generalization of results may not be possible due to varying environmental parameters that may affect the reproductive cycle of ascidians at different geographic locations. Further investigations are needed to predict diel spawning activity of ascidians and improve larvae sampling techniques for monitoring purposes.

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Table 1: One-way ANOVA examining the effects of time of day on larvae concentrations on 27 August of a) *S. clava*; and b) *Molgula sp.* Multiple comparisons were carried out with a Fisher's Least-Significant-Difference Test. Time of the day for a) *S. clava* and b) *Molgula sp.* are presented in increasing order of mean larvae concentration. Non-significant differences among treatments are underlined.

	Source of variation	SS	df	MS	F	P
a)	Time	0.319	14	0.023	16.584	**<0.001**
	Error	0.040	29	0.001		
	Total	0.359	43			
b)	Time	0.344	14	0.025	4.934	**<0.001**
	Error	0.145	29	0.005		
	Total	0.489	43			

Multiple comparisons:

a) 18:00 17:00 6:00 16:00 5:00 8:00 10:00 4:00 9:00 7:00 11:00 15:00 14:00 13:00 12:00

b) 4:00 5:00 6:00 7:00 10:00 8:00 9:00 11:00 12:00 13:00 18:00 16:00 17:00 14:00 15:00

Table 2: One-way ANOVA examining the effects of time of day on larvae concentrations on 22 September of a) *S. clava*; and b) *Molgula sp.* Multiple comparisons were carried out with a Fisher's Least-Significant-Difference Test. Time of the day for a) *S. clava* and b) *Molgula sp.* are presented in increasing order of mean larvae concentration. Non-significant differences among treatments are underlined.

	Source of variation	SS	df	MS	F	P
a)	Time	0.018	6	0.003	3.273	**0.032**
	Error	0.013	14	0.001		
	Total	0.031	20			
b)	Time	0.005	6	0.001	9.000	**<0.001**
	Error	0.001	14	0.000		
	Total	0.006	20			

Multiple comparisons:

a) 14:30 15:30 16:30 13:30 10:30 11:30 12:30

b) 16:30 15:30 11:30 12:30 14:30 10:30 13:30

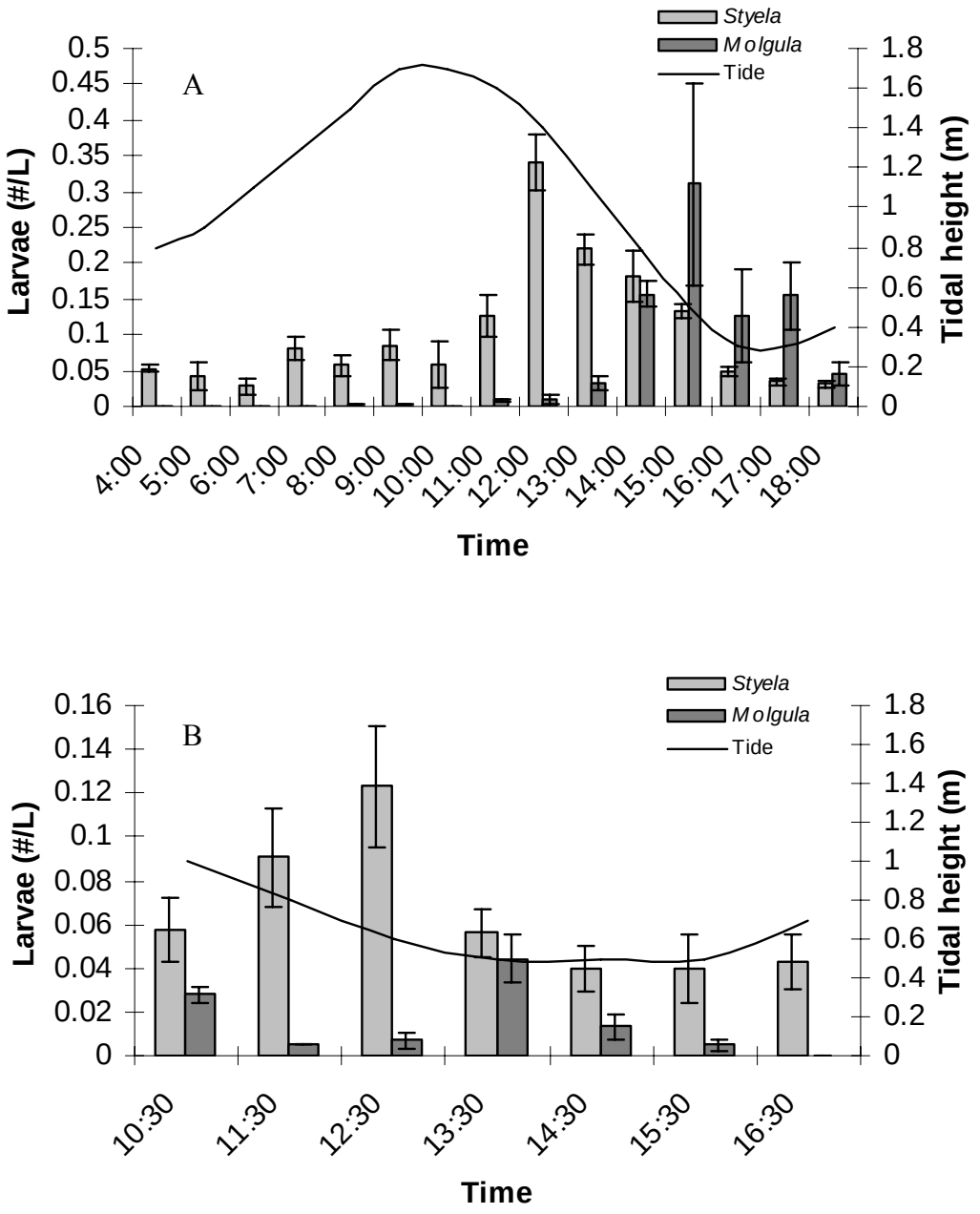

Figure 1: Number of ascidian larvae, *S. clava* and *Molgula sp.*, per liter of water (mean±SE, n=3) and tidal height on A) 27 August and B) 22 September.