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MONITORING RECRUITMENT PATTERNS OF MUSSELS AND FOULING TUNICATES IN MARICULTURE

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ABSTRACT Methods to increase the precision of spat collection and strategies to mitigate fouling are greatly needed in aquaculture production. As such, larval recruitment of mussels and a common tunicate species was investigated. Recruitment was measured in shallow (1–2 m) and deeper (4–5 m) water at three sites during the summer of 2012. In addition, to evaluate the importance of timing in deployment of mussel ropes, differences in mussel yield were examined. The settlement plates provided a good description of the settling community with high temporal resolution. Peaks in recruitment were observed for both mussels and tunicates but recruitment rates and the timing of peaks differed among sites. Although mussel larvae preferred shaded substrates at some sites and times, these substrates were consistently preferred by tunicates. Mussels preferred to settle at shallow depths, whereas tunicates were consistently more abundant deeper. In contrast to predictions, there was no positive relationship between the yield of mussels on ropes and settlement rates on corresponding weeks. Somewhat surprisingly, the final abundance of mussels and tunicates were not related to the length of the recruitment and growth period. These results indicate that not only initial recruitment, but also mortality and repeated recruitment events are important processes shaping these dynamic assemblages. Combining the results, a minimum recommendation for monitoring larval settlement is to use, at two depths, one monitoring unit with several dark-surfaced sampling plates. Considering the fact that timing of deployment of mussel ropes in relation to mussel and tunicate settlement has been identified as a problem, it is believed that such methods can be used to optimize production of mussels. Thus, studies like these can also contribute to optimize farming techniques and practices in a broader context.

KEY WORDS: larval recruitment, *Mytilus edulis*, *Ciona intestinalis*, biofouling, monitoring

INTRODUCTION

The expansion of the human population is continuing to put strain on already burdened fish stocks. Global fishery landings have maintained a stable level during the last decades, whereas most stocks of the 10 most commonly fished species are either fully or overexploited (FAO 2012). The increased demand for seafood must be met by other, more sustainable, means of production. Shumway et al. (2003) advocates the sustainability of shellfish farming where cultured stocks feed on naturally occurring phytoplankton, directly from the base of the food chain. Thus, in contrast to farming of many conventional fish species, shellfish aquaculture contributes to a large net gain in world fish supply (Naylor et al. 2000) whereas also providing benefits for the marine environment and socioeconomic development in coastal communities (Shumway et al. 2003).

The phytoplankton used by cultured shellfish thrives in eutrophicated coastal waters. This excess of phytoplankton and its impact on coastal environments through deep-water oxygen deficiency and loss of benthic fauna due to increased deposition of organic material were highlighted during the 1980s (Rosenberg 1985, Rosenberg & Loo, 1988, Rosenberg et al. 1990). Many different actions, such as the restriction of the use of fertilizers, improvement of waste water treatment plants, restoration of wetlands, and construction of sedimentation ponds, have been taken or are under way to counter these problems, and particularly around the Baltic Sea these actions are high on the political agenda (e.g., the Baltic Sea Action Plan). Lindahl et al. (2005) identified mussel farming as a potentially effective measure to reach the environmental goals established whereas Gren et al. (2009) calculated that mussel farming could be up to 11% more cost effective than other abatement measures in the Baltic Sea. Farming of filter-feeding mussels as a tool to counteract eutrophication has, however, not yet reached its full potential, mainly due to various socioeconomic and profitability reasons. Thus, the benefits of expanding the shellfish aquaculture production are numerous.

The most common method for cultivating mussels in Sweden is to collect naturally occurring mussel larvae on ropes deployed into the water column from ''long-line'' farms. Recruitment of larvae may, just like growth of mussels (Bergström et al. 2013), vary among years and locations. Although the recruitment varies, it is generally well above the typical numbers of around 400–500 individuals per meter rope at harvest of a well-functioning unit (Loo & Rosenberg 1983). Thus, the supply of larvae is generally not considered a limiting factor in production of mussels (Smaal 2002). Nevertheless, availability of wild seed may be very inconsistent (Kamermans & Smaal 2002) and dependence on natural sources poses a risk for the industry if spat recruitment fails (de Vooys 1999). Therefore, hatchery production and other methods for increasing seed availability could provide an important resource, enabling expansion of the mussel industry (Galley et al. 2010).

Since the ropes also offer an attractive substrate for a number of fouling organisms, knowledge about timing of recruitment of mussels and fouling organisms may provide opportunities for optimization of farming procedures (Sievers et al. 2014). The impact of biofouling on the aquaculture industry can be immense (see reviews by Adams et al. 2011, Fitridge et al. 2012, Lacoste & Gaertner-Mazouni 2014). Cultured shellfish may be affected by fouling in several ways: by competition for

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food and space (Lesser et al. 1992, Petersen 2007, Su et al. 2008, Daigle & Herbinger 2009), infliction of physical damage (Kaehler 1999), mechanical interference (Arakawa 1990), and by increased weight and drag from currents resulting in loss of mussel stock (Mallet & Carver 2006). Although problems with fouling can come from a wide range of organisms, perhaps the most frequent and serious problems worldwide are those caused by tunicates (LeBlanc et al. 2007, Locke & Carman 2009, Locke & Hanson 2011, Rolheiser et al. 2012, Aldred & Clare 2014).

Reports from mussel farmers and others show that the tunicate *Ciona intestinalis* (Linnaeus, 1767) is a particularly common fouling species with large impacts on mussel cultures (Aldred & Clare 2014, Bullard et al. 2013, Sievers et al. 2013). The tunicate *C. intestinalis* is a strong competitor, which outcompete many other species (Svensson et al. 2007, 2009) and prevent settlement of other species (Blum et al. 2007). The rapid growth and high fecundity of the species (Yamaguchi 1975), combined with suitable hydrodynamic (Havenhand & Svane 1991) and physical conditions (Rius et al. 2010) offered by long-line mussel farms make these structures particularly vulnerable to invasions by *C. intestinalis*. On the other hand, *Mytilus edulis* (Linnaeus, 1758) is also generally described as a strong competitor in both natural and artificial communities in this area (Berntsson & Jonsson 2003, Svensson et al. 2007) and even if the colonization of other species seems to facilitate a successful colonization of *M. edulis*, earlier colonists are not essential for this colonization (Chalmer 1982, Dean & Hurd 1980). Berntsson and Jonsson (2003) concluded that *M. edulis* was the dominant competitor in the fouling community and that it inhibited settlement of other species within 3 mo after colonization and that once established, could generally resist invasion of other fouling species. Thus, observations from farmers and scientific observations both suggest that the relative timing of settlement of *M. edulis* and *C. intestinalis* is an important determinant for whether the ropes will ultimately be dominated by mussels or tunicates.

In line with these observations, it has been suggested that avoidance of natural settlement is a feasible strategy for preventing problems with fouling organisms, particularly when recruitment of these organisms are variable but predictable in time and space (see reviews by Cyr et al. 2007, Sievers et al. 2014). Furthermore, such strategies highlight the need for and potential use of accurate information on the occurrence of larvae of the target species and potential fouling organisms (e.g., Arakawa 1990, Cyr, et al. 2007, Willis et al. 2011). For example, Cyr et al. (2007) showed that weekly monitoring of recruitment could provide farmers with valuable information to avoid peak settlement of fouling mussels on scallop collectors. They were also able to determine a deployment period that resulted in higher yields at retrieval 1 y later, compared with other periods. Thus, if recruitment of fouling species is high, deployment of farm equipment might be postponed or, if already deployed, measures to mitigate the infestation might be taken before substantial loss has occurred.

Therefore, the aim of this study was to monitor the recruitment of blue mussels (*Mytilus edulis*) and the most important fouling species (*Ciona intestinalis*) using a simple standardized method consisting of transparent and shaded petri dishes for identification of optimal time for deploying ropes. This was done by estimating spatial and temporal patterns of settlement at a range of scales and at different depths and light conditions as *C. intestinalis* is thought to have reduced settling on surfaces exposed to light (Rius et al. 2010). As a second step, the extent to which production of mussels and tunicates on ropes could be predicted from observations of settlement was tested.

MATERIALS AND METHODS

Pilot Study—Developing Sampling Method

To develop cost-efficient, objective subsampling procedures with sufficient accuracy, a pilot study was designed. The pilot study used a number of existing plates (transparent polystyrene petri dishes, ø = 90 mm), deployed for 3–4 days in the same general area as the main study, with varying densities of newly settled mussel larvae (up to ~500 µm) to estimate mean and SD and to calculate coefficient of variation. The abundance of settled mussel larvae was analyzed on 12 plates using three different subsampling methods (1, 0.25, and 0.01 cm^2, respectively) and measurements estimated for each of the methods. Coefficients of variation was plotted against means values ($\bar{x}$) and a function was fitted to obtain an equation for calculation of coefficients of variation. Noting that the relative error of the mean, i.e., the SE relative to the mean, can be calculated as $\frac{s}{\sqrt{n}\bar{x}}$, where s is the SD and n the number of samples, and using a target value of relative error of less than or equal to 0.3, the number of subsamples (n_{target}) needed to ensure the targeted accuracy at different abundances could be determined by the expression:

$$n_{\text{target}} = \left(\frac{\text{CV}}{0.3}\right)^2.$$

Based on the results of this preliminary study a practical strategy, which ensured that the counted area at any average density was larger than that required for the targeted maximum error was determined. Thus, if initial assessments showed that the abundance was less than or equal to 4 per square, the whole sample area (32 cm^2) was counted. When the abundance was between 4 and 40 per square, a 10-cm^2 area was counted and when abundance exceeded 40 per square, a 2.5-cm^2 area was counted. These results of this preliminary experiment were then used as a standardized sampling method for the monitoring of settlement experiment.

Monitoring of Settlement

The main study was conducted in collaboration with mussel farmers at three sites on the west coast of Sweden (Fig. 1). The settlement of larvae was monitored during 8 wk, with on average two sampling days per week, in May to July 2012, using monitoring units constructed from standard polyvinyl chloride -pipes. The pipes were cut vertically creating a slot with an angle of 150°. The monitoring units (Fig. 2) were attached to mussel rigs at Tjärnö and Mollösund, and at Gullholmen to a jetty. Two monitoring units, each consisting of four removable sampling plates (two from each shading treatment) were placed at two depths (1–2 m and 4–5 m) at all sites. Each sampling plate consisted of two standard petri dishes (ø = 90 mm, transparent polystyrene, VWR art no. 391–0893), mounted back to back. The downward surfaces were used to assess the abundance of settling larvae whereas the upward

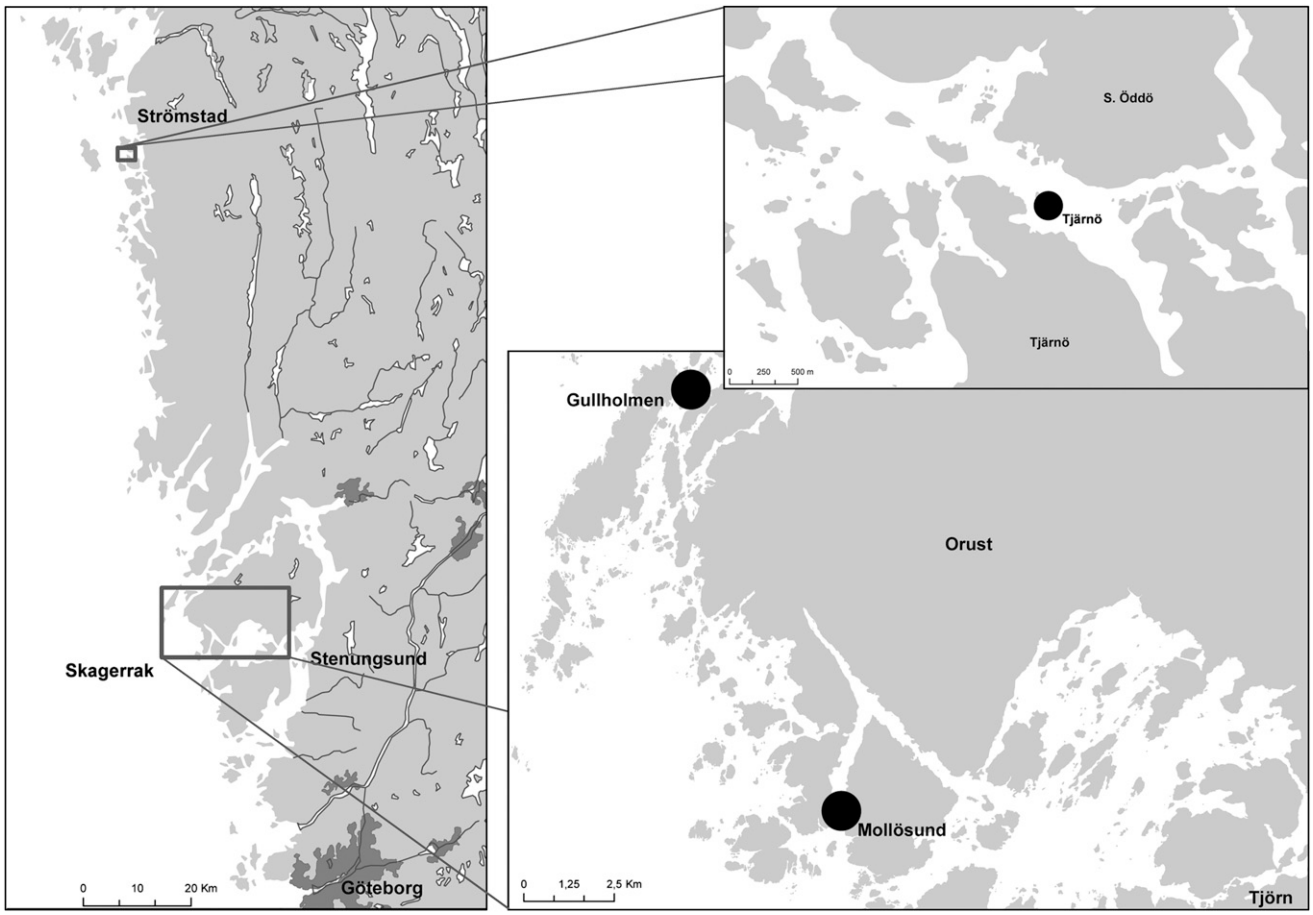

Figure 1. Map showing the county of Bohuslän on Swedish west coast and the three studied sites.

facing surfaces (referred to as background) separated the plates into different shading treatments (transparent or dark background). All sampling plates were rugged with a steel-wire brush (i.e., creating a less smooth surface) in a standardized pattern to increase the suitability of the surface as settling substrate. No conditioning of the settlement surfaces was done prior to deployment. On 16 occasions, sampling was performed by collecting the sampling plates and replacing them with new plates. The samples were individually preserved in 80% ethanol and stored in a fridge until analysis. All samples were individually examined using a stereomicroscope, and newly settled mussels (*Mytilus edulis*) and tunicates (*Ciona intestinalis*) were counted according to the assessment strategies determined in the pilot study.

Yield and Fouling on Mussel Ropes

To evaluate the relationship between settlement on plates and yield of mussels at different deployment times, the abundances of mussels and tunicates were measured on 5-m-long and 4.5-cm wide mussel ropes that were deployed weekly during the monitoring period (8 wk). The ropes were deployed in duplicates from long lines adjacent to the monitoring units at the Tjärnö and Mollösund sites. All deployed ropes were collected in October and stored at –25°C until analysis. Ropes were thawed and three 30-cm sections from both depths (1–2 m and 4–5 m) were cut from each rope. For every section of rope the proportion of area covered by mussels was estimated. All tunicates were counted per section and the abundance per meter rope calculated.

Data Analysis

Data on larval settlement on the sampling plates were standardized by immersion time (varied from 2 to 5 days) and analyzed using standard procedures for analysis of variance at each site separately (e.g., Underwood 1997). Initial inspections of residuals and tests for homogeneity of variances showed that $\log(X + 1)$ transformations sufficiently improved the distributional properties of the data.

At each site, data were analyzed using a four-factor mixed model with ''Shading'' and ''Depth'' as fixed factors and ''Days'' and ''Monitoring unit'' as random factors. Since each monitoring unit was located in the same place within a particular depth at each day of deployment, and each monitoring unit had replicate plates of each shading treatment, the random factor ''Monitoring unit'' was nested within ''Depth'' but crossed with "Day" and "Shading" (Underwood 1997, Quinn & Keough 2002). Note that complex designs like these may in some instances not allow for the *a priori* construction of valid F ratios for some of the sources of variation (e.g., Underwood 1997). As a consequence of this, tests for main effects of and

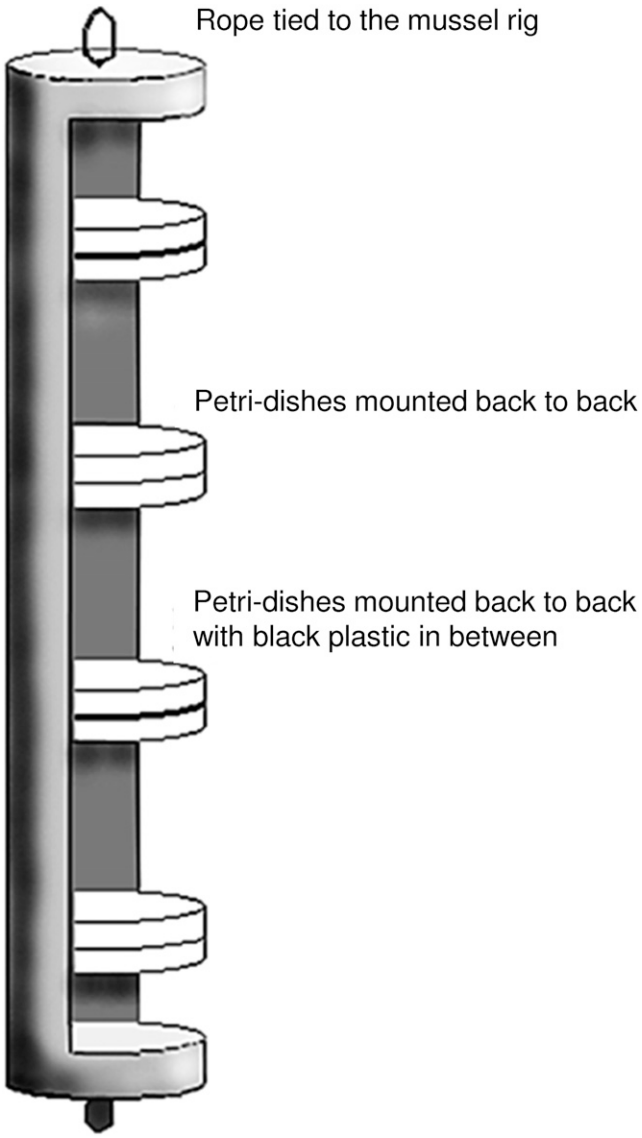

Figure 2. Schematic figure of the monitoring unit used in the experiments.

interactions between the two fixed factors can in some instances not be tested unless some of their interactions with the random factors can be shown to be without importance (conventionally at $P > 0.25$; Underwood 1997). This is, however, not a large problem because the existence of such interactions show that the effects of "Depth" and "Shading" vary among for instance "Days", which in turn show that simple main effects are not predominant.

Trends and differences among sites in the proportional area covered by mussels and abundance of settled tunicates on the ropes were also analyzed using analysis of variance. The relationships between settlement rates on plates and the yield on ropes at the end of the experiment were evaluated using correlative analyses. These analyses were performed using (1) instantaneous settlement (using settlement data from plates deployed at the same time as a particular rope) and (2) cumulative settlement (using settlement data from all subsequent plates after the deployment of a rope). All statistical analyses were performed using a significance level of $\alpha = 0.05$ and performed in R (R Core Team 2014) using the "aov" function.

RESULTS

Pilot Study—Development of Sampling Method

The average count of mussels in the pilot study varied between 0.2 and 80 individuals per sampling square of the petri dish measured (Fig. 3A). Data on average counts and coefficient of variation from all square sizes were fitted to a power function, which explained ~60% of the variation (Fig. 3A). For comparison it can be noted that the dispersion of data is generally larger than that, which can be expected from a Poisson distribution. The reason for this deviation is beyond the scope of this paper, but it was concluded that any sampling strategy based on the fitted data is likely to be conservative in relation to the expected Poisson. Thus, using the fitted equation, the subsampling area needed to maintain a relative error of less than or equal to 0.3 was calculated as (Fig. 3B):

$$n_{\text{target}} = \left(\frac{1.27x^{-0.28}}{0.3}\right)^2.$$

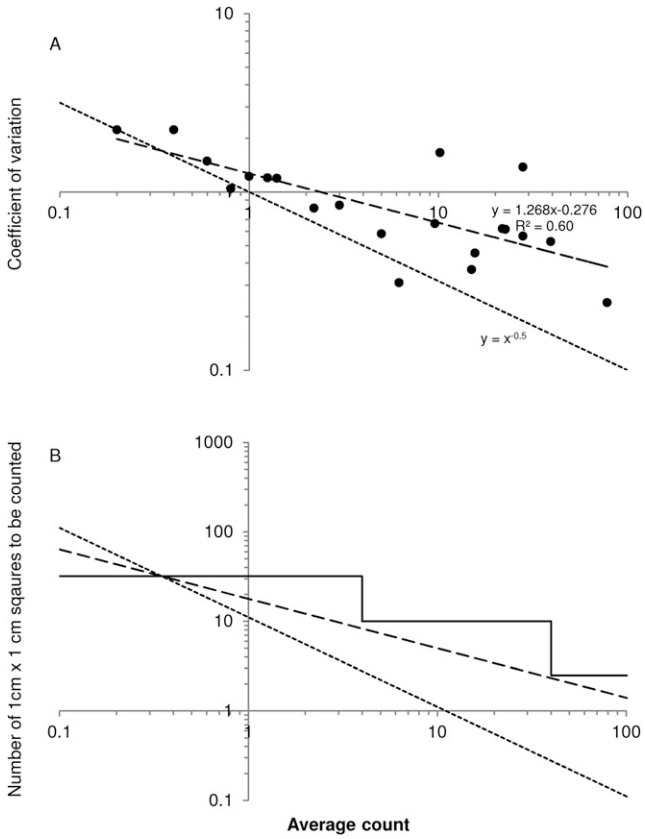

Figure 3. (A) Coefficient of variation as a function of average count in pilot study. Dashed line indicates the fitted relationship and dotted line represent that expected if the larvae were Poisson distributed. (B) Number of 1 cm^2 (or total area counted per plate) needed to maintain a relative error of less than or equal to 0.3. Dashed line calculated using fitted data, dotted line using the Poisson distribution and solid line represent the chosen subsampling strategy (maximum area is 32 cm^2 per plate).

Monitoring Study

Settlement of *Mytilus edulis*

At all sites there were strong and significant variability in settlement rates of *Mytilus edulis* among sampling days (Table 1, Fig. 4). Settlement followed the same general pattern at both Tjärnö and Mollösund, increasing during mid-June, peaking on sampling day 4 and 6, respectively, after which relatively high rates were recorded for 3 wk before decreasing again. Very low settlement was observed at Gullholmen during the whole study period where the highest number of mussels was found in mid-July (Fig. 4). Maximum settlement varied from 0.14 per cm^2 per day at Gullholmen to 0.6 per cm^2 per day at Mollösund. Differences among sites were observed, but the general temporal pattern regarding timing of settlement was similar at Tjärnö and Mollösund.

Settlement of mussels was affected by shading at Tjärnö and Gullholmen, but the effect varied among days (S × Da; Table 1). No effect of shading was evident at Mollösund. Nevertheless, in a qualitative sense the mean settlement of mussels was larger on dark compared with transparent background dishes at 10–11 days (of 16 possible) at all sites and only on two dates in Mollösund was the average settlement substantially larger on transparent plates (these dates were also associated with large variability). In summary, the average settlement rates were 45% and 35% higher on dark compared with a transparent background at Tjärnö and Gullholmen (Fig. 4). At both Tjärnö and Gullholmen, the effect of shading on mussel recruitment was most evident at days with relatively high abundance. Similarly, there were effects of depth at all sites, but these varied among days (Table 1). Again the differences were most consistent at Tjärnö and Gullholmen, where more mussels were found in shallow areas at 14 and 12 days, of a possible 16, respectively, whereas at Mollösund the patterns were more inconsistent (Fig. 4). Furthermore, at the former sites the average rates were ~350% and 250% larger at shallow areas than at the deeper ones. Finally, it is notable that the random variation due to monitoring unit within depths and its interactions with other factors was generally of limited importance (Table 1). The exception here is Tjärnö where the variability among monitoring units is significant. At Mollösund, the large variability among plates is notable.

Settlement of *Ciona intestinalis*

At all sites there were strong and significant variability in settlement rates of tunicates among sampling days (Table 1). Even though spatial and temporal differences among sites were not tested explicitly, some patterns were evident (Fig. 5). Inspection of temporal patterns of recruitment showed that the abundance of tunicates (mainly *Ciona intestinalis*) varied among the different sites. At Mollösund, the abundance was generally lower than that at the other sites with settlement rates ranging from 0 to 0.06 larvae per cm^2 per day. At Gullholmen, three separate peaks were identified at sampling day 1, 7, and 12 with a maximum recruitment of 0.55 larvae per cm^2 per day. The largest numbers of *C. intestinalis* recruits were consistently found at Tjärnö with the highest recorded recruitment of 1.40 larvae per cm^2 per day. Spatial differences among sites were also evident. The recruitment rates and temporal variation in recruitment was considerably larger at the sites Tjärnö and Gullholmen compared with Mollösund.

The effects of shading and depth on the settlement of tunicates were more consistent among sites than those observed for mussels (Table 1). At all sites, there were significant effects of shading, which were variable in size among days (S × Da). Nevertheless, these patterns were very consistent in a qualitative sense with larger recruitment rates on shaded plates in 44 out of the 48 combinations of dates and sites (Fig. 5). Furthermore, the rates were on average 200%–300% higher on shaded surfaces compared with the transparent. Recruitment of tunicates also differed strongly between the two depths (Fig. 5). Despite significantly variable effects among dates, settlement was almost consistently qualitatively larger in deeper areas and the mean abundance was generally 250%–450% larger in the deeper areas (Fig. 5). A significant interaction between "Shading" and "Depth" was also found at Tjärnö and Mollösund ($P < 0.001$; Table 1). Inspections of means suggest that these

TABLE 1.

Analysis of variance on recruitment of *Mytilus edulis* and *Ciona intestinalis* at individual sites.

		Mytilus edulis						*Ciona intestinalis*					
		Tjärnö		Mollösund		Gullholmen		Tjärnö		Mollösund		Gullholmen	
Source	df	MS*	*P*	MS	*P*	MS	*P*	MS	*P*	MS	P	MS	*P*
Shading, S	1	11.9	nt	2.42	0.48	0.67	nt	268	**0.001**	1.28	**0.001**	51.9	nt
Depth, D	1	133	nt	0.36	nt	5.3	**0.02**	1247	nt	1.03	nt	116	**0.001**
Day, Da	15	10.5	**0.001**	33.2	**0.001**	2.42	**0.001**	42.1	**0.001**	0.36	**0.001**	22.6	**0.001**
S × D	1	0.95	nt	0.59	0.71	0.05	nt	109	**0.001**	0.32	**0.01**	8.75	nt
S × Da	15	2.13	**0.001**	1.57	0.56	0.17	**0.001**	1.94	**0.01**	0.07	**0.01**	1.92	**0.001**
D × Da	15	6.88	**0.001**	6.05	**0.001**	0.8	**0.001**	21.7	**0.001**	0.08	**0.001**	6.35	**0.001**
Monitoring unit, Mu(D)	2	2.22	**0.01**	1.68	0.09	0.06	0.51	6.3	0.07	0.03	0.16	0.11	0.66
S × D × Da	15	0.82	0.07	2.01	0.35	0.11	**0.02**	2.25	**0.01**	0.03	0.22	0.43	**0.001**
S × Mu(D)	2	1.44	0.05	3.26	0.17	0.15	0.05	0.31	0.62	0.01	0.67	0.58	**0.01**
Da × Mu(D)	30	0.37	0.82	0.64	1.00	0.09	0.74	2.21	**0.001**	0.02	0.53	0.26	**0.02**
S × Da × Mu(D)	30	0.44	0.65	1.72	0.76	0.05	1.00	0.63	0.65	0.02	0.14	0.11	0.84
Residual	128	0.5		2.15		0.11		0.72		0.02		0.15	

* $MS \times 10^{-3}$

"nt" indicates that no unambiguous *F*-ratio could be constructed for "Shading," "Depth," or "S × D" (see text for further explanations). Values in bold represent significant values (i.e. where the *P*-values < 0.05).

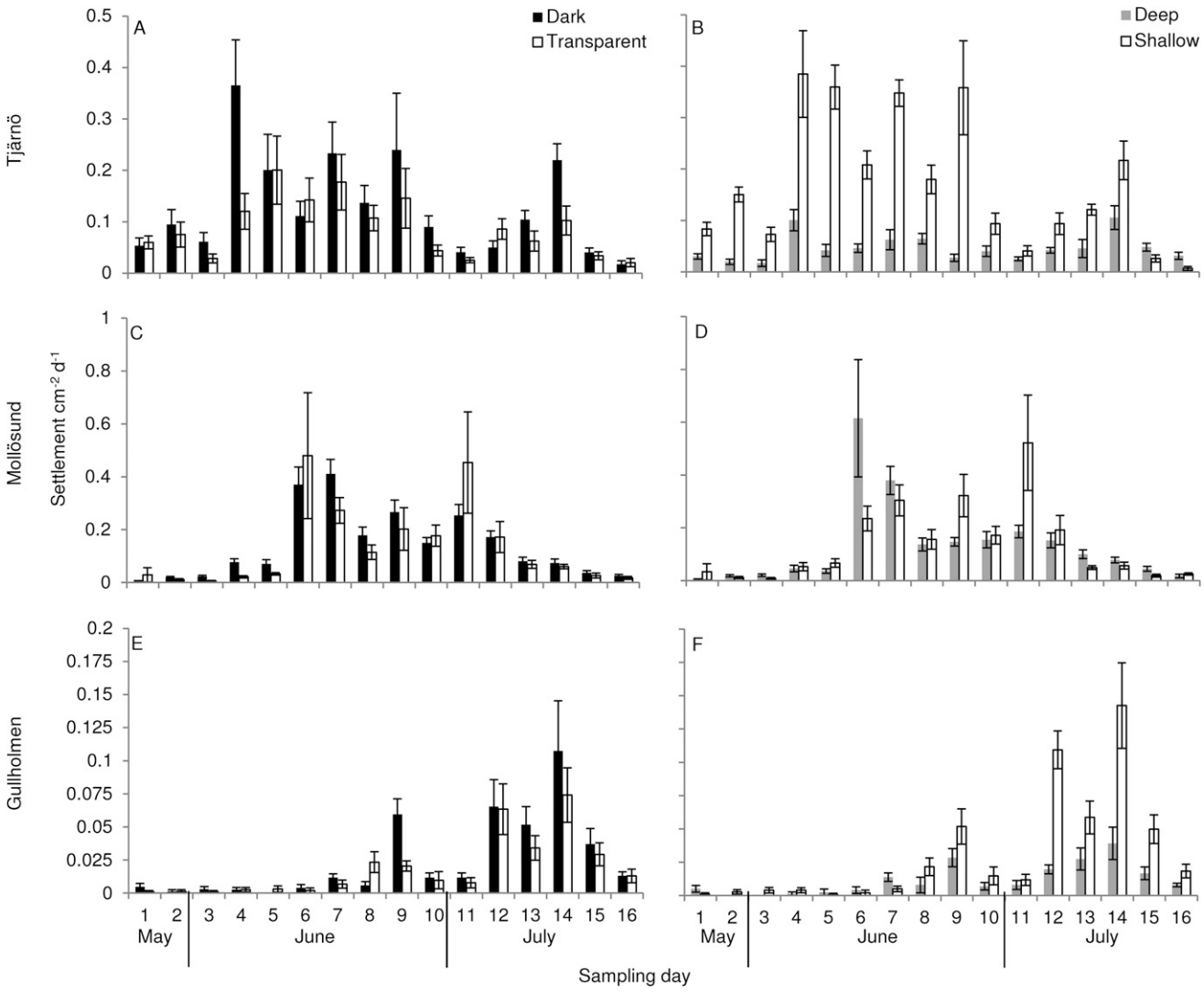

Figure 4. Settlement of *Mytilus edulis* over 16 sampling days on dark and transparent surfaces on (A) Tjärnö, (C) Mollösund and (E) Gullholmen. Settlement of *Mytilus edulis* over 16 sampling days on deep and shallow units on (B) Tjärnö, (D) Mollösund, and (F) Gullholmen. Error bars represent standard error.

patterns are due to the fact that the effect of shading was stronger in deeper units compared with shallow ones. These conclusions do not, however, invalidate previous conclusions about effect of shading and depth. The variability in recruitment associated with monitoring units and their interaction terms were generally small. Significant interactions between days and monitoring units at Tjärnö and Gullholmen indicate that small-scale variability among monitoring units may sometimes be important but that it is not consistent among days (Table 1).

Yield and Fouling on Mussel Ropes

Spatial and Temporal Patterns

The abundance of mussels and tunicates on ropes deployed during the experimental period was very variable between sites, depths, weeks, and ropes (Fig. 6). A general observation is that there appear to be common features of mussels and tunicates at the individual sites. At Tjärnö abundances tended to be larger on ropes deployed weeks 2–6, whereas at Mollösund deployment during the entire period resulted in substantial abundances of both species. At Mollösund, however, both mussels and tunicates were generally most abundant in shallow sections of the ropes. Despite substantial variability among ropes and replicate sections, analyses of mussel coverage and abundance of tunicates also revealed several patterns, which were different among species and sites (Table 2).

The distribution of mussels on the ropes appeared to be uneven both in terms of mussel size, depth, and mussel density with some parts of the ropes being completely free of mussels whereas others were covered with a uniform layer. These observations were manifested at Tjärnö as significant variability among ropes, including interactive effects with depth (Table 2). Such differences were not evident at Mollösund, where the yield was consistently larger at shallow compared with deep parts of the ropes (Table 2, Fig. 6). There were significant differences in yield among weeks, but contrary to

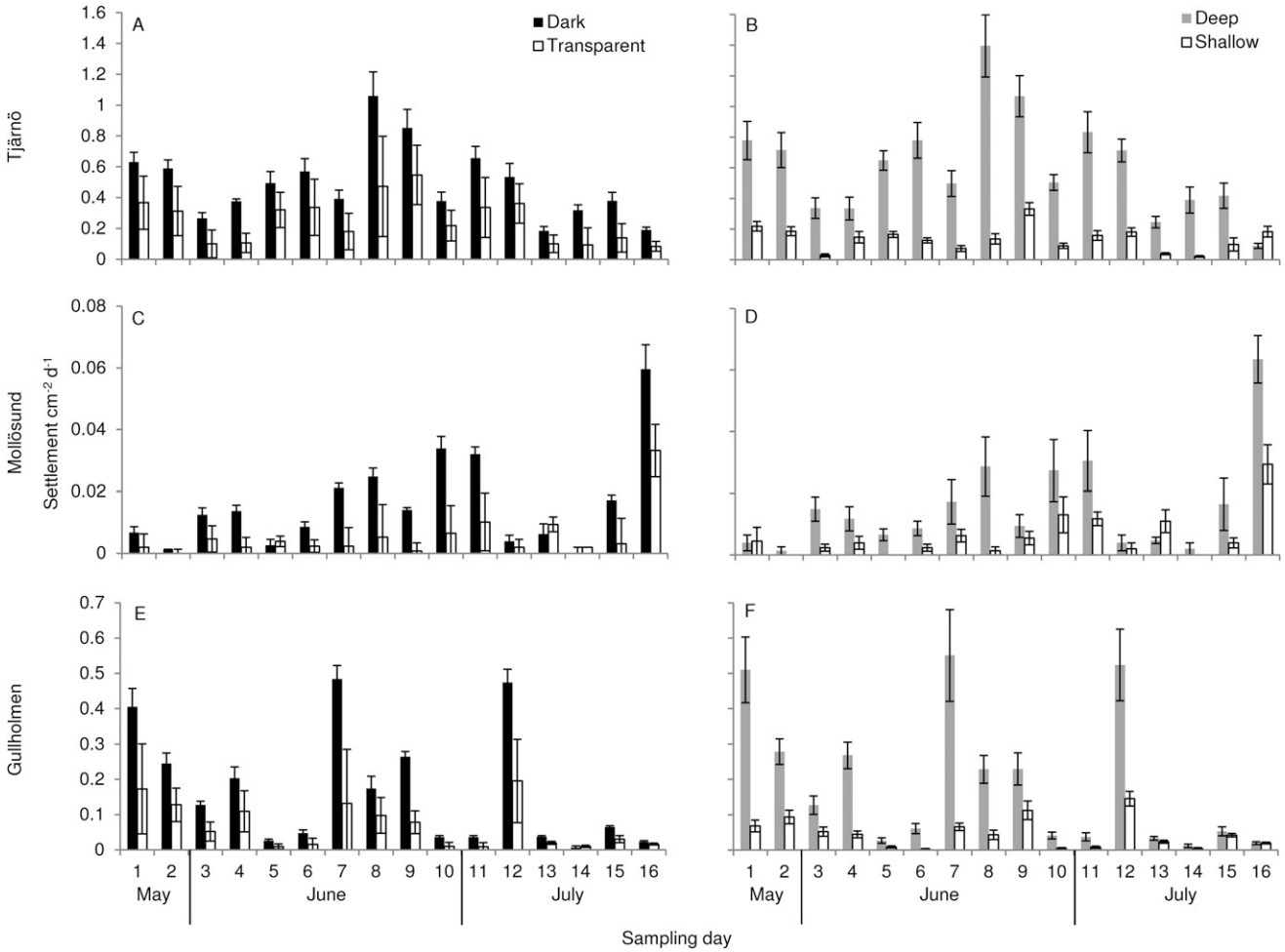

Figure 5. Settlement of *Ciona intestinalis* (±SE) over 16 sampling days on dark and transparent surfaces on (A) Tjärnö, (C) Mollösund and (E) Gullholmen. Settlement of *Ciona intestinalis* over 16 sampling days on deep and shallow units on (B) Tjärnö, (D) Mollösund, and (F) Gullholmen.

what was expected, there was no evident positive relation between the time in the water and the yield of mussels. Thus, ropes with a potentially longer recruitment and growth period did not harbor more mussels than those deployed for shorter periods (Fig. 6). Abundance of mussels was much lower than expected for ropes deployed in these areas and the most successful ropes typically averaged about 10% area covered at Tjärnö and 25% to 46% covered at Mollösund. The average area covered by mussels varied from 0.2% to 11.5% (Tjärnö) and 6% to 46% (Mollösund) on ropes deployed during the least and most successful weeks.

Patterns of tunicate abundance at the two sites showed many similarities to those of mussels (Table 2). At Tjärnö, abundances were largely unpredictable with large differences among ropes and sections, whereas in Mollösund patterns were more easily attributed to depths and weeks. Nevertheless, the abundance of tunicates was significantly affected by depth at both sites, but this effect was reversed in the sense that at Tjärnö, larger abundances were found on deeper sections of the ropes. At Mollösund the opposite was observed, but here the average abundances were also much smaller. The largest abundance of *Ciona intestinalis* was found at Tjärnö where the average reached 60 individuals per meter rope.

Relationship between Recruitment and Abundance on Ropes

The relationship between settlement rate and the resulting abundance of mussels and tunicates on ropes 3 mo after deployment date could be observed at a number of spatial and temporal scales. In qualitative terms, the observation of larger average recruitment rates at Mollösund compared with Tjärnö was reflected by a similar difference in cover of mussels on ropes. Similarly, at Tjärnö the larger settlement of mussels recorded on shallow monitoring units was reflected by larger yield on ropes at the corresponding depth. This pattern was, however, not observed at Mollösund where clear differences in mussel yield were found despite inconsistent differences in recruitment between depths. Tunicate abundance on ropes also reflected the differences in recruitment recorded between sites as well as between different depths at the site Tjärnö. At Mollösund, the higher abundance on shallow parts of ropes contradicted the differences in recruitment between depths.

Quantitative analyses of temporal relationships were more inconclusive. Correlations between the average weekly recruitment at a particular week of deployment and the amount of *Mytilus edulis* on ropes deployed in the same week were inconsistent and weak (Fig. 7). A positive trend was observed at Tjärnö ($r^2 = 0.22$), whereas a negative trend was found at

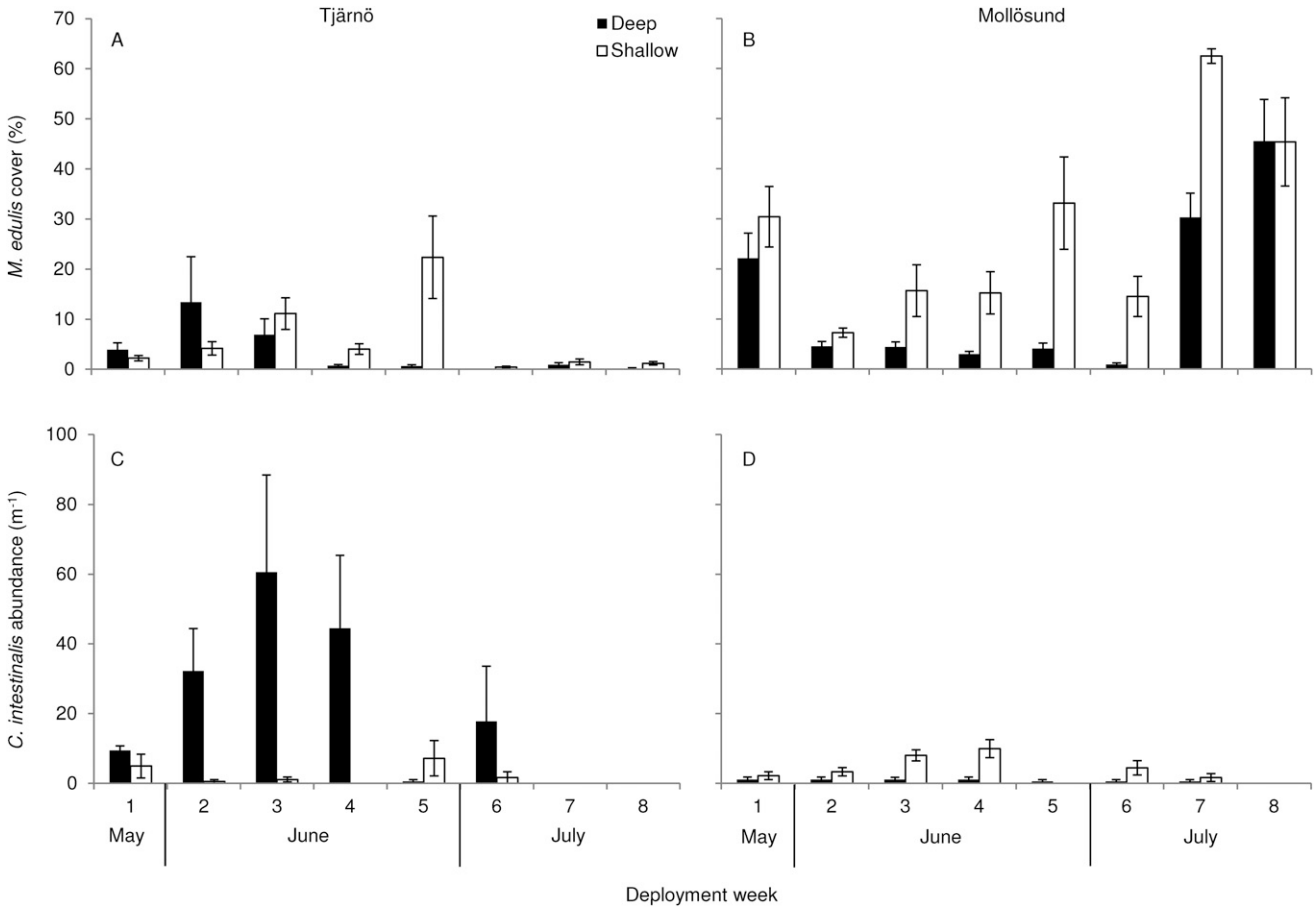

Figure 6. Average mussel coverage ±SE at two depths on mussel ropes deployed on different weeks on (A) Tjärnö and (B) Mollösund. Abundance of *Ciona intestinalis* per meter on deep and shallow parts of mussel ropes deployed on different weeks on (C) Tjärnö and (D) Mollösund.

Mollösund ($r^2 = 0.19$). Similarly, abundance of *Ciona intestinalis* showed a significant positive correlation at Tjärnö ($r^2 = 0.39$), whereas a weak negative relationship was found at Mollösund ($r^2 = 0.22$).

Relationships between abundance on ropes and the cumulative settlement of each species (i.e., the settlement at a site was summed over all dates of settlement from the deployment date to the end of the experiment) showed no correlation between cumulative recruitment of mussels and yield at Tjärnö, whereas at Mollösund a significant negative correlation was observed ($R^2 = 0.31$). The abundance of tunicates was positively correlated with cumulative recruitment at Tjärnö ($R^2 = 0.49$), whereas no relationship was observed at Mollösund (Fig. 7).

DISCUSSION

Using an extensive sampling design involving sampling of more than 750 settlement plates, the spatial and temporal patterns of settlement at a detailed resolution was quantified.

TABLE 2.

Analysis of variance on *Mytilus edulis* cover and *Ciona intestinalis* abundance on mussel ropes at individual sites.

	M. edulis								*C. intestinalis*							
		Tjärnö			Mollösund					Tjärnö			Mollösund			
Source	df	MS	*F*	*P*	df	MS	*F*	*P*	df	MS	*F*	*P*	df	*MS*	*F*	*P*
Depth = D	1	8.15	4.66	0.06	1	22.25	34.43	**0.00**	1	16.48	10.17	**0.01**	1	3.85	34.39	**0.00**
Week = W	7	4.96	4.47	**0.03**	7	10.00	23.20	**0.00**	7	2.75	1.52	0.28	7	0.99	3.71	**0.04**
D × W	7	2.15	1.23	0.39	7	1.58	2.45	0.12	7	2.97	1.83	0.21	7	0.62	5.55	**0.01**
Rope(W)	8	1.11	5.32	**0.00**	8	0.43	1.27	0.27	8	1.80	5.33	**0.00**	8	0.27	1.66	0.13
Rope(W) × D	8	1.75	8.39	**0.00**	8	0.65	1.90	0.07	8	1.62	4.80	**0.00**	8	0.11	0.69	0.70
Residual	64	0.21			76	0.34			64	0.34			64	0.16		

After inspection of residuals, all data were transformed to $\log(X + 1)$. Values in bold represent significant values (i.e. where the *P*-values < 0.05).

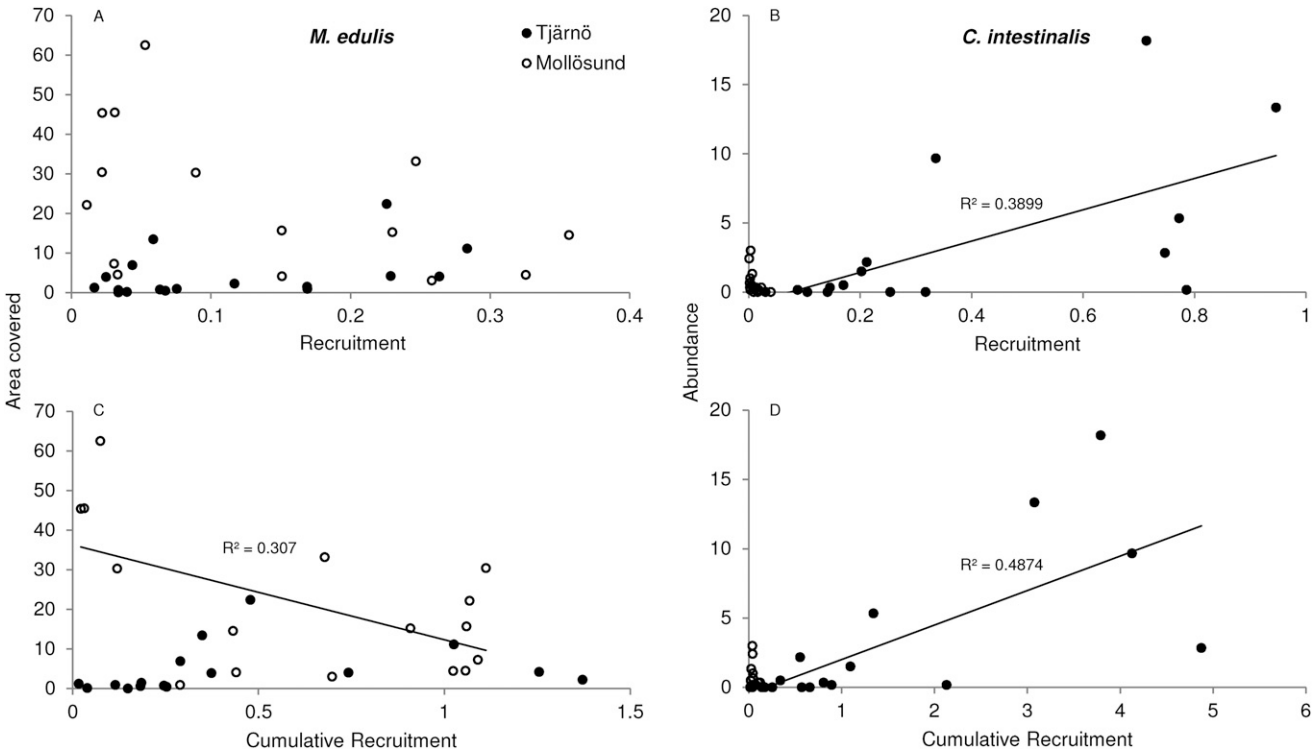

Figure 7. Relationships between (A) recruitment of mussels and area covered on ropes. (B) Recruitment of tunicates and abundance on ropes. (C) Cumulative recruitment of mussels and area covered on ropes. (D) Cumulative recruitment of tunicates and abundance on ropes. Trend lines shown for significant correlations.

These data were compared with recruitment of mussels and tunicates on authentic mussel ropes, to test hypotheses about the importance of settlement for the subsequent yield of mussels and fouling problems. First, based on these experiments it was concluded that methods like the one used in this study is in fact feasible for monitoring recruitment of mussel and fouling tunicates, and could be used as a tool for enhancing settlement of mussels and for avoiding competing fouling organisms. Second, the experiments also provided insights about the ecology of these organisms, which can be used to improve routines for mussel farming in Scandinavian waters and possibly elsewhere. These insights involve knowledge about species-specific settlement behavior, and information about the relative importance of settlement and mortality for the final yield in a farming situation.

In all aquaculture operations relying on natural recruitment or spat collection, the timing and spatial variability in settlement is crucial for its success and economic output. Therefore, methods for monitoring of settling larvae has been suggested as an important component for obtaining more reliable and stable supply of spat, particularly regarding commercially important species of bivalves (O'Beirn et al. 1995, Cyr et al. 2007, Maguire et al. 2007, Peteiro et al. 2007) and for developing biofouling management strategies (Campbell & Kelly 2002, Ramsay et al. 2009, Sievers et al. 2014). Different methods involving artificial materials (e.g., polyvinyl chloride, fiber material, and nylon scouring pads) have been used to assess settlement and recruitment of these organisms (e.g., Ramsay et al. 2009, Bullard et al. 2013). The petri dish method used was selected to provide a highly standardized method where newly settled larvae could easily be detected and counted without extensive handling or sorting. Overall, the method was simple, fast (less than 5 min per plate) and precise enough to serve as a realistic tool for monitoring the target species *Mytilus edulis* and its most conspicuous fouling competitor, *Ciona intestinalis*.

Although the subsampling procedures used to standardize sampling efforts ensured that sufficient precision was achieved on individual plates at an affordable cost, the sampling design also allowed for recommendations regarding monitoring designs. The variability among individual petri dishes was significantly greater than that among monitoring units, indicating that the use of multiple sampling plates was more important than using many monitoring units. Thus, together with observations of the importance of surface transparency and depth preferences, it is recommended that the settlement of *Mytilus edulis* and *Ciona intestinalis* is monitored with one monitoring unit at each of two depths. Because dark-surfaced sampling plates were generally preferred by both species, the use of transparent plates appears superfluous.

The results presented here provide ecological insights that may directly be used to improve existing practices for farming of *Mytilus edulis*. For example, knowledge about preferences and timing of settlement may be used to optimize collection of spat and or deployment of farming gear. These experiments showed that *M. edulis* and *Ciona intestinalis* show species-specific demands on the settling conditions and the settling rates varied between sites and times. Even though there was large variability, *M. edulis* larvae usually preferred settling on shallower placed monitoring units whereas the background properties were less important. In accordance with observations that light

levels (Miller & Etter 2008) and phototactic behavior (Nakagawa et al. 1999, Tsuda et al. 2003) of *C. intestinalis* larvae influence the tunicate community structure, the settlement of tunicate larvae was significantly higher on shaded surfaces than on transparent.

Observed recruitment rates of *Mytilus edulis* followed the general patterns described; peaking in mid-June followed by lower levels for a prolonged period but were generally low compared with normal recruitment rates (Loo & Rosenberg 1983) which could be due to the downward facing sampling surface. The tunicate *Ciona intestinalis* usually has two settling peaks in temperate waters resulting from differences in the reproduction cycles between shallower and deeper populations (Dybern 1965, Carver et al. 2003, Howes et al. 2007). In this study, only one peak was identified this might be attributed to the unusually cold winter resulting in delayed spawning as recruitment of *C. intestinalis* have been shown to closely correspond to water temperature (Ramsay et al. 2009). Although *C. intestinalis* clearly preferred shaded surfaces compared with transparent it is not yet feasible to use transparent ropes or nets as a method for preventing tunicate fouling in mussel farms. Differences in settlement found between depths are consistent with observed depth distribution of *C. intestinalis* on mussel farms (Howes et al. 2007, Rius et al. 2010) and the preferred depth reported for *M. edulis* (Dobretsov & Miron 2001). Settlement of both species is also known to vary between place and time (Ramsay et al. 2009, Bullard et al. 2013) and in accordance with a higher tunicate abundance in more sheltered areas (Howes et al. 2007, Rius et al. 2010). This study found both higher recruitment rates and final abundances at Tjärnö, which is the most sheltered site in this study.

One important result of this study was that in contrast to the predicted positive relationships, between settlement at the time of deployment (and subsequent cumulative settlement) and abundance of mussels on the ropes at the end of the summer season, was nonexisting for *Mytilus edulis*. This strongly suggests that mortality due to predation and dislodgement were important processes shaping the structure and determining the final yield of these dynamic assemblages. Although not investigated in this study, observations from both experiments (settling and yield) indicate that settling of the common starfish *Asterias rubens* (Linnaeus, 1758) on the settled mussels can generate both a lower abundance of mussels and spatial patchiness as well as complete removal of mussel recruits. Although the insight that there is substantial mortality of mussels in farming situations is not new (e.g., Smaal 2002, Kirk et al. 2007, Fitridge et al. 2012), this clear result strongly highlights the potential benefits of developing research and methods for reducing mortality. The fact that the largest yield was observed for some of the later deployment dates also suggests that a promising strategy may be to delay mussel rope deployment and avoid the peaks in settlement of the fouling organisms without reduced mussel density on harvest.

In contrast to the findings by Bullard et al. (2013) the abundance of *Ciona intestinalis* was positively correlated with recruitment at deployment date whereas no such correlation could be found for yield of mussels. This suggests that, for a successful farming practice, it is more important to avoid settlement peaks in fouling tunicate than identifying the maximum settlement peaks in *Mytilus edulis*. Utilizing recruitment of mussels occurring before the major settlement peak of *C. intestinalis* would only increase costs associated with fouling organisms without improving final yield of mussels. Many factors other than settlement influence yield patterns and further investigations into this are needed.

Utilizing the partial habitat segregation to reduce fouling *Ciona intestinalis* on mussel farms would imply a substantially increased farm size, compensating for reduced depth range, thus this is no clear-cut solution; however postsettlement, *Mytilus edulis* have shown to have a reducing effect on the settlement of *C. intestinalis* and other fouling organisms within 3 mo (Berntsson & Jonsson 2003, Ramsay et al. 2008), thus temporarily reducing depth and increasing surface area of the farm during deployment and settlement might have an important role to play in reducing fouling as most of the settling of *C. intestinalis* would be avoided whereas at the same time not significantly influencing the *M. edulis* settlement.

In conclusion, this study has taken some important steps toward developing useful monitoring tools for recruitment of *Mytilus edulis* and its common fouling organism *Ciona intestinalis*. The results further indicate that although it is simple, a method based on the use of settlement plates at different depths can, with low costs, successfully provide a good description of the settling community with a high temporal resolution. Thus, monitoring settlement of bivalves and fouling organisms using a method like the one tested in this study can be an important tool in developing both mussel farming as an industry and as a method for mitigation of eutrophied coastal waters.

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