



# Role of fish farm fouling in recolonisation of nearby soft-bottom habitats affected by coastal aquaculture

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## ABSTRACT

Organic loading from fish farming constitutes a significant disturbance to marine sediment, normally reducing species abundance and richness and creating disturbed patches in soft-bottom communities. In contrast, floating fish farms harbour a high abundance of invertebrates associated with fouling communities, particularly amphipods. Changes in macrofaunal recolonisation induced by fish farms were researched using amphipod assemblages as a useful representative group. The objectives of this experiment were: (1) to test the effect of fish farms on amphipod colonisation processes in defaunated sediments and (2) to define the influence of surrounding natural and artificial habitats as sources of initial colonisers. Experimental design included 36 plastic trays placed on the sea bottom (25–28 m depth) in fish-farming and control areas and retrieved one month after placement date. Significant differences were found in amphipod assemblage composition in control versus farm sites. While the recolonisation process in control areas depended on the species present in the sediments adjacent to the experimental trays, in fish-farming areas the recolonisation was strongly dependent on the input of amphipods from fouling communities. It is the first time that a spillover effect from fouling communities on floating habitats into the benthos is detected in the coastal areas. If the organic enrichment of aquaculture sediments is moderate, the ecological services of benthic habitats may be balanced, at least partly, by biomass exported from fouling communities on the same fish farms.

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## 1. Introduction

High organic loading on the sea bottom is a major anthropogenic disturbance to the marine benthos that constitutes a significant problem in coastal-zone management (Cloern, 2001). Coastal aquaculture perturbations are mainly derived from surplus fish feed and waste products accumulated on marine sediment (Gowen and Bradbury, 1987; Kalantzi and Karakassis, 2006) which cause silting, increased oxygen demand, anoxic sediment generation and toxic gases on the seabed (Wu, 1995; Borja, 2002). These negative effects may defaunate and disturb patches in soft-bottom communities (Berge, 1990; Lu and Wu, 1998; Pereira et al., 2004). Indeed, benthic assemblages are often eliminated or reduced below the cages (Edgar et al., 2005; Tomassetti et al., 2009; Martinez-Garcia et al., 2013). This process maintains spatio-temporal mosaics of affected sediment patches on the sea bottom (Hargrave et al., 1997), which are reflected as a wide variability in benthic communities affected by fish farming, on a scale of metres (Fernandez-Gonzalez et al., 2013).

While benthic fauna in offshore fish-farming areas are negatively affected under the cages, these structures, namely in the nets, ropes and buoys, harbour a high abundance of invertebrates (Fitridge et al.,

2012), which are potential colonisers to the neighbouring habitats. Amphipods are numerically dominant organisms among fish-farm fouling epifauna (Greene and Grizzle, 2007; Fernandez-Gonzalez and Sanchez-Jerez, 2014) and are among the most diverse and abundant groups of soft-bottom fauna (Gómez-Gesteira and Dauvin, 2000; De-la-Ossa-Carretero et al., 2010; Carvalho et al., 2012). They constitute an important link in the food web, since they are primary productivity consumers and also predators of larvae and adult organisms, being in turn a preferential prey of small crustaceans, polychaetes and many fish species (Bellan-Santini et al., 1998; Jiménez-Prada et al., 2015). Amphipods lack planktonic larvae so, as in most peracarids, embryos are carried by females in a ventral brood pouch or marsupium, from which fully-developed juveniles emerge (Sainte-Marie, 1991; Thiel, 1999). This impossibility of recruitment by pelagic larvae means that the colonisation must have been by juveniles or adults via crawling or swimming from nearby populations (Guerra-García and García-Gómez, 2006) or via drifting from distant communities (Cummings et al., 1995; Thiel, 2003).

Settlement and colonisation processes are important for soft-bottom benthic species and may be affected by external stressors such as organic enrichment (Snelgrove and Butman, 1994). Some ecological aspects of these processes have been studied through field experiments on artificially defaunated sediment in intertidal (Smith and Brumsickle, 1989; Negrello Filho et al., 2006) and subtidal habitats (Bell and Devlin, 1983;

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Wu and Shin, 1997; Lu and Wu, 2000; Hercul et al., 2006; Ferrero-Vicente et al., 2013). Moreover, experimental trays have also been used to elucidate environmental impacts such as dredging (Bolam et al., 2004; Guerra-García and García-Gómez, 2006), oil-spills (Berge, 1990; Lu and Wu, 2006), fish-farming (Lu and Wu, 1998), heavy metals and industrial wastes (Lu and Wu, 2003, 2007a; Trannum et al., 2004) on colonisation by benthic species. These experiments focused on recovery once the cause of defaunation had disappeared, therefore the dynamics of soft-bottom disturbed patches subsequently recolonised during the ongoing disturbance are still poorly known.

Initial colonisation of macrobenthos depends largely on local species availability at the moment when the substrate first becomes available (Wu and Shin, 1997; Diaz-Castañeda et al., 1993; Lu and Wu, 2007b). In this study, the changes of macrofaunal recolonisation induced by fish farms were researched using amphipod assemblages as a useful representative group. We hypothesised that artificially defaunated sediments would be recolonised by local soft-bottom populations but also affected by amphipod species from fouling communities attached to floating cage structures. The objectives of this experiment were: (1) to test the effect of fish farms on amphipod colonisation processes in defaunated sediments, and (2) to define the influence of the surrounding natural and artificial habitats as sources of the initial colonisers entering experimental trays.

## 2. Material and methods

### 2.1. Study area

Manipulative experiments were carried out off the coast of Guardamar del Segura (Alicante, SE Spain: 38° 5'45.88"N; 0°36'15.84" W) during June–August 2010. In this bay, 3 fish-farm facilities grow European sea bass *Dicentrarchus labrax* and gilthead sea bream *Sparus aurata*. The cage structures are located 3 to 4 km offshore at depths ranging from 23 to 30 m. The sea bottom in the Guardamar bay is homogeneous in terms of sediment particle size, in fact muddy sediments are mainly found (Fernandez-Gonzalez and Sanchez-Jerez, 2011; Fernandez-Gonzalez et al., 2013).

### 2.2. Experimental performance

Sediments for manipulative experiments were collected in situ by divers. Sediment particle size of these sediments was determined as silty-clay (Sand:  $6.84 \pm 1.39\%$ ; Silt:  $48.80 \pm 1.16\%$ ; Clay:  $44.36 \pm 2.56\%$ ) using Bouyucos method (Buchanan, 1984) and organic matter content was  $5.86 \pm 0.31\%$  (Loss-on-Ignition: 450 °C 4 h, Buchanan, 1984). Method for sediment defaunation was based on Guerra-García and García-Gómez (2006), thus sediments were frozen at  $-20$  °C for three days, and dried first under natural sunlight for one day and then at 40 °C in an oven until constant weight. This method was repeated twice.

Thirty-six experimental units (EUs, consisting of  $24 \times 15 \times 6$  cm plastic boxes) were filled with defaunated sediment, and their lids closed. The EUs were submerged and placed by divers on the sea bottom at 25–28 m depth, with their tops lying flush with the sediment surface, and then the lids were removed.

To test the effect of aquaculture facilities on recolonisation, the experimental design included two fish farms with three random sites each and three replicated EUs at each site, located below the cages. Analogously, two control areas with three sites each and three replicated EUs were selected, considering similar spatial scales as at fish-farm facilities.

After one month (35 days), EUs were closed and retrieved by divers. A subsample of sediment was taken from each to determine sediment particle size, organic matter and total free sulphides (TFS, Wildish et al., 1999), so real surface area of sampling was  $0.0315$  m<sup>2</sup>. The rest

of the sediment in each tray was sieved through a 0.25 mm mesh, in order to ensure the retention of small specimens, and the residues retained were preserved in a 10% formalin seawater solution. In the laboratory, amphipods were sorted, identified to species level whenever possible, and counted according to sex and life-history stage: males, females, brooding females and juveniles.

### 2.3. Source of colonisers data

To determine the source of colonisers, data obtained on amphipods in soft-sediment sampled using Van Veen grabs ( $20.5 \times 19$  cm or  $20.5 \times 13$  cm) were taken from previous studies (Fernandez-Gonzalez and Sanchez-Jerez, 2011; Fernandez-Gonzalez et al., 2013). This enabled natural assemblages on the surrounding sea bottom to be characterised.

Additionally, fouling communities at the two studied fish farms were sampled by scraping fouling organisms from mooring ropes. From each farm, six replicates 20 cm in length per rope were cleared using an air-lift device to ensure a quantitative method in high hydrodynamic conditions. The diameter of each rope was recorded to calculate an accurate sampled surface. The samples were sieved, preserved and amphipods sorted and identified in the same way as from EUs.

### 2.4. Statistical analysis

The data were analysed according to a 3-factor hierarchical design: 'Control and Farm' (fixed and orthogonal; two levels), 'Locality' (random and nested in 'Control-Farm' with two levels) and 'Site' (random and nested in 'Locality' with three levels) and three replicates.

To explore differences in amphipod assemblage recolonisation, non-metric multidimensional scaling (MDS; Clarke and Warwick, 1994) and the percentage similarities procedure (SIMPER) were used. A permutational multivariate analysis of variance based on the Bray–Curtis dissimilarities of the log ( $x + 1$ ) transformed data (PERMANOVA; Anderson, 2001a; McArdle and Anderson, 2001) was performed to analyse the differences in the overall species composition. The analysis was tested using 4999 random permutations of residuals under a reduced model (Anderson, 2001b), with appropriate units as required by the design (Anderson and ter Braak, 2003). When the number of possible permutable units was not enough to get a reasonable test by permutation, a *p*-value was obtained using a Monte Carlo test (Anderson and Robinson, 2003).

Additionally, sediment variables, total abundance and number of species of recolonising amphipods, and abundance of the most

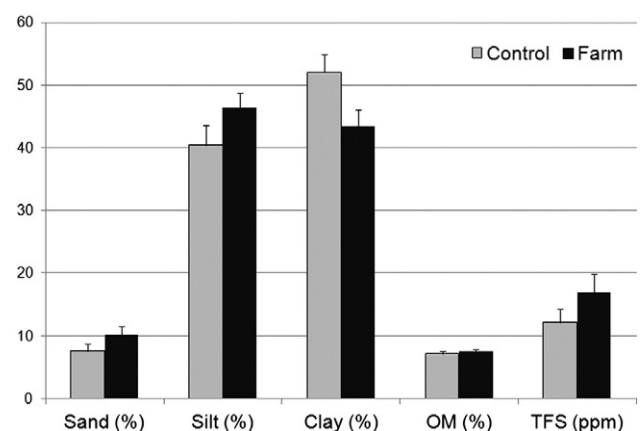


Fig. 1. Mean values ( $\pm$  standard error) of sediment variables in control and farm experimental trays after 35 days. OM: organic matter content and TFS: total free sulphides.

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