Benthic metabolism over the emersion - immersion alternation in sands colonized by the invasive Manila clam *Ruditapes philippinarum*

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ARTICLE INFO

ABSTRACT

The effect of an invasive infaunal suspension-feeding bivalve, the Manila clam *Ruditapes philippinarum*, on benthic inorganic carbon and nutrient fluxes was examined through *in situ* incubations. Measurements were performed in spring and summer on a tidal sandflat of the Rance estuary (south part of the Western English Channel) colonized by the Manila clam after its deliberate introduction in the 1990's. Benthic inorganic carbon fluxes were measured using light and dark benthic chambers both at emersion and immersion. Benthic nutrient fluxes were measured using dark benthic chambers at immersion. Inorganic carbon (IC) and ammonium sediment release under darkness at immersion reached 5.60 mmol m^{-2} h^{-1} and 441 μmol m^{-2} h^{-1} respectively for a clam density of 291 ind m^{-2}. The sediment IC-release under darkness was lower during emersion than during immersion, probably due to the reduced activity of infauna at low tide. Under ambient light, a sediment IC-uptake was systematically measured at emersion, indicating a net autotrophy under the condition of measurements (125 < surface PAR < 1670 μmol m^{-2} s^{-1}), while either sediment IC-uptake or release was measured at immersion according to light variation (20 < underwater PAR < 990 μmol m^{-2} s^{-1}). The highest gross community primary production, calculated from highest IC-fluxes at light (i.e. net community production) and highest IC-fluxes at dark (i.e. community respiration), was similar at emersion and immersion and reached 6.2 mmolC m^{-2} h^{-1}. These results suggest that the metabolic activity of the invasive Manila clam *Ruditapes philippinarum* contributes to increase inorganic C and ammonium sediment release. These regenerated nutrients may support microphytobenthic production which appeared particularly high on this intertidal sand flat.

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1. Introduction

Introduction of exogenous species concerns all ecosystems and is considered as one of the most serious threats on the conservation of natural biodiversity. In marine areas, accidental introductions increase inexorably with shipping traffic, and add to deliberate introductions for aquaculture (Carlton and Geller, 1993). Locally, the introduction of exogenous species may simply result in the addition of a new species, without apparent effect on autochthon communities (Reise et al., 2006). Introduced species may also proliferate (becoming invasive) and profoundly change the local diversity (by competitive exclusion of native species, through habitat modification, etc.) and strongly influence ecosystem functioning (Ruiz et al.,

1997). The infaunal suspension-feeding bivalve *Ruditapes philippinarum*, indigenous to sub-tropical and temperate coastal waters of the western Pacific and Indian oceans, has been purposefully introduced in Europe from the 1970s (Chiesa et al., 2017) because of its high commercial value as seafood species. Initially reared in areas spatially limited, the Manila clam rapidly spread and has established naturalised populations (Humphreys et al., 2015). In several disturbed estuaries or lagoons, the Manila clam has supplanted the European native carpet shell clam *Ruditapes decussatus* by occupying almost entirely its ecological niche (Bidegain et al., 2015).

Suspension-feeding bivalves are recognized to play an important role in benthic-pelagic coupling in many coastal ecosystems by removing particles from the water column and transferring undigested material in their biodeposits to the sediment surface (Newell, 2004). *R. philippinarum* has been shown to produce large amounts of faeces and pseudofaeces (Han et al., 2001). In sediments

in which the species is farmed, organic matter enrichment has been observed (Nizzoli et al., 2011) and sediment-water column fluxes of dissolved materials have been suggested to be stimulated through bacterial mineralisation of biodeposits (Bartoli et al., 2001). Infaunal species also stimulate sediment-water column fluxes through their bioturbation (Welsh, 2003) and influence the pathways of nutrient recycling through associated bacteria (e.g. Welsh and Castadelli, 2004). The role of *R. philippinarum* in stimulating ammonium release from the sediment is well documented (Magni and Montani, 2006; Nizzoli et al., 2011; Stief, 2013; Welsh et al., 2015). Farmed Manila clams have also been shown to modify nitrate and nitrite fluxes, stimulating both nitrification and nitrate reduction (Nizzoli et al., 2006b; Welsh et al., 2015), and to influence phosphorous recycling (Nizzoli et al., 2006a). Macrofauna also directly influences benthic fluxes through its metabolism: inorganic carbon is released through respiration and metabolic end-products, such as ammonium, are excreted to the water column. Isolated *R. philippinarum* individuals have been shown to exhibit high rates of respiration and ammonium or phosphate excretion (Magni et al., 2000; Welsh et al., 2015). This direct influence is likely to be density dependent. However, suspension-feeding by reducing water column turbidity may increase light penetration to the sediment surface and stimulate microphytobenthos production which in turn may reduce the nutrient efflux from the sediment (Newell, 2004). In support of this, stimulation of intertidal microphytobenthic production at higher densities of suspension-feeding bivalves was shown by field manipulation of the New Zealand cockle (*Austrovenus stutchburyi*) density (Sandwell et al., 2009). However, the analysis of compiled data from nine estuaries in the North Island of New Zealand led to a weak linkage between cockle abundance and primary production (Pratt et al., 2014a). Indeed, interactions between suspension-feeding bivalves and microphytobenthos imply complex relationships among benthic and pelagic processes that depend of environmental conditions.

There is then still a need for field studies to evaluate *in situ* influences of natural dense infauna populations on benthic metabolism (Welsh, 2003). In particular, there is a need to consider the metabolism of intertidal populations in the two contrasting environments they are alternatively exposed to (air and sea water). So far published studies on the influence of *Ruditapes philippinarum* on benthic fluxes concerned mainly shallow water populations (e.g. Welsh et al., 2015) and the few studies dealing with intertidal populations were based only on underwater measurements (e.g. Magni et al., 2000). In this context, the present study aimed to examine the effect of *R. philippinarum* on inorganic carbon fluxes in colonized intertidal sand flat, both at emersion and immersion. For that purpose, light and dark benthic chambers were used *in situ* and without any manipulation, during two seasons. The effect of *R. philippinarum* on dissolved nutrient fluxes was also examined in dark incubations at immersion. It was intended (1) to investigate the effect of clam density on benthic metabolism, (2) to provide an estimate of primary production, and (3) to compare aerial and underwater benthic metabolism.

2. Materials and methods

2.1. Study site

Measurements were performed in a mid-tidal sand flat of the upstream Rance estuary (48°52′N, 1°98′W), located in the southern part of the western English Channel (France). This estuary is characterized by the presence of a tidal power plant at its mouth, which imposes specific tidal conditions. Alternation of tide levels are not as regular than in the open sea and there is a particularly long high water stand (Retière, 1994). Tidal range varies between 4.0 and

7.0 m instead of 13.5 m in the open sea (Desroy and Retiere, 2001). Mean high water level is almost unmodified while mean mid and low water levels are elevated by approximately 2.5 and 3.5 m respectively (Desroy, 1998). The Manila clam *Ruditapes philippinarum* has been introduced in the Rance estuary at the end of the 1990's and has rapidly colonized all tidal sand flats, reaching densities of 500 ind. m^{-2} in the upstream part in 2002 (unpublished data from the Museum National d'Histoire Naturelle).

2.2. Sampling strategy

Benthic metabolism was measured during neap tides, with morning low tide, in summer (July 2010) and spring (April 2011). In July 2010, two stations were selected as being located at the same tidal level (about 8 m above chart datum) and as being, a priori, characterized by contrasted density of *Ruditapes philippinarum*. The two stations (A and B, 20 m apart) were investigated during two following days (A: July 05, B: July 06). At each station, three benthic chamber cores were positioned about 2 m apart at the beginning of emersion (predicted to occur for 8 m above chart datum at 8:48 the 05th and at 9:24 the 06th). Successive triplicate incubations were performed to assess sediment-air inorganic carbon fluxes until flooding. The first incubation was done in darkness (after the sediment has been shaded for a while) and the 3 or 4 next were done at ambient light. At the beginning of immersion (predicted to occur at 12:06 the 05th and at 13:24 the 06th), successive triplicate incubations were performed on the left standing cores to assess sediment-water inorganic carbon fluxes. Two incubations were done at ambient light and the last one was done in darkness. Nutrient fluxes were also assessed during this dark underwater incubation. Benthic chambers were opened at the end of each incubation, to allow complete air or seawater replenishment before the beginning of a new incubation. The sediment isolated by each core was sampled during the following ebb tide for macrofauna identification and counting and subsamples of each core were used for sediment characterization. In April 2011, the same investigation (except for the sediment characterization) was done on two other stations situated 150 m apart from the stations visited in July. The two stations (C and D, 20 m apart) were investigated the 11th and the 12th of April respectively. At these dates, the emersion of the 8 m level was predicted to occur at 8:06 and 8:42 respectively and its immersion at 11:18 and 13:00 respectively. During emersion, the first incubation was done in darkness and the 3 or 4 next were done at ambient light. During immersion, one or two incubations were done at ambient light and the last one was done in darkness.

2.3. Inorganic carbon and nutrients fluxes

Three sediment areas of 0.071 m^2 were enclosed down to a 10-cm depth using stainless-steel cores on which were later sealed acrylic hemispheres trapping a volume of 10.5 L. Incubations were performed under darkness to estimate benthic community respiration (CR) and under natural light condition to estimate net community production (NCP, the balance between community gross primary production and community respiration).

During emersion, CO_2 fluxes were measured at the sediment-air interface using the closed-chamber method described in Migné et al. (2002). Changes in air CO_2 concentration (ppm) in the benthic chambers were measured with infrared gas analysers (LiCor Li-800) for 15–35 min. CO_2 concentrations were recorded in data loggers (LiCor Li-1400) with a 15 s frequency. CO_2 flux was calculated as the slope of the linear regression of CO_2 concentration (μmol mol^{-1}) against time (min) and expressed in mmolC m^{-2} h^{-1} assuming a molar volume of 22.4 l at standard temperature and pressure.