1 Effect of submerged time of collector ropes on the settlement capacity
2 of *Mytilus galloprovincialis* L.

3 Laura G. Peteiro, Ramón Filgueira, Uxio Labarta and María José Fernández-
4 Reiriz*

5 Instituto de Investigaciones Marinas CSIC, Eduardo Cabello 6, 36208 Vigo, Spain.

6 *Correspondence to M.J. Fernández-Reiriz: tel.: +34 986 231930; fax: +34 986 292762;
7 e-mail: mjreiriz@iim.csic.es

8
9 Running title: Conditioning ropes for mussel spat settlement

10 Keywords: artificial spat collector, biofilm, mussel, *Mytilus galloprovincialis*.

11
12
13
14
15
16
17
18
19
20
21
22
23
24

1 Introduction

2 The supply of mussel seed is critical for the continued development of industrial mussel
3 cultivation, traditionally located in areas where mussel spat are readily available (Mason
4 1976). Direct seed gathering from shorelines is the method mostly used by farmers
5 (66% of mussel seed employed). However, several studies recommend mussel seed
6 from artificial collectors, since their previous adaptation to permanent submersion leads
7 to higher growth rates when subsequently transferred to the raft (Babarro *et al.* 2000).

8
9 Benthic invertebrate larval settlement is affected by several endogenous and exogenous
10 factors which collectively determine the most suitable substratum for larval survival
11 (Hunt & Scheibling 1996). In fact, several species can delay metamorphosis for months
12 until a suitable substratum is located (Lane *et al.* 1985). The substratum characteristics
13 which influence settlement of *Mytilus galloprovincialis* include the physico-chemical
14 properties (Pulfrich 1996), the presence of filamentous structures (Pulfrich 1996), and
15 biofilm development (Todd & Keough 1994; Satuito *et al.* 1995). Changes in the
16 successional composition, physiological condition and growth phase of the biofilm
17 community can also influence larval settlement (Bao *et al.* 2007).

18
19 In view of the fact that the number of ropes per raft is restricted by Galician legislature
20 (maximum 600 ropes of 12 m length during the larval settlement season), there is a clear
21 need to increase the seed yield from artificial collectors. This study investigates the
22 effect of submerged time of collector ropes on the settlement capacity of *Mytilus*
23 *galloprovincialis*.

Material and methods

Experimental collector ropes were hung from the prow of a raft located in Arnela (Ría de Ares-Betanzos, Galicia, NW Spain). Three ropes were weekly hung from the first week of February 2006 to the first larval settlement event (8th May 2006). The ropes were covered with jute to facilitate the sampling of recently settled larvae. When new ropes were hung, the previous ones were sampled (6 x 2 cm of jute material) to identify the first larval settlement event. Samples were taken in triplicate when the first larval settlement event occurred and preserved in 70% alcohol until laboratory processing. The mussels were detached from the jute using dilute bleach and ultrasonic bath treatment, then washed, dried at 80°C and counted using a binocular microscope.

Degradation of the jute on ropes submerged for more than 46 days prevented accurate quantification of the surface area. Therefore, the experiment was repeated in 2007 using domestic pan scourer instead of jute. The ropes were hung in the first week of February and the first larval settlement event was observed on 16th April.

The effect of submerged time on larval settlement abundance during the first larval settlement event was investigated using the Kruskal-Wallis test followed by pairwise analyses (U Mann-Whitney; SPSS 14.0).

Results

Figure 1 shows the mean settlement density on ropes submerged between 11 and 45 days prior to the first larval settlement event in 2006. The significant differences observed allow four homogeneous groups to be established (Fig. 1). The ropes submerged for more than 40 days showed significantly higher settlement densities

(121.39 ± 18.65 indiv. cm^{-2}) than the other ropes. Similarly, the ropes submerged between 27 and 35 days showed significantly higher densities (59.03 ± 13.62 indiv. cm^{-2}) than those observed for the ropes hung for 11 days (31.82 ± 5.32 indiv. cm^{-2}). Finally, ropes submerged 21 days showed intermediate density values (40.76 ± 12.22 indiv. cm^{-2}) with ropes hung for 11 and 27 days.

Figure 2 shows the settlement density on ropes submerged between 12 and 80 days prior to the first larval settlement event in 2007. As for the 2006 data, four homogeneous groups can be established (Fig. 2). The ropes hung for more than 46 days showed significantly higher settlement densities (13.1 ± 1.64 indiv. cm^{-2}) than the other ropes. Similarly, the ropes submerged between 27 and 40 days showed significantly higher densities (4.9 ± 0.04 indiv. cm^{-2}) than the 19 day ropes (3.6 ± 0.92 indiv. cm^{-2}) which, in turn, registered higher values than those for the 12 day submerged ropes (2.1 ± 0.40 indiv. cm^{-2}).

Discussion

Under natural conditions, biofilm formation can be described as a succession of processes beginning with the formation of an organic film (amino acids, glycoproteins and humic materials), followed by colonization of bacteria, diatoms, fungi and protozoans (Characklis & Cooksey 1983). In general, the magnitude of larval settlement increases with biofilm age (Keough & Raimondi 1995; Bao *et al.* 2007), although some species does not follow this trend (Keough & Raimondi 1995; Rahim *et al.* 2004). The present study shows a significant increment of *Mytilus galloprovincialis* settlement density with biofilm age, which agrees with observations by Bao *et al.* (2007) using the same species.

Increased submerged time favors greater bacterial and diatom densities, which is considered to be an important factor for larval settlement and metamorphosis (Rahim *et al.* 2004; Bao *et al.* 2007). In addition to quantitative aspects related to biofilm age, qualitative aspects such as metabolic activity (exuded products, Satuito *et al.* 1995; Bao *et al.* 2007) or community structure (Wieczorek & Todd 1997) might be also important.

The interannual variability of the settlement density recorded might be related to differences in the substratum employed although variability in settlement could also be attributed to changes in the quantity or seasonality of the spawning, as well as the interaction between environmental factors (temperature, food or hydrographic conditions) (Porri *et al.* 2006). The environmental variability may influence biofilm development and, therefore, its inductivity capacity (Bao *et al.*, 2007), as well as partly explaining the differences between the minimum submerged time in both experiments (41 and 46 days in 2006 and 2007, respectively). Although more specific investigations on factors influencing biofilm development and its inductivity capacity on *Mytilus galloprovincialis* larval settlement would be interesting, the results presented seem to state a minimum submerged time required to optimize the mussel seed yield on artificial collectors.

Acknowledgements

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

References

- Babarro J.M.F., Fernández Reiriz M.J. & Labarta U. (2000). Growth of seed mussel (*Mytilus galloprovincialis* Lmk): Effects of environmental parameters and seed origin. *Journal of Shellfish Research* **19**, 187-193.
- Bao W.Y., Satuito C.G., Yang J.L. & Kitamura H. (2007). Larval settlement and metamorphosis of the mussel *Mytilus galloprovincialis* in response to biofilms. *Marine Biology* **150**, 565-574.
- Characklis W.G. & Cooksey K.E. (1983). Biofilms and microbial fouling. *Advances in Applied Microbiology* **29**, 93-138.
- Hunt H.L. & Scheibling R.E. (1996). Physical and biological factors influencing mussel (*Mytilus trossulus*, *M. edulis*) settlement on a wave-exposure rocky shore. *Marine Ecology Progress Series* **142**, 135-145.
- Keough M.J. & Raimondi P.T. (1995). Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. *Journal of Experimental Marine Biology and Ecology* **185**, 235-253.
- Mason J. (1976). Cultivation. In: *Marine mussels: their ecology and physiology*. (ed. By B. L. Bayne), pp. 385-410, Cambridge University Press, Cambridge UK.
- Lane D.J.W., Beaumont A.R. & Hunter J.R. (1985). Byssus drifting and the drifting threads of young postlarval mussel *Mytilus edulis*. *Marine Biology* **84**, 301-308.

1 Porri F., McQuaid C.D. & Radloff S. (2006). Spatio-temporal variability of larval
2 abundance and settlement of *Perna perna*: differential delivery of mussels. *Marine*
3 *Ecology Progress Series* **315**, 141-150.

4
5 Pulfrich A. (1996). Attachment and settlement of post-larval mussel (*Mytilus edulis* L.)
6 in the Schleswig-Holstein Wadden Sea. *Journal of Sea Research* **36**, 239-250.

7
8 Rahim, S.A.K.A. & Kitamura J.-Y.L.H. (2004). Larval metamorphosis of the sea
9 urchins, *Pseudocentrotus depressus* and *Anthocardis crassispina* in response to
10 microbial films. *Marine Biology* **144**, 71-78.

11
12 Satuito C.G., Natoyama K., Yamazaki M. & Fusetani N. (1995). Induction of
13 attachment and metamorphosis of laboratory cultured mussels *Mytilus edulis*
14 *galloprovincialis* larvae by microbial film. *Fisheries Science* **61**, 223-227.

15
16 Todd C.D. & Keough M.J. (1994). Larval settlement in hard substratum epifaunal
17 assemblages: a manipulative field study of the effects of substratum filming and the
18 presence of incumbents. *Journal of Experimental Marine Biology and Ecology* **181**,
19 159-187.

20
21 Wieczorek S.K. & Todd C.D. (1997). Inhibition and facilitation of bryozoan and
22 ascidian settlement by natural multi-species biofilms: effects of film age and the roles of
23 active and passive larval attachment. *Marine Biology* **128**, 463-473.

Figure Legends

Fig. 1 Mean and standard deviation of the settlement density (indiv. cm⁻²) on collector ropes hung between 11 and 46 days prior to the first larval settlement event in 2006. Homogeneous groups were calculated using the non parametric ranking test of U Mann-Whitney and are indicated by the labels a, b, c and d.

Fig. 2 Mean and standard deviation of the settlement density (indiv. cm⁻²) on collector ropes hung between 12 and 80 days prior to the first larval settlement event in 2007. Homogeneous groups were calculated using the non parametric ranking test of U Mann-Whitney and are indicated by the labels a, b, c and d.

Fig.1

Conditioning ropes for mussel spat settlement

Peteiro *et al.*

Fig. 2

Conditioning ropes for mussel spat settlement

Peteiro *et al.*