



## The native rock shrimp *Rhynchocinetes typus* as a biological control of fouling in suspended scallop cultures

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### ABSTRACT

Scallop aquaculture in Chile suffers from intense fouling on culture facilities by invasive species such as the ascidian *Ciona intestinalis* and the bryozoan *Bugula neritina*. We examined the grazing effect of the rock shrimp *Rhynchocinetes typus* on fouling species, which colonize scallop pearl nets. We placed different densities of shrimp (0, 2, 5 and 10 individuals) in pearl nets with juvenile scallops (mean shell height 4.9 cm) at Tongoy Bay in northern-central Chile. We sampled the nets after 4 months (January–May 2007) and recovered 35–50% of the shrimp from the different treatments. The nets with 10 initial shrimp had a lower cover of the bryozoan *B. neritina* and lower densities of the ascidians *C. intestinalis* and *Pyura chilensis*, which resulted in a 50% decrease in biomass of fouling on nets. Low scallop mortality and slightly higher (yet not significant) growth in treatments with high shrimp densities suggest a positive interaction between the shrimp and scallops. The native rock shrimp is therefore considered a good candidate as a biological control of fouling communities and this could have a potential for polyculture.

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### 1. Introduction

Suspended culture systems deployed in the sea represent open habitats for fouling communities (Glasby and Connell, 1999) and niche opportunities for invasive species (Stachowicz et al., 1999). Fouling species, which frequently include invasive species, are considered pests in aquaculture because they reduce the culture efficiency by damaging equipment (abrasion, increased load) and reducing water flow through nets that, in some cases, directly affect growth and mortality of cultured species (Claereboudt et al., 1994; Cronin et al., 1999; Lodeiros and Himmelman, 2000). Given the recurrent problem of fouling in aquaculture facilities, a number of strategies have been proposed to prevent or reduce recruitment of fouling species and the most commonly used are highly labor-intensive mechanical cleaning procedures and toxic antifouling coatings (Braithwaite and McEvoy, 2005). The latter is not applicable to shellfish culture because the toxic chemicals would contaminate the suspension-feeding bivalves.

Reducing the load of fouling with biological control mechanisms (e.g. grazers) is a promising alternative but this method remains

largely undeveloped. Several studies indicate that grazers such as gastropods, crabs and sea urchins successfully reduced fouling on bivalve culture facilities (Hidu et al., 1981; Enright et al., 1983; Cigarria et al., 1998; Lodeiros and Garcia, 2004; Ross et al., 2004). Ideally, this method could turn into a polyculture if biological control species have a commercial value.

Several invasive species dominate the fouling community in northern Chile, namely the cosmopolitan bryozoans *Bugula neritina* and *B. flabellata* and the solitary ascidian *Ciona intestinalis*. The high tolerance of *Bugula* spp. to antifouling paints on vessels and metal pollution in harbors resulted in a widespread distribution around the world on anthropogenic structures (Piola and Johnston, 2006), and *B. neritina* and *B. flabellata* are common introduced bryozoans along the Chilean coast (Castilla et al., 2005). *C. intestinalis*, native from northern Europe, has been reported as a pest for bivalve aquaculture in France (Mazouni et al., 2001), Scotland (Karayucel, 1997), South Africa (Hecht and Heasman, 1999), Chile (Uribe and Etchepare, 2002), and more recently in Nova Scotia (Carver et al., 2003; Howes et al., 2007). Life-history traits of this solitary ascidian, such as fast growth, hermaphroditism and daily reproductive activity, are considered responsible for it becoming a prominent fouling species (Lambert, 2007). Very little, though, is known about potential predators of these fouling organisms both in the artificial aquaculture environment and in natural benthic habitats. To assess the role of the native rock shrimp *Rhynchocinetes typus* as a biological control in scallop aquaculture in

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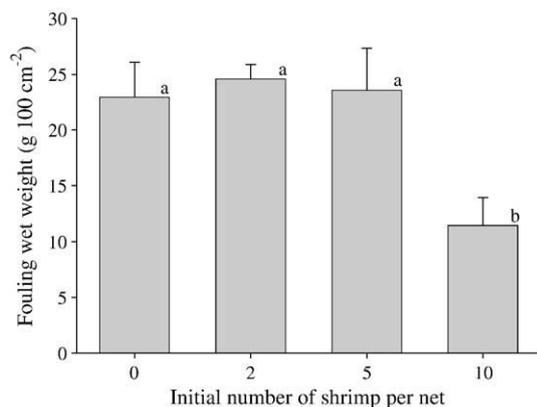
Chile, we evaluated the grazing effect of shrimp on fouling species colonizing pearl nets.

## 2. Materials and methods

Our study was conducted in Tongoy Bay (30°15'S, 71°35'W), in northern-central Chile. The eastern portion of the bay is used for suspended culture of the scallop *Argopecten purpuratus* on longlines and is the most important aquaculture area (23 km<sup>2</sup>) in northern Chile (Von Brand et al., 2006), but also suffers from intense fouling on culture facilities by the invasives *C. intestinalis* and *Bugula* spp., and the native ascidian *Pyura chilensis*.

The rock shrimp *R. typus*, which is a common benthic grazer in shallow subtidal habitats, can reach high abundances in wave-protected areas (Caillaux and Stotz, 2003). We collected shrimp from the shallow subtidal zone at La Herradura Bay (29°59'S, 71°22'W) and maintained them in the laboratory with flowing seawater for 1 day prior to the experiment. We selected shrimp with a size range of 12–16 mm in carapace length, corresponding to the stages *typus* or *intermedius* for males and likely ovigerous for females (Correa and Thiel, 2003). The frequency of males and females in our experiments was about equal, similar to the sex ratio observed in the field during winter (collection time) (Correa and Thiel, 2003). Scallops were obtained from spat collectors and then maintained in pearl nets on longlines of the scallop farm of Pesquera San Jose until the start of the experiment on 12 January 2007, corresponding to the period of the peak of fouling. Pearl nets used herein were square-based (30×30 cm), pyramidal-shaped nets with a 9 mm square mesh size. To avoid possible net shading and depth effects, only the first pearl net on a 3-net string was used for the experiment. We measured the shell height with a caliper (from the umbo to the ventral shell margin) to the nearest 0.1 mm, and individually tagged scallops with a plastic number that was glued on the upper (left) shell valve. Ten adult scallops (49.0 mm, SD±2.8) were placed in each of 20 fouling-free pearl nets. Five additional untagged scallops were added to each net in order to keep a density similar to that used in cultivation. We had four treatments corresponding to different densities (0, 2, 5 and 10) of shrimp in the 20 pearl nets (5 replicates per treatment).

The pearl nets were suspended for 4 months on a culture line at 7–8 m water depth and held in a vertical position with a small concrete weight at the bottom. Upon retrieval, bryozoan cover (*B. neritina*) and ascidian density (*C. intestinalis* and *P. chilensis*) were estimated immediately after bringing each pearl net aboard on the boat using 10×10 cm quadrats randomly placed on each side of the net (3 top inside and 3 top outside quadrats per pearl net, 2 bottom inside and 2 bottom outside quadrats per pearl net). Moreover, total fouling biomass



**Fig. 1.** Fouling biomass (mean±SE) on scallop pearl nets for different treatments consisting of four initial densities (0, 2, 5, 10 individuals per pearl net) of the rock shrimp *R. typus* per pearl net. Columns sharing the same letter are not significantly different (ANOVA,  $p>0.05$ ).

**Table 1**

ANOVAs comparing cover of bryozoans and the abundance of ascidians on pearl nets in 4 treatments (0, 2, 5, and 10 shrimp per pearl net).

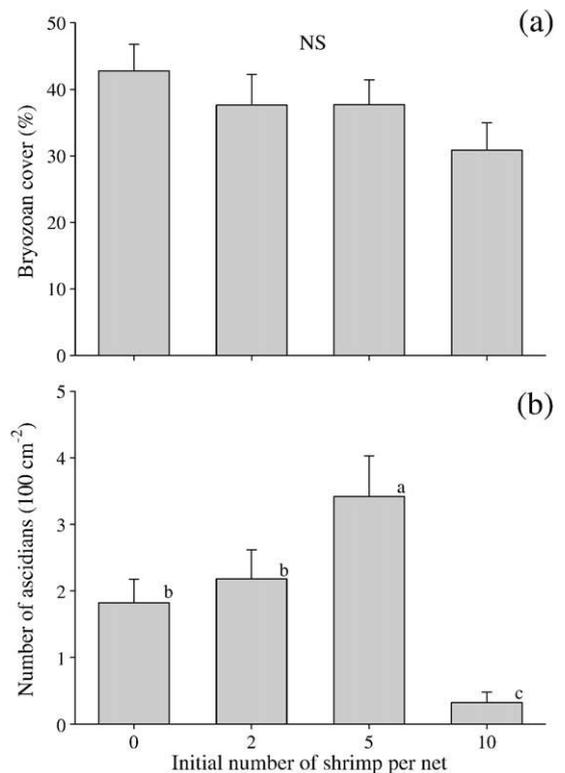
Source	Bryozoan cover				Ascidian number		
	df	MS	F	p	MS	F	p
Treatment	3	1051.58	0.56	0.65	68.74	4.62	<b>0.02</b>
Net (treatment)	15	1863.80	2.49	<b>0.002</b>	14.87	1.69	0.06
Residual	170	749.60			8.80		

Treatment was a fixed factor and net (quadrat within a replicate net) was random and nested within the factor treatment. Significant  $p$  values are in bold.

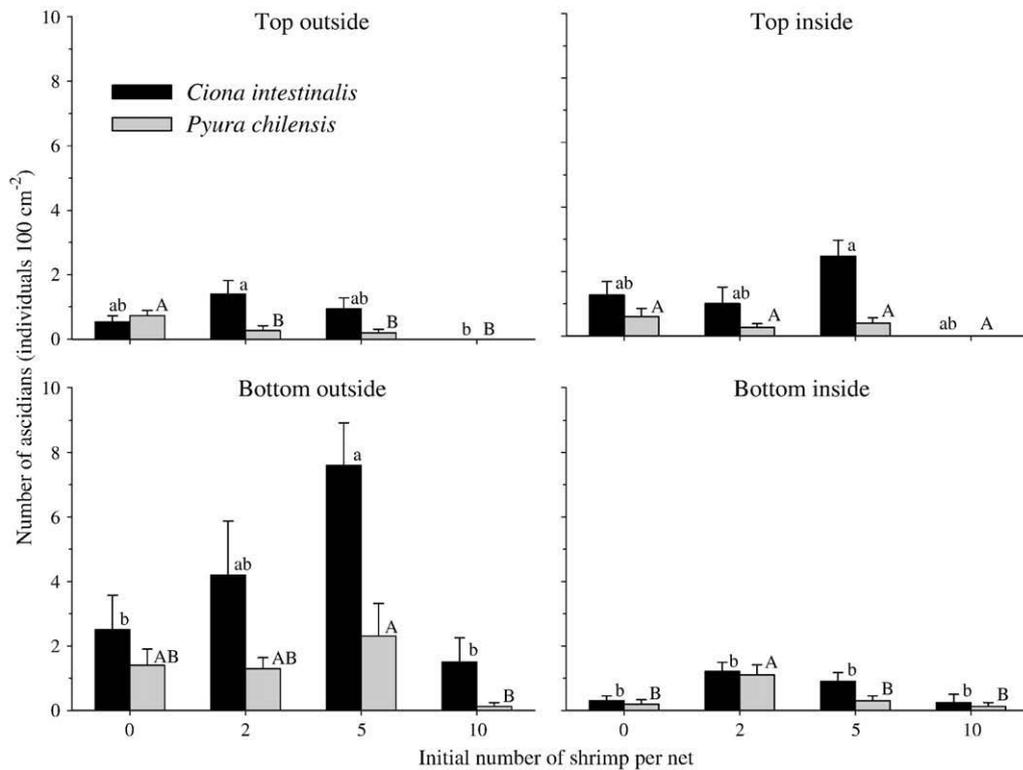
(wet weight to the nearest g) was quantified by removing all the fouling organisms within 2 quadrats on the upper outside surface of each net. The number of shrimp remaining in nets after 4 months was recorded, and we further evaluated mortality and growth of scallops.

To examine the behavioral interactions between shrimp and scallops, we conducted laboratory experiments where we positioned one adult scallop (shell height, 109.0 mm, SD±11.0) in a 30-l plastic tank with stagnant sea water. We then added 3 shrimp to the tank, and during 30 min, we measured the number of claps made by each scallop, as an indicator of scallop stress (Brokordt et al., 2006). Additionally, we registered if the shrimp were in contact with the scallop. We replicated this treatment 11 times and also had 11 control tanks to which no shrimp were added.

To evaluate the efficiency of shrimp in reducing the fouling on nets, fouling biomass (wet weight), percent cover of *B. neritina* and abundance of ascidians were examined separately using nested ANOVAs with the fixed factor treatment (shrimp number) and the random factor net nested within treatment. Multiple comparisons were made using SNK tests. Since one replicate was lost in two treatments, the analysis was run with 4 pearl nets per treatment. To evaluate the effect of shrimp on scallop growth we applied a nested ANOVA with the random factor pearl net nested in the factor



**Fig. 2.** (a) Bryozoan cover and (b) ascidian abundance on scallop pearl nets for different treatments consisting of four initial densities (0, 2, 5, 10 shrimp per pearl net) of the rock shrimp *R. typus*. Columns sharing the same letter are not significantly different (ANOVA,  $p>0.05$ ). NS indicates no significant difference among treatments.



**Fig. 3.** Number (mean  $\pm$  SE) of ascidians (*C. intestinalis* and *P. chilensis*) on scallop pearl nets for different treatments consisting of four initial densities (0, 2, 5, 10) of the rock shrimp *R. typus* in scallop pearl nets (4 months of duration; 5 replicates). Columns sharing the same letters (lower cases for *C. intestinalis* and upper cases for *P. chilensis*) are not significantly different (ANOVAs,  $p > 0.05$ ; see Table 2).

treatment (shrimp number). Assumptions of heteroscedasticity and normality were evaluated with Cochran's test and Kolmogorov–Smirnov test respectively.

### 3. Results

The percentage of shrimp recovered after 4 months varied between 35 and 50% among treatments. The final number of shrimp was still significantly different among treatments (Kruskal Wallis test,  $H_{3, 15} = 30.53$ ,  $p = 0.001$ ). The shrimp in all treatments were in good condition.

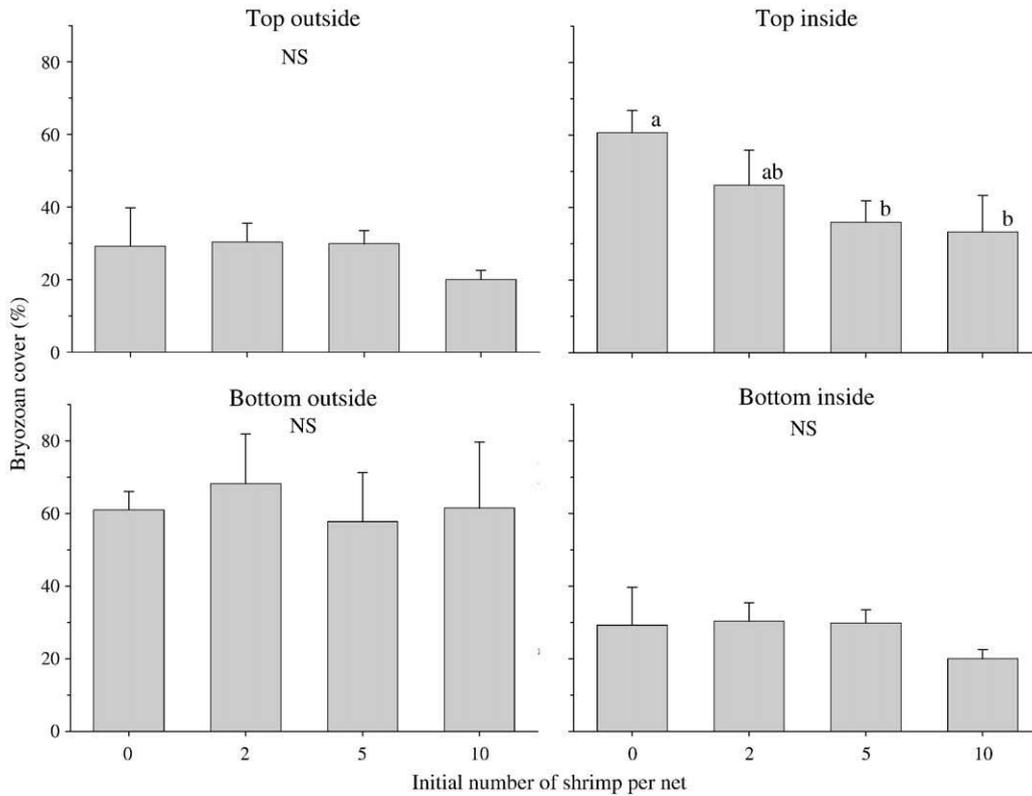
Overall, shrimp significantly suppressed the biomass of fouling communities on pearl nets with a ~50% decrease in the nets with 10 initial shrimp compared to nets without shrimp (ANOVA,  $F_{3, 12} = 4.76$ ,

$p = 0.02$ ; Fig. 1). The percent cover of the most abundant organism on the nets, the bryozoan *B. neritina*, decreased with 10 shrimp per net but the significant effect of the random factor pearl net nested within treatment revealed a higher variability in percent cover within pearl nets than treatments (Table 1, Fig. 2a). The abundance of ascidians was ~15% lower on nets with 10 shrimp than with no shrimp, but an increase of ascidians was observed with 2 and more importantly with 5 shrimp per net (Table 1, Fig. 2b). The number of ascidians was highest on the bottom outside of the pearl nets but lowest on the bottom inside (Fig. 3). No ascidians were observed on the top outside and top inside of the pearl nets in the presence of an initial number of 10 shrimp but this was not significantly different from the low recruitment on pearl nets with no shrimp (Table 2, Fig. 3). Similarly, the lowest cover of bryozoans (<30%) was encountered on the bottom

**Table 2**  
ANOVAs comparing cover of the bryozoan *B. neritina* and the abundance of ascidians *C. intestinalis* and *P. chilensis* on pearl nets in 4 treatments (0, 2, 5, and 10 shrimp per pearl net).

Source	<i>B. neritina</i>				<i>C. intestinalis</i>			<i>P. chilensis</i>		
	df	MS	F	p	MS	F	p	MS	F	p
					Bottom outside					
Treatment	3	213.24	3.17	0.113	69.67	16.42	<b>0.005</b>	7.10	5.52	<b>0.046</b>
Net (treatment)	5	63.86	0.07	0.997	4.19	0.23	0.945	1.28	0.31	0.906
Residual	29	975.58			17.98			4.18		
					Bottom inside					
Treatment	3	794.30	4.72	0.082	2.04	7.38	<b>0.041</b>	1.97	10.85	<b>0.021</b>
Net (treatment)	4	167.38	0.36	0.832	0.27	0.43	0.783	0.18	0.43	0.788
Residual	30	459.93			0.63			0.43		
					Top inside					
Treatment	3	2180.00	2.94	<b>0.043</b>	13.96	4.65	<b>0.006</b>	0.84	2.02	0.124
Net (treatment)	8	426.13	0.57	0.793	1.18	0.39	0.918	0.42	1.00	0.451
Residual	45	741.26			3.00			0.42		
					Top outside					
Treatment	3	261.34	0.53	0.663	4.76	3.55	<b>0.022</b>	1.35	5.42	<b>0.003</b>
Net (treatment)	8	301.08	0.61	0.763	1.23	0.92	0.510	0.13	0.54	0.823
Residual	45	491.51			1.34			0.25		

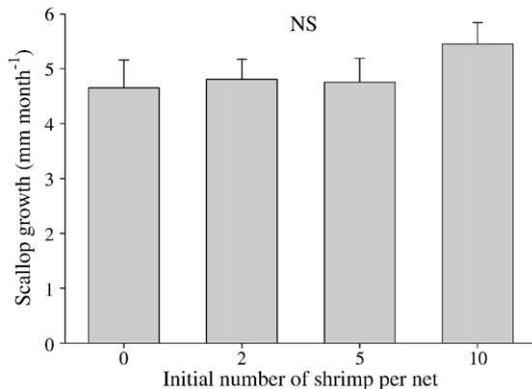
Treatment was a fixed factor and net (quadrat within a replicate net) was random and nested within the factor treatment. Significant  $p$  values are in bold.



**Fig. 4.** Percentage cover (mean  $\pm$  SE) of the bryozoan *B. neritina* on scallop pearl nets for different treatments consisting of four initial densities of the rock shrimp *R. typus* (0, 2, 5, 10 individuals) in scallop pearl nets (4 months of duration; 5 replicates). Columns sharing the same letters are not significantly different (ANOVAs,  $p > 0.05$ ; see Table 2). NS indicates no significant difference among treatments.

inside of nets (Fig. 4). The presence of 5 or 10 shrimp resulted in a significant difference in percent cover on the top inside of pearl nets with a decrease from 60% to ~35% cover of bryozoans (Table 2, Fig. 4).

Scallop mortality was low (1.3–4%) in all treatments with no change with increased shrimp density (1-way ANOVA,  $F_{3, 15} = 0.60$ ,  $p = 0.63$ ). Mean growth rate of scallops was higher in the presence of shrimp compared to control scallops but differences were not significant (nested ANOVA:  $F_{3, 122} = 1.20$ ,  $p = 0.11$ , Fig. 5), even though scallops with 10 shrimp grew an average of 5.4 mm/month in contrast to 4.7 mm in the control treatment. The significant effect of the random factor pearl net nested within treatment ( $F_{12, 122} = 1.97$ ,  $p = 0.03$ ) revealed a higher variability of scallop growth rate within pearl nets than among treatments.



**Fig. 5.** Growth rate of scallops (mean  $\pm$  SE) in pearl nets for different treatments consisting of four initial densities (0, 2, 5, 10 shrimp per pearl net) of the rock shrimp *R. typus*. NS indicates no significant difference among treatments.

In the laboratory, shrimp stayed in close proximity to the scallop with at least one of the three shrimp always on the upper shell throughout 95.2% (SE  $\pm$  2.18) of the 30-min observation time. This shrimp–scallop interaction did not lead to a significant change in number of claps (paired  $t$ -test,  $t_{10} = 0.18$ ,  $p = 0.43$ ) with  $0.26 \pm$  SE 0.39 and  $0.28 \pm$  0.10 claps  $\text{min}^{-1}$  in the presence and absence of shrimp, respectively. Further, scallops were filtering most of the time (except when they were generating claps) either in the presence or absence of shrimp.

#### 4. Discussion

Our study showed that the native rock shrimp *R. typus* is a good candidate for biological control of fouling communities, particularly against the ascidians *C. intestinalis* and *P. chilensis* and the bryozoan *B. neritina*, which correspond to the large majority of the fouling biomass on scallop aquaculture. The reduction of fouling was most successful with the highest shrimp density (10 individuals per net). However, after 4 months the number of shrimp in the nets in all treatments was reduced by ~50% of the initial abundance. The initial number of 10 shrimp per pearl net at the start of the experiment was apparently sufficient to significantly reduce fouling. Shrimp mortality could be the result of aggressive interactions with conspecifics (Correa and Thiel, 2003), cannibalism during molts (I. Hinojosa, personal communication) or food limitation during early stages of the experiment, when fouling was absent. Shrimp disappearance may not have exclusively been due to mortality as during the final sampling several shrimp were found on the outside of the nets, which could have escaped. Thus, the mesh size (9 mm mesh opening) was probably not small enough to keep shrimp inside the nets and the use of larger individuals might be worthwhile considering.

Shrimp are highly mobile organisms that rapidly move from one side to the other of pearl nets. The grazing activity of shrimp suppressed the abundance of ascidians on nets and to a lesser extent the cover of

bryozoans (Fig. 2). Using their slender and tweezer-like second chelipeds shrimp can select food and reach distant spaces including the outside of the nets. Shrimp might not select ascidians as preferred food and might only consume them when alternative food sources are limited. This could explain the increase in abundance of ascidians in treatments with 2 and 5 shrimp (Fig. 2b). The diet of the shrimp *R. typus* is largely unknown, but it has been observed to actively graze on ascidian recruits when offered in the laboratory or prevent recruitment of bryozoans in cages (C. Dumont et al., unpublished data). Further, scallops on the bottom likely prevented shrimp from actively grazing on the outside bottom. Similarly, Ross et al. (2004) suggested that fouling remained on the bottom outside of scallop pearl nets because a potential biological control (sea urchins) had difficulty in reaching and cleaning these surfaces. Therefore, the high cover of fouling encountered on the outside bottom might be caused by the combined effects of hampered access to the bottom due to scallops laying on the bottom and a preference of fouling species to recruit on shaded substrata (Howes et al., 2007).

The significant reduction of fouling resulted in a 17% (although not significant) increase in growth of juvenile scallops. The dominant fouling species, *C. intestinalis*, is a filter-feeder and consequently competes directly with scallops for food (Petersen, 2007). Fouling organisms can also reduce water flow through nets and subsequently reduce the flow of food particles (Claereboudt et al., 1994, but see Ross et al., 2002). Further, the presence of shrimp did not affect the survival of scallops. Our laboratory observations revealed no interruption of filter-feeding activity when shrimp were climbing on the shells of the adult scallops. Interestingly, *R. typus* has been observed to successfully prey on sea urchins *Loxechinus albus* and introduced abalones *Haliotis discus hannai* (Stotz, 2003; Stotz et al., 2006), but we did not observe any aggressive behavior against adult scallops *A. purpuratus* in the laboratory. Since no difference in mortality of small scallops was found in pearl nets with and without shrimp it appears reasonable to assume that shrimp also do not prey on large juvenile scallops. Kamermans and Huitema (1994) reported a disturbance of the siphon activity of the clam *Macoma bathica* by the shrimp *Crangon crangon*, which resulted in a reduction of clam growth. Overall, similar growth rates of scallops among treatments indicate that there were no negative interactions between shrimp and scallops, at least during our 4-month experiment.

Recent studies have considered sea urchins as potential biological control of fouling for suspended cultures of scallops (Lodeiros and Garcia, 2004; Ross et al., 2004). While being an efficient grazer in removing fouling on nets, there are several disadvantages in using sea urchins as a biological control. The spiny structure of sea urchins is painful for farmers manipulating the nets. Furthermore, the powerful and non-selective teeth of sea urchins have the potential to damage nets, which will require a more frequent replacement. Additionally, sea urchins are heavy animals, which significantly increase the total weight of pearl nets. For all these reasons, scallop farmers in Bahía de Tongoy have been reluctant to use sea urchins as biological controls of fouling after preliminary trials (Pesquera San Jose, pers. comm.). Finally, sea urchins feeding exclusively on fouling species have a low gonad size and quality (Kelly et al., 1998), which reduce the benefit of this polyculture by the devaluation of sea urchins as a resource.

The rock shrimp is a promising biological control organism because of its small size and important commercial value. An artisanal fishery with traps occurred until the 1950s before the start of intensive shrimp trawling off the Chilean coast (Vasquez and Castilla, 1982). The latter fishery decreased dramatically in the late 90s and extraction is now restricted to allow resource recuperation, and thus rock shrimp could become a valuable alternative resource. This highlights the opportunity for a likely successful polyculture. However, further studies are needed to evaluate the cost of such a polyculture and this will involve knowledge of the complex shrimp behavior. *R. typus* is an abundant benthic species (Caillaux and Stotz, 2003) that can be easily captured with baited traps but an evaluation of such a fishery should

be made. Most importantly in the context of shrimp–bivalve polyculture, shrimp could play a role as intermediate hosts of scallop parasites (e.g. Lafferty et al., 2004).

The present study reveals the potential benefits in using the rock shrimp *R. typus* as a biological control, but careful studies must first understand the diet and life cycle of the shrimp and evaluate the risk of disease/parasite transmission from shrimp to scallops. To date, there are no sustainable and cost-effective solutions to the fouling problem in aquaculture (Braithwaite and McEvoy, 2005). Future research should therefore focus on environmentally friendly antifouling methods such as biological control employing native grazers and predators that will suppress populations of non-native fouling species such as e.g. *C. intestinalis* and thereby hopefully minimize the risk of invasion into surrounding natural areas.

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