



The use of mussels for mitigating the noxious effect of phytoplankton spring blooms on farmed fish



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ABSTRACT

The possibility of using the natural biofiltration power of blue mussels (*Mytilus edulis*, $0.37 \pm 0.08 \text{ g ind}^{-1}$ dry weight) to dampen the potential detrimental effect of phytoplankton blooms on juvenile farmed sea bass (*Dicentrarchus labrax*) was tested in a fish farm during a 35-day mesocosm experiment. Mussel effective clearance rates averaged $41.15 \pm 14.19 \text{ m}^3 \text{ h}^{-1}$ and led to a 6.3–13.1-fold reduction of the phytoplankton abundance as well as comparable decreases in chlorophyll *a* and turbidity. This improvement in seawater quality significantly enhanced fish physiological performances: weight-based growth rates were significantly higher ($2.87 \pm 0.43\% \text{ d}^{-1}$) compared to control exposed to non-filtered (bulk) seawater ($2.55 \pm 0.44\% \text{ d}^{-1}$). The same observation holds for the Fulton condition index and the metabolic activity (RNA:DNA ratio). For fish reared in bulk seawater, diatoms embedded in gills (*Rhizosolenia imbricata*, *Thalassiosira* sp.) and mucus overproduction indicated a stress (i.e. mechanical damages) induced by phytoplankton exposure which, in turn, may have affected fish energy balance. The use of mussels as a satisfying mitigation tool reducing phytoplankton bloom impacts is discussed with regard to phytoplankton bloom magnitude and ashore marine fish farming in coastal ecosystems.

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1. Introduction

Harmful algal blooms (HABs) are responsible for massive fish mortalities in coastal aquaculture worldwide (Bruno et al., 1989; Brusle, 1996; Kent et al., 1995; Landsberg, 2002; Park et al., 2013; Thangaraja et al., 2007). They can also have sub-lethal effects such as appetite loss leading to a reduced fish growth (Rodger et al., 2011; Treasurer et al., 2003). In the 90s, Black et al. (1991) suggested four deleterious mechanisms to be caused by HABs: (i) physical damage to mucous membranes, (ii) asphyxiation due to oxygen depletion, (iii) gas bubble trauma resulting from photosynthesis driven oxygen hyper-saturation, and (iv) ichthyotoxicity (see review by Landsberg, 2002). Although sharp diatoms such as *Chaetoceros* sp. were proven to be nutritionally, and cost efficient in fish aquaculture and shellfish hatcheries (see review by Brown, 2002); their excessive proliferation was responsible for massive salmonid kills in fish farms located in British Columbia and Scotland by causing irritation, mucus overproduction and clogging of gills leading to oedema, necrosis, and asphyxiation (Albright et al., 1993; Bruno

et al., 1989; Kent et al., 1995; Treasurer et al., 2003; Yang and Albright, 1992). Other studies suggest that changes in seawater rheological properties induced by some phytoplankton blooming species (e.g. *Phaeocystis* sp. – Kesaulya et al., 2008; Seuront and Vincent, 2008; Seuront et al., 2006) can cause fish mortalities through respiration, and excretion hindering (Jenkinson, 1989).

For several years, coinciding with the phytoplankton spring bloom, mass mortalities in sea bass (*Dicentrarchus labrax*) rearing stocks have regularly been observed in a southern North Sea fish farm (Aquanord S.A., Gravelines, France). These mortalities, reaching about 20% of the annual fish mortality, were not explained by classical diseases such as parasitism, bacterial or viral infections. However a deleterious effect of the phytoplankton spring bloom has been suspected since potentially noxious species such as *Phaeocystis globosa*, sharp, and needle-shaped diatoms (e.g. *Chaetoceros* sp., *Rhizosolenia* sp., *Thalassiosira* sp.), and potentially toxic taxa (*Pseudo-nitzschia* sp., Lelong et al., 2012) were commonly observed in rearing seawater and fish gills.

Typical mitigation methods against HAB usually consist in modifying the rearing environment via physical means such as seawater aeration increase, water circulation modification, screen filtration, and ozone and UV radiations (Rodger et al., 2011). Chemical means have also been experimented (sodium hypochlorite, clay, and surfactants; Kim, 2006; Park et al., 2013) but they can be expensive,

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and detrimental to the environment (Rodger et al., 2011). Finally, the only developed biological technique tested so far is represented by micro-algae killers (e.g. bacteria from *Cytophaga*, *Alteromonas* and *Pseudoalteromonas* genus; Fukuyo et al., 2002; Imai, 2005; Imai et al., 1993) although its application is limited to enclosed systems. The use of filter-feeding organisms (e.g. bivalves or copepods) for HAB mitigation has already been considered in Korea but, due to the natural hydrodynamism, they did not represent a satisfying solution in open aquaculture systems (e.g. fish cages; Fukuyo et al., 2002). Quantitatively, blue mussels (*Mytilus edulis*) are known to be very efficient filter feeders with high clearance rates (i.e. $1\text{--}7\text{ L h}^{-1}\text{ g}^{-1}$, Petersen et al., 2004), and are able to significantly reduce seston (Dame et al., 1991; Davenport et al., 2000; Riisgård et al., 2011), and phytoplankton abundance (Dame et al., 1991; Grange and Cole, 1997; Ogilvie et al., 2000; Riisgård et al., 2011; Trottet et al., 2008). In fact, up to 60% of phytoplankton biomass can be removed from the water passing through a mussel farm (Gibbs et al., 1992; Waite, 1989). Qualitatively, mussel filtration is also reported to induce shifts in phytoplankton community composition from micro to pico-nanophytoplankton dominance. In fact, mussels efficiently retain (100%) particles larger than $7\text{ }\mu\text{m}$ although smaller particles ($1\text{--}4\text{ }\mu\text{m}$) can occasionally constitute a significant dietary component (Olsson et al., 1992; Prins et al., 1995; Strohmeier et al., 2012).

Given their possible noxious effects, dampening phytoplankton blooms would result in a subsequent improvement of fish condition and growth. This hypothesis was tested by using blue mussels as a screen filter of juvenile sea bass (*D. labrax*) rearing seawater in a southern North Sea ashore fish farm during a 35-day mesocosm experiment. Mussel filtration activity was estimated, and water quality improvement induced by this biological filtration was assessed by changes in hydrobiology, and phytoplankton components. Effects on reared juvenile sea bass physiological performances were determined, and results discussed with regard to bloom magnitude, existing mitigation tools and possible improvements of the developed experimental design.

2. Materials and methods

2.1. Experimental design

In order to assess seawater quality improvement through mussel biofiltration, and its consequences on the growth, and the condition of juvenile sea bass, a 35-day mesocosm experiment was carried out during the 2013 phytoplankton spring bloom (April 16th–May 21st, Fig. 1). The experimental setting consisted in three 5 m^3 mesocosms (95 cm height, 230 cm length, and width, Fig. 1). The fish farm, and thus the experimental system, was supplied with bulk seawater, i.e. a mix between natural coastal North Sea water, and heated ($+10^\circ\text{C}$ compared to in situ temperature) and chlorinated (1%) seawater released from the Gravelines Power Plant cooling system (France). Bulk seawater therefore comprised both fresh and degraded natural plankton components. Bulk seawater was filtered by mussels in a biofiltration tank (M) before fuelling a test tank (T) containing juvenile sea bass. The third tank was used as a control (C), and contained juvenile sea bass reared in bulk seawater (i.e. not biofiltered). In all the tanks, the flow rate was set at $5\text{ m}^3\text{ h}^{-1}$ allowing for a total water renewal every hour, and photoperiod followed natural day:night cycle. Six weeks before the experiment, mussels were collected at the entrance of the fish farm, in the seawater fuelling system. Mussels were rinsed with bulk seawater, and acclimated in a seawater flow-through tank (5 m^3 ; $10\text{ m}^3\text{ h}^{-1}$) using plastic mesh (10 mm) bags (see supplementary materials). At t_0 (beginning of the experiment), mussels were rinsed and living individuals were conditioned in 8 kg mesh bags so as

to reach 300 kg mussels ($n = 31,496$; shell length: $47.55 \pm 3.85\text{ mm}$, fresh weight: $9.53 \pm 2.16\text{ g}$) in the biofiltration tank (M, 60 kg m^{-3}), a density consistent with densities usually experimented during the depuration phase in mussel culture (Savary and Blin, regional shellfish farming comity, North Sea – Normandie). In order to optimize water residence-time in the biofiltration tank, and thus the mussel filtration efficiency, mesh bags were suspended one above the other and PVC walls inside the tank created a concentric water flow.

Juvenile sea bass (13,000 individuals, $4.98 \pm 0.76\text{ g}$, five-month old) were supplied by the Gravelines Marine Hatchery (Ecloserie Marine de Gravelines, France). The experiment focused on the youngest stages reared at the fish farm i.e. the more sensitive stage with regard to biotic and abiotic stress (Albright et al., 1993; Rodger et al., 2011). Furthermore this stage was considered as it is the most impacted one during the wane of the phytoplankton spring bloom in the fish farm. Fish were randomly and equally distributed into C and T tanks, and acclimated for one week in bulk seawater. Fish density in C and T tanks was 7.5 kg m^{-3} , which is consistent with the density used in the fish farm at the beginning of the pre-growing stage.

Fish were hand-fed twice a day with commercial food (2–3% of fresh weight; Skretting, Mar-Perla-MP-L). At t_0 , 60 fish from each tank were randomly sampled, anesthetized (clove oil, 30 mg L^{-1} , Mylonas et al., 2005), measured (total length, $\pm 0.1\text{ mm}$), and weighted ($\pm 0.01\text{ g}$) to define initial fish condition.

Experimental tanks were flushed every day (10–20% of total volume) to remove particles settled at the bottom (mostly mussels faeces/pseudo-faeces, and fish faeces). For biofiltration tanks, an additional complete flush was carried out once a week, and mussels mesh bags were thoroughly rinsed with bulk seawater before being resuspended. Each tank was aerated through the fish farm oxygen circuit in order to maintain oxygen concentration to $8.92 \pm 4.47\text{ mg L}^{-1}$ ($\sim 100\%$ saturation).

Given the logistic constraints, such as the number of available tanks, their respective volume (5 m^3) and consequently the large amount of organisms needed (mussels and fish), a single experiment was carried out. However, considering the innovative nature of the experimental protocol (i.e. mussels used upstream from the rearing fish tanks), and the larger number of parameters monitored (see next section) compared to previous studies, this study should be considered as a test-case experiment for phytoplankton blooms noxious effects mitigation.

2.2. Sampling strategy

2.2.1. Hydrobiological parameters

Hydrobiological parameters were assessed twice a week. Sampling and measurements were always carried out before the first food supply. Hydrological parameters were measured with probes placed at the middle of the tank (50 cm depth – Fig. 1): temperature ($^\circ\text{C}$), and salinity were measured with an Aanderaa Instruments probe whereas pH, and dissolved oxygen (mg L^{-1}) were obtained using a HANNA pH probe, and an oxymeter (Handy Polaris Oxy-guard), respectively.

Seawater was sampled in C and T tanks for turbidity (EUTECH Instruments Waterproof), chlorophyll *a*, and pheopigments. Chlorophyll *a* and pheopigments were estimated by fluorimetry (Trilogy, Turner Designs) following Lorenzen (1966). Briefly, triplicate seawater samples (500 mL) collected in C and T tanks were immediately filtered on glass fibre filters (Whatman GF/F), and frozen (-20°C) until analysis. In the laboratory, filters were extracted overnight in 90% acetone, and fluorescence values converted to pigment concentrations (chlorophyll *a* and pheopigments, $\mu\text{g L}^{-1}$) using a standard chlorophyll *a* solution (*Anacystis nidulans*, Sigma).

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