

The role of fish predation on recruitment of *Mytilus galloprovincialis* on different artificial mussel collectors

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A B S T R A C T

Fish predation interferes with mussel seed population dynamic and is an important limiting factor on seed supply in several areas of mussel farming production. In the present study we assessed the impact of fish predation in a mussel farm sited in the Ria de Ares-Betanzos (Galicia, NW Spain). To assess fish impact on recruitment, we have quantified mussel recruitment densities with or without excluding fish predation. The experiment was carried out using four different collector rope designs that could contribute to decrease the fish predation impact on the amount of mussel seed collected for cultivation.

The unprotected long-line (fish exposed treatment) showed lower recruitment densities than the protected one (fish exclusion treatment) for every collector design tested (between 38 and 58%; ANOVA, $p < 0.001$) with the exception of non-filamentous loop complement ropes (NF-L), which showed similar density values (9104 ± 316 and 7855 ± 375 indiv/m in the protected and unprotected long-line, respectively; Tukey $p > 0.05$). In addition, in the protected long-line recruitment densities were homogeneous between collector designs (8820 ± 635 indiv/m; ANOVA $p > 0.05$) whereas in the unprotected one statistically significant differences between collectors were observed. These results pointed out the influence of fish predation in the amount of mussel seed collected and its different effect between collector designs. The homogeneous density recorded in the protected long-line between collector designs would suggest the presence of another regulation factor of population size when fish predation is excluded. This regulation factor could be the intra-specific competition derived by space and food limitations of the studied area. With regard to the collector design, differences in recruitment density in the unprotected long-line would suggest different degree of protection from predators depending on collector texture and lacing complexity which could enhance the strength of seed attachment and create space refuges from fish.

1. Introduction

Gathering of mussel seed from collector ropes for industrial cultivation has increased in recent years (Perez-Camacho and Labarta, 2004), principally due to its higher growth rate when cultivated on the raft (Perez-Camacho et al., 1995; Babarro et al., 2000, 2003). Nonetheless, larval settlement shows high spatial and temporal variability which has been attributed to several biotic and abiotic factors involved in both larval dispersion and settlement (Pulfrich, 1996; Alfaro, 2006; Porri et al., 2006; Pineda et al., 2009). In addition to this variability, and as consequence of post-settlement mortality and emigration processes, settlement density might not be directly related to recruitment density (Hunt and Scheibling, 1997), defined in this study as the amount of viable individuals for cultivation with lengths around 20 mm.

Several factors may contribute to post-settlement mortality (Hunt and Scheibling, 1997). Fish predation on bivalves has been reported in last few years as a major cause of spat loss in commercial farms (Hortle and Cropp, 1987) and specifically in mussel cultivation (Hayden, 1995 in Schiel, 2004; Brehmer et al., 2003). Suspended cultivation systems provide three-dimensional structures with complex substrata. This may act as fish aggregation devices, which may attract pelagic fishes and may be used by common demersal species present in the area (Morrisey et al., 2006). Fish are relatively large and can move rapidly between habitats across a seascape and therefore have the potential to be more effective predators on a larger spatial scale than intertidal-dwelling invertebrate predators (Robles and Robb, 1993; Rilov and Schiel, 2006a,b). In our study area, Ria de Ares-Betanzos, predation of mussel spat by *Spondyllosoma cantharus* is commonly observed in early summer (PROINSA mussel farm experimental reports 1992–2007; Filgueira et al., 2007; Peteiro et al., 2007). *Spondyllosoma cantharus* belongs to the Sparidae family and has a differential seasonal distribution characterized by migrations to inshore waters in the summer (Perodou and Nedelec, 1980 in Gonçalves and Erzini, 2000; Veiga et al., 2006). All individuals belonging to Sparidae family are classified as generalist predators, feeding opportunistically on a wide variety of prey (Pita et al., 2002; Gamito et al., 2003) and being heavy predators on mussel seed in suspended culture (Brehmer et al., 2003).

Collector designs may interfere in post-settlement mortality linked to predation (Filgueira et al., 2007). Structural complexity may promote protection from predators (Moreno, 1995; Walters and Wethey, 1996; Frandsen and Dolmer, 2002; Lekang et al., 2003; Filgueira et al., 2007). In addition, the physicochemical and textural characteristics of collector rope designs can alter the strength of seed attachment, thereby modifying the probability of detachment by physical disturbance (Lekang et al., 2003).

In order to test fish predation impact on mussel recruitment, we quantified mussel seed recruitment densities with or without fish exclusion. In addition, we have tested four different collector rope designs in each treatment (protected and unprotected from fish predation). The different collector designs offer different textural properties and structural complexity which may enhance the strength of seed attachment and supply refuges for spat from predation.

2. Materials and methods

Four different collector rope designs were deployed in Arnela, a location commonly used as an experimental mussel seed collection area by the PROINSA mussel farm in the Ria de Ares- Betanzos (Galicia, NW Spain; Fig. 1) on 8th February 2007. Besides the traditional collector ropes (non-filamentous texture and lacing without loops; NF-NL) usually employed for mussel seed collection in the industrial cultivation (supplied by Interma nets, S.A.; www.intermas.com), three new rope designs with different lacing (with or without a loop complement) and textures (filamentous or non-filamentous) were evaluated; ropes with a filamentous loop complement (F-L; supplied by Itsakorda S.L.; www.itsakorda.es), ropes with a non-filamentous loop complement (NF-L; supplied by Interma nets, S.A.; www.intermas.com) and filamentous ropes without loops (F-NL; supplied by Itsakorda S.L.; www.itsakorda.es). Six ropes of each design (Fig. 2) were randomly distributed in a long-line. All ropes had a length of 6 m.

On 30th May 2007, two samples from each rope were taken at 1.0–1.5 m depth to quantify density and length of recruits of *Mytilus galloprovincialis*. Sampling involved the removal by scraping of all individuals from a 2 cm section of each rope. Samples were preserved in 70% alcohol until laboratory processing. Since scraping involved the

mussels attached to fibers from the collectors, the mussels were detached from one another using a 20% bleach dilution (Davies, 1974) and ultrasonic bath treatment (Fuentes and Molaes, 1994) for 5 min. Samples were washed through a series of successively finer mesh sieves (2300, 1000, 600, 355 and 125 μm of nominal aperture) to make the counting easier. Each sieved fraction was dried at 80 $^{\circ}\text{C}$ for easier examination and counted using a binocular microscope (Caceres-Martinez et al., 1993). Average shell length in each sieved fraction was calculated by measuring along the anterior–posterior axis with an ocular micrometer under a binocular microscope. A minimum of 30 individuals per sieved fraction or the number needed to obtain a coefficient of variation inferior to 10% was measured. The effect of collector design on mussel seed average shell length (mm) and density expressed as individuals per meter (indiv/m) was tested using a mixed nested ANOVA. Collector design was considered a fixed factor and ropes on each design a random factor nested to collector design. Tukey's HSD test was performed as post hoc test (Zar, 1984). Density values were square root transformed to accomplish homogeneity of variances that was tested for density and length values using the Levene's test (Zar, 1984). No significant effect of random factor "rope" on density (indiv/m) or length (mm) was detected ($p > 0.05$). Therefore, three ropes of each collector design were randomly selected and moved to a contiguous longline (25 m apart) which was whole surrounded with a mesh to exclude fish predation. The remaining three ropes of each design were left on the initial longline, which was exposed to fish predation. Collector designs were randomly distributed in both long-lines.

On 11th September 2007 another sampling took place to evaluate the recruitment when "early thinning-out" was performed. "Early thinning-out" is an industrial procedure in which mussel seed is detached and placed again in the culture at lower densities. In the present study the recruitment is defined in this moment because we assess the amount of viable individuals for fattening in rafts. Each sample involved the removal by scraping of all individuals from a 20 cm section of each rope at 1.5–2.0 m depth. Samples were weighted and density was estimated by counting all the individuals. From each sample, a sub-sample of 250–300 mussels were measured to the nearest 0.1 mm along the anterior–posterior axis using calipers (Mitutoyo1) for the average shell length calculation. The effect of protection from fish predation (fixed factor) and the effect of collector design (fixed factor) on mussel seed density and average shell length were

tested using a mixed two-level nested factorial ANOVA. Ropes of each collector design were considered as a random factor nested to collector design and to protection from predation. Tukey's HSD test was performed as post hoc test. Density values were square root transformed to accomplish homogeneity of variances that were tested for density and length values using the Levene's test (Zar, 1984). All data analyses were carried out using the statistical package Statistica 6.0.

3. Results

Table 1 shows average values of shell length and density of mussel seed of each collector design prior to transfer to the protected long-line and at recruitment for protected and unprotected long-lines. Mixed nested ANOVA showed no significant effect of the random factor (rope) associated to the collector design on density or length ($p > 0.05$; Table 2A) prior to transfer ropes to the protected long-line (30th May 2007). The collector design showed a significant effect on settlement density ($p < 0.001$; Table 2A). The post hoc test illustrated higher density values ($p < 0.05$) in ropes with a loop complement ($194,642 \pm 41,162$ and $174,950 \pm 43,801$ indiv/m for NF-L and F-L, respectively) as compared to ropes without loops ($131,700 \pm 27,775$ and $53,920 \pm 13,060$ indiv/m for F-NL and NF-NL, respectively) which in turn showed also differences between them ($p < 0.001$). With regard to mussel length, no significant differences between collector designs were detected ($p = 0.922$; Table 2A).

Nested factorial ANOVA in the final sampling (11th September) showed no significant effect of the random factor (rope) associated to collector design on density or length ($p > 0.05$; Table 2B). With regard to density, significant effects of protection, collector design and their interaction were detected ($p < 0.001$; Table 2B and Fig. 3). The significant interaction between fixed factors showed an interference of protection treatment in the recruitment density of collector designs (Table 2B; Fig. 3). The results of the post hoc test showed similar values of recruitment density in every collector design in the protected long-line ($p > 0.05$; Fig. 3) but not in the unprotected one ($p < 0.001$; Fig. 3). Excluding the NF-L design, all unprotected ropes showed a significant reduction in the recruitment mean density with regard to the protected ones, but the amount of that reduction varied with the collector design. Unprotected NF-NL ropes showed the lowest density ($p < 0.001$; Fig. 3) and therefore the highest reduction with

regard to the protected ones (58.16%). Unprotected F-L and F-NL designs showed a similar reduction in recruitment mean densities with regard to those designs in the protected long-line (42.33 and 37.75% for F-NL and F-L, respectively). With regard to the unprotected NF-L ropes, no significant density reduction with regard to the protected ones was observed ($p > 0.05$; Fig. 3). With regard to average shell length, no significant effects of protection, collector design or its interaction were detected ($p > 0.05$; Table 2B). Nevertheless, in the protected long-line, a negative correlation between the recruitment density and the recruitment length ($N = 24$, $r = -0.665$, $p < 0.001$) was observed, whereas in the unprotected long-line no significant relationship between both variables was recorded ($N = 24$, $r = 0.152$, $p = 0.478$).

4. Discussion

In the last few years mussel seed of the study area has been strongly affected by predation of the fish *Spondyliosoma cantharus* (PROINSA mussel farm experimental reports 1992–2007; Peteiro et al., 2007), which seems to be related to the coupling between mussel settlement in the area (Peteiro et al., 2007) and the arrival of marine fishes that use shallow-water areas at summer (Reina- Herva s and Serrano, 1987; Faria and Almada, 2006; Veiga et al., 2006). *Spondyliosoma cantharus* distribution is characterized by migrations to inshore waters in the summer (Perodou and Nedelec, 1980 in Gonçalves and Erzini, 2000; Veiga et al., 2006). Settlement pattern of *Mytilus galloprovincialis* in the study area is characterized by a main episode in early summer (Peteiro et al., 2007).

Fish predation has been reported as a major cause of spat loss in bivalve commercial farms (Hortle and Cropp, 1987) and specifically in mussel spat (Hayden, 1995 in Schiel, 2004; Brehmer et al., 2003). In agreement, results of the present study showed that fish predation exclusion leads to an increase in mussel seed obtained even 60% depending on the collector rope design. Although there can be several causes of mussel post-settlement mortality, the design of the present study, in which all ropes were placed in the same location, with the same trophic and hydrodynamic conditions, allows to isolate the effect of fish predation.

Although fish exclusion increased significantly the amount of mussel seed obtained, collector design also determine recruitment density but only in presence of fish predation. Differences in recruitment density between collector designs were only detected in the unprotected long-line. Traditional collector design (NF-NL) showed the lowest recruitment density in the unprotected long-line with a reduction of almost 60% with regard to the protected long-line. Unprotected rope designs with filamentous texture showed significantly lower density reductions (~40% for F-NL and F-L). Thready surfaces provide more attachment points for mussel seed (Pulfrich, 1996) which could diminish detachment associated to fish predation. However, only the non-filamentous loops design (NF-L) did not show a significant reduction in recruitment density between protected and unprotected long-lines. Non-filamentous loops (NF-L) have a certain stiffness that could improve protection from predators as was suggested by Filgueira et al. (2007). High complexity and heterogeneity of a substrate reduce predation pressure and physical disturbances associated by increasing the number of spatial refuges (Shanks and Wright, 1986; Walters and Wetthey, 1996; Frandsen and Dolmer, 2002). Fishes fed intensively on small mussels (<20 mm) (Rilov and Schiel, 2006a, b), therefore rope lacing complexity could provide spatial refuges for spat until mussel seed reaches the “size refuge”.

In the protected long-line, no differences in density were detected between collector designs. A possible explanation could be that increase in mussel density caused by higher survival could intensify intra-specific competition for space and food. The increase of the individual size in a population implies an increment of the space and food requirements. The self-thinning process describes the negative relationship between body size and population density when the individual growth rate involves mortality by intra-specific competition (Westoby, 1984; Guíñez and Castilla, 1999; Guíñez, 2005; Filgueira et al., 2008). This mechanism adjusts the population biomass to the ecosystem carrying capacity and has been reported to act in regulating density in industrial mussel cultivation (Filgueira et al., 2008). The negative correlation between the recruitment density and the recruitment length ($N = 24$, $r = -0.665$, $p < 0.001$) observed in the protected long-line would suggest a population regulation based on self-thinning processes. The latter processes could explain the homogenization of recruitment density in the protected long-line between collector designs despite the differences observed previously to predation exclusion treatment. Accordingly, greater

pressure of intra-specific competition in collector designs which showed higher density previously to the predation exclusion could explain the homogenization of density values at recruitment in the protected long-line. The homogenization of density values could suggest equilibrium in population density limited by the ecosystem carrying capacity. Conversely in the unprotected long-line, where fish predation was not excluded, homogenization of densities at recruitment was not observed. The different density patterns observed between collector designs previously to fish exclusion treatment were maintained at recruitment in unprotected long-line. Density reduction caused by predation would imply a decrease of intra-specific competition, which can cause the non-significant correlation between length and recruitment density in the unprotected long-line ($N = 24$, $r = 0.152$, $p = 0.478$). This would suggest that these populations were not regulated, or not only regulated, by self-thinning processes. These results are in good agreement with [Connell \(1985\)](#) who suggested that recruitment will reflect settlement only when early post-settlement mortality is density independent. Nonetheless, further experiments with different densities of different recruit size classes should be performed to determine the effect of intra-specific competition on population size regulation and its interaction with predation pressure.

In conclusion, fish predation may interfere with population dynamic independently of mussel density values and is a major cause of spat loss for cultivation in the study area. Increasing complexity and heterogeneity of collector ropes may provide space refuges for mussel seed which could increase the survivorship in areas where fish predation represents an important cause of post-settlement mortality. In absence of predation, density dependent processes might become relevant in regulating recruits population size.

Acknowledgements

We wish to thank PROINSA mussel farm and their employees, especially H. Regueiro, M. García, C. Brea and O. Fernandez-Rosende for technical assistance. This study was supported by the contract-project CSIC-PROINSA, Code CSIC 20061089, Galicia PGIDIT06RMA018E.

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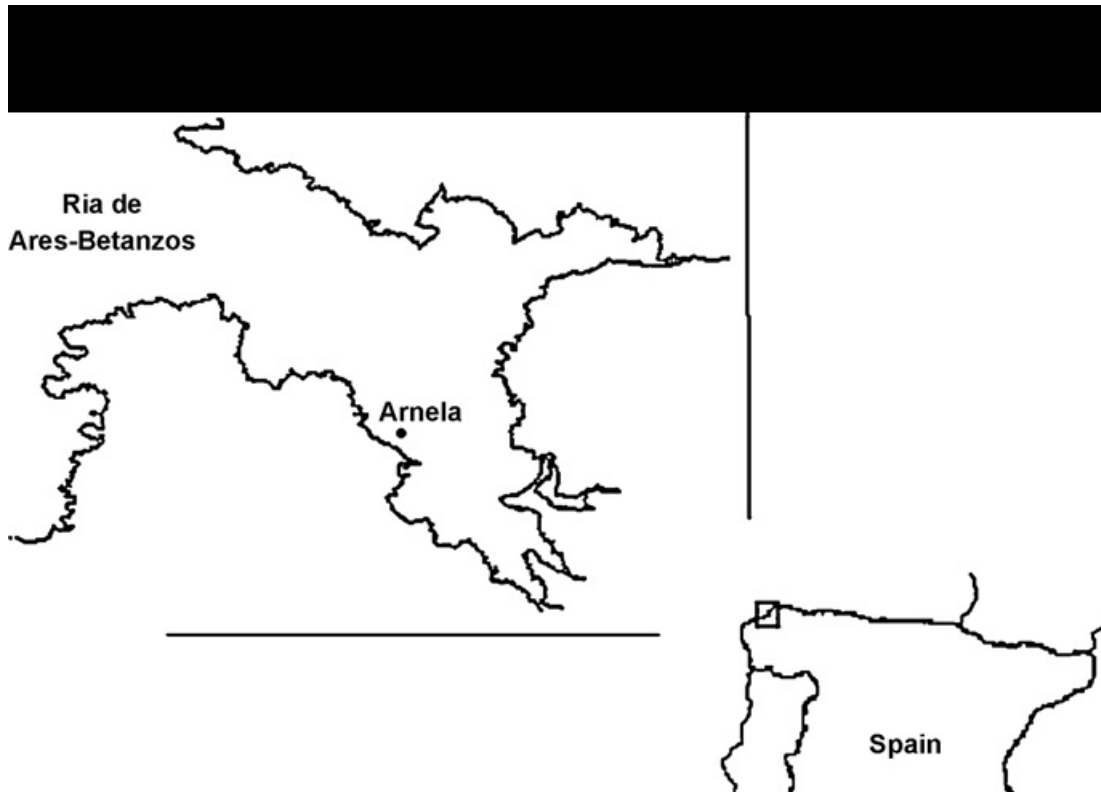


Fig. 1. Map of the Ria de Ares-Betanzos showing the Arnela area under study.

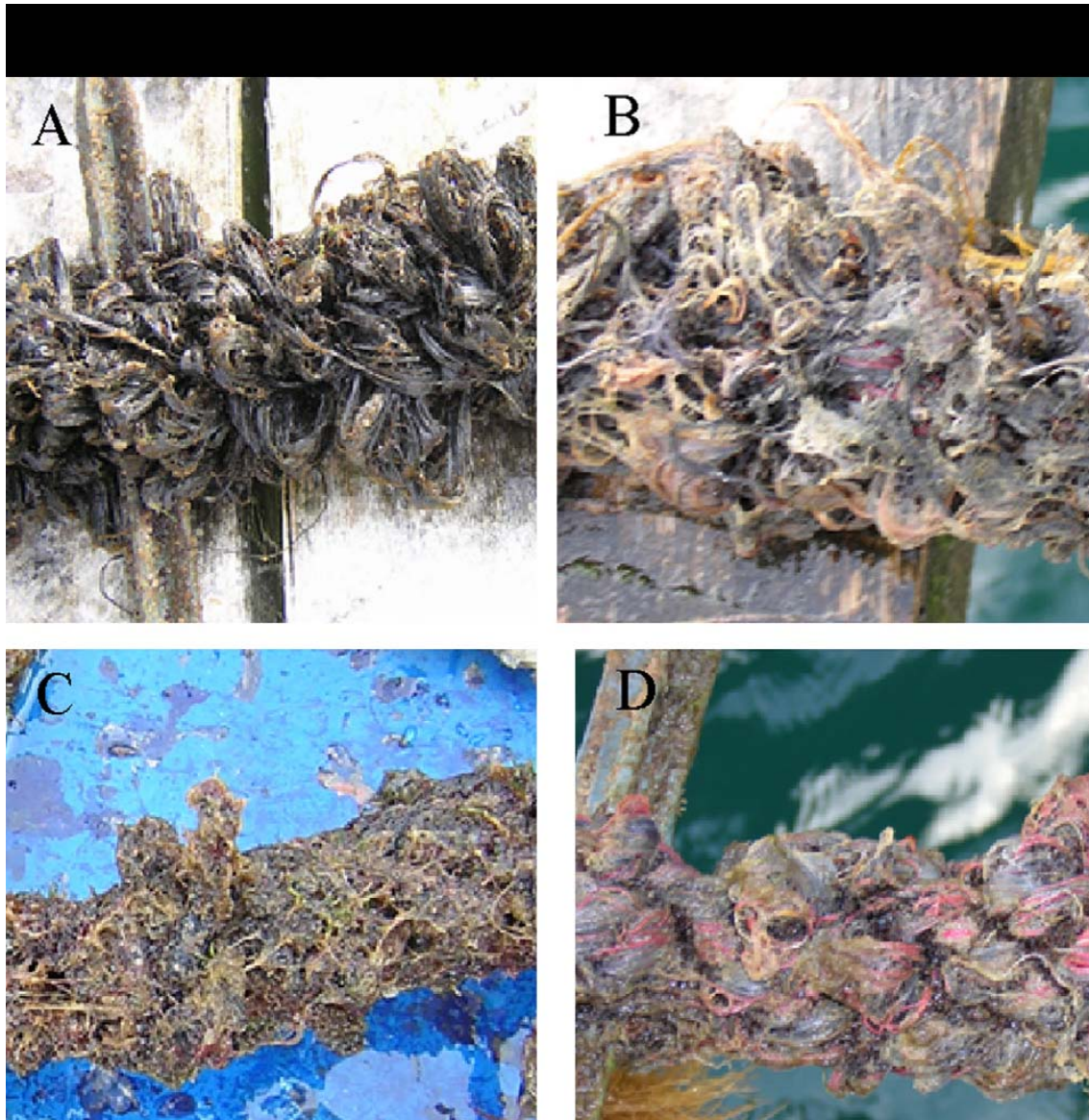


Fig. 2. Collector designs. (A) Ropes with a non-filamentous loop (NF-L), (B) ropes with a filamentous loop complement (F-L), (C) non-filamentous ropes without loops complement (NF-NL) and (D) filamentous ropes without loops (F-NL).

Table 1

Average values of shell length (mm) and density (indiv/m) of mussel seed of each collector design (non-filamentous without loops; NF-NL, filamentous without loops; F-NL, filamentous loops; F-L, and non-filamentous loops; NF-L) prior to transfer to the protected long-line (30th May 2007) and at recruitment (11th September 2007) for protected and unprotected long-lines.

Prior to protection	Collector design			
	NF-L	F-L	F-NL	NF-NL
Density (indiv/m)	194,642±41,162	174,950±43,801	131,700±27,775	53,920±13,060
Length (mm)	1.25±0.02	1.26±0.01	1.25±0.02	1.25±0.02
Recruitment				
Protected long-line				
Density (indiv/m)	9,104±316	8,910±197	8,617±832	8,663±914
Length (mm)	20.16±0.82	20.91±0.88	21.82±0.97	21.68±1.09
Unprotected long-line				
Density (indiv/m)	7,855±375	5,547±1,067	4,969±195	3,625±355
Length (mm)	21.24±1.53	21.81±0.69	21.90±1.01	20.69±1.04

Table 2

Mixed nested ANOVA test to determine the effect of collector design (non-filamentous without loops; NF-NL, filamentous without loops; F-NL, filamentous loops; F-L, and non-filamentous loops; NF-L) on density (indiv/m) and average shell length (mm) prior to fish predation protection treatment (30th May 2007) (A). Mixed two-level nested factorial ANOVA test to determine the effect of protection treatment (protected or unprotected from fish predation) and collector design on recruitment density and average shell length (11th September 2007) (B).

Sources of variation	Total density (indiv/m)					Shell length (mm)				
	d.f.	SS	MS	F	p	d.f.	SS	MS	F	p

				value					value	
(A) Prior to protection treatment										
Collector design	3	311,862	103,954	45.34	<0.001	3	0.00023	0.00008	0.16	0.922
Rope (collector design)	20	45,857	2,293	1.77	0.090	20	0.00945	0.00047	1.87	0.071
Error	24	31,038	1,293			24	0.00605	0.00025		
Total	47									
(B) Recruitment										
Protection	1	5,052	5,052	300.30	<0.001	1	0.85	0.85	0.70	0.417
Collector design	3	1,497	499	29.66	<0.001	3	8.32	2.77	2.28	0.119
Protection x collector design	3	1,029	343	20.38	<0.001	3	8.12	2.71	2.22	0.125
Rope (collector design (protection))	16	269	17	1.27	0.291	16	19.47	1.22	1.27	0.291
Error	24	318	13			24	23.01	0.96		
Total	43					43				

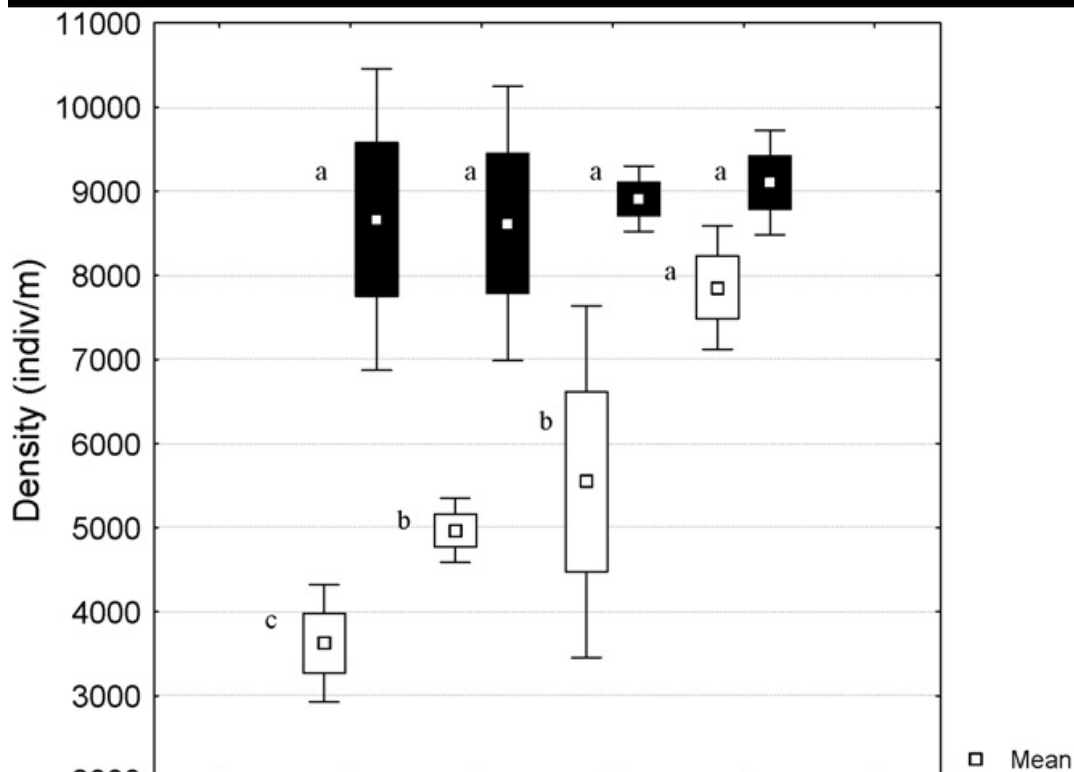


Fig. 3. Recruitment densities (indiv/m) in the protected (black squares) and unprotected (white squares) long-lines for the different collector designs tested (non-filamentous without loops; NF-NL, filamentous without loops; F-NL, filamentous loops; F-L, and non-filamentous loops; NF-L). Post hoc results for the interaction between factors (protection and collector design) are illustrated with different letters for significant differences in density