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Effects of seasonal variations in phytoplankton on the bioenergetic responses of mussels (*Mytilus galloprovincialis*) held on a raft in the proximity of red sea bream (*Pagellus bogaraveo*) net-pens

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ABSTRACT

The seasonal variability of the physiological components of the Scope for Growth (SFG) of mussels *Mytilus galloprovincialis* was investigated in a raft adjacent (170 m) to fish net-pens and compared with a raft 550 m distant from the cages in Ría Ares-Betanzos (Galicia, Spain). Chlorophyll and phytoplankton size-classes were determined in the field, simultaneously with SFG. Average chlorophyll-*a* was 0.65 ± 0.24 μg l^{-1}, while nanophytoplankton (2–20 μm) was the most abundant size-class, ranging from 50 to 70% of the total chlorophyll. The temporal pattern found for chlorophyll-*a* and phytoplankton size-classes reflected the upwelling–downwelling events and were correlated with the feeding, digestive and metabolic rates. Nanophytoplankton and microphytoplankton were preferentially cleared and ingested by mussels. There were no significant differences between the chlorophyll and phytoplankton size-classes among rafts. The lack of any enhancement in food availability resulted in no significant increase in the SFG of mussels beside the fish cages. Maximum SFG corresponded with the autumn (16.60 ± 7.90 J h^{-1}) and spring (12.72 ± 9.32 J h^{-1}) chlorophyll maximums. An abnormally hot summer and reduced chlorophyll levels resulted in lower energy intake, significantly higher metabolic expenditure and a negative SFG (-34.57 ± 12.55 J h^{-1}). Any particulate wastes and potential fish-derived chlorophyll enhancement would be rapidly diluted by the currents, while the placement of bivalves too distant from the fish farm in an environment with high supplies of natural seston may explain the lack of an augmented SFG of the co-cultured mussels.

1. Introduction

Since the 1980s the rapid population growth coupled with a rising seafood demand has led aquaculture to become the fastest growing food sector in the world. Shellfish farming is one of the most important mariculture products and represents 42.8% of the global production (FAO, 2009). The Galician Rías (N.W. Spain) have a thriving mussel industry. Galicia is the third largest producer of mussels (*Mytilus galloprovincialis*) in the world, with 3300 floating rafts that produce 250,000 tons year^{-1} worth more than $165 million US (Labarta et al., 2004). Mussel farming started in 1946 and provides 9000 and 20,000 direct and indirect jobs (Labarta et al., 2004). The high production of phytoplankton during the upwelling season (March–October) provides food of high quality (50% organic content) that is efficiently absorbed (60%) by the cultured mussels (Figueiras et al., 2002). The sheltered coasts of the Galician Rías also provide a suitable environment for open-water intensive sea cage fish-farming, but environmental concerns and limited space to allocate the cages are the main issues restraining the expansion of this activity. Caged fish-farming releases large amounts of solid organic nutrients (i.e. organic C, N and P contained in undigested feed pellets and feces) and dissolved inorganic nutrients (i.e. NH^{3+}, PO_4^{3-} and CO_2 through excretion and respiration) (Wang et al., 2012) that have been associated with phytoplankton blooms near the fish cages (Pitta et al., 2009; Sarà et al., 2012). Several studies have indicated that mussels could be cultured alongside fish cages to utilize the additional phytoplankton production, the unconsumed feed and fish feces as an additional food source, while simultaneously offering environmental, economical and social benefits (Handå et al., 2012a, 2012b; Lander et al., 2012; MacDonald et al., 2011). The synergistic culture of finfish (fed aquaculture) in close proximity to mussels or other organic (e.g. sea cucumbers) or inorganic (e.g. seaweed) extractive species, is a practice known as Integrated Multi-Trophic Aquaculture (IMTA) (Chopin et al., 2008, 2012). IMTA is considered a potential strategy to recycle surplus organic and inorganic nutrients released from fish farms and simultaneously increase the growth of the extractive species.

Most investigations assessing mussels' ability to uptake fish unconsumed feed and feces have measured the growth (Lander et al., 2012; Stirling and Okumus, 1995), the fatty acid (Handå et al., 2012a, 2012b) and isotopic profile of the mussels (Gao et al., 2006; Redmond

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et al., 2010). Conversely, very little is known about the physiological energetics of bivalves cultured in the proximity to fish cages, since previous studies have focused mainly on the absorption efficiency of the fish particulate surplus (Irisarri et al., 2013; MacDonald et al., 2011; Reid et al., 2010). Measurements of the different physiological rates of a bivalve (clearance, ingestion, absorption, respiration, excretion) can be integrated to determine the net energy balance (difference between the energy absorbed from the ingested food and the energy lost in respiration and excretion), which is commonly referred to as the "Scope for Growth" (SFG) (Winberg, 1960). SFG variations reflect spatio-temporal fluctuations in environmental conditions (Albentosa et al., 2012). To our knowledge, no previous studies have investigated the SFG of bivalve species cultured in proximity to fish cages either in the field or under laboratory conditions. However, SFG is one of the main approaches to model bivalve growth and has been successfully used in a range of different mytilid species exposed to varying environmental conditions. Hence, several studies have measured the SFG of the mussel *M. galloprovincialis* (Albentosa et al., 2012; Fernández-Reiriz et al., 2012; Helson and Gardner, 2007; Navarro et al., 1991, 1996; Pérez-Camacho et al., 2000; Sarà and Pusceddu, 2008).

Previous studies on the utilization of fish effluents by mussels have been performed with a limited number of individuals, which does not allow for a general conclusion on the potential contribution of commercial-scale mussel farming mitigating fish nutrient impacts (Handå et al., 2012a). In this study, this issue was overcome by selecting a commercial mussel raft operating in the proximity of a fish farm of red sea bream in the Ría Ares-Betanzos (Galicia, NW Spain).

This study investigated the seasonal variability in energy uptake and utilization by the edible mussel *M. galloprovincialis*. The primary objective was to determine if any particulate food enhancement from fish wastes increased the SFG of the commercial mussels compared to mussels contained on a reference raft distant from the fish farm. Measurements of clearance, ingestion, absorption respiration and excretion rates were determined in the field under natural conditions of food availability and were integrated to determine O:N ratio, SFG and net growth efficiency. A second objective was to analyze temporal and spatial variations in total phytoplankton biomass (chlorophyll-*a*) and phytoplankton size-classes, to investigate: (1) their importance in the mussels' diet, (2) the physiological responses of mussels to natural dietary fluctuations and (3) to determine if any dissolved inorganic nutrients contained in fish waste enhanced the local phytoplankton biomass.

2. Material and methods

2.1. Study site

Field studies were carried out in the Lorbé raft polygon in the Ría Ares-Betanzos, NW Spain (Fig. 1; latitude 43°23′ 24.74″ N; longitude 8°17′ 48.30″ W). All mussels *M. galloprovincialis* used in this study had the same origin and were cultured on 12 m long ropes at a stocking density of 700–1000 mussels m^{-1}. Water sampling and physiological measurements were conducted at two commercial rafts. Raft P-14 (43° 23.3328′ N; 8° 17.2878′ W) was the culture unit closest to the fish cages in the Lorbé raft polygon (170 m north of a red sea bream farm), while raft P-46 (43° 23.4876′ N; 8° 17.109′ W) was used as a reference station and was situated 550 m north from the net-pens. Raft P-14 had an average annual population of 3856 $\times 10^3$ mussels, while raft P-46 had an average population of 4299 $\times 10^3$ mussels (Table 1).

The fish farm of red sea bream (*Pagellus bogaraveo*) consisted of 48 net-pens, with an extra 2 empty cages. Each pen is 28.5 m in diameter, 6 m in depth and has an approximate volume of 3692.64 m^3 (Guisado et al., 2007). The fish farm has an estimated annual stocked biomass of 450 tons, and the estimated stocked biomass during the sampling period was around 70 tons, with an approximate culture density of 0.4 kg m^{-3}. Fish were fed ad libitum, with a constant daily feeding regime representing 0.5–0.7% of their fresh body weight (Guisado et al., 2007). Fish were hand-fed a commercial diet of heat extruded pellets (Skretting B4 power 2 P). During the course of this study the fish farm was stocked with more than a single cohort of fish, implying that there were no major seasonal variations in feed use.

The general pattern of water circulation in the Ría Ares-Betanzos consists of oceanic water from the continental shelf entering along the southern margin of the Ría, from where it moves towards the east, then southwards, north-easterly and finally westward into the Atlantic (Sánchez-Mata et al., 1999). The Ría has a prevalent positive circulation scheme with a two-layered residual circulation pattern, this means that the upper layer moves seaward, while denser and deeper layers of oceanic water move landward (Sánchez-Mata et al., 1999 and references therein). Predominant north-easterly winds during spring and summer usually enhance this positive circulation pattern, whereas south-westerly winds blowing during autumn and winter can reverse the positive estuarine circulation (Bode and Varela, 1998).

The Ría has a semi-diurnal tidal frequency, with spring and neap mean tidal ranges of 4.14 and 0.02 m, respectively (Sánchez-Mata et al., 1999). Tidal currents are more important than wind-induced or wave-induced currents in the Ría Ares-Betanzos (Sánchez-Mata et al., 1999) and maximum tidal current speed in the middle of the Ría at 3 m depth is 2.2 cm s^{-1} (Piedracoba et al., 2014). Tidal currents are rectilinear, and accommodate to the shape of the Ría, flowing with a mean along-channel orientation in an anticlockwise angle of 139° (i.e. 0° starts on the east) (Piedracoba et al., 2014; Fig. 1C). Tidal currents explain 53.4% of the total variance of surface currents in Lorbé raft polygon and have an average speed of 1.7 cm s^{-1} at 1 m depth (Piedracoba et al., 2014). Average residual current speeds at P-14 and P-46 are 3.70 and 3.64 cm s^{-1}, respectively (Zuñiga et al., submitted for publication). During the sampling period, maximum total current speeds at the raft next to the cages ranged from 5.1-13.1 cm s^{-1}, whereas maximum total current speed at the reference raft ranged from 5.3-11.5 cm s^{-1}. During the ebb tide, water flows from the fish cages to the mussel rafts, whereas during the flood tide it flows from the rafts towards the cages (Fig. 1). Hence, mussels might potentially be within the transport pathway of fish particulate wastes during a large percentage of the tidal cycle.

All measurements were conducted within two consecutive days during five seasonal campaigns. The campaigns were selected to represent typical oceanographic scenarios of the Rías: 1) summer upwelling (6th and 7th July 2010), 2) autumn bloom (5th and 6th October 2010 and 24th and 25th October 2011), 3) winter mixing (7th and 8th February 2011) and 4) spring bloom (2nd and 3rd May 2011). The two sampling campaigns in October were executed to test for interannual variance. Physiological rates and environmental parameters were determined simultaneously in the field, onboard a boat moored to each raft to maintain ambient conditions of temperature, salinity, and food availability.

2.2. Chlorophyll-a and phytoplankton size-classes determination

Seawater from 3 m depth within each raft was supplied by a peristaltic pump. Three replicates of 1 l of seawater (n = 30) were collected from the outflow of an empty chamber used as control during the physiological experiments. Seawater collected from each site and sampling date was filtered through a serial polycarbonate filtration unit for determination of chlorophyll-*a* concentrations within three phytoplankton size-classes according to their equivalent spherical diameter (ESD). Phytoplankton were fractionated into picophytoplankton (0.2–2 μm ESD), nanophytoplankton (2–20 μm ESD) and microphytoplankton (>20–50 μm ESD) size-classes and the total chlorophyll-*a* (chl-*a*, μg l^{-1}) was calculated as the sum of the chlorophyll determined in each of the size classes. All filters were frozen at −20 °C to facilitate cellular lysis and enhance chlorophyll extraction. Pigments were extracted using 5 ml of 90% acetone as a solvent, and left in the dark for 12 h. The solution