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Influence of increased sediment exposure on suspension-feeder assemblages in a temperate seagrass meadow

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by

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ABSTRACT

The impact of increased sediment exposure on coastal marine ecosystems is one of the most important contemporary environmental issues. Sediment exposure is defined as the concentrations of sediment particles suspended in the water column and the amount of sediment depositing on the seabed. In addition to natural events, such as land erosion, rainfall, and tidal currents, anthropogenic activities such as land use, road building, logging, mining, port maintenance and dredging, contribute to the discharge of a great amount of sediment in the water column. As sessile suspension-feeder assemblages play a critical role in marine ecosystems through their active transfers of organic and inorganic between the water column and the seabed (i.e. benthic-pelagic coupling), increased sediment exposure generated by anthropogenic activities may negatively impact these organisms. This study set out to evaluate the influence of increased sediment exposure on suspension-feeder assemblages in a temperate seagrass habitat. Most of the research was conducted within *Posidonia sinuosa* meadows near Woodman Point (32◦7'S, 115◦44'E), south of Fremantle, Western Australia. At this location, persistent sediment plumes from a cement manufacturer's wash plant provided an opportunity to study the mechanisms through which increased sediment exposure can affect suspension-feeder assemblages. This study was examining: the relationship between sediment exposure and suspension feeder assemblage composition; the influence of increased sediment exposure on the contribution of potential food sources to suspension feeder diets; and the influence of sediment exposure on suspension-feeding mechanisms (filtration and retention rates) and strategies (food particle selection).

The descriptive work, presented in Chapter 1, revealed a strong gradient in sediment exposure with decreasing sediment deposition with distance from the wash plant. There was little dissimilarity, in term of species diversity and biomass, among suspension-feeder assemblages under high sediment exposure and those experiencing natural sedimentation regimes. These findings indicated that the suspension-feeder assemblages at the study site were resistant to high sediment exposure and that some species could potentially display compensatory mechanisms. Thus, the degree to which increased sediment exposure influences suspension feeders was more likely to be species specific and depend on the resilience of their feeding mechanisms and strategies. Those findings underlined the need to test the causal parameters underlying responses to suspension-feeding activity and selectivity due to increased sediment exposure.

Results presented in Chapter 2 indicated that the three most conspicuous suspension feeder in term of biomass and abundances presented distinct isotopic signatures, implying dissimilarities in their diets. Differences in $\delta^{13}C$ and $\delta^{15}N$ can be explained by consumption of different types of picoplanktonic particles and the degree to which sedimentary organic matter contributed to their diets. Increased sediment exposure had no influence on the natural diets of the ascidian

Herdmania momus and the bivalve *Pinna bicolor*. For the sponge *Tethya* sp, the contribution of sedimentary organic matter to its diet increased at sites with high sediment exposure, suggesting a potential benefit to its diet. Overall, the influence of sediment exposure on suspension feeder diets was species specific and dependent on the nature (e.g. organically rich versus organically depleted) and concentrations of the sediment.

In Chapter 3 data are presented to show that feeding mechanisms and strategies of three species of suspension feeders best representing the benthic assemblages were influenced by sediment exposure. Under high sediment exposure, the ascidian *Herdmania momus* had lower pumping rates, but maintained a relatively constant food retention rate, and optimised its food intake by expanding its food sources from mainly cyanobacteria (*Synechococcus*) to a wider range of food sources. The bivalve *Pinna bicolor* also had lower filtration activity with high sediment exposure, again, maintaining relatively constant total retention rates and displaying a change in particle selection from bacteria and *Synechococcus* to larger picoeukaryotic cells of higher carbon content. The sponge *Tethya* sp. appeared to benefit from elevated sediment concentrations, as filtration and retention rates increased, potentially related to a lack of food selectivity.

In Chapter 4, a short-term laboratory experiment was combined with a field transplant experiment to investigate the response of suspension feeders to increased sediment exposure. Under elevated sediment exposure, *Herdmania momus* and *Pinna bicolor* modulated their particle selection to optimise food intake, while the non-selective suspension feeder *Tethya* sp increased its particle retention rate and efficiency. Both the laboratory and transplant experiment findings corroborated observations made in Chapters 1 and 2; compensatory adaptations associated with the feeding activity, such as the modulation of pumping rates and the optimisation of food intake by particle selection, help to explain the potential resistance of suspension feeder community structure to high sediment exposure.

A major outcome of this research is that it informs shallow coastal ecosystems stakeholders of the possible consequences of anthropogenic activities that increase sediment exposure, particularly those in the order of TSS at 40 mg·l^{-1} and deposition rates in the order of 10 g·cm^{-2}·month^{-1}. Despite this magnitude of sediment exposure had little influence on suspension feeder assemblage composition, the effects on the suspension-feeding function, including the increase of the filtration activity and transfers of carbon to the benthos, potentially influence benthic-pelagic coupling and other ecosystem-scale processes. Given the variation in sensitivity to sediment exposure among suspension feeder species, meaningful criteria to limit the effects of anthropogenic sediment loading on shallow coastal ecosystems should take into account the whole species assemblage present at any given site.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

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When the seagulls follow the trawler, it is because they think sardines will be thrown into the sea (Cantona 1995).

Gant ar boan hag an amzer a-benn a bep tra e teuer (Breton proverb).

TABLE OF CONTENT

GENERAL INTRODUCTION

Despite representing only 7% of the world's oceans, coastal zones contribute 38% of the economic value of the world ecosystems and 60% of the economic value of all marine ecosystems (Costanza et al. 1998). As well as providing food, habitat and protection for a wide diversity of marine fauna (Costanza et al. 1998), coastal zones also provide services to humans including nutrient cycling, disturbance regulation, food production and locations for recreational and cultural activities. However, with over one-third of the world's human population living close to the coast, coastal ecosystems experience significant anthropogenic pressure. As is the case with most other ecosystems, shallow marine ecosystems are exposed to four major threats: climate change, increased nutrient inputs, destruction of physical and biogenic habitat and over-exploitation of biota (Scheffer et al. 2001). Concern over changing ecological conditions has fostered a need to assess the impacts of human disturbances and to develop mitigation measures

The impacts of sediment deposition and suspended sediments on marine ecosystems is one of the most important contemporary environmental issues. As sand is used in a wide range of industry, including production of glass, wine, paper, paint, electronic and aeronautic, there is an ever-increasing demand for sedimentary aggregates. Due to the decline of the inland resource and the expansion of the construction industry, which is the main consumer of sediment, marine aggregates became the principal source of sediment (Steinberger et al. 2010; Krausmann et al. 2009). Dredging required for marine aggregates extraction, sediment transportation and washing process to remove salt, contribute to the discharge of a great amount of sediment in the water column. Among other anthropogenic activities such as land use, road building, logging, dredging program for mining and port maintenance, the marine aggregates industry is one of the main contributor of increased sediment concentrations in coastal waters. As sediment loads, from anthropogenic activities end up, sooner or later on the seabed, they result in major changes to sessile benthic macrofauna. Therefore, there is an urgent need to understand changes within marine benthic community in term of species composition, abundance, biomass and also, ecological function performed. Natural events, such as land erosion, rainfall, and tidal currents, can lead to the discharge of sediment in coastal waters (Szostek et al. 2013) and aquatic organisms subject to these natural variations are adapted to them. However, in addition to natural episodes, high levels of sediment exposure induce by anthropogenic activities may cause significant environmental changes in shallow coastal ecosystem beyond natural variation (Hewitt & Pilditch 2004). Sediment exposure is defined by the concentrations of sediment particles in suspension in the water column and the amount of sediment depositing on the seabed. Being immobile, and due to their feeding behaviour, sessile suspension feeders are among the organisms most likely to be negatively impacted by increased sediment exposure.

This thesis presents the findings of research into the effect of increased suspended sediment and sediment deposition on sessile suspension-feeding assemblages. Particular emphasis was given to suspension feeders in seagrass habitat, a critical benthic habitat often characterised, in the temperate region of Australia, by abundant suspension-feeder assemblages (Lemmens et al. 1996). Seagrass beds, are highly productive ecosystems providing important ecological and economic functions in coastal zone, through their significance to fisheries and the prevention of coastal erosion (Erftemeijer & Lewis 2006; Duarte 2002). Increased sediment exposure, from anthropogenic activities such as dredging and sand mining, threatens the functioning of seagrass ecosystems through its direct influence on suspension-feeder assemblages (Szostek et al. 2013; Safi et al. 2007), but also on the substrate that represents the seagrass habitat (Erftemeijer & Lewis 2006).

1. Suspension feeders

The term "suspension feeder" gathers species that feed from particles suspended in the water column (Dame et al. 2001). Among suspension feeders, the term "filter feeder" is only appropriate for organism employing specialized filtering structure (e.g. mucus nets, setae, rakes) that retain particles according to size and shape and in certain instances, capable of creating a feeding current (Riisgård and Larsen 2010; Jørgensen 1966). Therefore, the term "suspension feeder" defines the origin of the food, while the term "filter feeder" defines a sub-group of suspension feeders with specific capture mechanisms. For example, crinoids are suspension feeders that use tube-feet passive suspension-feeding to catch particles, but they are not filter feeders because this feeding mode does not allow them to select particles and/or control water flow. In comparison, all ascidians are suspension feeders and specifically filter feeders. At the ecosystem level, suspension feeders form a functional group composed of organisms with distinct feeding mechanisms and strategies (Dame et al. 2001).

Suspension-feeding species represent nearly a quarter of all known aquatic species (Gruner 1963) and include a diverse array of marine animal classes such as corals, bryozoans, sponges, gorgonians, sea pens, echinoderms, brachiopods and bivalves (Nikora et al. 2002; Green et al. 1998). These species are mainly heterotrophic organisms but they may include mixotrophs associated with zooxanthellae, such as sponges and octocorals (Fabricius et al. 1995). Suspension feeders can represent more than 70% of the biomass on coastal temperate reefs (Newell et al. 1982) and between 30 and 60% of the biomass in intertidal ecosystems (Ricciardi & Bourget 1999). Sessile benthic suspension feeders are a common trophic group inhabiting hard- and soft-bottom habitats in shallow coastal waters worldwide (Gili & Coma 1998). They include most epifaunal invertebrates as well as some infauna (Bullivant 1968). According to FAO (2012), suspension feeders, mostly oysters, mussels, clams, cockles, arkshells and scallops, contribute to

the majority of aquaculture production in shallow coastal ecosystems (75.5 percent, 13.9 million tonnes).

1.1 Ecosystem functions

Suspension-feeder assemblages are a critical component of shallow coastal ecosystems, performing a wide range of ecological functions (Cooper et al. 2008). Through their filtration, suspension feeders play a major role in benthic/pelagic coupling by removing organic and inorganic particles from the water column, excreting nutrients and excreting faeces and pseudo-faeces in the sediment (Wildish & Kristmanson 2005; Dame et al. 1998). Sessile benthic suspension feeders are able to filter large amounts of water (up to 100 $m^3 \cdot d^{-1} \cdot m^{-2}$; Jørgensen 1996), transferring suspended organic and inorganic matter from the water column to the sea floor. Their role in benthic-pelagic coupling has a significant influence on the organic matter and nutrient pools available to pelagic and benthic organisms (Lawton & Jones 1995; Jones et al. 1994). The capture of nutritive particles can directly regulate primary production (phytoplankton biomass and/or diversity) and indirectly influence secondary production (Kimmerer et al. 1994), by modulating the availability of resources to other organisms (Pile 2005; Ostroumov 2005; Dame et al. 2001; Dolmer 2000; Riera and Richard 1996; Lemmens et al. 1996;)). Suspension feeder driven fluxes of particulate nutrients toward the sediment promotes mineralisation and enhances fluxes of inorganic nutrients toward the water column (Dame et al. 2001). Moreover, suspension feeders have been recognised as important recyclers of pelagic particulate organic matter (Jiménez & Ribes 2007) as they can effectively excrete nutrients (ammonium and nitrate) in association with heterotrophic bacteria (Jiménez & Ribes 2007; Ostroumov 2005).

Sedimentation rates can be significantly enhanced by suspension feeder activity (Dame et al. 2001). Following filtration, suspension feeders excrete faeces and pseudo-faeces, organic-rich detritus, which can be available for other benthic organisms, such as detritus feeding polychaetes and oligochaetes (Jiménez & Ribes 2007; Ostroumov 2005; Commito 2001). This sediment biodeposition, as well as bioturbation, can stimulate microbial activity (recycling of oxygen, carbon and nutrients) (Dame et al. 1998) and the sequestration of harmful substances (Cooper et al. 2008).

Suspension feeders can significantly modify local hydrodynamics due to their morphology (Nikora et al. 2002; Green et al. 1998). Their physical structure changes can act to slow current flow, thereby increasing the residence time of particles (Gili & Coma 1998). Consequently, epibenthic suspension feeders have an important function as bioengineers. The habitat created by these organisms, provides essential nursery area, feeding grounds and firm settling structure that attract a wide range of organisms and can increase local and regional biodiversity (Asmus 2005).

The array of functions performed by suspension feeders is more significant in epibenthic than in endobenthic (or in-fauna) communities (Dame et al 2001). Furthermore, many studies indicate a

correlation between diversity of epibenthic assemblages and factors such as the nature of substrata, near-bed tidal velocity, water depth and temperature (Freeman & Rogers 2003; Callaway et al. 2002) making them ideal candidates as indicators of environmental conditions. Epibenthic suspension feeders are employed as a means of detecting environmental perturbation (Brown et al. 2001; Veale et al. 2000; Kaiser & Spencer 1994), and are included in national and international monitoring programmes (Callaway et al. 2002; Ellis et al. 2000; Rees et al. 1999).

1.2 Feeding activity

Suspension feeders have adopted feeding strategies for a wide spectrum of food particle types and sizes (Pile et al. 1997; Coma et al. 1995). They feed mostly on phytoplankton (pico and nanoplankton) but also on zooplankton (Bode et al. 2006), dissolved organic matter (DOM) and bacteria (Bell 2008; Yahel et al. 2007; Ostroumov 2005; Pile 2005). Resuspended microphytobenthos and detritus (drifting macroalgae, faeces and so on), can also form part of the diet of suspension feeders (Miller & Page 2012; Lebreton et al. 2011; Riera & Richard 1996). Where nutrient availability and pelagic primary production is low, some suspension feeders establish symbioses with green algae, dinoflagellates, or cyanobacteria from which they derive nutrients (Hawkins & Klumpp 1995; Klumpp 1992; Fankboner et al. 1990). Suspension feeding involves the capture of food items that are either large enough to be individually seized or so small that they are only obtained in sufficient quantity by processing the surrounding water. Therefore, many benthic sessile suspension feeders have developed distinct feeding strategies and special mechanisms that enable them to feed on the suspended food particles present in their environment (Riisgård & Larsen 2010; Shimeta & Jumars 1991), as summarised in Table 1. Despite the many differences in morphology and living conditions, suspension feeding organisms can be divided in four broad categories on the basis of their feeding behaviours: active, passive, facultative and combined feeding strategies (Labarbera 1984).

Active suspension feeders use metabolic energy to drive currents that bring food to and through the feeding apparatus (Riisgård and Larsen 2010). In active benthic suspension feeders, food-particles capture mechanisms include: cirri trapping with gill's cirri that beat against the feeding current (Kiorboe & Mohlenberg 1981; Jorgensen 1975); ciliary sieving where pumped particles are retained by a filter formed by a band of stiff cilia (Riisgård & Ivarsson 1990; Riisgård & Nielsen 2006); mucus-net where ciliary or muscular pumps drive water through a mucus net retaining suspended food particles (Riisgård and Larsen 2010).

Passive suspension feeders depend exclusively on ambient water movements to drive water past their filtering structures (Ribes et al. 1999; Riisgård and Ivarsson 1990; Labarbera 1984; Jørgensen 1966). In passive benthic suspension feeders, food-particles capture mechanisms include: ciliary spike bring suspended food particles into contact with the tentacles (Riisgård and Nielsen 2006); tube feet where particles encountered and retained on the tube feet are transported

between adjacent tube feet along the arm towards the mouth (Labarbera 1984); cnidae with "needle cells" where the prey is caught by the tentacles, paralysed by nematocysts, and carried to the mouth (Riisgård and Larsen 2010).

Facultative suspension feeding is performed by suspension feeders capable to switch between active and passive feeding depending on ambient water velocity conditions (Wildish & Kristmanson 2005). For example, barnacles can switch between an active suspension-feeding mode in slow ambient water flow and a passive suspension-feeding mode in fast flow (Trager et al. 1990).This feeding behaviour include organisms with setal feeding mechanism capturing particles by means of cirri (Riisgård and Larsen 2010).

Unlike in facultative suspension feeding, combined suspension feeders continuously perform both passive and active feeding mechanisms simultaneously (Wildish & Kristmanson 2005). This feeding behaviour is found on organisms with collar sieving structure, where a beating flagellum creates a feeding current through a collar filter that passively captures food particles (Simpson 1984; Jørgensen 1983; Frost 1978; Bergquist 1978; Reiswig 1975).

Table 1: Feeding behaviour and particle capture mechanisms in sessile epibenthic suspension feeders.

Feeding behaviour	Food-particle capture mechanisms	Subdivision	Taxa	Mechanisms principle	Particle size	Main diet
Active	Cirri trapping	Eu-laterofrontal cilia	Most bivalves (Riisgård & Larsen 2005)	Particles are retained by the alternating beat pattern of the bands of gill's laterofrontal cirri (Riisgård & Larsen 2010)	**~0.5 µm up to 3 mm**, depending on species and particle concentrations (Lehane & Davenport 2006; Beninger et al. 1994). Typically between **~2 to 40 µm** (Ward & Shumway 2004; Sobral & Widdows 2000)	Bacteria, detritus, phytoplankton and zooplankton (Davenport et al. 2011; Arapov et al. 2010)
Active	Cirri trapping	Pro-laterofrontal cilia	Bivalves: Pectinidae, Anomiidae and Pteriidae (Riisgård & Larsen 2005)	Particles are retained by the beating of a single row of prolaterofrontal cilia inside gill (Riisgård & Larsen 2010)	**~2 to 72 µm** (Brillant & MacDonald 2000)	Bacteria, detritus, phytoplankton (Lorrain et al. 2002; MacDonald & Ward 1994; Shumway & Selvin 1987)
Active	Ciliary sieving	Upstream collection	Bryozoans, phoronids and brachiopods (Riisgård & Larsen 2005)	Tentacles with lateral ciliary bands produce feeding currents directed straight towards the mouth (Riisgård & Larsen 2010)	**5 to 30 µm** (Lisbjerg & Petersen 2001)	Phytoplankton (Lisbjerg & Petersen 2001; Bullivant 1968)
Active	Ciliary sieving	Downstream collection	Diverse group of invertebrates, including some polychaetes (Riisgård & Larsen 2010)	Compound cilia constitute a pump to catch and transfer food particles to the mouth (Riisgård & Larsen 2010)	**~1 to 10 µm** (Riisgård et al. 2000)	Bacteria and phytoplankton (Riisgård et al. 2000)
Active	Mucus-net		Polychaetes, sorberaceans and all ascidians (Riisgård & Larsen 2010)	Suspension feeding by means of an efficient mucus net which sieves the feeding current (Riisgård & Larsen 2010)	**0.06 µm to >100 µm** (Petersen 2007; Coma et al. 2001; Armsworthy et al. 2001; Riisgård & Larsen 2001)	Bacteria, detritus and phytoplankton (Petersen 2007; Coma et al. 2001; Armsworthy et al. 2001;)

Feeding behaviour	Food-particle capture mechanisms	Taxa	Mechanisms principle	Particle size	Main diet
Passive	Ciliary-spike	Polychaete: *Sabellaria alveolata* (Dubois et al. 2009)	Tentacles with ciliary spikes (compound cilia) retain food particles from ambient currents (Riisgård & Larsen 2010)	**>5 to 20 μm** (Dubois et al. 2005)	Phytoplankton (Dubois et al. 2005)
	Tube feet	All crinoids (Riisgård & Larsen 2010)	Particles encountered and retained on the tube feet are transported between adjacent tube feet along the arm towards the mouth (Riisgård & Larsen 2010)	**10 to 200 μm** (Messing 1997)	Detritus, phytoplankton and zooplankton (Kitazawa et al. 2007)
	Cnidae	Scyphozoans, hydrozoans and anthozoans (Riisgård & Larsen 2010)	Presence of nematocysts (cnidae, 'nettle cells') capturing suspended particles (Riisgård & Larsen 2010)	**<1 to 200 μm** (Orejas et al. 2001)	Bacteria, detritus, phytoplankton and zooplankton (Orejas et al. 2001)
Facultative (shift between active and passive)	Setal	Crustaceans such as barnacles (Riisgård & Larsen 2010)	Particles are captured from appendages, which have developed into filtering organs, with plumate 'filter setae' (Riisgård & Larsen 2010)	**~1 μm to 2000 μm** (Anderson 1993; Trager et al. 1990; Lewis 1976; Crisp & Southward 1961)	Bacteria, detritus, phytoplankton and zooplankton (Dubois & Jean-Louis 2007; Dolenec et al. 2006; Wang & Rainbow 2000)
Combined (passive and active)	Collar sieving	All sponges (Riisgård & Larsen 2010; Coma et al. 2001)	A choanocyte with a flagellum pumps water through a collar of microvilli acting as a sieve that capture food particles (Riisgård & Larsen 2010)	**0.1 to 70 μm** with higher retention efficiencies on particle smaller than 10μm (Leys & Eerkes-Medrano 2006; Ribes et al. 1999)	Phytoplankton, bacteria, detritus and DOM (Miller & Page 2012; Hanson et al. 2009; Yahel et al. 2005; Pile 2005;)

2. Impact of increased sediment exposure induced by anthropogenic activities on suspension feeders

Anthropogenic activities such as land reclamation, road building, logging, sand mining and dredging program lead to adverse impacts on the marine environment. Dredging for port maintenance and marine aggregates excavation can directly affect benthic communities through the loss of their benthic habitats and the reduction of abundance, biomass and diversity (Dalfsen & Essink 2001; Sarda 2000). The indirect effects of these human activities are generally related to the associated resuspension of sediment into the water column, which increases turbidity, light attenuation, sediment deposition and smothering of the benthos (Szostek et al. 2013; Wenger et al. 2012; Lohrer et al. 2006). Depending upon the nature of the sediment, materials can be released to the water column, including toxicants, nutrients and organic matter (Erftemeijer et al. 2012). The nature of the sediment and the characteristics of the seabed, such as sediment type, and hydrodynamics, influence the spatial and temporal extent of such pressure (Long et al. 1995; Stickney & Perlmutter 1975). The impact of these pressures on sessile suspension feeders (Hewitt & Pilditch 2004) can be manifest through pelagic and benthic pathways (Fig. 1).

Pelagic pressures (Fig. 2) result from the increased concentrations of total suspended sediment (TSS) leading to increased light attenuation and an associated reduction in phytoplankton primary production (Gartner et al. 2010), thereby altering the availability of food particles for suspension feeders. Light reduction also negatively affects suspension feeders associated with autotrophic symbionts (Fabricius 2005). Increased TSS can also affect the feeding activity and behaviour through mechanical abrasion that physical damages the gills and reduces feeding rates (Shin et al. 2002) or clogs the filtering apparatus (Ayukai & Wolanski 1997).

Benthic pressures (Fig. 2) on sessile suspension-feeder assemblages result from increased sediment deposition leading to smothering of burial of organism. Smothering or burial can significantly reduce abundance, diversity and biomass for many sessile suspension feeders (Carballo 2006; Skilleter et al. 2006; Blanchet et al. 2005; Macdonald & Perry 2003). In addition, changes in the nature of freshly deposited sediment may also alter the suitability of the substrate for some taxa (Harrison et al. 2007). Loss of suspension feeders or changes in assemblage composition triggers modification of seabed topography, potentially speeding up current flow and decreasing the residence time of food particles (Gili & Coma 1998). Furthermore, abundances of potential food sources such as bacteria, epilithic and epiphytic primary producer may reduce when covered by sediment (Licursi & Gómez 2009). Therefore, elevated sediment deposition might also impair feeding activity through a reduction of food particles available for suspension feeders, but also the clogging of suspension feeders apparatus (Ayukai & Wolanski 1997). Often, elevated sediment deposition is associated with an increase in local sediment resuspension (Lohrer et al. 2006), feeding back into pelagic pressures.

Both pelagic and benthic pressures (Fig. 1&2) involve changes in suspension feeder assemblage composition and feeding activity (Skilleter et al. 2006; Kruger et al. 2005). As sessile suspension feeders play a key role in benthic-pelagic coupling, increased sediment exposure can exert a significant influence in the availability of organic matter and nutrients for other marine organisms (Pile 2005; Ostroumov 2005; Dame et al. 2001) and bio-geochemical fluxes between benthic and pelagic compartments (Shin et al. 2002).

The degree to which sediment exposure (TSS and sediment deposition) influence suspension feeders appears to be highly dependent on the nature and particle size distribution of the sediment (Thrush & Dayton 2002). Some suspension feeders possess physiological and behavioural mechanisms that allow them to compensate for increased concentrations of suspended sediment and sediment deposition. Those compensatory mechanisms include modification of feeding behaviour (use of a different portion of the resource pool), reduction the diameter of their feeding apparatus (shift from large particles to small particles) or adaptive mechanisms to expel sediment settled on them, through mucus production, ciliary action or polyp expansion (Pile 2005; Armsworthy et al. 2001; Gili & Coma 1998). Organism morphology also influences the sensitivity to increased sediment exposure, with erect organisms are more likely to be resilient than prostrate suspension feeders, which accumulate more sediment on their surfaces (Rogers 1990). Furthermore, suspension feeders living in more turbid and low energy waters are better conditioned to cope with increased sediment deposition compared to species living in clearer and high energy waters (Mallela 2007).

Figure 1: Example of an anthropogenic activity inducing increased suspended sediment and sediment deposition and its impact on suspension feeder assemblage and ecosystem processes.

Figure 2: Impact of increased sediment exposure on ecosystem processes and suspension feeder's structure and functions. Circled sections correspond to this project's area of focus.

3. A case study from a temperate seagrass meadow of south-west Australia

The study was located near Woodman Point (32°7'S, 115°44'E; Fig. 3), south of Fremantle, Western Australia. The climate in the region is Mediterranean and seasonally variable, with a short winter period defined by frequent storms and seawater temperatures around 17 ℃, and longer summer period with calm conditions and water temperatures rising up to 24.5 ℃ (Bettignies & Wernberg 2013; Smale & Wernberg 2009). Due to the absence of major upwelling, the coastal water in the region is considered oligotrophic (Hanson et al. 2005) and picoplankton that are able to utilize the low levels of nutrients represent >85% of phytoplankton biomass (Hawkes 2006).

Figure 3: Study area

This location was chosen as it is affected by persistent sediment plumes (Fig. 4) induced by a local cement manufacturing company's activities (Cockburn Cement Ltd; hereafter CCL). CCL obtains its main raw material for lime and cement production from dredging shell sand banks nearby Owen Anchorage. Dredged shell sand is dumped at the wash plant jetty, and then pumped onshore to CCL wash plant, where it is washed before being pumped elsewhere for processing into lime and cement. Spent wash water overflow from the wash plant is discharged to Owen Anchorage via a pipe suspended under the wash plant jetty that discharges close to the end of the jetty. Consequently, vessel movements (propeller wash), dumping of shell sand, operation of the reclaimer, discharge of wash water from the wash plant and re-suspension by waves of fine material that deposited in the area generate additional sediment exposure to natural turbidity experienced in the region. The main cause of increased sediment exposure is the wash plant discharge, which operates continually and produces the largest and most persistent plume, covering an area up to approximately 30 ha.

Previous investigations into the turbidity associated with the wash plant include the modelling work by Steedman Limited (1987), which predicted that sand-sized fractions would settle out within 20–30 m of the discharge, with fine to very fine silts remaining suspended for 100–200 m and the clay fraction 1–2 km from the discharge. They also concluded that the direction and extent of sediment plumes at the study site was primarily influenced by wind speed and direction. Plumes drift away from the wash plant jetty (generally in a north-westerly direction) or simply radiate out from the jetty in response to prevailing wind (Fig. 4). Wind directions observed at Woodman Point (Fig. 5) follow a pattern of easterly winds ("offshore") in the early to mid-morning, followed by south/south-westerly winds ("onshore"; "seabreeze") in the early and late afternoon. During "onshore" conditions, sediment plumes drift in an east/north east direction, but remain within a localised area, with the furthest extent just to the south of the southern-most jetty on Coogee Beach. During 'offshore' conditions, plumes drift in a west/north-west direction, but remain within a localised area, with the furthest extent just to the west of the tip of Woodman Point. South-western Australia is influenced by both swell and wind, with strong seasonal variations (Lemm et al., 1999). In spring and summer (October-March), regular strong sea breezes ("onshore" winds) generate moderate waves whereas in autumn and winter (April-September), frequent storms generate large swell and waves locally. As a result, sedimentation regimes vary seasonally with strong resuspension events reported during the winter period, especially in the relative shallow water column of Woodman Point (2 – 5 m).

Figure 4: Extent of sediment plumes: a) offshore conditions; b) onshore conditions.

Photos courtesy of BMT OCEANICA Ltd.

(a)

9 am

6139 Observations

3 pm

6141 Observations

(b)

9 am

513 Observations

3 pm

514 Observations

(c)

9 am

522 Observations

9 am

521 Observations

Figure 5: Wind Roses (direction versus wind speed in km/h) for the Woodman Point study site covering the period 10th September 1993 to 30th September 2010 for 9 am (Left) and 3 pm (Right). (a) Mean per annum; (b) mean for January (Summer); (c) mean for July (Winter). An asterisk (*) indicates that calm is less than 0.5%. Document released by the Australian Climate Centre of the Bureau of Meteorology.

The study was undertaken within the extensive meadows of seagrass surrounding the CCL wash plant jetty (Fig. 6). Across the study area, meadows characterized by dense covers of *Posidonia* spp (>200 shoot·m^{-2}; BMT OCEANICA Ltd report 2011) support high densities of macro-epibenthic suspension feeders, predominantly represented by ascidians, sponges and bivalves, but also epifaunal suspension feeders such as hydroids, spirorbids, bryozoans, barnacles and amphipods (Lemmens et al. 1996 ; cf. Appendix A). Elevated abundance and of macro and epifaunal suspension feeders can be explained by a combination of factors, including enhanced rates of recruitment and colonization within seagrass canopies (Boström & Bonsdorff 2000; Edgar 1992), shelter from predators (Peterson and Heck 2001) and higher abundance of food availability (Peterson et al. 1984).

Figure 6: Map of seagrass coverage for Owen Anchorage derived from aerial photography classification (Cockburn Cement Ltd, 2004).

In addition to natural turbidity experienced in the region, CCL activities in the vicinity of the wash plant generate supplemental sediment exposure that may have detrimental environmental impact, especially on seagrasses. Indeed, seagrasses in the region may experience reduced light, potentially resulting in losses of primary producer communities. Therefore, CCL and governmental agencies developed various management plans in order to monitor and mitigate this environment issue (BMT OCEANICA Ltd report 2011). However, none of those programs include the influence of increased sediment exposure on suspension-feeder assemblages.

4. Aims and significance

4.1 Aims

The objective of this research was to investigate the composition of suspension-feeder assemblages, and the response of selected suspension feeders to increased sediment exposure induced by anthropogenic activities. To achieve this objective, this thesis considers functional groups of epibenthic suspension feeders which perform the most significant array of ecological functions, based on their feeding behaviour and food-particle capture mechanism (described on Table1), in addition to the traditional species-level approach. This study focused on 5 specific aims:

More specifically, the research aimed to:

1. Examine the relationship between increased sediment exposure and the composition of suspension-feeder assemblages (Chapter 1).

2. Compare the contribution of potential food sources to the diet of co-occurring suspension feeders with distinct feeding mechanisms and strategies (Chapter 2).

3. Describe the influence of increased sediment exposure on the food resource exploitation and the trophic relationships among co-occurring suspension feeders (Chapter 2, 3, 4).

4. Estimate the sensitivity to increased sediment exposure of the species that best characterised a suspension feeder assemblage in a temperate seagrass meadow (Chapter 1, 2, 3, 4).

The aims of this study were achieved through four data chapters. While conserving the sequential construction of the thesis, the four chapter were written to facilitate the publication of three peer-reviewed articles:

- An article that combined the surveys of sediment exposure and assemblage composition (chapter 1) with results on filtration and retention rates (chapter 3).
- An article that covers both results from the stable isotope study (chapter 2) and results on phytoplankton retention efficiency (chapter 3).
- An article that incorporates the experimental work and some survey work that uses filtration/retention rates and selectivity (chapter 3 and 4) to talk about resilience.

Because the study site is common to all chapters and identical methods were used, there is some repetition of themes in introduction and methods of each chapter.

4.2 Significance

Seagrass beds fulfil a key role in the coastal zone, notably through the habitat they provide for a wide range of organisms, including suspension feeders (Duarte 2002). Globally, the estimated loss of seagrass from direct and indirect human impacts is 33,000 km2 over the last two decades (Short and Wyllie-Echeverria, 2000). The primary cause of seagrass degradation and loss globally is reduction in water clarity (Walker and McComb, 1992; Duarte, 2002; Short, 2003). Increases in catchment runoff, elevated sedimentation and water column turbidity are symptoms of worldwide coastal development, which may lead to various adverse impacts on the marine environment, especially when carried out near sensitive habitats such as seagrass beds (Erftemeijer and Lewis, 2006). In many cases, increased sediment exposure has contributed to loss of seagrass vegetation, directly due to the burial of beds, or indirectly as a consequence of lethal or sublethal stress to seagrasses caused by elevated turbidity and sedimentation (Erftemeijer & Lewis, 2006). In Australia, *Posidonia*, also known as ribbon or strap weed, is one of the most significant meadow-forming seagrasses (Meehan & West 2000; Kirkman & Kendrick 1997). *Posidonia* seagrasses are distributed all around subtropical and temperate Australian waters and also in the Mediterranean Sea (Aires et al. 2011). A decline in *Posidonia* beds may also mean loss of habitat and a consequent decrease of suspension feeder biomass (Lemmens et al. 1996), influencing benthic-pelagic coupling and other ecosystem-scale processes. Therefore, the nature of sediment plumes and the environmental conditions present in the study area are ideal to relate the influence of increased sediment exposure to suspension feeders inhabiting seagrass meadows. Due to the extensive global distribution of seagrass meadows (Green and Short 2003) and sessile suspension feeders (Gili & Coma 1998), as well as increasing concerns worldwide about sediment exposure on coastal ecosystems, understanding gained from this study could be highly transferable to other suspension-feeders assemblages in Australia and other region in the world.

5. Thesis overview

<u>CHAPTER 1:</u>

This chapter describe sedimentation patterns with increasing distance from a source of suspended sediment at the selected study site. From this descriptive work, this chapter examines whether suspended sediment is affecting the taxonomic composition and biomass of suspension-feeder assemblages within seagrass meadows. Being immobile, and due to their feeding behaviour, sessile suspension feeders are among the organisms most likely to be negatively impacted by increased sediment exposure. Despite the large body of literature describing potential effect of sediment exposure on coastal marine ecosystem or commercial species of suspension feeders, few have examined the impact of sediment on co-occurring species or whole assemblages. This chapter addresses the contributing parameters behind suspension feeder distributions in relation to sediment exposure. The degree to which increased sediment exposure influences suspension-feeder assemblages is carried throughout this dissertation and the chapter concludes with an outline of the need to test, in further chapters, the causal parameters underlying responses to suspension-feeding activity and selectivity due to increased sediment exposure

<u>CHAPTER 2:</u>

Sharing a same habitat, the diversity of feeding structures amongst suspension feeders is expected to lead to dissimilarities in their diets in order to partition the food resource. Isotopic mixing models established from natural abundances of stable isotopes of carbon and nitrogen, were used to assess the contribution of potential food sources (seagrass detritus, organic matter in the sediment and particulate organic matter) to the diet of the most conspicuous suspension feeders within the study area (*Pinna bicolor*, *Herdmania momus* and *Tethya* sp). From these models, chapter 2 examines whether increase sediment exposure influence the diet of co-occurring suspension feeders. This chapter identifies main food sources for suspension-feeder assemblages and also provides baseline knowledge about food particles retention and selection examined later in chapter 3 and 4.

<u>CHAPTER 3:</u>

During events of increased sediment exposure, a trade-off can arise between negative and positive influence of increased sediment exposure, depending on the sensitivity and resilience of each suspension feeder specie and their feeding mechanism. This chapter tests whether suspension-feeding activity and the plasticity of feeding mechanisms help them to cope with high sediment exposure. Using the InEx method from Yahel et al. (2005), instantaneous measurement of the filtration and food particles retention rates were conducted to assess the relationship between the variety of feeding mechanisms and the different types of picoplanktonic food particles consumed. These observations provide insight regarding the influence of increased sediment exposure on the

selective uptake of food particles by the three co-occurring suspension feeders with distinct feeding mechanisms (*Pinna bicolor*, *Herdmania momus* and *Tethya* sp). While this chapter provides some initial and fundamental information on food utilization and specialized adaptions to sediment exposure, it also emphasises the need to investigate in details the response of suspension feeding to acute increases in sediment exposure as conducted in chapter 4.

CHAPTER 4:

The last data chapter consist on a short-term laboratory experiment combined with a field transplant experiment to investigate the response of distinct suspension-feeding mechanisms to increased sediment exposure. This experimental approach tests hypothesis formulated in the previous chapters about the influence of sediment exposure on suspension-feeding activity and its implications on suspension feeder community structure. Chapter 4 describes the capacity for suspension feeder to use compensatory adaptations associated with their feeding activity that could explain the potential resistance of their community structure toward high sediment exposure.

GENERAL DISCUSSION

Finally, in the general discussion, the results from these studies are drawn together to discussion highlights how each chapter are inter-related and further our understanding on the flow-on effects of sediment exposure on suspension-feeder assemblages. The recent concerns over the worldwide impacts of sediment exposure on marine ecosystems and the widespread distributions of studied species make the results of this study transferable to a wide range of suspension-feeder assemblages in temperate seagrass meadows and rocky reefs. This chapter concludes on the relevance of this research to inform managers of the possible consequences of anthropogenic activities that increase sediment exposure and propose directions for future research on suspension feeders.

CHAPTER 1
Relationship between increased sediment exposure and suspension-feeder assemblage's composition.

PREAMBLE

Chapter 1 presents the results from the field survey conducted to determine sedimentation patterns with increasing distance from a source of suspended sediment and to assess whether suspended sediment is affecting the taxonomic composition and biomass of suspension-feeder assemblages within seagrass meadows. This chapter addresses the contributing parameters behind suspension feeder distributions in relation to sedimentation rates.

ABSTRACT

Sessile suspension feeders perform a broad range of ecological functions (Cooper et al. 2011; Dame et al. 2001). They play a key role in benthic-pelagic coupling which has a significant influence in making organic matter and nutrients available to other marine organisms (Pile 2005; Ostroumov 2005; Dame et al. 2001). Increases in sediment loads to coastal ecosystems triggered by anthropogenic activities often result in elevated sedimentation rates and suspended sediment levels (Ellis et al. 2002). This sediment can negatively affect the health, abundance and distribution of benthic fauna and particularly sessile suspension feeders (Szostek et al. 2013; Bolam 2011; Ellis et al. 2002; Thrush & Dayton 2002; Long et al. 1995). The aim of this study was to test whether sessile suspension feeder composition and abundance showed patterns that reflected strong sedimentation gradient. Study sites were located within a seagrass meadow affected by sediment plumes induced by a local cement manufacturing company near Woodman Point, south of Fremantle, Western Australia. This location provided an opportunity to understand the mechanisms through which increased suspended sediment can affect suspension-feeder assemblages. Results indicate a persistent gradient of sedimentation defined by decreasing values with distance from the cement wash plant. Whereas high levels of sediment exposure were observed, results suggested that increased sediment exposure did not reach lethal and sub-lethal threshold levels, allowing suspension-feeder assemblages to cope with this anthropogenic stressor. From the higher abundance and biomass of *Herdmania momus*, *Coelosphaera* sp, *Pinna bicolor*, and *Tethya* sp observed at sites near the wash plant (200 and 500 m) and the absence of *Tethya* sp at reference sites, it seems plausible that some of the suspension feeder taxa benefit from the increased sediment exposure. However, other species such as the ascidians *Polycarpa veridis* and *Phalusia obesa* were more abundant at sites away from the peak of sediment perturbation (800 m and reference sites), indicating that the nature of the influence of increased suspended sediment on suspension feeders is more likely to be species specific. Responses might depend on their feeding activity and the resilience of feeding mechanisms.

1.1 INTRODUCTION

Suspension feeders represent nearly a quarter of all known aquatic species (Gruner 1963) and comprise a large proportion of the biomass and abundance of coastal benthos (Lemmens 1996). They represent more than 70% of biomass on some coastal temperate reefs (Newell 1982) and 30 - 60% of biomass in some intertidal ecosystems (Ricciardi & Bourget 1999). This group includes a wide range of marine animal classes such as corals, bryozoans, sponges, gorgonians, sea pens, ascidians, and bivalves (Nikora et al. 2002; Green et al. 1998). The term "suspension feeder" gathers species that feed from particles suspended in the water column. At the ecosystem level, suspension feeders form a functional group composed of organisms with distinct feeding mechanisms and strategies (Dame et al. 2001).

Sessile suspension feeders perform a broad range of ecological functions (Cooper et al. 2011; Dame et al. 2001). They play a key role in benthic-pelagic coupling, which has a significant influence in making organic matter and nutrients available for other marine organisms in the ecosystem (Pile 2005; Ostroumov 2005; Dame et al. 2001). Ostroumov (2005) reported that suspension feeders are often able to from 0.1 to 5 $m^3 \cdot m^{-2} \cdot day^{-1}$ of water. With such a capacity for filtering water and assimilating organic material, suspension feeders have the potential to filter the total volume of many water bodies within days (e.g. Lake Tuakitoto, New Zealand; Bay of Brest, France; South San Francisco Bay, USA) to weeks (e.g. Chesapeake Bay, USA) and to control phytoplankton biomass (Dame et al. 2001; Hily 1991; Cloern 1982; Newell et al. 1982; Frost 1978). Suspension feeders can also modify substrate hydrodynamics and the associated transport, deposition, and entrainment of sediment, influencing both the structure and diversity of macrofaunal assemblages (Cummings et al. 2001).

Increases in sediment loads to coastal ecosystems can occur naturally in response to events such as erosion, rainfall, and tidal currents. However catastrophic events triggered by anthropogenic activities, such as road construction, logging, mining, farming, dredging and inadequate land management practices, often result in elevated sedimentation rates and suspended sediment concentrations (Ellis et al. 2002). Depending on the sensitivity of the biota to increased sediment exposure, changes in community composition can occur (Skilleter et al. 2006; Kruger et al. 2005; Koslow et al. 2001). Changes in substrate composition and sediment bio-geochemistry induced by high level of sediment input can alter substrate suitability for specific taxa (Harrison et al. 2007; Skilleter et al. 2006; Carballo 2006; Blanchet et al. 2005; Macdonald & Perry 2003). Light attenuation induced by increased sediment exposure can alter primary production and nutrient cycles and decrease food particle availability (Gartner et al. 2010; Ellis et al. 2002). Mechanical abrasion and clogging events induced by increased suspended sediment can impair the feeding and respiratory functions of suspension feeders due to physical damage of their feeding and breathing apparatus (Shinet al. 2002; Ayukai & Wolanski 1997). Some macro invertebrates are

able to compensate for high sediment exposure, by changing feeding behaviour (use of a different portion of the food resource pool), reducing the diameter of their filtering apparatus (shift from large particles to small particles), or using mechanisms to expel sediment settled on them, through mucus production, ciliary action or polyp expansion (Pile 2005; Armsworthy et al. 2001; Gili & Coma 1998). Despite these animals possessing mechanisms to cope with high level of suspended sediment, their feeding activity may still be affected by a reduction in food value and the density of food items (Harrison et al., 2007) decreasing energy available for benthic macro invertebrate assemblages and affecting the diversity and composition of other benthos (Ellis et al. 2002). From this, broader scale changes in the environment can be expected through habitat modification and impacts on the health, abundance and distribution of benthic fauna, particularly sessile suspension feeders (Szostek et al. 2013; Bolam 2011; Ellis et al. 2002; Thrush & Dayton 2002; Long et al. 1995).

Despite the large body of literature describing potential effect of sediment exposure on coastal marine ecosystem or commercial species of suspension feeders, few have examined the impact of sediment on co-occurring species or whole assemblages. This study investigated responses of suspension-feeder assemblages to increased sediment exposure. There is growing evidence that species functional traits link changes in species composition and shifts in ecosystem processes (Villéger et al. 2010). Indeed, sessile suspension feeders play a significant part in the overall functioning of many shallow coastal systems through their different feeding activity and behaviour (Dame et al. 2001). For example, two functional groups such as active and passive suspension feeders are able to share the same habitat and partitions resources although having different requirements. However active suspension feeders which feed more effectively on small particles requiring higher flow velocities are affected differently by increased sediment exposure than passive suspension feeders which are better at remaining larger and more energy-rich particles requiring smaller volume of water (Gili & Coma 1998). This study investigated whether responses to sediment were reflected in functional groups based on feeding behaviour (active, passive, facultative, combined) and food-particle capture mechanisms (described on Table1 in the general introduction).

The principle aim of this study was to test whether sessile suspension feeder composition and abundance showed patterns that reflected strong sedimentation gradient. This study was performed in an area where sediment plumes generated by anthropogenic activity were expected and so an additional aim was to test for the pressure and magnitude of such patterns.

1.2 MATERIALS AND METHODS

1.2.1 Study sites

Figure 1: Study sites.

Eight sampling sites were located within *Posidonia sinuosa* meadows near Woodman Point (32∘7'S, 115∘44'E), south of Fremantle, Western Australia. Seagrass beds in this region provide habitat for a range of organisms including suspension feeders (Lemmens et al. 1996). The average shoot densities measured in the *Posidonia sinuosa* meadow was 525 ±107.5(SE) shoots·m^{-2} in 2013 and 546 ±79.75(SE) shoots·m^{-2} in 2014 (Mohring & Rule 2014 & 2013). This location was chosen as it is affected by sediment plumes induced by a local cement manufacturing company's (Cockburn Cement Ltd; hereafter CCL) activities, covering an area up to approximately 30 ha. This location provided an opportunity to study the mechanisms through which increased suspended sediment can affect suspension-feeder assemblages.

Considering the previous information on sediment plumes direction and extent (cf. general introduction), eight study sites were located at increasing distances from the CCL wash plant, and were oriented along two different directions: east and north-west (Fig. 1). Steedman Limited's (1987) model predicted that sand-sized fractions would settle out within 20–30 m of the discharge, with fine to very fine silts remaining suspended for 100–200 m and the clay fraction up to 1 km from the discharge. Thus, study sites were selected as follows: two sites located 200 m from the wash plant jetty and considered as heavily impacted by increased suspended sediment; two sites 500 m from the discharge and moderately affected by suspended sediment; two sites 800 m away

and less impacted; and two sites more than 1 km away from the jetty, presumably not subject to sediment from the wash plant (reference sites). Each study site was located within a natural bed of *Posidonia sinuosa* and varied in depth from 1.7 to 3.2 m.

To confirm the minor influence of the sediment plumes on the most distant sites (1 km away from the jetty), two additional sites were surveyed in Marmion Lagoon (31°48'19.02"S, 115°42'53.80"E) and Garden Island (32°10'5.98"S, 115°40'42.13"). These supplementary sites were selected as they were not exposed to any anthropogenic source of sediment and presented similar ecological characteristics (*Posidonia sinuosa* meadow with similar shoot densities, depth from 1.7 to 3.2 m) as those located at Woodman Point. Suspension feeder composition from additional sites were compared with Woodman Point distant sites to assess whether sites located 1 km away should be considered as reference sites not influenced by the sediment plumes or only regular station along the transect.

1.2.2 Field survey
1.2.2.1 Suspension feeder composition

A pilot study was conducted to determine the optimum size and number of replicate samples to examine species richness and biomass at each site. Determining minimum sample size is important in order to ensure that the statistical tests are sufficiently powerful to detect changes in composition. Using Bros and Cowell's (1987) method, the minimum sample size and the minimum detectable difference (MDD) (α = 0.05, 80% power) was determined from data collected on the 4th of February 2013 using 20 randomly-placed 0.0625, 0.25 and 1m^2 quadrats in a seagrass meadow located in the vicinity of the wash plant (Woodman Point). From each quadrat, all sessile epi-benthic suspension feeders were collected in calico bags, returned to the laboratory and then identified, oven dried and weighed to obtain the species richness and biomass. From the data examined in this pilot study, a sample size of 10 replicates of 0.25 m^2 quadrat was an acceptable sampling effort (mean MDD = 2.5 % for species richness and 10.9 % for biomass), ensuring a statistical power of 81% to detect changes in biomass, but conservative decision to use 20 was made instead. This was considered acceptable, as no significant gains would be achieved (e.g. resolving power and reducing MDD) by an increase in replicates (i.e. sampling effort). Quadrat size had no significant affect, so 0.25m^2 quadrats were subsequently used, as this size is the most commonly used in the literature.

To determine suspension feeder composition, sites located at Woodman Point were sampled twice, between the 5th and the 10th of May 2013 and between the 11th and the 14th of May 2014. Sites located in Marmion Lagoon and Garden Island were only sampled once, respectively on the 22nd and 27th of May 2014. Prior to each monitoring survey, shoot densities were measured at each site using 20 quadrats of 0.04m^2. Results from these monitoring surveys (cf. Appendix B)

did not reveal any significant differences between sites. From these findings, it appeared that seagrass shoot densities would have no or very little influence on suspension-feeder assemblage's composition. At each site, SCUBA divers, collected suspension feeders from 20 quadrats (0.25 m^2), randomly placed within the seagrass meadows habitat. All large suspension-feeders present at the surface of the sediment (e.g. ascidians, sponges, crinoids, bivalves; cf. Appendix A) were collected and transported to the laboratory and sieved over a 2 mm sieve before to be stored at -20°C. Infaunal species (e.g. tubiculous suspension-feeding polychaetes) and epibionts (e.g. hydroids, spirorbids, bryozoans, barnacles, amphipods) were not included in the survey. Suspension feeders were identified to genus and, when possible to species level, and then each specimen was assigned to the appropriate functional group level as defined by their feeding behaviour and food-particle capture mechanism (cf. Table 1 in general introduction). Each organism was carefully cleaned to remove any carbonate debris, attached epifauna and exoskeletons (e.g. shells). The dry flesh weight (DW) of animals was measured by drying to a constant weight in a 60°C oven for 72 h.

1.2.2.2 Environmental parameters

At each site, three sediment traps were deployed to estimate monthly gross sedimentation rates, from 10th May 2013, until 9th September 2014. Due to weather conditions or fieldwork impairments, data for November 2013, February and May 2014 are missing. Following t he design described by Jurg (1996), sediment traps were 30 cm high and were made of PVC tubing with an internal diameter of 5 cm, the aperture positioned above the seagrass canopy and fixed to a steel rod buried in the seagrass mat. Storlazzi (2010) indicate that settled sediment collected in traps, using appropriate standard deployment, would not be re-suspended. Baffles were positioned 3 cm below the top of the pipe to stop unwanted animals or objects getting into the trap. The sediment traps were collected at approximately monthly intervals and brought back to the laboratory where deposited material was sieved into three size classes: >63 µm; >20 µm and < 20 µm, dried at 60 °C for 72 h and weighed. Specific ranges of particle sizes were classified following the USDA (United States Department of Agriculture) classification as clay (particles with diameter < 4 µm); very fine and fine silt (4-20 µm); medium and coarse silt (20-63 µm); and sand (>63 µm). The term 'fines' can be used to group all particles with a diameter <63 µm (clay and silt). These different size classes were also selected for the potential interaction with suspension-feeding behaviour. It is known that suspension feeders are variably able to capture and assimilate organic and inorganic particle depending on their sizes (Pile et al. 1997; Coma et al. 1995). Gross sedimentation rates (mg·cm^{-2}·month^{-1}) were calculated by dividing sample dry weight by the internal disc surface of the trap and the number of days of deployment. Organic matter content was measured as loss on ignition (400°C for 4 h) as a relative measure of organic carbon, and

expressed as a % of the total sediment collected in traps. In addition, one light logger (Hobbo®) was deployed at each site, to record light intensity (Lux), every hour.

1.2.3 Data analysis

Statistical analyses were performed on environmental (sedimentation rates, organic matter deposited and proportion of particle size distribution) and biological (taxonomic survey) data using Primer v6 and R software. Each of the environmental variables were assessed for equality of variance and normality. In all cases, the data were heteroscedastic, so a log (X+1) transformation was used to correct for significant differences among variances, prior to statistical analyses.

In order to test for spatial patterns in total sediment and organic particle deposition rates during the field survey period, permutational multivariate analyses (PERMANOVA) were performed for the factors Distance (200, 500, 800 and >1000 m; Fixed factor), Orientation (East, North-West; Fixed factor) and their interaction. These analyses included the influence of each month (random factor) nested within season (spring/summer: October and December 2013 and January and March 2014; autumn/winter: July, August and September 2013, April, June, July and August 2014; fixed factor). To test for any influence of season as a priori, a pairwise contrast tests (PERMANOVA) for distance among month nested with season was also conducted. The pairwise tests allowed identifying differences between sites close to the wash plant and those close to reference sites to be examined. A multiple factorial analysis was also performed on the monthly water quality data, using sedimentation rates, amount of organic matter, organic matter contribution to sediment mass and sedimentation rates for each size class of sediment: clay and fine silt (<20 µm), medium and coarse silt (20<x>63 µm), sand (>63 µm).

For the biological data set (suspension feeder biomass), a square root transformation was applied to correct for significant differences among variances, prior to statistical analyses. In order to identify differences on suspension feeder composition amongst sites and distances from the wash plant, a PERMANOVA analysis, based on the Hellinger dissimilarity matrix, was performed. The results were visualised by principal coordinate analysis (PCO) and constrained analysis of principal coordinates (CAP). These descriptive statistical analyses enable the identification of clusters among sampling sites and distances (200, 500, 800 and >1000 m). In order to test for significant differences between clusters, PERMANOVA (permutations = 9999) were performed and the the similarity of percentages procedure (SIMPER) was conducted to break down the contribution of each variable (cut-off 60%) to the observed dissimilarities. As there is growing evidence that species functional traits (feeding behaviour and feeding mechanisms) link changes in species composition and shifts in ecosystem processes (Villéger et al. 2010), three different SIMPER analyses were performed according to three different classifications of the biota: 1) by taxonomic group; 2) by their feeding behaviour (active,

combined, passive); and 3) by their feeding apparatus (Mucus-net filter-feeding, Cirri trapping, Collar sieving and Tube-feet passive suspension feeding).

1.3 RESULTS

1.3.1 Environmental parameters

1.3.1.1 Sediment

The amount of sediment collected monthly in the sediment traps generally decreased with distance from the discharge point (Fig. 2a). The most obvious difference was between sites at 200 m from the wash plant and the reference sites. Over the field survey period, mean sedimentation rates were 8 times higher in the vicinity of the wash plant than at the reference sites (Fig. 3a). The lowest sedimentation rates were during spring/summer period (October and December 2013, January and March 2014). The rates in autumn/winter (Jul, Aug, Sep 2013, plus Apr, Jun, Jul and Aug 2014) were 128 times higher, but with large variations among months.

Estimates of components of variations (ECV) from the PERMANOVA (Table 1) indicated that, by far, the most of the variability in sedimentation rate was explained by the factor Season ($p = 0.0037$), though distance also contribute to the variance ($p = 0.0001$), albeit to a lesser extent than season. Transect orientation (East; North-West) accounted for little of the variation in comparison to Season (ECVs of 1% vs 74%) and distance (ECVs of 1% vs 8%). The variation in sediment regime among distance varied depending on the transect orientation ($p = 0.0002$). The magnitude of the difference in sedimentation rates among distances and/or orientation varied among months. Overall, PERMANOVA results confirmed the presence of a spatial pattern of sediment rates. However, the influence of this pattern was weak relative to the seasonal trend and not consistent from month to month. Pairwise comparisons of sedimentation rates between each distance among months (Table 2) indicated significant differences between sites 200 m from the discharge point and reference sites, every month of each season and for each transect.

The spatial and temporal variation in sedimentation was similar for each size class of sediment particle (<20 µm; 20<x>63 µm; 63 µm; Fig. 3b, c, d). The contribution of each size class of sediment particle to the total sedimentation rate was dominated by the highest sediment size fraction (sand: >63 µm). Nonetheless, a slight increase of the contribution of the two other size fractions (clay/fine silt; medium/coarse silt) was observed during the summer period (Fig. 4).

Figure 2: Data average (a) for total gross sedimentation rates (g (dry weight) ·cm^{-2}· month^{-1}; ± standard error; left) and total organic matter deposition rates within the sediment (g (dry weight) ·cm^{-2}· month^{-1}; ± standard error; right), but also for each size class: (b) <20 µm; (c) 20<x>63 µm; (d) >63 µm, from sediment traps collected monthly at each site from July 2013 to August 2014 (at the exception of November 2013, February and May 2014).

Figure 3: (a) Mean gross sedimentation rate (g (dry weight) $\cdot cm^{-2} \cdot month^{-1}$; ± standard error), (b) mean amount of organic matter deposition rate (g (dry weight) $\cdot cm^{-2} \cdot month^{-1}$; ± standard error) and (c) contribution of organic matter to total sedimentation (%; ± standard error) in relation to distance from the discharge point: 200, 500, 800 and over 1000 m (REF).

Table 1: PERMANOVA, testing for differences in sedimentation rates for each distance (200, 500, 800 and >1000 m; fixed factor), for each orientation: Orientation (East, North-West; fixed factor) and for each month (random factor) nested within season (spring/summer: October and December 2013 and January and March 2014; autumn/winter: July, August and September 2013, April, June, July and August 2014; fixed factor).

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	Estimates components of variation
Se	1	828.6	828.6	96.331	0.0037	2238	6.7331
Di	3	135.45	45.149	27.648	0.0001	9962	0.71463
Or	1	18.036	18.036	6.1521	0.0372	9831	0.12403
Mo(Se)	9	77.227	8.5808	30.794	0.0001	9921	0.34789
SexDi	3	5.6966	1.8989	1.1631	0.3408	9956	0.0087497
SexOr	1	0.33645	0.33645	0.11499	0.731	9846	0.042612
DixOr	3	28.723	9.5742	11.586	0.0002	9950	0.28733
Mo(Se)xDi	27	44.007	1.6299	5.8491	0.0001	9901	0.22636
Mo(Se)xOr	9	26.323	2.9248	10.496	0.0001	9940	0.22176
SexDixOr	3	2.3804	0.79348	0.96081	0.4269	9951	0.0021272
Mo(Se)xDixOr	27	22.27	0.82481	2.96	0.0001	9912	0.18299
Res	175	48.765	0.27865				0.27865
Total	262	1257.5					

Table 2: Summary of results of pairwise (PERMANOVA) comparisons of sedimentation rates and organic matter deposition rates between each distance and between transects. Numbers and percentage of month where there was a significant effect of distance.

Comparison	Sedimentation rates			Organic matter deposited		
	autumn/winter	spring/summer	total	autumn/winter	spring/summer	total
200, 500	7 (100%)	3 (75%)	10 (91%)	1 (14%)	3 (75%)	4 (36%)
200, 800	7 (100%)	2 (50%)	9 (81%)	2 (28%)	2 (50%)	4 (36%)
200, REF	7 (100%)	4 (100%)	11 (100%)	7 (100%)	4 (100%)	11 (100%)
500, 800	6 (86%)	3 (75%)	9 (81%)	0 (0%)	2 (50%)	2 (18 %)
500, REF	5 (71%)	2 (50%)	7 (63%)	4 (57%)	1 (25%)	1 (9%)
800, REF	5 (71%)	4 (100%)	9 (81%)	3 (43%)	3 (75%)	3 (27%)
East, North West	5(71%)	2(50%)	7 (63%)	1 (14%)	2(50%)	3(27%)

Figure 4: Average contribution of each size class (%): <20 µm; 20<x>63 µm; >63 µm to total gross sedimentation rate (g (dry weight) $\cdot$ cm^{-2} $\cdot$ month^{-1}) every month from July 2013 to August 2014 (at the exception of November 2013, February and May 2014).

1.3.1.2 Organic matter

Over the field survey period, the mass of organic matter deposited was a highly correlated with gross sedimentation rate (R^2= 0.84, p<0.0001) while the contribution of organic matter to the total sediment mass had a negative correlation (R^2 = 0.69, p<0.0001). Following a similar pattern to gross sedimentation rates, the mass of organic matter settling in the sediment traps was higher at sites near the jetty than other sites, especially the reference sites, while the contribution of the organic fraction displayed the inverse pattern. For sites 200 m from the wash plant a mean of 3.63×10^{-1} g$\cdot$cm^{-2} of organic matter settled every month, contributing 8% of total deposition, while at the reference sites the 1.10 g$\cdot$cm^{-2} of organic matter settling every month, contributed to 22% of total deposition (Fig. 3b, c). The mass of organic matter depositing on the eastern transect was four times higher at 200 m from the wash plant that at the reference site. On the western transect, site on the vicinity of the discharge point (200 m) organic matter deposited was two and a half times higher than for reference site (Fig. 2a). As for the total sedimentation rates, organic matter sedimentation varied seasonally, with autumn/winter period 15 times higher with important variations between months, in comparison to the spring/summer season (Fig. 2a).

Patterns in organic matter deposition rates were similar to overall sedimentation rates (Table 3), with differences between seasons accounting for most of the variation and a highly significant influence of the two periods (p = 0.0018). Distance also accounted for a significant amount of variation, albeit to a lesser extent than season (p = 0.0001) but there was no significant influence (p = 0.1204) of the orientations. Variation among distances varied depending on the transect orientation (p = 0.0003). Time was the principal factor of variations, as Months nested within Season have a significative influence on amount of organic matter settled and also, as its interactions with distance and orientation. Month and season interactions indicate that the magnitude of the difference in organic matter deposited among distances and orientation vary each month and season. Overall, results confirmed the presence of a spatial pattern of organic matter deposition rates. However, the influence of this pattern was weak in comparison to the seasonal variation. Despite the absence of a clear spatial pattern, pairwise comparison of organic matter deposited between each distance among month nested with season (Table 2) indicated significant differences between sites 200 m away from the discharge point and reference sites, every month of each season and for each transect.

Table 3: PERMANOVA, testing for differences in organic matter deposition rate for each distance (200, 500, 800 and >1000 m; fixed factor), for each orientation: Orientation (East, North-West; fixed factor) and for each month (random factor) nested within season (spring/summer: October and December 2013 and January and March 2014; autumn/winter: July, August and September 2013, April, June, July and August 2014; fixed factor).

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	Estimates components of variation
Se	1	407.55	407.55	104.04	0.0018	8157	3.358
Di	3	53.08	17.693	25.052	0.0001	9955	0.282
Or	1	4.74	4.74	2.9796	0.1204	9842	0.026
Mo(Se)	9	35.252	3.9168	11.084	0.0001	9931	0.151
SexDi	3	1.8661	0.62204	0.88073	0.4556	9934	0.003
SexOr	1	1.6868	1.6868	1.0604	0.3218	9826	0.002
DixOr	3	12.79	4.2635	8.7899	0.0003	9954	0.126
Mo(Se)xDi	27	19.078	0.70659	1.9996	0.0043	9900	0.060
Mo(Se)xOr	9	14.316	1.5907	4.5014	0.0001	9931	0.105
SexDixOr	3	4.3619	1.454	2.9976	0.0442	9958	0.064
Mo(Se)xDixOr	27	13.099	0.48516	1.3729	0.1127	9907	0.045
Res	173	61.133	0.35337				0.279
Total	260	646.36					

1.3.1.3 Light intensity

There was only a slight correlation between total sediment deposition rates and the light reaching the seagrass habitat ($R^2 = 0.36$, p value < 0.0001, n=288). Also, average light intensity values did not reveal any particular spatial pattern along the distance transects (Fig. 5). Indeed, there were no significant differences on the average light intensity between reference sites and impacted sites (p >0.5). Light intensity was four times higher during summer (October and December 2013 and January and March 2014), than winter (July, August and September 2013, April, June, July and August 2014).

Figure 5: Light intensity (Lux.day-1) at each site from September 2013 to August 2014 (at the exception of November 2013, February and May 2014).

1.3.2 Suspension feeders composition

Fifteen different macro suspension feeder taxa were identified during the surveys (2013 and 2014). The number of taxa (Fig. 6) showed no strong spatial trends, with the exception of the east reference site, which had the lowest species diversity in both surveys. In order to confirm suitability of the location of sites REF E and REF NW as reference sites, the composition of suspension-feeder assemblages at those sites was compare with those from two additional sites located in Marmion Lagoon and Garden Island. These sites were similar to Woodman Point reference sites (natural bed of *Posidonia sinuosa* and depth from 1.7 to 3.2m). The composition of suspension-feeder assemblages (Table 4) was similar between the two surveys (2013 and 2014; p = 0.3729), but varied significantly between sites (p = 0.0001). Pairwise comparisons (Table 5) revealed strong differences between REF E and REF NW and the two additional sites (P < 0.05). The eastern reference sites contained the lowest abundance, biomass and species diversity (Fig. 7).

Figure 6: Number of taxa identified per sites in 2013 and 2014.

Table 4: PERMANOVA, testing for differences in suspension-feeder composition for each site and for each monitoring period (2013 and 2014).

Source	df	SS	MS	Pseudo-F	P (perm)	Unique permutations
Year	1	0.16136	0.16136	0.9622	0.3729	9935
Site	9	11.479	1.2754	2.9959	0.0001	9827
Res	190	80.884	0.42571			
Total	199	92.363				

Table 5: Pairwise PERMANOVA comparisons of suspension feeder biomass in between non impacted sites: Marmion, Garden Island (GI), reference sites (REFNW and REF E) and sites directly impacted by the wash plant (200NW and 200E).

Groups	t	P(perm)	Unique permutations
GI, MARMION	0.8391	0.6932	9933
GI, REFE	1.649	0.0125	9720
GI, REFNW	0.97591	0.4458	9939
MARMION, REFE	2.0777	0.0001	9943
MARMION, REFNW	0.60673	0.9423	9933
200E, GI	1.612	0.0126	9928
200E, MARMION	1.4562	0.044	9917
200NW, GI	2.0171	0.0018	9925
200NW, MARMION	1.7165	0.0116	9926
200E, 200NW	1.4387	0.0608	9942
REFE, REFNW	2.361	0.0001	9931

The mean suspension feeder biomass per quadrat was highest at sites near the wash plant (200 m), with maximum values observed in 2014 (Fig. 7). The mean biomass tends to reduce toward the furthest sites, especially for the eastern transect. During the two monitoring periods, the ascidian *Herdmania momus* and the bivalve *Pinna bicolor* were the most dominant species in term of biomass and presence at each site (Table 6). Along each transect, other species, such as the sponges *Coelosphara* sp and *Tethya* sp, the crinoid *Comatula purpurea* and the holothuridae *Cercodemas anceps*, were also commonly found with relatively high biomass. The composition of suspension-feeder assemblages (Table 7) did not vary significantly between the two sampling periods (2013 and 2014; $p = 0.4739$), but varied significantly between distances ($p = 0.0084$) and Orientation ($p = 0.0268$). For Distance, the dissimilarities among groups were generally higher than within groups (Table 8). The highest dissimilarities (>50%) were among the group of stations located more than 1000 m from the wash plant and the group closest to the jetty (200 and 500 m). The magnitude of dissimilarities between these two groups depended on orientation; the highest dissimilarity was observed on the eastern transect, while the difference between 200 m and reference sites was minimal at the north-western transect (Fig. 8).

Figure 7: Mean suspension feeder biomass per quadrat (g; ±standard error) at each site for 2013 and 2014.

Table 6: Total biomass for each suspension feeder taxa at each site in 2013 and 2014.

Principal Taxa	200E		200NW		500E		500NW		800E		800NW		REFE		REFNW	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Herdmania momus	163.98	333.17	182.50	200.01	27.69	31.69	25.76	4.50	33.72	5.55	51.88	25.17	9.30		78.25	73.49
Pinna bicolor	30.27	17.46	64.50	78.08	16.29	18.29	83.41	174.78	31.18	34.18	35.63	43.53	7.15		89.61	140.67
Coelosphaera sp	16.85	76.69	1.37	17.85	47.90	2.69	9.71	20.65	16.33		44.72	9.32			10.28	20.93
Comatula purpurea	39.78	11.21	8.39	6.80	2.01	0.72	3.57	10.76	10.96	3.21	9.53	1.17	3.26	2.33	3.51	9.43
Tethya sp	17.31		19.44	18.02	9.67	6.40	2.10	7.41	14.78			9.18				
Cercodemas anceps	14.46	12.44	4.27	1.98		3.53	11.08		25.79		10.28	2.63			5.20	
Polycarpa veridis	6.09	7.27	2.13	5.62				5.10			4.10	5.87	5.34	9.99	4.24	8.94
Phalusia obesa		0.32	1.41	1.35	13.35						16.22	3.57			12.38	11.20
Oyster spp			8.07	12.32			0.82	1.05			0.12	1.10			2.17	2.63
Spondylus sp		0.53		0.36	0.58	0.13	2.00	9.40	1.37	0.87	0.66	2.16			0.96	3.16
Botrylloides schlosseri		1.23		0.12	0.21		4.64	6.57				1.09				
Chondrilla australiensis				9.79	0.21			0.81	2.85							
Mimachlamys asperrima	0.03	0.91							0.68	2.01	0.09	0.08			3.15	1.63
Cardita incrassatta					1.75		0.68	0.15				1.37			0.54	0.89
Mytilus sp				0.22											0.85	1.33

Table 7: PERMANOVA, testing for differences in suspension feeder's composition for each distance (200, 500, 800 and >1000 m; fixed factor), for each orientation (East, North-West; fixed factor) and for each monitoring period (2013 and 2014).

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	Estimates components of variation
Year	1	0.16136	0.16136	0.9622	0.4739	9935	0.0008
Distance	3	1.2697	0.42324	2.5238	0.0084	9937	0.0639
Orientation	1	0.43169	0.43169	2.5742	0.0268	9937	0.0330
Ye x Di	3	0.46352	0.15451	0.92131	0.5862	9940	0.0066
Ye x Or	1	0.19139	0.19139	1.1413	0.3432	9934	0.0059
Di x Or	3	0.87396	0.29132	1.7371	0.0742	9926	0.0618
Res	3	0.5031	0.1677				0.1677
Total	15	3.8947					

Figure 8: Hellinger dissimilarities (%) based on suspension feeder's biomass between the distance at 200m from the jetty and each distance, for each transect (East; North-West). The dissimilarity between 200E and 200NW is represented in the middle of the histogram.

Table 8: Hellinger dissimilarities (%) based suspension feeder assemblage biomass within and between the group distance (200 m; 500 m; 800 m; >1000 m (REF)).

Dissimilarity within distance (%)		Dissimilarity between distances (%)	
200	20.27	200 vs 500	44.18
500	31.31	200 vs 800	40.03
800	40.13	200 vs REF	51.83
REF	30.29	500 vs 800	39.98
		500 vs REF	55.07
		800 vs REF	43.72

Between 2013 and 2014, there were no significant differences on the composition of suspension-feeder assemblages in terms of biomass and abundances. Therefore, results from the two monitoring periods were combined to estimate the mean biomass of the most conspicuous species, giving more power to subsequent statistical analyses.

Based on both taxonomic and functional composition, the descriptive statistical analyses (Fig. 9) identified two clusters of stations. From the data on suspension-feeder assemblage biomass and composition, sites near the jetty (200 m and 500 m) were significantly distinct from more distant assemblages ($p = 0.001$), especially those of reference sites (Fig. 9a). Assemblages from sites located 800m away from the discharge point were not so clearly distinguished. Almost 60% of the dissimilarities between sites "near the jetty" (200 and 500 m) and site "distant from the jetty" (800 and Reference sites), was explained by the presence and higher abundance and biomass of the ascidian *Herdmania momus*, the sponges *Coelosphaera sp* and *Tethya* sp and the bivalve *Pinna bicolor* at sites close to the discharge point (SIMPER, Table 9). The mean biomass for the ascidian *Herdmania momus* and the sponges *Coelosphaera sp* and *Tethya* sp (Fig. 10) were significantly higher (p values < 0.05) at sites closest to the discharge point. Indeed, average biomass for *Herdmania momus*, *Coelosphaera* sp, and *Tethya* sp, was from 2 to 3.5 times higher at sites near the jetty (Fig. 10). The average biomass for the bivalve *Pinna bicolor* was higher near the discharge point but relatively close of those observed further away (Fig. 10). As for sites further away from the wash plant, their group was characterised by ascidians *Polycarpa veridis* and *Phalusia obesa*.

A slight distinction based feeding mechanisms (Fig. 9c) existed between a group including sites 200 m away from the jetty and reference sites and another group including sites 500 and 800 m away ($p = 0.0008$). Mucus-net filter-feeding organisms contributed the most to the first group, while cirri-trapping animals mainly contributed to the second one (Table 10). Indeed, ascidians (mucus-net filter-feeding mechanisms) *Herdmania momus* and *Polycarpa. veridis* respectively, contribute the most in sites at 200 m away and reference sites. Whereas *Pinna bicolor*, cirri-trapping organisms characterised sites distant by 500 and 800 m. No significant distinction between groups of distances ($p = 0.15$) could be observed based on species feeding behaviour (Fig. 9b). Each distance groups were dominated by active suspension feeders, which explain why no clear pattern could be observed (Table 10).

Figure 9: Principal coordinates analysis (PCO; top) and constrained analysis of principal coordinates (CAP; bottom) for differences in (a) species composition of suspension feeder assemblages, (b) feeding behaviour (active, passive and combined) and (c) feeding mechanisms, for each site and each monitoring period (2013 and 2014).

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Table 9: Suspension feeder's contribution to dissimilarities (SIMPER; %; cut-off 60%) between the two distance groups: "near jetty" (200, 500 m) and distant to jetty (800 m, reference sites (>1000 m)).

Taxonomic group Average dissimilarity = 62.25	Group near jetty Average Abundance	Group distant to jetty Average Abundance	Contribution %	Cum. %
Herdmania momus	121.16	34.67	41.46	41.46
Pinna bicolor	60.39	47.74	25.92	67.38
Coelosphaera sp	24.21	12.7	10.62	78
Tethya sp	10.04	3	4.67	82.67
Cercodemas anceps	5.97	5.49	3.71	86.38
Comatula purpurea	10.41	5.43	3.69	90.07

Figure 10: Average biomass (g; ± standard error) of the most conspicuous species: *Herdmania momus*, *Coelosphaera sp*, *Pinna bicolor* and *Tethya* sp at sites near the jetty (200 and 500 m) and sites further away (800 and >1000 m); ★ indicates significant difference between the 2 groups of stations.

Table 10: Feeding behaviours and feeding mechanisms contribution to dissimilarities (SIMPER; %, cut-off 60%) between the four distances from the wash plant: 200, 500, 800, >1000 m (REF).

	200	500	800	REF
feeding behaviour				
Active	**73.07**	**45.47**	**53.42**	**60.67**
Passive	16.76	25.01	24.38	20.27
Combined	10.18	29.52	22.2	19.07
feeding mechanism				
Mucus-net filter-feeding	**46.26**	28.34	31.22	**49.7**
Cirri trapping	22.39	**38.9**	**41.93**	22.34
Collar sieving	17.92	19.39	13.6	
Tube-feet passive suspension feeding	13.42	13.37	13.26	20.82

1.4 DISCUSSION

1.4.1 Organic and inorganic particles

A clear and persistent gradient of higher sedimentation near the wash plant was present. The size, shape, direction and intensity of the wash plant generated plume are largely controlled by wind speed and direction. As a result, there is a considerable spatial and temporal variation in the rates of sedimentation affecting seagrass habitats adjacent to the discharge jetty. There was a high seasonal variability with a distinct autumn/winter period (July, August and September 2013, April, June, July and August 2014), when sedimentation rates and the amount of organic matter settling were highest. During this period, organic and inorganic particles settled in greatest mass near the discharge location. In the autumn/winter season, frequent storm occurs in south-western Western Australia and generate large swell and waves locally (Lemm et al., 1999). Storm events enhance the resuspension of sediments, especially in the relative shallow water column (2–5 m). Acute resuspension events could explain the high variation in sedimentation rates and organic matter inputs observed between autumn/winter months. During summer (October and December 2013, January, March 2014), sediment plumes drifted away from the jetty in response to prevailing wind (west and south-west) and sedimentation patterns spread to a further extent. Accordingly, particles from the wash plant would settle over a larger area explaining the lower sediment rates recorded and reduced amount of organic matter collected within the sediment traps. Also in summer, regular strong sea breezes generate moderate waves (Lemm et al. 1999), reducing the chance of resuspension events.

Underwater observations made during the field survey revealed that turbidity plumes formed a relatively thin (approximately 1 m deep) layer at the top of the water column, below which water still fairly clear. Therefore, it appeared that, despite the surficial turbidity plume, light loggers were receiving substantial reflected and refracted light. Indeed, little correlation existed between sediment exposure and the light reaching the seagrass habitat ($R^2 = 0.36$). Extensive seagrass bed is still present in the vicinity of the wash plant and there was no obvious spatial pattern of light attenuation with distance from the wash plant (Fig. 5).

Overall, the findings indicated that suspension feeders experienced high level of sediment exposure, linked with an elevation of organic matter input, in the vicinity of the wash plant. The survey also revealed that the extent and intensity of sediment and organic matter exposure was highly influenced by seasonal variations of environmental conditions, particularly wind and water movements. Spatial and temporal variations of sediment and organic content regimes at Woodman point are representative of many shallow coastal marine habitat experiencing increased sediment loading induced by anthropogenic activities (Fabricius et al. 2013; Diaz & Rosenberg 2008; Cloern 2001).

1.4.2 Suspension feeders

The nature of the sediment (large dominance of the sand fraction) and the intensity of sedimentation rates near the wash plant seemed to exert no negative effect on the suspension-feeder assemblages. Despite experiencing high levels of sediment exposition, all but rare species were present, some in greater quantities. Consequently, it appeared that suspension feeders were able to maintain feeding and respiration functions to meet their basic metabolic requirements.

Active suspension feeders were the dominant feeding group at each site. Active feeding behaviour is more efficient at retaining smaller food particles (< 5 μm) (Gili & Coma 1998) which dominate pelagic phytoplankton assemblages in the region (Koslow et al. 2008). The ascidian *Herdmania momus* and the bivalve *Pinna bicolor* were the most conspicuous species in term of biomass and abundance. Both are erect organisms which are more likely to be resilient than prostrate suspension feeders, which accumulate more sediment on their surfaces (Irving & Connell 2002). Mucus-net filter-feeding organisms, such as ascidians were relatively more abundant at sites 200 m away from the jetty and reference sites, while cirri-trapping bivalves were relatively more abundant at sites 500 and 800 m away. Both feeding groups possess a number of physiological and behavioral mechanisms to compensate for events of increased sediment exposure (Armsworthy et al. 2001; Norkko et al. 2001).

For mucus-net suspension feeders, squirting plays a key role in compensating high sediment exposure. Jørgensen (1966) measured an increase in squirting frequency by the Stolidobranch ascidian, *Phalusia* sp, when delivered graphite, but not algae. Squirting serves to expel unwanted particles, and also reducing particle retention by the mucus and the subsequent chance of clogging (Petersen 2007; Armsworthy et al. 2001; Klumpp 1984). Additional mechanisms of active rejection of overload in inorganic particles may also include changes in siphon-opening diameter, structure and transport of mucus, and particles retention efficiency to maintain constant clearance rates. Klumpp (1984) observed a reduction of the inhalant-siphon diameter by the Stolidobranch ascidian, *Pyura stolonifera* in addition to non- nutritious bead. Armsworthy (2001) reported that the ascidians *Halocynthia pyriformis* maintained a constant clearance rate that permits maximum absorption of organic material despite increasing sediment exposure.

For cirri-trapping bivalves, the rejection of sediment particles is ensured by the production of mucus-bound pseudofaeces. In this case, particles intercepted by the gills are transported toward the labial palps to be process and inorganic particles are rejected as pseudofaeces (Beninger 2009). In addition to increase particles rejection, some bivalves increase their pumping rates and the food sources to be consumed, mitigating the increase in inorganic particle concentrations. These compensatory mechanisms have been observed for the bivalve species *Austrovenus stutchburyi*, *Paphies australis*, *Macoma liliana* and *Atrina zelandica*. For these four species, laboratory and field experiments found an increase in clearance rates and food source used following suspended

sediment addition (Safi et al. 2007; Hewitt 2001;). The modification of pallial organ size to tolerate sediment exposure is another adaptive mechanism that could occur for cirri-trapping bivalves (Dutertre et al. 2009).

While compensatory mechanisms might explain the presence of the suspension feeders near the wash plant, this hypothesis does not refute the negative influence of sediment plumes. Physiological and behavioral mechanisms found in the literature appeared to be closely tied to suspension feeder's filtration and retention rates. Based on this premise, increased sediment exposure is likely to have a negative effect on suspension feeder energy allocation and levels. For instance, Hewitt & Pilditch (2004) showed that increased energy expended on compensatory mechanisms had detrimental effects on the physiological condition of the bivalve *Atrina zelandica*.

Despite the absence of a strong biomass and species composition gradient to match the patterns in sedimentation rates, the survey revealed dissimilarities (Fig. 9 & 10) between group of sites near the most impacted area (200 and 500 m away from the discharge points) and those further away (800 m and Reference sites). The suitability of the site REF NW as a reference site not influenced by sediment plumes could be demonstrated by the similarities of suspension feeder composition with the additional sites (Marmion and Garden island). However, the same conclusion could not be addressed for the site REF E (Table 6). Even if sediment plumes appeared to have no effect on suspension feeders inhabiting this site, other factors independent from the wash plant activities appeared to determine species composition and low biomass and species diversity. Nevertheless, suspension feeder biomass and abundance for the group of sites "near the jetty" (200 and 500 m) tended to be higher that at the group of sites "distant from the jetty" (800 and Reference sites). Almost 60% of the dissimilarities between these two groups were explained by the presence, higher abundance and biomass of *Herdmania momus*, *Coelosphaera* sp, *Pinna bicolor*, and *Tethya* sp at sites close to the discharge point (Table 10). This might be related to the increased organic material present in suspended sediment near the wash plant, potentially providing a valuable food source for suspension feeders (Ellis et al. 2002; Marta Ribes et al. 2000; Gili and Coma 1998; Grant 1996; Klumpp 1984). Increased suspended inorganic material can also enhance food availability to suspension feeders via the incorporation of small organic particles, such as picoplankton and bacteria, into larger inorganic particles (Newell et al. 2005; Waite et al. 2000). Depending on their feeding mechanisms, retention efficiency of suspension feeders varies as a function of the nature and size class of food particles (Riisgård & Larsen 1995; Riisgård & Ivarsson 1990). Therefore, aggregates of organic and inorganic particles allow certain suspension feeders to ingest efficiently food-size particles that would otherwise be too small to access (Kach & Ward 2008). Furthermore, marine aggregates have much higher settling rates than individual organic particle and contribute significantly to inputs of particulate organic ingested

by suspension feeders (Newell et al. 2005; Waite et al. 2000). Organic matter embedded into large inorganic particle could represent an important food resource (Newell et al. 2005; Waite et al. 2000).

1.5 CONCLUSION

This study confirmed that a clear and persistent gradient of higher sedimentation near the wash plant was present in the study area. Overall, results suggested that suspension-feeder assemblages were strongly resistant to high sediment exposure. It was hypothesized that physiological and behavioural mechanisms explained the presence of suspension feeders at sites highly impacted by increased sediment exposure. From the higher abundance and biomass of *Herdmania momus*, *Coelosphaera sp*, *Pinna bicolor*, and *Tethya* sp observed at sites near the jetty (200 and 500 m) and the absence of *Tethya* sp at reference sites, it even seems plausible that some of the suspension feeder taxa benefit from a diet supplemented with sediment. However, other species such as the ascidians *Polycarpa veridis* and *Phalusia obesa* were more abundant at sites away from the peak of sediment perturbation (800 m and reference sites). Under those circumstances a trade-off can arise between negative and positive effects depending on the sensitivity of each specie (Kruger et al. 2005; Skilleter et al. 2006). Thus, the degree to which increased sediment benefit or harm suspension feeders is more likely to be species specific and dependent on their feeding activity and the resilience of feeding mechanisms.

CHAPTER 2
Food sources and trophic relationships among co-occurring suspension feeders within a *Posidonia* seagrass meadows influenced by increased sediment exposure.

PREAMBLE

This chapter describes the exploitation of a broad range of potential food sources by the most conspicuous suspension feeders (in term of biomass and abundance) within the study area. Using natural abundances of stable isotopes of carbon and nitrogen, this chapter investigates suspension feeder food resources exploitation by comparisons between their stable isotope ratios and those of the available food sources. Stable isotope analysis of suspension feeders and their potential food sources provides a longer time integration of the influence of sediment exposure on suspension feeder feeding activity and allow to characterize their diets.

ABSTRACT

Suspension feeders are an essential component of benthic marine food webs because they actively participate in organic matter and nutrient recycling, as well as in energy transfer between pelagic and benthic compartments. Increases in sediment triggered by anthropogenic activities can have detrimental effects on suspension feeder distribution and abundance, but also their feeding activity, reducing the energy available for benthic assemblages. The aim of this work was to determine the influence of sediment exposure on the diet of three co-occurring species of suspension feeders by comparisons between species stable isotope ratios and those of potential food sources, at sites with different sedimentation regimes. The study was performed at a location where the benthic suspension feeder assemblage experienced spatial patterns of strong sedimentation gradient. Although POM was likely to be the main contributors to suspension feeder diets, the co-occurring suspension feeders presented distinct isotopic signatures, which suggested dissimilarities in their diets. Differences in δ^{13}C and δ^{15}N between the three suspension feeders could be explained by consumption of different types of picoplanktonic particles and also the degree to which sedimentary organic matter contributed to their diets. Increased sediment exposure had no influence on the natural diets of the ascidian *Herdmania momus* and the bivalve *Pinna bicolor*, both possessing compensatory mechanisms. For the sponge *Tethya* sp, there was a strong relationship between its diet and sediment and organic deposition rates. For sponges experiencing high sediment exposure, sedimentary organic matter represented an additional food source. Overall, the influence of sediment loads on suspension feeder diets appeared to be to be species specific and dependent on the nature and concentrations of the sediment.

2.1 INTRODUCTION

Suspension feeders comprise a large proportion of the biomass and abundance of the benthic fauna and are a major conduit in benthic-pelagic coupling (Gili & Coma 1998) due to their ability to filter large amounts of water (up to $100m^3 \cdot d^{-1} \cdot m^{-2}$; Jørgensen, 1996). In coastal zones, suspension feeders actively participate in organic matter and nutrient recycling, as well as in energy transfers between pelagic and benthic compartments (Lefebvre et al. 2009; Ostroumov 2005; Pile 2005). The array of food particles potentially exploited by suspension feeders incudes bacteria (Hanson et al. 2009), picoplankton (0.2-2 μm) and nanoplankton (2–20 μm) (Pile 2005), resuspended detritus (Smit et al. 2006), larger phytoplankton and microzooplankton (20-200 μm) (Dupuy et al. 1999) and even very large zooplankton (>200 μm) (Kreeger & Newell 2001). In marine ecosystems, particulate organic matter (POM) represented by phytoplankton is an important contributor to suspension feeder diets (Cloern 2001). POM can be broadly divided into food particles <20 μm representing bacteria, pico and nanoplankton and particles >20 μm comprised of larger phytoplankton (Kang et al. 2009). Seagrass and macroalgal detritus, as well as organic matter within resuspended sediment can also represent an significant part of their diets (Miller & Page 2012; Leal et al. 2008; Smit et al. 2006; Kreeger & Newell 2001; Riera & Richard 1996). Because they are able to exploit a wide spectrum of food particles of different types and sizes, benthic suspension feeders exhibit distinct feeding structures and strategies. Hence, there is a need to investigate the diversity of feeding mechanisms among suspension feeders to better understand the functioning of coastal ecosystems, and the importance of benthic–pelagic coupling.

Quantifying the contribution of different food sources to suspension feeder diets in shallow coastal ecosystems is often difficult due to this diversity of organic matter sources (OMS) (Leal et al. 2008). Natural abundances of stable isotopes $\delta^{15}N$ and $\delta^{13}C$ are now commonly used to identify food sources contributing the most to consumer diets (Topçu et al. 2010; Carlier et al. 2009; Hanson et al. 2009; Lefebvre et al. 2009; Dubois et al. 2009; Leal et al. 2008). Based on the assumption that stable isotope ratios ^{13}C / ^{12}C and ^{15}N / ^{14}N exhibit relatively predictable discrimination between OMS and consumers (Caut et al. 2009; Vanderklift & Ponsard 2003), stable isotope analysis can provide evidence of the diet composition of organisms (Fry 2007). Discrimination values can be found in the literature even if they are still subject to debate because these values are highly specific and linked to the origin of the food source and the feeding level (Gaye-Siessegger et al. 2004; McCutchan et al. 2003). The contribution of each OMS is often estimated using isotopic mixing models (Leal et al. 2008; Connolly et al. 2004; McCutchan et al. 2003; Zanden & Rasmussen 2001). From this method, the trophic relationships among co-occurring suspension feeders in coastal zones has been investigated in numerous studies (Lefebvre et al. 2009; Leal et al. 2008; Riera 2007; Decottignies et al. 2007; Dubois et al. 2007).

These authors found that resource partitioning due to fundamental differences in food particle selection capability within multispecific assemblages can limit interspecific competition for food. However, ecosystem characteristics of the organic matter sources are of primary importance to determine the extent to which suspension-feeders potentially compete for food (Lefebvre et al. 2009). Because, suspension feeders may modify their diet depending on food resource availability and quality (Safi et al. 2007; Petersen 2007; Armsworthy et al. 2001), the trophic relationships among species influenced by changes in environmental conditions (Topçu et al. 2010; Schaal et al. 2008).

Increases in sediment exposure to coastal ecosystems can occur naturally but its impact on the benthic fauna is often enhanced when triggered by anthropogenic activities such as road building, logging, mining, aquaculture, port maintenance and dredging (Ellis et al. 2002). In addition to the detrimental effect on suspension feeder distribution and abundance, high sediment exposure may also negatively influence their feeding ecology and alter food resource partitioning. Potentially this can impact coastal ecosystem functioning by modifying food availability and by affecting ecosystem-level processes performed by suspension feeders (Safi et al. 2007; Newell et al. 2005; Armsworthy et al. 2001; Ellis et al. 2002; Kach & Ward 2001).

The aim of this work was to determine the influence of sediment exposure on the diet of three co-occurring suspension feeders by comparisons between species stable isotope ratios and those of potential food sources at sites with different sedimentation regimes. The study was performed at a location where the benthic suspension feeder assemblage experienced spatial patterns associated with a strong sedimentation gradient (see Chapter 1). The continuous sediment plumes, generated by a lime and cement manufacturer in this area, represented an adequate study model to understand the influence of increased sediment exposure on suspension-feeding activity. Among the suspension feeders present, three species were selected. These species comprised distinct feeding strategies: the solitary ascidian *Herdmania momus* actively captures food particles through a mucus net, the pteroid bivalve *Pinna bicolor* uses a cirri trapping mechanism to actively gather food particles and the demosponge *Tethya* sp combines active and passive feeding by the mean of a collar sieving structure.

2.2 MATERIAL AND METHODS

2.2.1 Study site

Six sampling sites were located within seagrass meadows located at Woodman Point (32°7'S, 115°44'E), south of Fremantle, Western Australia. As previously described in Chapters 1 and 2, high turbidity observed in this area was generated by shell sand dumping, reclaiming, washing and vessel movements associated with activities from a cement manufacturer wash plant. Seagrass beds in the area provide habitat for a range of organisms including suspension feeders (Lemmens et al. 1996). The six sites were located at increasing distances from the Cockburn Cement wash plant, and were oriented along two different directions: East and North-West (Fig. 1). These sites can be classified as follows: two sites located 200m of the wash plant jetty and considered as heavily impacted by increased suspended sediment, two sites 500 m from the wash plant and moderately affected by suspended sediment and two additional sites more than 1 km away from the jetty and subject to as little anthropogenic influence as possible (Reference sites). Each site was located within a natural bed of *Posidonia sinuosa* and varied in depth from 1.7 to 3.2 m.

Figure 1: Study sites

2.2.2 Sample collection and processing

2.2.2.1 Sedimentation rates

At each site, three sediment traps were deployed from 10th July 2013 until 9th September 2014 to estimate monthly gross sedimentation rates. Following the design described by Jurg (1996), sediment traps were 30 cm long and were made of PVC tubing with an internal diameter of 5cm, the aperture positioned above the seagrass canopy and fixed to a steel rod. Baffles were positioned 3cm before the top of the pipe to stop unwanted animals or objects getting into the trap. Sediment traps were collected at the end of each month, brought back to the laboratory where they were dried at 60°C for 72 hours and finally weighed. Gross sedimentation rates ($gDW \cdot cm^{-2} \cdot month^{-1}$) were calculated by dividing sample dry weight by the internal disc surface of the trap. Organic matter content was measured as loss on ignition (400 °C for 4 h) as a relative measure of organic carbon, and expressed as $gDW \cdot cm^{-2} \cdot month^{-1}$.

2.2.2.2 Suspension feeders

Collection of suspension feeders took place twice from 21st to 28th of September 2013 and from 25th to 31st of August 2014. At each site, 5 individuals of the ascidian *Herdmania momus*, the bivalve *Pinna bicolor* and the sponge *Tethya* sp were hand-collected by SCUBA divers. As far as possible, individuals of similar size and weight (appendix C) were collected to limit the potential effect of age and size on feeding behaviour. Specimens were transported to the laboratory where they were placed in filtered and aerated seawater overnight to clear their gut contents (or pores for the sponge), before being killed by freezing. Each organism was carefully cleaned with distilled water to remove any carbonate debris and remove any attached epifauna. Adductor muscle tissue of bivalves, muscle tissue of ascidian body and a mix of internal cortical tissue and external endosome tissue for sponges were dissected. As none of these samples contained calcium carbonates, there was no need for acid treatment (Kaehler & Pakhomov 2001). All samples were dried at 60[0]C for 48 hours, homogenised to a powder using a ball and mill and weighed into tin capsules ready for stable isotope analysis.

2.2.2.3 Organic matter sources (OMS)

Potential sources of organic matter available to suspension feeders in the study area (POM >20 µm, POM <20 µm, organic matter in the sediment and seagrass detritus) were collected at each site every 2 months from June 2013 to September 2014. Each potential food sources are considered non limited in the study area (Smit et al. 2005). Because isotopic signature of an organism tissues integrate assimilation of food over long time periods, annual means of OMS stable isotope ratios were considered rather than time specific values (Vanderklift & Ponsard 2003).

At each site 20l of water was collected 1 m above the seagrass bed and passed through a 180 µm Nitex mesh to remove zooplankton and large particles, and stored in acid-washed plastic bottles.

5l triplicates of seawater samples were then passed through a 20 μm sieve to obtain suspended particulate organic matter comprised of larger phytoplankton (POM>20). 1.5l triplicates of the remaining seawater was filtered through precombusted (450 °C, 4 h) Whatman GF/F glass-fiber filters (nominal pore size = 0.7 μm) to obtain suspended particulate organic matter representing pico- and nanoplankton (POM<20). Both POM>20 and POM<20 filters were desiccated and designated for either $\delta^{13}C$ or $\delta^{15}N$ analysis. A 12h exposure to HCL (35%) fumes in a desiccator was performed on $\delta^{13}C$ filters in order to remove carbonates (Carabel & Godínez-Domínguez 2006). Next, both type of filters was dried and packed into 12 x 10 mm tin capsules.

At each sites, three samples of the first 2 cm of sediment were collected using a PVC cores (i.d. 47 mm). Sediment samples were passed through a 250 μm screen to remove the largest inorganic particles and then dried for 3 days at 60 °C. Sediment subsamples for isotopic carbon analyses were acidified with 4% HCl to remove inorganic carbon, washed in deionised water then centrifuged (3400 revolutions per minute, for 5 minutes) and the supernatant with acid residues carefully removed by pipette. Subsamples for isotopic nitrogen analyses were not acid-treated because this would affect the $\delta^{15}N$ values (Ryba & Burgess 2002). Both type of subsamples was dried and then homogenised to a thin powder using a ball and mill grinder and weighed into tin capsules.

From sediment samples collected at each site, seagrass detritus samples were extracted by density centrifugation using a sediment: Ludox HS-40% proportion of 1:2 and recovering the overlying detritus with a Pasteur pipette, a method slightly modified from Blanchard et al. (1988). Seagrass detritus were rinsed with deionised water, dried at 60 °C for 24 h, then ground using a ball and mill and weighed into tin capsules ready for stable isotope analysis.

2.2.3 Stable isotope analysis

All samples were analysed at UC Davis Stable Isotope facility. Suspension feeders and seagrass samples were analysed for $\delta^{15}N$ and $\delta^{13}C$ using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

POM filters and sediment samples were analysed using an Elementar Vario EL Cube or Micro Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Stable isotopic data are expressed as the relative per mil (‰) differences between the samples and the conventional standards, Pee Dee Belemnite (PDB) for carbon and atmospheric N_2 for nitrogen, according to the following equation:

$$\delta(‰) = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where R is ^{13}C / ^{12}C for carbon and ^{15}N / ^{14}N for nitrogen. Sample precision was 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$. The internal standard was the USGS 41 of the International Atomic Energy

Agency (δ^{13}C = -37.626; δ^{15}N = 47.6). The typical precision in analyses was ±0.09‰ for carbon and ±0.17‰ for nitrogen.

2.2.4 Data analysis

All statistical analyses were conducted using the permutational analysis of variance (PERMANOVA+) package in PRIMER v6 (Plymouth Routines in Multivariate Research), based on Euclidian distances.

To confirm the presence of a spatial patterns of sedimentation gradient and estimate its magnitude, variations in sedimentation rates and organic matter deposition rates was compared between sites defined by their orientation and distance from the wash plant. A two-way PERMANOVA was performed with both distance and orientation considered as fixed factor.

To test the influence of such patterns on the potential food sources for suspension feeders, annual mean δ^{15}N and δ^{13}C of each OMS were compared among sites, depending on distance and orientation from the wash plant. A three-way PERMANOVA was conducted for the factors food sources, distance and orientations, all considered as fixed factors. A pairwise comparison was also realized in order to identify differences in δ^{15}N and δ^{13}C between each potential food sources.

The influence of sediment exposure was also investigated on suspension feeder isotopic signatures. The differences in δ^{15}N and δ^{13}C between the three species was compared over the two monitoring periods (2013 and 2014) and among sites, depending on distance and orientation from the wash plant. PERMANOVA was performed using distance, orientation, time and species as fixed factor and pairwise comparisons were made to identify differences in suspension feeder isotopic signature within and between sites.

OMS contributions (POM, sedimentary organic matter and seagrass detritus) to each suspension feeders according to their distance from the wash plant was estimated by using the mixing model developed in the R package SIAR (Parnell et al. 2010). Despite the strong recommendation for using accurate estimation of trophic enrichment in between food sources and consumers, very few values are available regarding marine suspension feeders (Lefebvre et al. 2009). Therefore, the same fractionation values were used for all species, with a trophic enrichment of 1‰ (±0.15‰) for δ^{13}C and 3‰ (±0.25‰) for δ^{15}N. Those values were selected averaging values from McCutchan et al. (2003): 0.4 ± 0.1‰ for δ13C and 2.3 ±0.3‰ for δ^{15}N, and those of Dubois et al. (2007): 1.9 ± 0.2‰ for δ^{13}C and 3.8 ± 0.2‰ for δ15N. Results from mixing model included Credibility intervals (CI) of 0.95, 0.75 and 0.25 but only the lowest and highest limits of 0.95 CI were used to illustrate the range of contribution values for each OMS.

2.3 RESULTS

2.3.1 Sedimentation and organic matter deposition rates

Sedimentation and organic matter deposition rates decreased with distance from the discharge point toward reference sites (Fig. 2). Those rates did not tend to vary significantly between east and northwest orientations (p=0.279 and 0.182; Table 1), but they were significantly different between distances (p = 0.0001). Sedimentation rates in the vicinity of the wash plant were twice the amount recorded 500 m away and almost 8 times higher than at reference sites. Organic matter deposition rates near the wash plant were approximatively 1.5 times more elevated than those at 500 m away and more than 3 times higher than at reference sites. Sedimentation rates and organic matter deposition were highly correlated (R^2 = 0.74; p<0.0001).

Figure 2: Data average for gross sediment (a) and organic matter (b) deposition rates (g(DW)·cm^{-2}·month^{-1}; ± standard error) from sediment traps collected monthly at each site from July 2013 to August 2014 (at the exception of November 2013, February and May 2014).

Table 1: PERMANOVA, testing for differences in gross sedimentation and organic matter deposition rates for each distance (200, 500 and >1000m; fixed factor) and orientation: Orientation (East, North-West).

Source	df	Sedimentation rates			Organic matter		
		MS	P	permutations	MS	P	permutations
Distance	2	15.315	0.0001	9950	13.842	0.0001	9936
Orientation	1	0.977	0.279	9838	1.621	0.182	9808
DixOr	2	1.418	0.179	9944	0.708	0.464	9953
Res	167	0.831			0.921		
Total	172						

2.3.2 Potential food sources

Mean $\delta^{13}C$ ranged from -22.3‰ to -21.1‰ for POM<20, from -21.5‰ to -20.7‰ for POM>20, from -18.9‰ to -18.3‰ for sedimentary organic matter and from -10.3‰ to -9.9‰ for seagrass detritus (Fig. 3). Mean $\delta^{15}N$ ranged from 5.4‰ to 6.3‰ for POM<20, from 4.7‰ to 5.3‰ for POM>20, from 5.2‰ to 5.8‰ for sedimentary organic matter and from 4.7‰ to 5.06‰ for seagrass detritus (Fig. 3). No clear spatial pattern was observed for either $\delta^{15}N$ or $\delta^{13}C$ of each OMS (Table 2&3, Fig. 3). However, both $\delta^{13}C$ and $\delta^{15}N$ were significantly different between each OMS (p = 0.0001; Table 2&3). Because $\delta^{13}C$ and $\delta^{15}N$ of POM>20 and POM<20 overlapped, they could be pooled (hereafter referred to as POMtot).

96% of the total variation in $\delta^{13}C$ was attributed to the differences between food sources, but only 15% for $\delta^{15}N$. $\delta^{13}C$ of sedimentary organic matter, seagrass detritus and POMtot were significantly different (p = 0.0001). $\delta^{13}C$ of POMtot was lower than sediment which was lower than seagrass detritus.

2.3.3 Suspension feeders

$\delta^{13}C$ of the suspension feeders ranged from -21.2‰ to -20.4‰ for *Herdmania momus*, from -19.7‰ to -19.1‰ for *Pinna bicolor* and from -21.4‰ to -18.6‰ for *Tethya* sp (Fig. 3). $\delta^{13}C$ and $\delta^{15}N$ were similar between the two periods (p > 0.05; Table 4&5). $\delta^{13}C$ varied significantly among species (p = 0.0001) and with distance from the wash plant (p=0.0001), with a significant interaction between species and distance (p=0.001; Table 4). $\delta^{13}C$ of ascidians and bivalves remained were not significantly different between sites (p > 0.05), while $\delta^{13}C$ of *Tethya sp* (Fig.3; Table 6) tended to decrease from the wash plant (200 m) toward reference sites (>1000 m). $\delta^{13}C$ of *Tethya* was correlated to sedimentation rates ($R^2 = 0.55$; p = 0.0001) and the amount of organic deposited on the seabed ($R^2 = 0.46$, p = 0.0002).

$\delta^{15}N$ ranged from 8.2 to 9.0‰ to for *Herdmania momus*, from 7.1 to 8.1‰ for *Pinna bicolor* and from 8.2‰ to 8.8‰ for *Tethya* sp (Fig. 3). $\delta^{15}N$ varied significantly among suspension feeder species (p=0.0001), with a significant interaction between species and distance from the wash plant (p=0.005; Table 5). Because $\delta^{15}N$ was not significantly different between each distance (Table 6), no clear spatial pattern could be identified.

Figure 3a: Dual isotopic plots of mean $\delta^{13}C$ and $\delta^{15}N$ (± standard deviation) for co-occurring suspension feeders (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) in 2013 and 2014 and annual mean $\delta^{13}C$ and $\delta^{15}N$ for potential food sources. Values recorded at sites 200m away from the discharge point.

Figure 3b: Dual isotopic plots of mean $\delta^{13}C$ and $\delta^{15}N$ (± standard deviation) for co-occurring suspension feeders (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) in 2013 and 2014 and annual mean $\delta^{13}C$ and $\delta^{15}N$ for potential food sources. Values recorded at sites 500m away from the discharge point.

Figure 3c: Dual isotopic plots of mean δ¹³C and δ¹⁵N (± standard deviation) for co-occurring suspension feeders (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) in 2013 and 2014 and annual mean δ¹³C and δ¹⁵N for potential food sources. Values recorded at sites >1000m away from the discharge point (reference sites).

Table 2: Results of three-way PERMANOVA testing for differences in $\delta^{13}C$ between the four potential food sources (POM >20µm, POM<20µm, SOM and seagrass detritus) among distance and orientation from the wash plant, including estimated variance component (and % variance). P values in bold and red indicate significance.

Source	df	MS	Estimate Variance	(%)	P	Unique permutations
Food sources	3	56.682	1.180	96.02	**0.0001**	9942
Distance	2	0.158	0.001	0.11	0.057	9956
Orientation	1	0.006	-0.001	-0.03	0.745	9839
FoxDi	8	0.017	-0.002	-0.19	0.959	9935
FoxOr	4	0.035	-0.001	-0.06	0.626	9949
DixOr	2	0.005	-0.001	-0.10	0.912	9952
FoxDixOr	8	0.041	-0.002	-0.13	0.640	9933
Res	210	0.054	0.054	4.39		
Total	239					

Table 3: Results of three-way PERMANOVA testing for differences in $\delta^{15}N$ between the four potential food sources (POM >20µm, POM<20µm, SOM and seagrass detritus) among distance and orientation from the wash plant, including estimated variance component (and % variance). P values in bold indicate significance.

Source	df	MS	Estimate Variance	(%)	P	Unique permutations
Food sources	3	8.025	0.149	15.04	**0.0001**	9941
Distance	2	1.447	0.007	0.71	0.200	9943
Orientation	1	0.794	-0.001	-0.08	0.349	9840
FoxDi	8	1.342	0.028	2.87	0.151	9927
FoxOr	4	0.110	-0.032	-3.28	0.976	9953
DixOr	2	0.272	-0.015	-1.56	0.732	9931
FoxDixOr	8	0.607	-0.035	-3.56	0.704	9928
Res	210	0.889	0.889	89.88		
Total	239					

Table 4: Results of four-way PERMANOVA testing for differences in $\delta^{13}C$ between the three suspension feeders (*Herdmania momus, Pinna bicolor* and *Tethya* sp) among distance and orientation from the wash plant, including estimated variance component (and % variance). P values in bold indicate significance.

Source	df	MS	Estimate Variance	(%)	P	Unique permutations
Year	1	1.992	0.019	1.621	0.072	9830
Species	2	29.127	0.570	48.700	**0.0001**	9951
Distance	2	1.251	0.013	1.068	0.138	9952
Orientation	1	0.658	0.000	0.039	0.313	9832
YexSp	2	1.500	0.035	2.988	0.095	9948
YexDi	2	0.670	0.002	0.153	0.349	9953
YexOr	1	0.817	0.005	0.454	0.263	9847
SpxDi	4	2.275	0.094	8.052	**0.0057**	9937
SpxOr	2	1.323	0.028	2.383	0.124	9948
DixOr	2	0.008	-0.025	-2.111	0.988	9950
YexSpxDi	3	0.491	-0.013	-1.151	0.499	9953
YexSpxOr	2	0.024	-0.048	-4.115	0.964	9954
YexDixOr	2	0.185	-0.035	-3.010	0.742	9953
SpxDixOr	4	0.126	-0.057	-4.873	0.942	9959
YexSpxDixOr	3	0.412	-0.043	-3.641	0.574	9940
Res	136	0.626	0.626	53.443		
Total	169					

Table 5: Results of four-way PERMANOVA testing for differences in $\delta^{15}N$ between the three suspension feeders (*Herdmania momus, Pinna bicolor and Tethya sp*) among distance and orientation from the wash plant, including estimated variance component (and % variance). P values in bold indicate significance.

Source	df	MS	Estimate Variance	(%)	P	Unique permutations
Year	1	0.373	0.001	0.067	0.268	9832
Species	2	29.247	0.579	38.068	**0.0001**	9946
Distance	2	11.072	0.215	14.166	**0.0001**	9944
Orientation	1	0.001	-0.004	-0.273	0.955	9834
YexSp	2	0.379	0.003	0.210	0.288	9954
YexDi	2	0.300	0.000	0.002	0.366	9945
YexOr	1	1.430	0.031	2.065	0.290	9829
SpxDi	4	8.425	0.464	30.531	**0.0001**	9943
SpxOr	2	0.037	-0.011	-0.691	0.894	9951
DixOr	2	0.058	-0.010	-0.634	0.825	9930
YexSpxDi	3	0.244	-0.006	-0.362	0.484	9956
YexSpxOr	2	0.212	-0.007	-0.460	0.487	9954
YexDixOr	2	0.457	0.013	0.827	0.223	9962
SpxDixOr	4	0.138	-0.018	-1.212	0.763	9960
YexSpxDixOr	3	0.148	-0.030	-1.992	0.683	9946
Res	136	0.299	0.299	19.688		
Total	169					

Table 6: Summary of results of pairwise (PERMANOVA) comparisons for mean δ13C and δ15N between each suspension feeders (ascidian (A), bivalve (B) and sponge (S)) and between each distance from the wash plant. P values in bold indicate significance.

Groups	$\delta^{13}C$			$\delta^{15}N$		
	200	**500**	**>1000**	**200**	**500**	**>1000**
Ascidian, Sponge	**0.0001**	**0.0002**	**0.0025**	0.0642	**0.0304**	0.7048
Ascidian, Bivalve	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0012**	**0.0001**
Sponge, Bivalve	**0.0004**	**0.0001**	**0.0001**	**0.0004**	**0.0001**	**0.0001**
	A	**B**	**S**	**A**	**B**	**S**
200m, 500m	0.2786	0.2933	**0.0001**	**0.002**	0.4838	0.6122
200m, >1000m	0.6001	0.6443	**0.0001**	0.7391	**0.0008**	0.4716
500m, >1000m	0.5957	0.0694	**0.0001**	0.1457	**0.0028**	0.387

2.3.4 Contributions of food sources to suspension feeders

The contribution of POM (Fig. 4) dominated the diet of the ascidian *Herdmania momus* at each site, as the mixing model gave higher upper and lower limits of 0.95 CI from 88.3 to 100%. Likely, sedimentary organic matter and seagrass detritus contributions were extremely low, with respective 0.95 CI values ranging from 0.0 to 1.6% and from 0.0 to 0.2%. The range of food sources contributions to the diet of the bivalve *Pinna bicolor* were similar at all sites. However, the large range in proportion contribution between the 5th and 95th percentiles indicated uncertainty for individual food sources contribution. POM had the greatest potential contribution with 0.95 CI ranged from 50.7 to 93.4%. Sedimentary organic matter appeared to be the second contributor with 0.95 CI values ranging from 0.5 to 48.9% and seagrass detritus potential contribution was negligible (0.95 CI from 0 to 7.5%). Despite potential uncertainties, the diet of the sponge *Tethya* sp was characterised by variation in the potential contribution of each food source among distance from the wash plant. At sites located 200 m away from the discharge point the likely contribution of POM and sedimentary organic matter were of the same order of magnitude with 0.95 CI ranged respectively from 41.5 to 73.4% and from 18.7 to 58.1%. At sites 500m away from the wash plant POM contribution was higher (0.95 CI from 76.1 to 96.1%) and reduced for the sedimentary organic matter (0.95 CI from 0.9 to 22.9%). A similar trend was observed at reference sites in 2013 (sponges were absent from the meadows in 2014) with 0.95 CI ranged from 55.7 to 100% for POM and from 0.0 to 40.9% for the sediment. At all sites the potential contribution of seagrass detritus to the sponge diet was almost inexistent (0.95 CI from 0.0 to 8.7%).

Figure 4: Comparison of contributions POM (particulate organic matter), sediment (sediment organic matter (SOM)) and seagrass detrital matter to suspension feeder (*Herdmania momus*, *Pinna bicolor* and *Tethya*) isotopic signatures from the mixing model SIAR. 0.95, 0.75, 0.25 credibility intervals are in dark grey, light grey and white, respectively.

2.4 DISCUSSION

2.4.1 Organic matter sources

Four potential food sources were identified in this study: POM>20µm mainly represented by large phytoplankton, POM<20µm which comprised ultraplankton (bacteria, pico and nanoplankton), organic matter in resuspended sediment and seagrass detritus. $\delta^{13}C$ and $\delta^{15}N$ of the different organic matter sources were similar to values recorded for seagrass by Smit et al. (2006) and Hyndes et al. (2013), for sedimentary organic matter by Hyndes and Hanson (2009) and pelagic POM by Hanson et al. (2005) in south-western Australia. An important criterion for using stable isotopes at natural abundance levels as tracers is that potential food sources have isotopes signatures which are distinct from each other (Smit et al. 2005). Although several studies revealed isotopic variations across suspended POM size classes (Rolff 2000; Sato et al. 2006; Kang et al. 2009), for this study, the mean $\delta^{15}N$ and $\delta^{13}C$ of POM>20 and POM<20 were too similar to establish the contribution of each size fraction to the suspension feeder's diets. Isotope ratios for POM_{tot} which is comprised of the two size fractions, were significantly lower than those measured in resuspended sediment and seagrass detritus. While macroalgae detritus and benthic microalgae can be an important component of the suspended particulate organic matter pool (POM) present in in the region (Hyndes & Lavery 2005; Smit et al. 2005), this two organic matter sources appeared to be absent from the study area. Although Lenanton (1982) described possible transient accumulation of wrack in the vicinity of the study area, macroalgae detritus could not be find in the seagrass meadows during the monitoring period. Furthermore, examination by light microscopy on the supernatant liquid extracted by density centrifugation following the method of Blanchard et al. (1988) showed that benthic microalgae were absent from the sediment samples collected at each site. Detrital seagrass leaves appeared to be the only source of detritus present in the study area. However, isotope ratios for seagrass detritus were significantly distinct from those for POM suggesting that detrital leaves did not contribute to the suspended particulate organic matter pool. $\delta^{13}C$ for POM suggests that it largely originates from phytoplanktonic microalgae, which have $\delta^{13}C$ values between -23 and -21‰ (Moncreiff & Sullivan 2001). Values found in this study were similar to that obtained from a study on a low nutrient *Posidonia oceanica* meadow near Corsica where POM <50 µm had a value of -22.5 ± 0.8‰ (Lepoint et al. 2000). $\delta^{13}C$ of the sedimentary organic matter was significantly lower that of seagrass detritus, but slightly higher than that that of POM. This organic matter was therefore likely originated from the organic matter in the water column rather than a benthic input. This finding contrast with other studies on nearshore seagrass ecosystem in Western Australia, which suggested that sedimentary organic matter largely originated from detrital seagrass leaves, while POM did not accumulate in the sediment (Smit et al. 2005; Smit et al. 2006; Hyndes & Lavery 2005). However, these studies never attempted to determine the contribution of the POM fraction less than 20 µm, which represents more than 85% of phytoplankton biomass (Hawkes 2006).

Moreover, these studies only considered time specific values rather than annual means for POM, excluding the important influence of seasonal changes in the isotopic ratios and abundance of the phytoplanctonic compartment. Despite other studies show that sedimentary organic matter usually reflects the major macrophyte present (Adin & hytola 2003), the results of this study suggested that the contribution of seagrass detritus was unlikely. This finding could be explained by the relative rapid decomposition of seagrass leaves (Anesio et al. 2003; Holmer & Olsen 2002; Loneragan et al. 1997) and bacterial activity enhancing the dissolution of particulate seagrass, which become available for uptake by bacteria in the water column (Anesio et al. 2003) and benthic primary producers (Holmer & Olsen 2002). Instead of a benthic origin, isotope ratios for sedimentary organic matter appeared to derive from organic matter in the water column. However, Smit et al. (2006) recorded low sedimentary organic matter content in seagrass beds in the vicinity of the study area, which indicated a relatively low accumulation of POM in the sediment. Since the sediment discharged from the wash plant was associated with an increase of organic matter deposition, this additional source of organic matter could potentially replenish the sediment organic matter pool within the study area. The origin and nature of the sediment dredged offshore might explained the lower δ13C and δ15N observed. Further isotopic analysis on the sediment exploited by the wash plant could help to determine the exact nature of the sedimentary organic matter.

2.4.2 Natural diets of the suspension feeders

$\delta^{13}C$ and $\delta^{15}N$ of the three suspension feeders, and inferences about likely food sources were consistent with those estimated for the ascidian *Herdmania momus* by Hyndes et al. (2013), for the bivalve *Pinna bicolor* by Smit et al. (2005) and *Tethya* sp by Simister and Taylor (2013). Because suspension feeding activity rely on the capture of particles in suspension in the water column (J. M. Gili & Coma 1998), unsurprisingly the results of this study indicated that POM was likely the main contributor to suspension feeder diets among the six sites. According to $\delta^{13}C$ for POM, it appeared that the diets of the 3 species depend predominantly on phytoplankton, which best represented the POM fraction. Similar results were obtained in the coastal ecosystem of Normandy (France), where the contribution of phytoplankton remained the most important for all co-occurring suspension feeders, except *Sabellaria alveolata* (Lefebvre et al. 2009). In marine systems, phytoplankton represents the principal food source for suspension-feeders and constitutes the first biological level in the carbon and nitrogen cycles (Cloern et al. 2002). Coastal waters of south-western Australia are characterised by oligotrophic conditions (Koslow et al. 2008) and pico and nanoplankton (heterotrophic and autotrophic cells 0.2 – 20 μm in size) that are able to utilize low levels of nutrients represents more than 85% of phytoplankton biomass (Hawkes 2006). Therefore, it is likely that the 3 species mostly rely on pico and nanoplankton for their diets. *Pinna bicolor* and *Tethya* sp were also able to exploit sedimentary organic matter, albeit to a varying degree, but there was no clear evidence of an exploitation of detrital material from the seagrass by any of the three species. Several studies carried out in the same region concluded that detached seagrasses were

unlikely to be assimilated by suspension feeders (Smit et al. 2006; Smit et al. 2005; Hyndes & Lavery 2005). This lack of contribution to the suspension feeder diets might be explained by the relatively poor nutritional value and high lignocellulose content of seagrasses (Klumpp et al. 1992; Thayer et al. 1984).

Despite occupying a relatively similar trophic position, the isotopic signatures of the three suspension-feeding species were significantly distinct (Fig. 3, Table 7). Survival of multispecific assemblages of benthic suspension feeders depend on the faculty for each specie to maximize their food particles capture efficiency, which involve the partitioning of available food resources to limit competition on similar prey items (Gili & Coma 1998). Sharing a same habitat, the variety of feeding structures and strategies amongst the different suspension feeders was expected to lead to dissimilarities in their diets in order to partition the food resource (Kang et al. 2009; Lefebvre et al. 2009; Pile 2005). For example, Pile (2005) observed that co-occurring species of ascidians and sponges in temperate ecosystems retained the same particles but utilized different portions of the ultraplankton community (pico and nanoplankton). Similar observation were made in the study Kang et al. (2009), which examine the trophic relationship between the ascidian *Halocynthia roretzi* and the Pacific oyster *Crassostrea gigas*. While sharing the same habitat and capable to capture the same food particles, these two co-cultured suspension feeders exploited different POM size fractions. Isotope ratios highlighted the importance of pico-/nano-size fractions as their dietary components for the ascidians. Therefore, differences in $\delta^{13}C$ and $\delta^{15}N$ between the three suspension feeders might be explained by a consumption of different type of picoplanktonic particles and also the degree to which sedimentary organic matter contributed to their diets. The distinctive food particle capture mechanisms and feeding strategies employed by the 3 suspension feeders strengthen the hypothesis of different patterns in food resource exploitation.

2.4.3 Influence of sediment exposure on suspension feeder diets

Herdmania momus diet is likely to rely exclusively on POM and more specifically on pico and nanoplankton. This finding corroborates those of various studies defining ascidians as active suspension feeders that graze primarily on the ultraplankton (pico and nano size) of the plankton community (Lesser & Slattery 2015; Lohrer et al. 2006; Ribes & Coma 2005; Randløv & Riisgård 1979; Fiala-Médioni 1979). Indeed, the mucus net of ascidians deploys filter nets arranged in rectangular meshes of very fine filaments on the inner surface of the branchial basket (Petersen 2007), which can retain particle as small as bacteria (Bak et al. 1998; Randløv & Riisgård 1979; Jorgensen 1975). Because *Herdmania momus* presented relatively consistent isotopic signature at each site, increased sediment exposure from the wash plant appeared not having any particular influence on *Herdmania momus* diet. In intertidal ecosystems on the French coast, Dubois et al. (2007) found that regardless of sampling site or composition of available food resource, isotopic signatures of the analogous ascidian *Ascidiella aspersa* remained similar. The limited intraspecific

variability of ascidian signatures suggested that *Ascidiella aspersa* tend to be more trophic specialist than other suspension feeding such as bivalves. This lack of trophic plasticity might explain the consistent isotopic signature exhibited by *Herdmania momus* despite experiencing different sediment exposure levels. Furthermore, ascidians might be able to compensate for the adverse effects of high sediment exposure by altering siphon-opening diameter, squirting frequency, structure and transport of mucus, and potential particle selection (Petersen 2007; Armsworthy et al. 2001;). Although ascidians do not produce pseudofaeces (Klumpp 1984; Robbins 1983; Fiala-Médioni 1974), the constriction of the inhalant siphon accompanied by squirting play a major role to reject unwanted material when experiencing elevated sediment exposure (Petersen et al. 1999). Additionally, squirting serves to alter the retentive capabilities of the mucus sheet, reduce the distance of mucus transport, and reduce the probability of clogging (Armsworthy et al. 2001; Petersen & Riisgard 1992). Because ascidians cannot discriminate inorganic and organic particles they reject (Kang et al. 2009; Petersen 2007), these compensatory mechanisms could explain why sedimentary organic matter do not contribute to *Herdmania momus* diet.

If the results from the mixing model indicated that *Pinna bicolor* is predominantly feeding on POM, the contribution of sedimentary organic matter to its diet is also relatively important. The large contribution of POM has been previously established for the diet of the Mediterranean bivalve *Pinna nobilis* found in *Posidonia oceanica* meadows (Kennedy & Richardson 2001) and also for the diet of pinnid bivalve *Atrina zelandica* distributed around the coast of north-eastern New Zealand (Hewitt & Pilditch 2004). Numerous studies have reported that phytoplankton constitutes the greatest part of benthic suspension feeding bivalves (Lefebvre et al. 2009; Safi et al. 2007; Dupuy et al. 1999; Shumway & Selvin 1987). Suspension feeding bivalves have the ability to change their feeding depending on food availabilities (Leal et al. 2008; Ribes et al. 2003; Ropert & Goulletquer 2000; Kamermans & Huitema 1994). Because pico and nanoplankton dominates pelagic planktonic community in south-western Australia (Koslow et al. 2008), this fraction of the POM is likely to represent an important food resource for *Pinna bicolor*. Although many bivalves are often not efficient to capture small phytoplankton (<5 μm) (Sobral & Widdows 2000; Dupuy et al. 1999; Ward et al. 1998), some species are capable to exploit small food particles such as picoplankton and bacteria (Kreeger & Newell 2001). For example, Safi et al. (2007) reported that the pinnid bivalve *Atrina zelandica was able to access* bacteria and picoplankton. In addition to phytoplankton, sedimentary organic matter represented an alternative food resource for *Pinna bicolor*. The cirri trapping mechanisms gives bivalves the ability to sort and select particles before ingestion. Therefore, this preingestive selection to regulate the ingestion of particles by production of pseudofaeces can also discriminate organic particles embedded in inorganic sediment particles (Cognie et al. 2001; Navarro et al. 1997; Barillé et al. 1997; Smaal & Prins 1993). Such a qualitative

selection capability allows Pinna bicolor to exploit organic matter from the sediment. In the study area, sedimentary organic matter available to suspension feeders was not only associated with sediment resuspension but also the discharge activities from the wash plant. Episodic organic matter enrichment associated with increased sediment exposure could potentially enhance the amount of food available for bivalves. As for *Herdmania momus*, the bivalve *Pinna bicolor* presented relatively consistent isotopic signature at each site independently of sediment exposure levels. *Pinna bicolor* morphology and physiology is defined by a "mussel" form characterised by an open pallial cavity with the inhalant and exhalent chambers completely separated by eulamellibranch gills (Butler et al. 1993). Due to its open mantle cavity, *Pinna bicolor* is more vulnerable to sediment deposition. Despite this disadvantage, this species is able to compensate for increased sediment exposure by its rapid growth above the sediment and adaptive mechanisms (Yonge & Thompson 1976) that allow active rejection of overload in inorganic particles and the cleaning both chambers of the mantle cavity (Kach & Ward, 2008). Such compensatory mechanisms could explain the absence of influence of sediment exposure levels on *Pinna bicolor*'s diet observed in the mixing models. An alternative explanation to the consistent isotopic signature exhibited by *Pinna bicolor* despite experiencing different sediment exposure might result from a reduced trophic plasticity. This hypothesis is consistent with the work of Dubois et al. (2007) that compared the trophic plasticity of oysters with mussels sharing the same physiological characteristics than *Pinna bicolor*. In this study, oysters exhibited larger spatial variations in isotopic signatures than did mussels. Despite feeding on the same mixture, oysters were capable of a greater trophic plasticity than did mussels, which conserved a relatively similar diet independently of sampling sites.

Picoplankton, which best represented the POM fraction is likely to be a major contributor to *Tethya* sp diet. Many recent studies have confirmed the dominance of picoplankton (heterotrophic and autotrophic cells 0.2–2 µm size) to sponge diets (Hanson et al. 2009; Pile & Young 2006; Ribes & Coma 2005). Contrary to observations made for *Herdmania momus* and *Pinna bicolor*, contributions of OMS to *Tethya* sp diets were characterised by variation among distance from the wash plant. For the sponges experiencing the highest level of sediment exposure (200 m), the likely contribution of sedimentary organic matter to their diet was significantly more important. Differences for the exploitation of the food resources among sites located at different distance from the discharge point suggested a potential influence of increased sediment to sponge nutrition. If demosponges like *Tethya* may cope with high sediment exposure by developing periodic contraction of the exhalant system that help to effectively expel out debris (Ellwanger et al. 2007; Elliott & Leys 2007; Nickel & Brümmer 2004), sponges are not able to select food particles (Topçu et al. 2010; Pile & Young 2006; Duckworth & Brück 2006). Consequently, sponges found at sites near the wash plant likely captured higher concentrations of suspended sediment which potentially provided an additional source of organic matter. $\delta^{13}C$ of the sponge, reflecting origins of assimilated

carbon (Peterson & Fry 1987), were correlated to sedimentation rates ($R^2 = 0.55$; p = 0.0001) and the amount of organic deposited on the seabed ($R^2 = 0.46$, p = 0.0002). The relationship between $\delta^{13}C$ of *Tethya* sp and the sediment discharged from the wash plant reinforced the idea of a potential food enhancement induced by the anthropogenic activity.

2.5 CONCLUSION

Although POM was likely to be the main contributor to suspension feeder diets, the co-occurring suspension feeders presented distinct isotopic signatures, which suggested dissimilarities in their diets. Because POM is mostly comprised of small heterotrophic and autotrophic cells (0.2 – 2 μm in size; Hawkes 2006), it seems plausible that the three suspension feeders consume different types of picoplanktonic particles. Also, the variation in likely contributions of sedimentary organic matter participated to the contrasts observed between the 3 suspension feeder diets.

Analyses of the C:N ratios did not provide any particular valuable insight regarding either the quality of the food or the physiological state of the organisms. The gradient of sediment exposure had no influence on those ratios and on the isotopic signatures of *Herdmania momus* and *Pinna bicolor*. Presumably, the relatively low trophic plasticity associated with their specific feeding mechanisms and strategies allowed the conservation of relatively consistent diets independently of changes in their environment. These findings corroborate the hypothesis formulated in chapter 1 regarding possible physiological and behavioural mechanisms explaining the presence of suspension feeders at sites with high sediment exposure. Conversely, spatial variations of the isotopic signatures of the sponge *Tethya sp* indicated changes in the food resource exploitation related to sediment exposure levels. Episodic organic matter inputs associated with sediment loading potentially provided an additional source of food to the sponge. The findings from this study consolidate the hypothesis mentioned in chapter 1 about the possibility for some suspension feeder species to benefit from a diet supplemented with sediment. This hypothesis could explain the total absence of *Tethya* sp from reference sites in 2014 (cf. chapter 1).

Overall, the influence of sediment exposure on suspension feeder diets appeared to be to be species specific and dependent on the nature of the sediment. Feeding ecology of the three suspension-feeders partly explained why a system experiencing increased sediment exposure could support an unexpectedly large numbers of suspension-feeders (cf. chapter 1). Consequently, information gained from this study could be improved by further studies focusing on the different fraction of the picoplankton exploited by suspension feeders and the influence of different type of sediment (e.g. organically rich versus organically depleted) and range of concentrations.

CHAPTER 3
Response of suspension-feeding activity and selectivity to increased sediment exposure

PREAMBLE

From the previous chapter, POM, principally represented by the picoplankton, was likely to be the main contributor to suspension feeder diets. However, the co-occurring suspension feeders presented distinct isotopic signatures, which suggested that the three species consume different types of picoplanktonic particles. Therefore, this chapter describe the relationship between the variety of feeding mechanisms of co-occurring suspension feeders and the selective uptake of picoplankton cells. In Chapter 1, suspension-feeder assemblages showed no strong patterns in composition related to the gradient in sediment exposure. One possible reason is that the species present are resistant to high sediment exposure. The work presented in this chapter examined, whether suspension-feeding activity and the plasticity of feeding mechanisms help them to cope with high sediment exposure.

ABSTRACT

Despite the key role suspension feeders have on coastal ecosystem functioning, there are relatively few studies on their feeding biology, particularly the ability and efficiency of suspension-feeder assemblages to selectively retain different types or size classes of particles. Nor is there much information on how sediment exposure affects their feeding biology. The purpose of this study was to describe the relationship between the variety of feeding mechanisms of co-occurring suspension feeders and the different types of picoplanktonic food particles that the assemblage utilised, and to investigate the influence of increased sediment exposure on their feeding rates and food resource partitioning. Study sites were located within a seagrass meadow affected by sediment plumes induced by a local cement manufacturing company near Woodman Point, south of Fremantle, Western Australia. Pumping rates and retention efficiencies on picoplankton were estimated for three species of suspension feeders that best characterise the benthic assemblages, using the InEx method used by Yahel et al. (2005). Overall, this study demonstrated that increased sediment exposure had an influence on the feeding activity of all three species. At high levels of sediment exposure, the ascidian *Herdmania momus* decreased pumping rates, thereby maintaining a constant retention rate, and optimised its food intake by expanding its food selection to include a wider range of food sources. *Pinna bicolor* had lower filtration activity under elevated sediment exposure, again, maintaining relatively constant total retention rates and displaying a change in particle selection from bacteria to larger cells of better nutritive quality. The sponge *Tethya* appeared to benefit from elevated sediment concentrations, as filtration and retention rates increased, potentially related to the lack of food selectivity in this demosponge. Even though the sediment exposure in the study area did not appear to cause mortality, it influenced feeding activity depending on feeding mechanisms and ability to cope with sediment exposure. Changes in particle selection and ingestion due to sediment exposure could potentially have a negative influence on food resource partitioning within suspension-feeder assemblages and limit the food available to other benthic organisms.

3.1 INTRODUCTION

Suspension feeders comprise a large proportion of the biomass and abundance of the benthic fauna of coastal marine ecosystems (Lemmens et al. 1996; Ricciardi & Bourget 1999; Newell et al. 1982) and are represented in almost all marine animal classes (Riisgård & Larsen 2010). Because of their biomass, abundance and ability to filter large amounts of water (up to 100 $m^3 \cdot d^{-1} \cdot m^{-2}$; Jørgensen 1996), suspension feeders can exert a major influence on the ecosystems they inhabit (Cooper et al. 2011; Dame et al. 2001). Suspension feeders have the potential to filter the total volume of water bodies within days (e.g. Lake Tuakitoto, New Zealand; Bay of Brest, France; South San Francisco Bay, USA) to weeks (e.g. Chesapeake Bay, USA). Consequently, they can control phytoplankton biomass (Dame et al. 2001; Hily 1991; Cloern 1982; Newell et al. 1982; Frost 1978) and may play a key role in shallow coastal ecosystems, by reducing seston concentrations (Cooper et al. 2011; Dame et al. 2001) and modifying the availability of organic matter and nutrients (Riisgård & Larsen 2010; Ostroumov 2005; Pile 2005; Dame et al. 2001).

Although picoplankton (0.2-2 µm) and nanoplankton (2–20 µm) are often considered to be the main contributors to suspension feeder diets (Lesser & Slattery 2015; Hanson et al. 2009; Pile 2005), potential food particles also including bacteria, protozoans, larger phytoplankton, zooplankton and detritus (Miller & Page 2012; Smit et al. 2005; Dupuy & Bel Hassen 1999).As their typical stochastic habitat is comprised of a wide spectrum of food particles, benthic sessile suspension feeders exhibit very diverse feeding behaviours (active, combined, passive) and particle capture mechanisms (Riisgård & Larsen 2010). Suspension feeding requires food items to be small enough to avoid the clogging of their feeding apparatus and large enough to be retained (Armsworthy et al. 2001). Whether a particle will be rejected or ingested varies between suspension feeders depending on their feeding ecology (Dubois et al. 2007). For example, Gili & Coma (1998) reported that particle retention efficiency varied between active feeding mode, in which suspension feeder actively creates a current to bring food particles through their feeding apparatus, and passive suspension feeding, which exclusively rely on ambient water movements. For instance, active suspension feeders are usually more effective at retaining smaller particles, while passive feeders preferentially ingest larger and more energy-rich particles. Morphological differences in feeding apparatus, such as mucus-net filter-feeding, cirri trapping, collar sieving or tube-feet passive suspension-feeding (Riisgård & Larsen 2010), also influence particle retention and selection. For example, Kang et al. (2009) found that the cirri trapping bivalve *Crassostrea gigas* and the mucus net ascidian *Halocynthia roretzi* within the same habitat feed on different size fractions of the phytoplankton despite. In the same manner, Pile (2005) noticed that sponges (using collar sieving capture mechanisms) and ascidians in an Australian temperate ecosystem retained the same particles but utilized different portions of the ultraplankton community. Due to species-specific physiological and behavioural differences, this partitioning of food resource potentially reduces

interspecific competition for food within multispecific assemblages (Lefebvre et al. 2009; Decottignies et al. 2007; Dubois et al. 2007; Riera et al. 2002). However, changes in environmental conditions may influence the pool of organic matter available for suspension feeders and suspension feeding processes. Despite their key role on coastal ecosystem functioning, there is a paucity of studies regarding the influence of environmental pressures on the trophic relationships among co-occurring suspension feeders.

Suspension feeding can be influenced by the proportion of suspended sediment in the water column (Safi et al. 2007; Newell et al. 2005; Kach & Ward 2001; Waite et al. 2000). Increases in sediment loads to coastal ecosystems can occur naturally (e.g. erosion, rainfall, and tidal currents) but its impact on benthic fauna is often enhanced when triggered by anthropogenic activities such as road building, logging, mining, aquaculture, port maintenance and dredging (Ellis et al. 2002). Previous investigations have shown that increased suspended sediment can negatively influence the suspension feeding activity of bivalves and ascidians (Ellis et al. 2002; Armsworthy et al. 2001). However, the degree to which increased suspended sediment influences suspension feeders is likely to be species specific and dependent on their feeding activity and resilience of feeding mechanisms. Because of the diversity of feeding strategies and mechanisms among suspension feeder species, dissimilarities in their uptake of picoplankton and food partitioning can be expected (Topçu et al. 2010; Hanson et al. 2007; Gili & Coma 1998). Hence, the purpose of this study was to determine the influence of increased sediment exposure on the selective uptake of food particles by co-occurring suspension feeders with distinct feeding mechanisms.

3.2 MATERIAL AND METHODS

3.2.1 Study site

The study was undertaken within *Posidonia sinuosa* meadows near Woodman Point (32°7'S, 115°44'E), south of Fremantle, Western Australia, which are an important habitat for the abundant benthic suspension feeders in this region (Paterson 2008; Lemmens et al. 1996). These coastal waters are oligotrophic with pelagic phytoplankton communities dominated by picoplankton and small ultraplankton (< 5µm) (Koslow et al. 2008), which represent the principal food resource for the suspension feeders (Hanson et al. 2009). This location was chosen as a continuous sediment plume induced by a cement manufacturer's wash plant is present, offering an opportunity to understand whether increased suspended sediment had an influence on the feeding rates of each species and for food resource partitioning. Suspension-feeder assemblages in these meadows have been characterised in terms of biomass and abundance (Chapter 1; Lemmens et al. 1996) and include species with distinct feeding mechanisms. Among the most conspicuous species present are *Pinna bicolor*, *Herdmania momus* and *Tethya* sp. The solitary ascidian *Herdmania momus* actively captures sestonic particles through a mucus net. The Pterioid bivalve *Pinna bicolor* operates a cirri trapping mechanism to actively gather food particles. The demosponge *Tethya* sp combines active and passive feeding using a collar sieving structure.

Six sampling sites were located within *Posidonia sinuosa* meadows located at Woodman Point (32°7'S, 115°44'E), south of Fremantle, Western Australia. The sites were located along a gradient of turbidity. As previously described in Chapter 1, the high turbidity observed in this area is induced by dredging, dumping, reclaiming, washing and vessel movements associated with the Cockburn Cement Limited (CCL) wash plant. Three of the sites were oriented eastward of the wash plant and three were oriented to the North-West (Fig. 1). From the data presented in Chapter 1, the sites can be classified as: heavily impacted by increased suspended sediment, the two sites located 200m from the wash plant jetty; moderately affected, the two sites 500m from the wash plant; reference sites, the two sites more than 1 km away from the jetty and subject to as little influence from the wash plant (Reference sites). The sites comprised depths ranging from 1.7 to 3.2m.

Figure 1: Study sites

3.2.2 Sampling strategy

Sampling was performed twice, first between 21st to 28th of September 2013 and then from 25th to 31st of August 2014, on five individuals of *Pinna bicolor*, *Herdmania momus* and *Tethya* sp, at each site (depth <4 m). At each site and for each species, specimens of similar size and weight (appendix C) were carefully selected to minimize the potential effect of age and size on suspension feeding activity. From this, biomass (flesh dry weight) of ascidians ranged from 15.1 to 25.8g in 2013 and 14.5 to 25.5g in 2014, biomass of bivalves ranged from 15.1 to 25.7g in 2013 and 16.2 to 20.9 g in 2014 and the sponge biomass ranged from 1.1 to 3.1g in 2013 and 1.2 to 2.6 g in 2014. Measurements of pumping rate and retention efficiency were conducted following the InEx method of Yahel et al. (2005), in which paired water samples from the water inhaled and exhaled by suspension feeders were simultaneously collected by two SCUBA divers. Samples of inhaled water (In) were collected using shortened polystyrene Sterilin © pipettes with the upper half of a microcentrifuge tube attached at one end and a syringe attached to the other end. Samples were slowly taken by a diver holding the tube inlet next to the animal's inhaling aperture. Simultaneously, exhaled water (Ex), was sampled using a pipette with microcentrifuge tube attached at both ends, by another diver holding inlet, from a second tube, within the exhalent jet and aligned with it, as close as possible (<2 mm) to the animal's exhaling aperture, but with no physical contact. Once sampling was accomplished, microcentrifuge tubes attached to the pipettes were simultaneously plugged. As far as possible, the study only concerned individuals of a similar size in order to limit the potential effect of age on feeding rates. The difference between the inhaled and the exhaled water samples (InEx pair) indicates uptake of picoplanktonic cell types (Yahel et al. 2005). Samples were kept cold and dark in an icebox until brought to shore, and then placed into

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sterile 1.8mL cryovials and preserved in freshly prepared paraformaldehyde (1% final concentration), before being frozen in liquid nitrogen and then transferred to a -80°C freezer for storage.

3.2.3 Flow cytometry

Picoplanktonic cell types (heterotrophic bacteria, *Synechococcus* cyanobacteria and autotrophic eukaryotes) were identified and counted in InEx water samples by flow cytometry following the methods of Marie et al. (1997). Measurements were carried out using a Beckman Coulter Gallios flow cytometer with 488 nm argon laser to excite ultraphytoplanktonic cells and heterotrophic prokaryotes. For each cell, forward scatter signal (FSC; cell size related) was collected on a photomultiplier tube (PMT) using a 488 nm band pass filter. The right angle light scatter signal (SSC; cell shape and structure related) was first transmitted by a 605 SP beam-splitter and then recorded on a PMT using another 488/10 nm band-pass filter after reflection by a 525/40 nm band pass dichroic mirror. Orange fluorescence (phycoerythrin related) was collected on a PMT after a 575/30 nm band pass filter and red fluorescence (chlorophyll related) after a 695/40 nm band pass filter on another photomultiplier tube. Amplification was logarithmic for all signals and data collection was triggered by the red fluorescence signal for ultraphytoplankton, and SSC for heterotrophic prokaryotes. Data for individual samples were collected in list-mode files, analysed using Kaluza® software.

A first run of flow cytometry analyses was performed on non-photosynthetic cells (heterotrophic bacteria). To visualize these cells, 80μl of water sample was incubated for 15 min at 80 °C in the dark, with the nucleic acid stain SYBR Gold (Molecular probe) at a 5×10^{-5} v/v final dilution of the commercial solution. This stain is excited by the 488 nm blue laser beam and its emission is collected on a PMT using a 525/40 nm band pass filter. Each sample was analysed during a 2min run with a low flow rate, and the discriminator was set to green fluorescence (Fl1). Heterotrophic bacteria were identified by their signatures on a plot of side scatter (SSC) versus green fluorescence. Particles with a low ratio of green fluorescence to SSC were considered as non-living seston (NLS). A second run of analyses were conducted on photo-autotrophic microorganisms (*Synechococcus* and autotrophic eukaryotes), which did not require the addition of a fluorescent dye. Taxonomic discrimination was made based on the orange fluorescence (Fl2) of phycoerythrin and the red fluorescence (Fl3) of chlorophyll a (Marie et al. 1997), and on side scatter (SSC, a proxy of cell volume; Simon et al. 1994) and forward scatter (FSC, a proxy of cell size; Cunningham & Buonnacorsi 1992; Robertson et al. 1998). Both *Synechococcus* and autotrophic eukaryotes emit a red fluorescence, due to chlorophyll, after excitation at 488 nm, but only *Synechococcus* possesses phycoerythrin capable of producing an orange fluorescence. Each sample was analysed for 4 min with a high flow rate, and the discriminator was set to red fluorescence. Fluorescence emissions were collected on three PMTs, after a 525/40 nm band pass filter, a 695/30 40 nm band pass filter

for chlorophyll, and a 575 nm/30 nm band-pass filter for phycoerythrin. 1μm yellow-green fluorescent beads (Polysciences Inc.) were added to the sample in order to control instrument quality and to normalize all cellular parameter using the following equation: $X_{rel} = X_{pop}/X_{beads}$ where X_{pop} is the average value of a cell parameter (scatter or fluorescence) for a given population and X_{beads} the same parameter for the beads.

Overall, flow cytometric analysis allowed identification and enumeration of one population of heterotrophic bacteria, based on their distinct levels of green (DNA) fluorescence, one population of autotrophic *Synechococcus* cyanobacteria on the basis of orange and red fluorescence and one population of picoeukaryotes (autotrophic picoplanktonic eukaryotic organisms) discriminated by the red fluorescence and reduced orange fluorescence emission (Marie et al. 1997).

Figure 2: a) and b) flow cytometric analysis of seawater stained with SYBR gold discriminating heterotrophic bacterioplankton based on green fluorescence; and c) and d) flow cytometric analysis of unstained seawater discriminating autotrophic picoeukaryotic cells differentiated by red (chlorophyll a) fluorescence and autotrophic Synechococcus cells differentiated by orange (phycoerythrin) fluorescence. Panels a) and c) are ambient (IN) water, while panels b) and d) are the matching exhalant (EX) sample illustrating cell removal from *Herdmania momus*.

3.2.3 Feeding activity estimates

3.2.3.1 Pumping rates

Ex-current flow velocity measurements were performed after each InEx sample using frame-by-frame video analysis of the speed of a small amount of fluorescein-dyed seawater (100 mg·l^{-1}) retained inside a transparent and graduated, cylindrical tube carefully positioned within the exhaling aperture. As the ex-current jet flow through the tube, the movement of the dye within the tube is videotaped, and the dye-front speed is determined using marks on the tube as a scale. The pumping rate of each suspension feeder is estimated from the instantaneous water flux (ml·s^{-1}) per ex-current aperture, which was calculated as mean dye front speed (ml·s^{-1}). This rapid measurement

was replicated 5 times for each individual. Also, a set of different diameter tubes (from 0.2 to 1 cm) were used to closely match suspension feeders exhalent and inhalant apertures (Yahel et al. 2005).

3.2.3.2 Retention rates

The difference between the inhaled and the exhaled water samples (InEx pair) indicated the uptake of picoplanktonic cell types (Pile 2005). A conservative estimate of food consumption was also calculated by converting the mean number of picoplankton and bacteria cells removed to an equivalent mass of carbon. Carbon in the form of heterotrophic bacteria can be estimated using the following relationship between biovolume and carbon content per cell (fgC·cell[-1]) from Azam and Simon (1989):

$$Carbon\ content\ per\ cell\ = 92\left(V_{bac}{}^{0.598}\right)$$

where V_{bac} represents bacteria biovolume in μm^3 estimated following the method of Servais et al. (2003):

$$V_{bac} = 0.717\ (relative\ SSC) + 0.014$$

An equivalent of cellular carbon conversion of 178 fgC.cell[-1] was selected for *Synechococcus*-type cyanobacteria (Charpy & Blanchot 1998; Heldal et al. 2003). For autotrophic eukaryote cells, carbon content per cell (fgC·cell[-1]) was determined using the formula:

$$Carbon\ content\ per\ cell\ = 433 \times (V_{euk})^{0.866}$$

where V_{euk} represents the average eukartote biovolume of 4.57 μm^3, as determined from epifluorescence microscopy (Campbell et al. 1994).

Retention rate (cells·s^{-1}; Yahel et al., 2005), which is defined by the number of food particles captured per unit of time, was calculated as the product of pumping rates (ml·s^{-1}) and the uptake of picoplanktonic cell types (cells·ml^{-1}). From the carbon equivalent conversion, retention rate could be also defined by the amount of carbon retained per unit of time ($\mu gC·s^{-1}$), which was calculated as the product of pumping rates (ml·s^{-1}) and the carbon content of picoplankton captured ($\mu gC·ml^{-1}$). Pumping and retention rates were calculated per individual, but were also standardized per gram of dry flesh weight (DW) to account for differences in mean size and between taxa. Indeed, studies on suspension feeders have shown that feeding activity (FA; pumping and retention rates) is related to body weight (*W*) according to the allometric equation:

$$FA = aW^b$$

where *a* is the rate for an animal of unit mass and *b*, the weight exponent, is the slope of log FA - log *W* plot (Bayne et al. 1985). Therefore, pumping and retention rates of suspension feeders were standardized to a 1 g dry organ weight animal using the equation (MacDonald & Ward 1994):

$$FA_s = \left(\frac{W_s}{W_o}\right)^b FA_0$$

where FA_s is the average pumping or retention rate for an organism of standard dry weight W_s (1 g), FA_o is the observed pumping or retention rate for an organism of dry weight, W_o (g), and b is the weight exponent specific to each species. Selected values for the weight exponent b were 0.67 for *Pinna bicolor* (Petersen 2007), 0.64 for *Pinna bicolor* (Bricelj & Shumaway 1991) and 1 for *Tethya* sp (Thomassen & Riisgard 1995).

3.2.3.3 Retention efficiencies and particle selection

The mean retention efficiency (R, expressed as %) for each type of cell was calculated as:

$$R = \frac{(cell\ count\ from\ inhalent\ water - cell\ count\ from\ exhalent\ water)}{cell\ count\ from\ inhalent\ water} \times 100$$

for the paired samples (n = 10) (Pile, Patterson, and Witman 1996). Food selectivity was determined using Chesson's selectivity index (α_i):

$$\alpha_i = R_i \left(\sum_{i=1}^{m} R_i \right)^{-1}$$

where *m* is the number of food particle types and R_i is the retention efficiency for the ith food particle type. Chesson's selectivity index (α_i) can be displayed as an electivity (selection index) describing a predator's selectivity for food particles. It scales from -1 to 1, with 0 representing no preference, -1 indicating a total avoidance and + 1 a total preference for a cell type. The electivity index was calculated as:

$$\epsilon_i = \frac{m\alpha_i - 1}{(m-2)\alpha_i + 1}$$

3.2.4 Environmental parameters

Sediment traps were deployed at each site from the 1st to the 30th of September 2013 and from the 1st to 31st of August 2014, to measure monthly sedimentation rates. Following the design described by Jurg (1996), sediment traps were 30cm long and were made of PVC tubing with an internal diameter of 5cm, the aperture positioned above the seagrass canopy and fixed to a steel rod buried in the seagrass mat. Baffles were positioned 3cm before the top of the pipe to stop unwanted animals or objects getting into the trap. Sediment traps were located at distance maximal of 5 m from each sampled specimen. Sediment traps were collected at the end of each month, brought back to the laboratory where they were dried at 60°C for 72 hours and weighed (g). Gross sedimentation rates ($g \cdot cm^{-2} \cdot month^{-1}$) were calculated by dividing sample dry weight by the internal disc surface of the trap.

For each monitoring period, 2 l of water were collected 10 cm above each suspension feeder. Total suspended sediment (TSS; $mg \cdot l^{-1}$) concentrations were estimated by filtering 1l of water sample through pre-combusted and pre-weighed GF/F Whatman filters (47 mm), rinsed with distilled water to dissolve sea-salt and stored frozen and then oven-dried (60°C, 48 h), before weighing them again

(mg). Particle size distribution from water samples was measured by laser diffraction (Malvern Mastersizer 2000 with 2000 g wet dispersion accessory).

3.2.5 Data analysis

3.2.5.1 Sediment characteristics

Two-way ANOVAs were used to test for differences in sediment characteristics (gross sediment deposition rates, organic matter deposition rates and total suspended sediment) of the study sites between the fixed factors, distance (distance of sites from the wash plant) and year (2013 and 2014). Tukey post-hoc tests were performed to identify the nature of differences between each distance from the wash plant.

3.2.5.2 Composition and variation of ambient water

Three-way ANOVAs were used to test for differences in total suspended sediment concentrations, picoplankton concentrations and carbon content for the three cell types (bacteria, *Synechoccocus* and picoeukaryote) between sites, years (2013 and 2014) and cell types (all fixed factors). Tukey post-hoc tests were performed to identify differences between each type of picoplankton. Results were visualised by principal coordinate analysis (PCO).

3.2.5.3 Pumping rates, retention efficiency and food particle selectivity

In order to identify differences for pumping rates, retention rates, retention efficiency and food particle selectivity amongst sites, a PERMANOVA analysis based on the Euclidean distance and a pairwise comparison were performed. These analyses were conducted with distance (from the wash plant), year (2013 and 2014) and taxa *(Herdmania momus*, *Pinna bicolor* and *Tethya* sp) as fixed factors. The results for pumping rates were visualised by principal coordinate analysis (PCO). The association between pumping rates and physical and biological environmental variables were investigated using distance-based multiple linear regression (DistLM) in PERMANOVA+ (add-on in PRIMER v6). DistLM does not assume normality of data distributions as hypotheses are tested by permutations (Anderson 2001). Draftsman plots were used to check for skewness and multi-collinearity in the predictor variables. Variables that were highly correlated with other variables were removed in order to maximise the parsimony of our models. The following 5 variables were considered in the DistLM analysis: sediment deposition rates, total suspended sediment, concentrations of bacteria, concentration of picoeukaryotes, and concentration of *Synechococcus* in ambient water. Except for sedimentation rates, the value of each predictor variable was specific to each suspension feeder specimen. Although there was a high correlation between sediment deposition rates and TSS, both predictor variables were kept in the model in order to discriminate the influence of increased sediment deposition and elevation of suspended sediment. The "Best" procedure based on modified Akaike's Information Criterion (AICc) was used to determine models that best fit the data with fewest predictor variables. Particular variables or models were not

necessarily causative as variables may be involved indirectly or simply acting as proxies for other underlying causes. The same procedure was performed for retention efficiency values.

All statistical analyses were performed with R software and PRIMER-E v6 with the PERMANOVA add-on.

3.3 RESULTS

3.3.1 Sediment characteristics

Sedimentation rates and TSS were correlated ($R^2 = 0.61$; $p<0.0001$) and were significantly affected by distance from the wash plant (Fig. 3; Table 1). Sedimentation rates did not differ significantly between the two sampling periods but TSS concentrations were significantly higher in 2014 (Table 1). Post-hoc tests indicated significant differences ($p < 0.05$) in sediment exposure among sites, with a general decrease of sedimentation rates and TSS concentration from sites near wash plant to reference. The most obvious difference was between sites at 200 m from the wash plant and the reference sites. Mean sedimentation rates over the full survey period around 7 times higher at the 200m site than at the reference sites and total suspended sediment 3.5 to 5 times higher. The D_{50} of the suspended sediment grain size ranged from 29.37 to 38.25µm, with values increasing with distance from the discharge point toward reference sites (Table 2).

3.3.2 Composition and variation of picoplankton concentrations

Ambient concentrations of picoplankton cells in inhalant water samples were relatively similar between 2013 and 2014 ($p= 0.336$; Table 3), averaging $1.23 \pm 0.21 \times 10^6$ cells·ml^{-1} for bacteria, $4.23 \pm 2.67 \times 10^4$ cells·ml^{-1} for *Synechococcus* and $1.66 \pm 0.67 \times 10^4$ cells·ml^{-1} for picoeukaryotes (Fig. 4). While picoplankton concentrations did not vary among sites ($p = 0.213$), there were significant difference in concentration among cell types ($p <0.001$), with a significant interaction among cell type, year and distance. Picoplankton concentrations were dominated by bacteria, which were significantly more abundant than cyanobacteria and picoeukaryotes ($p <0.001$). In 2013 and 2014, bacteria represented 94 and 98% respectively of the food particles (picoplanktonic cells) present in the ambient water. Bacteria were 19 to 66 times more abundant than cyanobacteria and approximately 61 to 95 times more abundant than picoeukaryotes (Fig. 4). In 2014, densities of cyanobacteria and picoeukaryotes were significantly lower ($p<0.001$).

Mean carbon content of picoplankton populations (Fig. 5) differed significantly with distance from the wash plant, sampling periods and cell types, with no interactions of these factors (Table 4). Mean carbon content of picoplankton populations was significantly higher in 2013. Carbon content of the cyanobacteria population ranged from 3.25 ± 0.49 in 2014 to 11.82 ± 2.28 µgC·l^{-1} in 2013, and was significantly lower than the bacteria population (from 24.26 ± 1.49 in 2014 to 27.00 ± 6.37 µgC·l^{-1} in 2013) and picoeukaryote population (from 20.55 ± 3.55 in 2014 to 32.94 ± 12.29 µgC·l^{-1} in 2013). Carbon content of bacteria and picoeukaryote populations were not significantly different

for each monitoring period. Despite the significant effect of distance, no clear spatial pattern for carbon content of the three picoplanktonic population could be identified.

Figure 3: Mean gross sedimentation rate (g·cm^{-2}·month^{-1}; top) and mean total suspended sediment (TSS; mg·l^{-1}; bottom) at each site in September 2013 and August 2014 (± standard deviation).

Table 1: Results of two-way ANOVAs and Tukey post-hoc tests performed on sedimentation rates, organic matter deposited and total suspended sediment in September 2013 and August 2014 at each distance from the wash plant. P values in bold indicate significance.

	Two-way ANOVA			Tukey post-hoc test	
	df	F	p value	Comparison	p value
Sedimentation rates					
Year	1	2.09	0.1590	*200m vs 500m*	<0.0001
Distance	3	47.37	**<0.0001**	*200m vs Ref*	<0.0001
YearXDistance	3	0.64	0.5360	*Ref vs 500m*	0.0005
Total suspended sediment					
Year	1	53.31	**<0.0001**	*200m vs 500m*	<0.0001
Distance	3	198.61	**<0.0001**	*200m vs Ref*	<0.0001
YearXDistance	3	1.5	0.2390	*Ref vs 500m*	0.0002

Table 2: Particle size distribution at each site in 2014. The D50, the mass median diameter, is defined as the diameter where half of the population lies below this value. Similarly, 90 percent of the distribution lies below the D90, and 10 percent of the population lies below the D10.

Site	D10 (μm)	D50 (μm)	D90 (μm)
REF NW	7.51	38.25	132.37
REF E	7.53	36.64	122.15
500NW	5.90	31.13	91.57
500E	6.27	32.79	180.19
200E	6.23	29.96	111.36
200W	5.48	29.37	112.07

Figure 4: Concentration of picoplankton population in ambient water (cells·ml^{-1}; ± standard deviation) at each site.

Table 3: Results of three-way ANOVAs and Tuckey post-hoc tests performed on total cell concentrations (cells·ml^{-1}) for each cell type (bacteria, *Synechococcus* and picoeukaryote) in September 2013 and August 2014 at each site. P values in bold indicate significance.

Three-way ANOVA				Tukey post-hoc test	
	DF	**MS**	**p value**	Comparison	**p value**
Year	1	1.187E+10	0.336	*Eukaryote-Bacteria*	**<0.0001**
Cell type	2	1.783E+13	**<0.0001**	*Synechococcus-Bacteria*	**<0.0001**
Distance	2	2.002E+10	0.213	*Synechococcus-Eukaryote*	0.419
YearXCell type	2	1.672E+09	0.877		
YearXDistance	2	1.251E+11	**0.0001**		
Cell typeXDistance	4	2.877E+10	0.068		
Cell typeXDistanceXYear	4	1.195E+11	**<0.0001**		

Figure 5: Mean carbon content of picoplankton (µgC·l^{-1}; ± standard deviation) at each site.

Table 4: Results of three-way ANOVAs and Tuckey post-hoc tests performed on mean carbon content (µgC.l^{-1}) for each cell type (bacteria, *Synechococcus* and picoeukaryote) in September 2013 and August 2014 at each site. P values in bold indicate significance.

Three-way ANOVA				Tukey post-hoc test	
	DF	**MS**	**p value**	Comparison	**p value**
Year	1	2.567E+16	<0.0001	*Eukaryote-Bacteria*	0.816
Cell type	2	8.503E+16	<0.0001	*Synechococcus-Bacteria*	<0.0001
Distance	2	8.973E+15	0.005	*Synechococcus-Eukaryote*	<0.0001
YearXCell type	2	3.614E+15	0.105		
YearXDistance	2	5.148E+14	0.7206		
Cell typeXDistance	4	2.503E+15	0.181		
Cell typeXDistanceXYear	4	5.131E+14	0.8587		

3.3.3 Pumping rates

Pumping rates measured in-situ for each suspension feeder taxon (Fig. 6) did not differ between September 2013 and August 2014 but were significantly different among taxa ($p < 0.001$; Table 5) and distances from the wash plant ($p = 0.001$), with an interaction between distance and taxa ($p = 0.0001$). For the ascidian *Herdmania momus,* pumping rates increased from the discharge point toward reference sites (Fig. 5). For the bivalve *Pinna bicolor*, pumping rates were similar at sites 200 and 500 m ($p = 0.2645$; Table 6) from the wash plant but were significantly higher at the reference sites ($p = 0.0001$), though only one individual was monitored at the eastern reference site in 2013 and 2014. *Tethya* sp, showed the opposite pattern in pumping rates to *Herdmania momus*, generally decreasing from the discharge point toward reference sites (Fig. 6), though rates could not be measured at every site and time; only one individual was monitored at the north-western reference site and no sponges could be found at reference sites in 2014.

DistLM Results (Table 7) for *Herdmania momus* showed that 82% of pumping rate variations was explained by sedimentation rates (75%), TSS (5 %) and the concentration of bacteria (2%). For *Pinna bicolor*, 53% of the pumping rate variations was explained by sedimentation rates (45 %) and the concentration of picoeukaryotes in the water column (8 %). For *Tethya* sp, 87 % of the total observed variations was best explained by a model including sedimentation rates (70 %), TSS (10 %) and concentration of *Synechococcus* in the water column (7 %).

Figure 6: Mean pumping rates (l·h⁻¹·gDW⁻¹; ± standard deviation) at each site for 2013 and 2014.

Table 5: PERMANOVA, testing for differences in pumping rates (L.h^{-1}·gDW^{-1}) between suspension feeders (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) at each sites in September 2013 and August 2014 (9999 permutations). P values in bold indicate significance.

Source	df	MS	P	Unique permutations
Year	1	0.004	0.7603	9831
Distance	2	0.601	**0.0001**	9956
Taxa	2	67.880	**0.0001**	9961
YexSi	2	0.058	0.2864	9940
YexTa	2	0.110	0.0955	9948
DixTa	4	2.503	**0.0001**	9946
YexDixTa	3	0.063	0.2489	9959
Res	137	0.046		
Total	153			

Table 6: Summary of results of pairwise (PERMANOVA) comparisons of pumping rates between each site for the three suspension feeders. P values in bold indicate significance.

Comparison	*Herdmania momus*		*Pinna bicolor*		*Tethya* sp	
	P	Unique permutations	P	Unique permutations	P	Unique permutations
200, 500	**0.0001**	9834	0.2645	9826	**0.0001**	9836
200, REF	**0.0001**	9825	**0.0001**	9833	**0.0001**	9808
500, REF	**0.0001**	9840	**0.0001**	9826	**0.0001**	9840

Table 7: Results of best distance based linear models (DistLM), based on modified Akaike's Information Criterion (AICc), to identify the physical and biological variables that best explain variation in pumping rates.

Herdmania momus			
Selected variables	R^2 adjusted	AICc	Direction
Sedimentation rates	0.74657	-47.281	Negative
+Total suspended sediment	0.80486	-54.305	Negative
+Concentrations of bacteria	0.82156	-54.985	Negative
Pinna bicolor			
Selected variables	R^2 adjusted	AICc	Direction
Sedimentation rates	0.44958	-15.526	Negative
+Concentrations of picoeukaryote	0.52679	-18.581	Positive
Tethya sp			
Selected variables	R^2 adjusted	AICc	Direction
Sedimentation rates	0.70178	-40.739	Positive
+Concentrations of *Synechococcus*	0.8026	-53.206	Positive
+Total suspended sediment	0.8699	-65.674	Positive

3.3.4 Variation of retention efficiency and food particle selectivity

Food particle retention rates varied between monitoring periods, and among the distances and species of suspension feeders, with a significant interaction between these three factors (Table 8). Retentions rates for each suspension feeder were significantly lower (p = 0.0001) in August 2014 than September 2013 (Fig. 7). At both times, retention rates from the ascidian *Herdmania momus* are significantly lower (p = 0.001) than for the bivalve *Pinna bicolor* and the sponge *Tethya* sp. Retention rates for the ascidians were similar among sites 200 m and 500 m away from the wash plant (p > 0.05; Table 9). In 2013 retentions rates were significantly higher, while those values were significantly lower in 2014. Despite the differences between reference sites and sites near the wash plant (200m and 500m), retention rates remained in the same order of magnitude with values ranging from 23.6 ±7.4 to 46.3 ± 17.0 µgC·h^{-1}·gDW^{-1} in 2013 and from 9.5 ± 3.1 to 18.0 ±3.2 µgC·h^{-1}·gDW^{-1} in 2014. Despite significant differences among reference sites and sites closer to the wash plant, the bivalve and the sponge were removing similar amount of carbon from the water column. Retention rates of *Pinna bicolor* ranged from 80.4 ±9.9 to 246.0 ±29.1 µgC·h^{-1}·gDW^{-1} in 2013 and from 31.8 ±11.2 to 69.7 ±n.a. (only one individual was monitored) µgC·h^{-1}·gDW^{-1} in 2014. Bivalve retention rates were significantly different between each distance with an exception in 2013 when reference sites and sites at 200m presented similar values. Despite those differences, no clear spatial patterns of carbon removal could be identified. For *Tethya* sp retention rates ranged from 11.9 ±n.a. (only one individual was monitored) to 199.9 ±44.8µgC·h^{-1}·gDW^{-1} in 2013 and from 20.2 ±2.4 to 67.6 ±10.6µgC·h^{-1}·gDW^{-1}. Retention rates values for the sponge were significantly different between each distance from the wash plant (p < 0.05) and exhibited a general spatial pattern characterised by decreasing values from the discharge point toward reference sites (Fig. 8).

Figure 7: Retention rates (μgC.h^{-1}·gDW^{-1}; ± standard deviation) for *Herdmania momus*, *Pinna bicolor* and *Tethya* sp at each site in September 2013 and August 2014.

Table 8: PERMANOVA, testing for differences in retention rates (μgC.h^{-1}·gDW^{-1}) for each taxon (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) at each sites in September 2013 and August 2014. P values in bold indicate significance.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique permutations
Year	1	35.574	35.574	270.890	**0.0001**	9826
Distance	2	20.547	10.274	78.233	**0.0001**	9952
Taxa	2	35.662	17.831	135.780	**0.0001**	9946
YexSi	2	6.306	3.153	24.011	**0.0001**	9941
YexTa	2	11.167	5.583	42.517	**0.0001**	9951
DixTa	4	21.185	5.296	40.331	**0.0001**	9959
YexDixTa	3	3.811	1.270	9.673	**0.0001**	9958
Res	136	17.860	0.131			
Total	152	152.000				

Table 9: P values of pairwise (PERMANOVA using 9999 permutations) comparisons of retention rates ($\mu g C.h^{-1} \cdot g DW^{-1}$) between each site for the three suspension feeders in September 2013 and August 2014. P values in bold indicate significance.

Comparison	Herdmania momus		Pinna bicolor		Tethya sp	
	2013	2014	2013	2014	2013	2014
200, 500	0.3603	0.9447	**0.0001**	**0.0122**	**0.0004**	**0.0001**
200, REF	**0.0228**	**0.0043**	0.0706	**0.0004**	0.0004	
500, REF	**0.0031**	**0.0038**	**0.0008**	**0.0003**	0.0009	

Retention efficiencies on the three different types of food particles also exhibited high variation among monitoring periods, sites and the suspension feeders (Fig. 8).

For *Herdmania momus,* the retention efficiency of picoeukaryotes was similar between 2013 and 2014 (p = 0.675; Table 10), but there was an interaction between years and distances (p < 0.001). The highest values were observed at sites 200 m from the wash plant but also at site REF NW in 2013 and at site 500E in 2014 (Fig. 9). Retention efficiency of *Synechococcus* was significantly higher in 2013 (p = 0.001; Table 11) though a pattern of increasing retention with distance from the wash plant, was detected for both times. Retention efficiency on bacteria was also higher in 2013 (p = 0.001), but with a similar pattern of decreasing values with distance from the wash plant in both monitoring years.

Pinna bicolor retention efficiency on picoeukaryotes was significantly higher in 2013 (p = 0.001), with highest values at sites 200 m from the wash plant in 2013. Overall, a spatial pattern with decreasing values from the wash plant toward reference sites, was identified for each monitoring period. Mean retention efficiency on *Synechococcus* did not differ among years (p = 0.164). In 2013, mean retention efficiency increased with distance from the wash plant but in 2014 this spatial pattern was only present on the north western transect, whereas the eastern transect had decreasing values toward the reference site. Retention efficiency of bacteria was significantly higher in 2013 (p = 0.001), with both showing a similar spatial pattern of increasing values with distance from the wash plant.

Tethya sp retention efficiency on picoeukaryotes was significantly higher in 2013 (p = 0.001), with lowest values at the reference sites and similarly high values at sites 200 and 500 m from the wash plant. Retention efficiency on *Synechococcus* was higher in 2013 (p = 0.001) but with the same pattern of decreasing values with distance from the wash plant toward reference sites in both years. Retention efficiency on bacteria was also higher in 2013 (p = 0.001), with a similar spatial pattern of decreasing values from the wash plant toward reference sites in both years.

Figure 8: Mean retention efficiency (%; ± standard deviation) by the three suspension feeders over the three food particles at each site in 2013 and 2014.

Table 10: PERMANOVA, testing for differences in picoplankton (picoeukaryote, *Synechococcus* and bacteria) retention efficiency for each taxon (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) at each sites in September 2013 and August 2014. P values in bold indicate significance.

Source	df	*Herdmania momus* MS	P	*Pinna bicolor* MS	P	*Tethya* sp MS	P
Retention efficiencies on Picoeukaryotes							
Year	1	0.6751	0.2237	13.497	**0.0001**	16.383	**0.0001**
Distance	2	12.556	**0.0001**	13.712	**0.0001**	16.493	**0.0001**
YexDi	2	4.3458	**0.0004**	6.8711	**0.0001**	1.646	**0.0001**
Retention efficiencies on Synechococcus							
Year	1	14.346	**0.0001**	1.4435	0.1639	19.621	**0.0001**
Distance	2	12.395	**0.0001**	7.9331	**0.0004**	13.603	**0.0001**
YexDi	2	0.50617	0.2485	1.1383	0.2263	0.26677	0.2963
Retention efficiencies on Bacteria							
Year	1	19.499	**0.0001**	23.126	**0.0001**	37.872	**0.0001**
Distance	2	12.607	**0.0001**	13.448	**0.0001**	7.7077	**0.0001**
YexDi	2	2.1624	**0.0001**	1.1621	**0.0006**	0.36785	0.0268

Table 11: P values of pairwise (PERMANOVA using 9999 permutations) comparisons of retention efficiency for each suspension feeder between each site and each picoplanktonic food particle in September 2013 and August 2014. P values in bold indicate significance.

Comparisons	*Herdmania momus* 2013	2014	*Pinna bicolor* 2013	2014	*Tethya* sp 2013	2014
Retention efficiencies on Picoeukaryotes						
200, 500	**0.0002**	0.5257	**0.0001**	0.5406	0.5047	**0.0001**
200, REF	**0.0003**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
500, REF	0.5904	**0.0001**	**0.0006**	**0.0012**	**0.0001**	**0.0001**
Retention efficiencies on Synechococcus						
200, 500	**0.0034**	0.7399	0.9167	0.3249	0.1109	**0.0045**
200, REF	**0.0003**	**0.0002**	**0.0023**	0.1096	**0.0001**	**0.0002**
500, REF	**0.0069**	**0.0001**	**0.0001**	0.2377	**0.0004**	**0.0001**
Retention efficiencies on Bacteria						
200, 500	0.2759	**0.0001**	**0.0182**	0.1099	0.7689	**0.0002**
200, REF	**0.0004**	**0.0001**	**0.0001**	**0.0002**	**0.0002**	**0.0001**
500, REF	**0.0029**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Herdmania momus food particle retention efficiency was significantly correlated with sedimentation rates and total suspended sediment. Mean retention efficiencies of *Synechoccocus* and bacteria were also positively correlated with the concentrations of the cyanobacteria available in the water column. The best distance-based linear model for *Herdmania momus* (Table 12) showed that 49% of the variation in retention efficiency on picoeukaryotes was explained by TSS which explained for 36% of the total variations and concentrations of *Synechococcus* in ambient water representing 13% of variation. For the retention efficiency on *Synechococcus,* 72% of the total observed variations was explained by the concentrations of *Synechococcus* (40 %), sedimentation rates (30%) and concentration of bacteria (2%). 76% of the variation in retention efficiency on bacteria was attributed to TSS (46%), concentration of *Synechococcus* (18%), concentration of bacteria for (4 %) and sedimentation rates for (3%).

Pinna bicolor food particle retention efficiency was significantly correlated with sedimentation rates and total suspended sediment. 50 % of the variations of the bivalve retention efficiency on picoeukaryotes was attributed to sedimentation rates (45%) and concentrations of picoeukaryotes in the water column (5%). For retention efficiency on *Synechococcus,* 52% of the total observed variations was explained by sedimentation (36%), concentrations of picoeukaryotes (12%) and TSS (4%). Retention efficiency on bacteria had 53% of the total observed variations explained by TSS (49%) and concentrations of picoeukaryotes (4%).

Tethya sp food particle retention efficiency was significantly correlated with sedimentation rates and total suspended sediment. 75 % of the total observed variations on the retention efficiency on picoeukaryotes was explained by sedimentation rates (56%), concentrations of picoeukaryotes (16%) and concentrations of *Synechococcus* (2%). 81% of the variation for retention efficiency on *Synechococcus* were attributed to the concentrations of *Synechococcus* (59%) of the total variations, sedimentation rates accounting (19%) and the concentration of bacteria (2%). Retention efficiency on bacteria had 85 % of the total observed variations explained by the concentrations of picoeukaryotes (68%), sedimentation rates accounting (11%) and the concentration of bacteria (6%).

Table 12: Results of distance based linear models (DistLM), based on modified Akaike's Information Criterion (AICc), to identify the physical and biological variables that best explain variation in retention efficiency.

			Herdmania momus		
Retention efficiency	**AICc**	**R² adjusted**	**Predictor variables**	**% of variability explained**	**Direction**
Picoeukaryote	-18.76	0.49	TSS	35.99	Positive
			+Synechococcus concentrations	+12.88	Negative
Synechococcus	-39.10	0.72	Synechococcus concentrations	39.94	Positive
			+Sedimentation rates	+29.68	Negative
			+Bacteria concentrations	+2.60	Negative
Bacteria	-37.13	0.76	Synechococcus concentrations	17.74	Positive
			+TSS	+46.33	Positive
			+Bacteria concentrations	+3.87	Positive
			+Sedimentation rates	+3.35	Positive
			Pinna bicolor		
Retention efficiency	**AICc**	**R² adjusted**	**Predictor variables**	**% of variability explained**	**Direction**
Picoeukaryote	-18.08	0.50	Sedimentation rates	45.09	Positive
			+Picoeukaryote concentrations	+5.31	Negative
Synechococcus	-28.99	0.52	Sedimentation rates	36.36	Negative
			+Picoeukaryote concentrations	+11.90	Negative
			+TSS	+4.01	Negative
Bacteria	-20.55	0.53	TSS	48.97	Negative
			+Picoeukaryote concentrations	+4.00	Positive
			Tethya sp		
Retention efficiency	**AICc**	**R² adjusted**	**Predictor variables**	**% of variability explained**	**Direction**
Picoeukaryote	-40.57	0.75	Sedimentation rates	55.64	Positive
			+Picoeukaryote concentrations	+15.95	Positive
			+Synechococcus concentrations	+2.93	Positive
Synechococcus	-53.35	0.81	Synechococcus concentrations	59.11	Positive
			+Sedimentation rates	+19.33	Positive
			+Bacteria concentrations	+2.12	Positive
Bacteria	-55.27	0.85	Picoeukaryote concentrations	67.77	Positive
			+Sedimentation rates	+11.00	Positive
			+Bacteria concentrations	+6.07	Positive

Selectivity by the suspension feeders for different food types (Fig. 9), as visualised using Chesson's electivity index (ε_i), was always significantly different at each sites (p = 0.0001; Table 13&14) and time (p < 0.05), with a significant interaction between site and time (p < 0.01).

Selectivity by *Herdmania momus* for picoeukaryotes was generally negative or close to zero at reference sites and 500 m from the wash plant, but consistently positive at 200 m from the wash plant. Selectivity for *Synechococcus* was always highly positive at reference sites and then, decreased at site at 500 m from the wash plant and was always negative at sites near the wash plant. Selectivity for bacteria was highly negative at reference sites and increased with proximity to the wash plant, being slightly positive in 2014 at site 200E. Overall results indicated a high preference for removing *Synechococcus* at reference sites and a slight preference for picoeukaryotes at sites 200 m from the wash plant. For the sites 500 m away the preference was variable among times and transects, with a preference for *Synechococcus* in 2013 but in 2014 a preference for picoeukaryotes at 500E and a slight preference for both eukaryotes and *Synechococcus* at 500NW. Also, results revealed a reduction of the order of magnitude for the selectivity values from the reference sites toward the sites near the wash plant.

Selectivity by *Pinna bicolor* for picoeukaryotes was always negative at reference sites and positive at 200 m sites but variable with time at the 500 m sites, with intermediate values in 2013 but in 2014 high values at 500NW but negative selectivity at 500NW. Selectivity by the bivalve for *Synechococcus* was positive at reference sites and negative at all other sites in 2013, while in 2014 there was a general reversal of this spatial pattern. For bacteria, the reference sites had the highest selectivity values being positive or slightly negative and generally negative at the other sites. Overall, results for September 2013 showed that the bivalve was preferentially removing picoeukaryotes in the vicinity of the wash plant, transitioning to a preference for *Synechococcus* and bacteria at reference sites.

Selectivity by *Tethya* sp for the three picoplanktonic population was close to zero. Nonetheless, in 2014 values of selectivity for bacteria were more often negative. Overall, the sponge appeared to possess very little selection on food types.

Figure 9: Mean feeding selectivity (± standard deviation) by *Herdmania momus*, *Pinna bicolor* and *Tethya* sp for three types of food particle as estimated by the electivity index (ε_i), where 0 represents no preference, +1 represents high positive preference and -1 high negative preference for the different food types (Chesson 1983).

Table 13: PERMANOVA, testing for differences in picoplankton (*Picoeukaryote*, *Synechococcus* and bacteria) electivity for each taxon (*Herdmania momus*, *Pinna bicolor* and *Tethya sp*) at each sites in September 2013 and August 2014. P values in bold indicate significance.

Source	df	Herdmania momus MS	P	Pinna bicolor MS	P	Tethya sp MS	P
Electivity for Picoeukaryote							
Year	1	0.5713	**0.0007**	6.4681	**0.0001**	0.7983	**0.0001**
Distance	2	1.4584	**0.0001**	8.9182	**0.0001**	0.4145	**0.0001**
YeXDi	2	0.6313	**0.0001**	0.8921	**0.0008**	0.0918	0.2724
Electivity for Synechococcus							
Year	1	0.0994	0.0782	6.4681	**0.0001**	0.7983	**0.0002**
Distance	2	2.3899	**0.0001**	8.9182	**0.0001**	0.4145	**0.0002**
YeXDi	2	0.2145	**0.003**	0.8921	**0.001**	0.0918	0.2771
Electivity for Bacteria							
Year	1	0.3382	**0.0001**	6.4681	**0.001**	0.7983	**0.0001**
Distance	2	0.7158	**0.0001**	8.9182	**0.001**	0.4145	**0.0002**
YeXDi	2	0.2914	**0.0001**	0.8921	**0.001**	0.0918	0.2746

Table 14: P values of pairwise (PERMANOVA using 9999 permutations) comparisons of electivity values for each suspension feeder between each site and each picoplanktonic food particle in September 2013 and August 2014. P values in bold indicate significance.

Comparisons	Herdmania momus 2013	2014	Pinna bicolor 2013	2014	Tethya sp 2013	2014
Electivity for Picoeukaryote						
200, 500	**0.0001**	0.3331	**0.0001**	0.2758	0.0823	0.776
200, REF	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0015**	**0.0046**
500, REF	0.9401	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.2317
Electivity for Synechococcus						
200, 500	**0.0002**	0.0877	**0.0001**	0.2713	0.0902	0.7824
200, REF	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0009**	**0.0052**
500, REF	**0.0351**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.232
Electivity for Bacteria						
200, 500	**0.035**	**0.0002**	**0.001**	0.245	0.0821	0.7722
200, REF	**0.0015**	**0.0001**	**0.001**	**0.001**	**0.0011**	**0.0052**
500, REF	**0.0001**	**0.0012**	**0.001**	**0.001**	**0.0001**	0.235

3.4 DISCUSSION

Globally, heterotrophic bacteria are the most abundant picoplankton with an average abundance of $1.23 \pm 0.21 \times 10^6$ bacteria·ml^{-1}, which is the range with those observed in other oligotrophic coastal waters (Fuks et al. 2005; Jochem 2001; Seuront et al. 2014; Seymour et al. 2005). The average abundance of cyanobacteria *Synechococcus* ($4.23 \pm 2.67 \times 10^4$ cells·ml^{-1}) in this study was of similar magnitude to south-western Australian coastal waters (Hanson et al. 2009; Paterson et al. 2013) and oligotrophic coastal waters elsewhere (Seuront et al. 2014; Seymour et al. 2005; Pile 2005; Matsumoto et al. 2004). Picoeukaryotes are the least abundant group of picoplankton with an average abundance of $1.66 \pm 0.67 \times 10^4$ cells·ml^{-1}, which is in the range of values obtained from other studies conducted in coastal oligotrophic waters (Matsumoto et al. 2004; Pile et al. 1997). Although concentrations of picoplankton were representative of south-western Australia coastal waters, values estimated at this location should only be considered as an approximation due to the temporal scale limitations. Indeed, as the monitoring was conducted at the end of the winter period when frequent storm occur in south-western Western Australia, which generate large swell and waves locally (Lemm et al. 1999), different values of picoplankton concentrations may be expected at different time of the year.

The results from this study did not reveal any significant differences in total concentrations of picoplankton from inhalant water samples between sites for both monitoring period in 2013 and 2014. This finding demonstrated that the picoplanktonic compartment was across the study sites and that increased sediment exposure across those years had no influence on the availability of the principal food resource for the suspension feeders. Nonetheless, natural variations in concentrations on some type of picoplanktonic cells could be observed. Indeed, changes in environmental conditions (temperature, wind, swell, nutrients, etc.) may explained the significantly lower ($p<0.001$) densities of cyanobacteria and picoeukaryotes in 2014.

The main findings from this study showed a strong influence of increased sediment exposure on the suspension feeding activity (pumping rates and particle retention efficiencies) of the three species that best represent suspension-feeder assemblages within the study area. Suspension-feeding activity can be characterised by the capture of suspended food particles through active and/or passive water filtration (Ribes et al. 1999; J. M. Gili & Coma 1998; Jørgensen 1983) and the exploitation of different particle types and size classes (Riisgård & Larsen 2010; Hanson et al. 2009; Ostroumov 2005; Armsworthy et al. 2001; Coma et al. 2001; Dame et al. 1998). The relationship between the availability of certain food particles and suspension feeding activity is contingent upon the great variations in particle capture mechanisms and pump design. For instance, lateral cilia on gill filaments of suspension-feeding bivalves, cilia on either side of the stigmata in ascidians and flagella in choanocytes of sponges allowed these suspension feeders to modulate their filtration activity in order to optimise food acquisition and maintain metabolic energy requirement

(Riisgård & Larsen 2010, 1995). From this study, the degree to which increased suspended sediment and sediment deposition benefit or harm suspension feeding activity was proved to be species dependent. Indeed, the three suspension feeders displayed contrasting patterns of pumping and retention rates, but also food particle selection, at increasing distances from the wash plant. These variations among species could be reconciled with an understanding of their distinctive filtering apparatus and feeding strategies. The solitary ascidian *Herdmania momus* actively captures sestonic particles through a mucus net, the Pterioid bivalve *Pinna bicolor* operates a cirri trapping mechanism to actively gather food particles and the demosponge *Tethya* combines active and passive feeding using a collar sieving structure.

3.4.1 The solitary ascidian *Herdmania momus*

The mean pumping rate of $1.51 \, l \cdot h^{-1} \cdot gDW^{-1}$ measured from *Herdmania momus* was similar to other solitary ascidians (Petersen 2007; Armsworthy et al, 2001). *Herdmania momus* had lower pumping rates at sites characterised by higher suspended and deposited sediment concentrations (Fig. 6). Filtrations rates of ascidians are influenced by factors such as temperature, oxygen concentration, food concentrations as well as body size (Ribes et al. 1998; Petersen & Riisgard 1992). For this study, specimens of similar size were selected, a low variability in picoplankton concentrations among sites was recorded and because of the limited spatial scale of the study area (~ 30 ha), physicochemical parameters (depth, temperature, oxygen, etc.) were assumed to be similar among sites. Therefore, sedimentation rates and TSS appeared to be the main factors explaining the variations of pumping rates among sites according to their distance from the wash plant, a deduction supported by the linear modelling (Table 8). The negative influence of high concentrations of suspended particles on pumping rates has been previously described for other ascidian species (Petersen et al. 1995, 1999; Petersen & Riisgard 1992) and attributed to increased squirting, diameter reduction of the inhalant siphon and decreased beat frequency of lateral cilia (Petersen 2007; Armsworthy et al. 2001; Petersen et al. 1999). Despite the obvious influence inherent to the increase in squirting and the subsequent period of reopening of the siphons, such mechanism account only for a minor fraction of the overall reduction in pumping rate (Petersen et al. 1999). Furthermore, ascidian pumping rates recorded in the study were measured apart from squirting events. Therefore, this mechanism cannot be associated with the reduction of pumping rates observed. Another means of regulating water filtration is by altering siphon-opening diameter. Siphon diameter constriction associated with higher sediment concentrations has been observed in *Pyurid* ascidians similar to *Herdmania momus* (Armsworthy et al. 2001). However, the main mechanism responsible for the variation in pumping rates is the modification in beat frequency of latera cilia located in the oral siphon of the ascidians (Petersen 2007). The decreased in cilia beat frequency at high particle concentration was observed for the *Enterogona* ascidians *Ciona intestinalis* (Petersen et al., 1999). The presence of complex sensory lateral cilia in Pyurid ascidians

(Caicci et al. 2010) suggest that similar mechanism is likely to occur for *Herdmania momus*. The decrease in beat frequency is likely triggered by gut fullness (Petersen et al. 1999; Petersen & Riisgard 1994).

Despite the reduction of pumping rates associated with increased values of TSS and sedimentation rates*, Herdmania momus* maintained constant food particle retention rates. Previous studies demonstrated that increased suspended particles above a minimum threshold negatively affected clearance rate of ascidians resulting in a constant ingestion rates (Petersen 2007; Petersen et al. 1999; Petersen & Riisgard 1992; Robbins 1984; Robbins 1983; Fiala-Médioni 1979), which is similar to the functional response demonstrated in many other suspension feeders. The diet composition of *Herdmania momus* was comparable to those reported from previous studies on ascidians (Slattery 2015; Petersen 2007; Pile 2005; Armsworthy et al. 2001; Lesser & Ribes et al. 1999). At sites not impacted by sediment plumes (reference sites), retention efficiency was high for *Synechococcus* (up to 66%) and much lower for picoeukaryotes and heterotrophic bacteria (as low as 10%), indicating that the ascidian is able to select food. Although ascidians are often described as non-selective suspension feeders, the possibility of particle selection has been previously reported (Armsworthy et al. 2001; Seiderer & Newell 1988; Klumpp 1984). For instance, several studies suggested that squirting for the purpose of particle rejection may be closely tied to particle selection (Armsworthy 2001) and based on particle size (Klumpp 1984) and quality (Millar, 1960; Jørgensen 1966; Suh and Hong 1982; Young 1988; Bingham and Walters 1989). With increasing TSS and sediment deposition, retention efficiencies for cyanobacteria significantly decreased while retention efficiencies for bacteria and picoeukaryotes increased. As bacteria and picoeukaryotes represented a greater source of carbon than cyanobacteria, it appeared that changes in particles selection allow the ascidians to optimize the nutritive quality of their diet (organic carbon enrichment) while experiencing increased sediment exposure.

These findings suggested that the maintenance of the retention rates under increased sediment exposure may be associated with the selective retention of food particles. This mechanism to compensate increasing sediment concentration was previously described for the ascidian *Halocynthia pyriformis* (Armsworthy et al. 2001). However, the physiological mechanism behind particle selection and its relation with increased sedimentation remain unclear. Although the reduction of the pumping rates may be considered as a physiological regulation in terms of optimization of energy expenditure in order to maintain constant retention rates (Petersen 2007), food particle selection might reflect the optimization of energy uptake by feeding on a wider range of food sources in order to extract energy from a low quality diet. Both mechanisms could explain the presence of the ascidian in the vicinity of the wash plant, where sediment exposure is maximum (see Chapter 1).

3.4.2 The Pterioid bivalve *Pinna bicolor*

The mean pumping rate of 6.01 $l \cdot h^{-1} \cdot gDW^{-1}$ measured from *Pinna bicolor* was similar to the mean pumping rate of the con-generic *Pinna nobilis* (5.99 $l \cdot h^{-1} \cdot gDW^{-1}$; Vafidis 2014). The higher pumping rates for *Pinna bicolor* at the reference sites indicated that increased sedimentation rates had adverse effects on pumping rates (Fig. 6). Several studies on suspension feeding bivalves, *Cerastoderma edule* (Widdows & Navarro 2007), *Mytilus edulis* (Petersen et al. 2004), *Crassostrea gigas* (Barillé et al. 1997; Troost et al. 2009), *Perna canaliculus* (Hawkins et al. 2001), *Mercenaria mercenaria* (Grizzle et al. 2001) and *Atrina zelandica* (Coco et al. 2006) demonstrated a strong inverse relationship between increasing total suspended particulates concentrations and pumping rates. Pumping rates generally increased up to a 'threshold' concentration, above which they decreased (e.g., Mohlenberg and Kiorboe, 1981; Kiorbe et al., 1980). These threshold concentrations vary depending on the bivalve species (e.g., Hawkins et al., 1999). From the results of this study, it appeared that *Pinna bicolor* bivalves at sites close to the wash plant (500 and 200 m away) were experiencing sediment concentrations above their tolerance threshold. Due to its relatively large water filtering structure and open mantle cavity, *Pinna bicolor* is able to maintain high pumping rates at low energetic cost (Bernard & Noakes 1990). However, the open mantle cavity increases the vulnerability to the deposition of sediment particles (Butler et al. 1993). Therefore, *Pinna bicolor,* like many others bivalves, possesses specialized adaptation to cope with increased suspended sediment and sediment deposition (Yonge & Thompson 1976). Compensatory mechanisms include fast growth to rise above the sediment, increased size of labial palps to select and sort particles and pseudo-faeces production (Hewitt & Pilditch 2004; Kiorboe & Mohlenberg 1981). The energetic cost of those adaptations is balanced by a reduction of energy dedicated to water filtration (Kiorboe & Mohlenberg 1981). This energy relocation could explain the reduced pumping rates recorded for *Pinna bicolor* at sites with high sediment exposure.

The range of food particles captured by bivalves is strongly related to the local resources available in the water column (Ward et al. 1998). In south-western Australia the pelagic planktonic community is dominated by small (<5 mm) cells (Koslow et al. 2008) which are an important food resource for the benthic suspension feeders (Hanson et al. 2009). Other studies reported that picophytoplankton and bacteria is part of the natural diet for some bivalves (Ward et al. 1998; Kreeger & Newell 2001). However, the continuous filtering activity performed by *Pinna* is an adaptation to low food concentrations (Rhodes & Thompson 1993; Butler et al. 1993; Jorgensen 1975), though larger food particles (phytoplankton >2 µm, detritus and zooplankton) can also be included in their diet when available (Safi et al. 2007; Hewitt & Pilditch 2004). From the differences in retention rates of picoeukaryotes, bacteria and cyanobacteria recorded in this study, *Pinna bicolor* appears to be one of the many suspension feeder bivalves that are highly selective grazers. This faculty to select food particles was also reported for Atrina zelandica (Hewitt & Pilditch 2004),

which also belongs to the same Pinnidae family. Increased TSS concentration and sedimentation rates had a negative effect on *Pinna bicolor* retention rates for bacteria and *Synechococcus* and positively increased the removal of picoeukaryotes. Under undisturbed conditions (reference sites) retention efficiencies were higher for bacteria and *Synechococcus*, but at sites with increased TSS and sedimentation rates, bivalves were feeding principally on picoeukaryotes. The selection of larger cells of better nutritive quality (organic carbon enrichment) appeared to be an adaptation to lower food quality at sites with higher sediment concentrations. This capacity to select particles of higher quality selection to compensate for the dilution of food sources with inorganic particles has been reported for various suspension feeding bivalves (Ward & Shumway 2004), including the pinnid bivalves *Atrina zelandica* (Safi et al. 2007). Thus, *Pinna bicolor* was able to compensate the reduction of pumping rates, affecting energy allocation, by retaining food particles of higher value, compounding the negative influence of increased sediment exposure.

3.4.3 The demosponge *Tethya* sp

The mean pumping rate for *Tethya* sp was $2.37 \, l \cdot h^{-1} \cdot gDW^{-1}$, which is in the range of values reported for *Tethya* and other demosponges (Mentel et al. 2014; Maldonado et al. 2012; Stabili et al. 2006; Thomassen & Riisgard 1995; Reiswig 1981; Reiswig 1975; Reiswig 1974;). Pumping rates generally decreased with distance toward the reference sites (Fig. 6). This contrast the results of studies in similar sponge species, where decreased pumping rates were observed at high sediment exposure (Bannister et al. 2012; Tompkins-MacDonald & Leys 2008; Gerrodette & Flechsig 1979). However, sponges have been reported to be able to regulate their pumping rates in response to environmental cues (Stabili et al. 2006; Simpson 1984), which could explain the differences in the water filtration activity observed among *Tethya* sp at each distance from the wash plant. Reiswig (1971) reported a relative tolerance to high turbidity in coastal waters for *Tethya crypta*. Indeed, some demosponges like *Tethya* may cope with high sediment exposure by developing periodic contraction of the exhalant system that help to effectively expel debris (Nickel & Brümmer 2004; Elliott & Leys 2007; Ellwanger et al. 2007). However, there is no evidence in the literature that demonstrates an increase of pumping rates with high sediment exposure. Data from the study suggest that sponges have a high degree of control over their filtration activity, though it is unclear whether the increased pumping rates in higher sediment load conditions are the result of adaptation or the consequence of a greater availability of food particles.

The diet and range of retention efficiencies recorded for *Tethya* sp were similar to those reported for other demosponges (Hanson et al. 2009; Ribes & Coma 2005; Pile et al. 1997). Retention rates for the different types of picoplankton were highly correlated with pumping rates observed at each site, and therefore increased with higher sediment exposure. At each site, the sponge performed little to no selection. Previous studies (Topçu et al. 2010; Pile & Young 2006; Duckworth & Brück 2006) have demonstrated that sponges are not able to select among food particles. Since selectivity

for the three picoplankton communities was minimal and retention efficiencies increased toward the discharge point, the high level of sediment concentration appeared to have a positive effect on feeding activity. The higher biomass and abundances of the sponge recorded in the vicinity of the wash plant and its absence from reference sites (see Chapter 1) further argues for a positive influence of TSS and sedimentation rates. Although demosponges inhabiting highly turbid habitat can present behavioural and physiological adaptations to expel sediment particles (Elliott & Leys 2007; Ellwanger et al. 2007; Nickel & Brümmer 2004; Reiswig 1971), the mechanisms behind this potential benefit from sediment exposure remain unexplained. Previous studies demonstrated that increased concentrations of suspended inorganic material could enhance food availability to suspension feeders (Newell et al., 2005; Waite et al., 2000). Indeed, suspended sediment can provide extensive surface for the colonization of microorganisms and formed "marine aggregates" comprised of picoplankton and bacteria incorporated into larger inorganic particles (Muschenheim et al. 1989). There is growing evidence that marine aggregates can contribute to suspension feeder diets (Newell et al. 2005). Also aggregates have much higher settling rates than individual picoplanktonic cells, enhancing the amount of food particles that reach the seabed (Newell et al. 2005; Waite et al. 2000; Muschenheim et al. 1989).

3.5 CONCLUSION

Despite the differences in particle capture mechanisms among the three species of suspension feeders, there was clear overlap of diets on the picoplankton cells targeted by each specie. The decrease of total retention rates in 2014 as a consequence of lower densities of cyanobacteria and picoeukaryotes confirmed the importance of picoplankton for the feeding activity of sessile suspension feeders inhabiting Posidonia meadows. From the temporal variations of retention rates, changes in particle selection were observed in 2014 but overall selection pattern remained similar and closely tied to sediment exposure levels. This study demonstrated that increased TSS and sediment deposition had an influence on the feeding activity of all three species. At high sediment exposure, the ascidian *Herdmania momus* decreased pumping rates, thereby maintaining a constant retention rate, and optimised its food intake by expanding its food sources from mainly cyanobacteria to a wider range of food sources. *Pinna bicolor* also had lower filtration activity under elevated TSS conditions, again, maintaining a relatively constant total retention rates and displaying a change in particle selection from bacteria and *Synechococcus* to picoeukaryotes. The sponge *Tethya* appeared to benefit from elevated sediment concentrations, as filtration and retention rates increased, potentially related to the lack of food selectivity in this demosponge. Overall, the findings from this study showed a strong relationship between the influence of high level of sediment exposure and compensatory mechanisms that some species may have developed to preserve energy allocation, notably those associated with food particle selection.

This study provided the first record of pumping and retention rates on picoplankton for *Herdmania momus*, *Pinna bicolo*r and *Tethya*. Overall, the results show that even though increased sedimentation rates were unlikely to cause mortality, sub-lethal effect influence feeding activity, which can in turn alter ecosystem processes. *Pinna bicolor* and *Herdmania momus* filter large volume of water and particulate matter. The suspension feeding activity occurring in the *Posidonia* meadows within the study area can alter the quality and quantity of suspended particles in the water column and significantly influence phytoplankton population (Lemmens et al. 1996). Hence, the reduction of pumping rates and the modification of particle selection would be expected to affect clearing of sediment from the water column, and the food supply for the local macrobenthic community (Hewitt & Pilditch 2004; Ellis et al. 2002; Dame et al. 1998; Cloern 1982). Furthermore, ascidians and bivalves produce large quantities of biodeposits (Hewitt & Pilditch 2004; Mazouni & Gaertner 2001) that enhance the sedimentation of sediment from the water column to the seafloor (Norkko et al. 2001) and influence composition of benthic assemblages (Safi et al. 2007). Changes in suspension feeding activity (e.g. pumping rates and particle selection) might alter rejection rates and the composition of faeces and/or pseudofaeces (Mazouni & Gaertner 2001; Fiala-Médioni 1974), affecting the surrounding benthic community. In conclusion, changes in feeding activity as a results of increased sediment exposure could alter organic and inorganic fluxes associated with the benthic-pelagic coupling.

The electivity index used in this study only provided information regarding the preference or the avoidance for organic particles. Future investigation on the retention of sedimentary particles could provide additional information. Indeed, several studies on suspension feeders showed that sediment exposure affects the size of the particle retained (Beninger et al. 2007; Barillé et al. 2006; Hawkins et al. 1996). Therefore, the use of coulter-counter analyses might help to determine changes in size of the particles 100% retained and demonstrate potential clogging of suspension feeder' filtering apparatus. A further consideration is that instant measurements do not provide a complete understanding of the influence of increased sediment exposure on suspension feeding activity and the consequences for ecosystem level processes. There is clearly a need for further studies integrating feeding activity over a longer time period. Also, reproducible measurement using controlled field and laboratory experiments are required to validate the applied methods and hypothesis formulated over the influence of increased sediment exposure. This experimental approach is undertaken in the following chapter. Nonetheless, the present study provides some initial and fundamental information on food utilization and specialized adaptions to sediment exposure.

CHAPTER 4
Influence of increased sediment exposure on suspension-feeding activity: an experimental assessment.

PREAMBLE

In Chapter 1 it was shown that the suspension feeder assemblage in a *Posidonia* meadow was potentially resistant to high sediment exposure. Findings from chapter 3 indicated that suspension feeders could cope with high concentrations of suspended sediment and rates of sediment deposition through compensatory adaptation of their feeding activity. Because these observations were made at a location experiencing continuous and persistent sediment plumes since 1972, it is not clear if the response of suspension feeders to elevation in sediment exposure is the result of long-term adaptation to a chronic pressure. The severity of impact from sediment disturbances is related to the intensity, duration and frequency of exposure to increased concentrations of suspended sediment and elevated rates of deposition. Therefore, it is important to investigate the response of suspension feeding to acute increases in sediment exposure induced by natural event such as flood associates with storm or anthropogenic activities such as dredging. In this chapter, a one-month transplant experiment and a laboratory experiment were conducted to determine the effect of a short-term increase in sediment exposure on the feeding activity and selectivity of three key suspension feeders.

ABSTRACT

The red-throated ascidian *Herdmania momus*, the razor clam *Pinna bicolor* and the golf ball sponge *Tethya* sp, are common suspension feeders in temperate Australian seagrass habitats. As suspension feeders, they are likely to be influenced by human-induced increases in suspended and depositing sediments. A short-term laboratory experiment was conducted to investigate whether their feeding mechanisms and strategies were altered by exposure to increased sediment loads. To validate the laboratory experiment findings and better understand the response of suspension feeding under field conditions, a one-month transplant experiment was also performed. This approach enabled investigation of the relationship between sediment exposure and feeding activity (feeding rates and food resource partitioning). Both experiments demonstrated that increased sediment exposure (up to 40 mg·l^{-1} of TSS and 8 g·cm^{-2}·month^{-1} of sediment deposition) influenced the feeding activity of the three species but in different ways. At low levels of sediment exposure, the ascidian *Herdmania momus* was preferentially removing 23 to 42% of *Synechococcus* cells, while only retaining 9 to 27% of bacteria and 9 to 10% of picoeukaryotes. When experiencing high sediment exposure, the ascidian *Herdmania momus* optimised its food intake by feeding less selectively on a wider range of food sources, retaining around 30% of each type of food particles. At low sediment exposure, the bivalve *Pinna bicolor* was removing more efficiently 47 to 57% of *Synechococcus* cells, while removing only 23 to 28% of bacteria and 12 to 14% of picoeukaryotes. With high sediment exposure *Pinna bicolor* was able to increase its organic carbon intake by shifting particle selection by retaining 32 to 34% of larger picoeukaryotic cells, but only 22 to 24% of Synechococcus and 1 to 5% of bacteria. Despite a total absence of particle selection, the feeding activity for the sponge *Tethya* sp appeared to benefit from elevated sediment concentrations, as food particle retention efficiency increased from 15 to 40% of food particles removed.

4.1 INTRODUCTION

Increases in catchment runoff, elevated sedimentation and water column turbidity are symptoms of coastal development and associated anthropogenic activities. Such increase in sediment exposure lead to changes in benthic communities over broad scales, altering coastal ecosystem-level process. Sessile suspension feeders are likely to be negatively impacted by increased sediment exposure. Previous investigations have shown that increased sediment resuspension and deposition can negatively influence feeding rates of suspension-feeding bivalves and ascidians (Ellis et al. 2002; Armsworthy et al. 2001). Through their feeding activity, suspension feeders are able to filter large amounts of water (up to 100 $m^3 \cdot d^{-1} \cdot m^{-2}$; Jørgensen 1996) and remove organic and inorganic particles from the water column, excreting nutrients and excreting faeces and pseudo-faeces in the sediment (Wildish & Kristmanson 2005; Dame et al. 1998). Due to this key role in benthic-pelagic coupling, alteration of suspension-feeding activity can reduce organic matter and nutrients available to other marine organisms (Pile 2005; Ostroumov 2005; Dame et al. 2001). However, some suspension feeders possess physiological and behavioural mechanisms that allow them to compensate for increased concentrations of suspended sediment and sediment deposition. Those compensatory mechanisms include modification of species feeding behaviour (use of a different portion of the resource pool), reduction of the diameter of their feeding apparatus (shift from large particles to small particles) and adaptive mechanisms to expel sediment settled on them, through mucus production, ciliary action or polyp expansion (Pile 2005; Armsworthy et al. 2001; Gili & Coma 1998). Thus, the degree to which high sediment exposure influences suspension feeders is likely to be species specific and depend on their feeding ecology and the resilience of their feeding mechanisms.

The purpose of this study was to elucidate the influence of increased sediment exposure on the uptake of food particles from co-occurring suspension feeders. Seagrass beds in south-western Australia are an important habitat for many benthic suspension feeders (Lemmens et al. 1996). Among them, the solitary ascidian *Herdmania momus*, the pteroid bivalve *Pinna bicolor* and the demosponge *Tethya* sp are conspicuous in term of biomass and abundance. The three species are characterised by very distinct feeding mechanisms: *Herdmania momus* actively captures seston through a mucus net; *Pinna bicolor* operates a cirri trapping mechanism to actively gather food particles; and *Tethya* sp combines active and passive feeding using a collar sieving structure.

The coastal waters of south-western Australia are characterised by oligotrophic conditions (Koslow et al. 2008). Picoplankton (heterotrophic and autotrophic cells 0.2 – 2 μm in size) that are able to utilize the low concentrations of nutrients represent more than 85% of phytoplankton biomass (Hawkes 2006) and form the principal food resource for suspension feeders (Hanson et al. 2009). The findings from Chapter 3 confirmed that that the three species of suspension feeders examined here were mostly exploiting picoplankton, and potentially selecting different fractions of this food

source. Hence, this study focused on the uptake of picoplankton by suspension feeders and whether or not it is influenced by increased sediment exposure.

This study tested the hypothesis that the influence of increased sediment exposure on suspension-feeding activity would vary among species, depending on the compensatory mechanisms associated with their distinctive feeding structures and strategies. To do this, a short-term laboratory experiment was conducted to test whether suspension feeding was affected by suspended sediments, and whether the response differed among the three organisms, which have different feeding apparatus. To validate the laboratory experiment results and better understand the response of suspension feeders under field conditions, a reciprocal transplant experiment was performed between sites with relatively high and low TSS and sediment deposition.

4.2 MATERIAL AND METHODS

4.2.1 Laboratory experiment

4.2.1.1 Experimental design and set-up

The effects of different levels of suspended sediment on the feeding activity (feeding rates and food selectivity) of the ascidian *Herdmania momus*, the bivalve *Pinna bicolor* and the sponge *Tethya* sp were investigated over 3-hour period experiment. Suspension feeders were collected from Owen Anchorage (32° 7'S; 115°44'E), a relatively clean water open coast site just outside Fremantle, Western Australia. At each site and for each species, specimens of similar size and weight (appendix C) were carefully selected to minimize the potential effect of age and size on suspension feeding activity. The ascidians ranged from 9.2 to 12.4 cm body length, the bivalves ranged from 23.1 to 28.2 cm shell length and the sponges ranged from 4.1 to 5.7 cm body diameter. Prior to the feeding experiments, the suspension feeders were allowed to acclimatise for 24 h and it was visually confirmed that the animals were continuously and actively filtering water. Temperature was held constant at 17 °C, and a 10:12 light/dark cycle was used.

Separate experiments were conducted for the three suspension feeder species. For each species, replicate aquaria were filled with 30 l of sterile seawater and continually aerated. Based on the measurement of the filtrations recorded during previous field survey, the volume of water was adapted to physiological characteristics (size and dry-weight) of the selected ascidians and the bivalves, but appeared inappropriate considering the significantly smaller sponges. Therefore, decision was made to conduct the experiment using six individuals of *Tethya* for each experimental treatment and suspension feeding measurements for each specie were standardized per gram of dry flesh weight (DW). Therefore, nine aquaria received one individual (except for *Tethya* which included 6 individual) held in their natural orientation on the floor of the aquarium with mesh baskets. Three replicate aquaria were subjected to one of three TSS treatments (i.e. 3 replicates per TSS level: 5, 20 and 40 mg·l^{-1}), encompassing the range of TSS concentrations recorded at Woodman Point (32°7'S, 115°44'E), a seagrass location affected by TSS generated by a local cement manufacturing company (see Chapter 2). The rate of food consumption was compared among treatments. To controls for changes in food concentrations not related to suspension-feeder activity, a complete set of aquarium were established as above but without animals. In each control aquarium a dummy specimen was placed into the empty reference to assess possible artefact of particle retention from suspension feeder's structures. 15 cm PVC bottles, empty shells of *Pinna bicolor* and golf balls were respectively used to mimic ascidians, bivalves and sponges.

Potential food sources for suspension feeders were added to all aquaria (including controls) at final concentrations of ~$1·10^6$ cells·ml^{-1} of bacteria (<1 μm cells size), ~$3·10^4$ cells.ml^{-1} of *Synechococcus* (1 μm cells size) and ~$1.5·10^4$ cells.ml^{-1} of *Isochrysis* (1μm cells size), at the same time as the sediment slurry. Bacteria were isolated from local seawater, *Synnechococcus* originated

from the CS 94 strain cultured by CSIRO and *Isochrysis* were obtained from commercial highly concentrated culture (Proaqua ©).

Sediment used in the experiment was collected from the impacted area at Woodman Point. This sediment was wet sieved to remove particles larger than 200 µm, oven dried at 450 °C for 4 h to remove organic materials and then homogenised to a thin powder using a ball and mill grinder. Particle size distribution of the sediment was measured by laser diffraction (Malvern Mastersizer 2000 with 2000 g wet dispersion accessory) to determine the mass-median-diameter (D_{50}) of suspended particles. The D_{50} value of the sediment was 38.39 µm, close of the mean D_{50} value of 33.03 µm recorded at Woodman Point (see Chapter 2). A "sediment slurry" was then made by adding 250 g of sediment to 1 l of sterile seawater. Before starting the experiment, the slurry was added to the treatment aquaria to achieve the required treatment levels. Each aquarium was fitted with a submerged stream pump to keep the particles in suspension. Several pilot trials over week-long periods were conducted to ensure TSS concentrations were consistent within tanks over time and between replicate tanks in a given treatment. TSS ($mg \cdot L^{-1}$) concentrations were estimated by filtering 1 l of water through pre-combusted and pre-weighed GF/F Whatman filters (47 mm), rinsed with distilled water to dissolve sea-salt and stored frozen and then oven-dried (60 °C, 48 h), before weighing them again (mg). Reciprocity of sediment treatment was tested between aquarium with animal and control aquarium without animals, and between each replicate.

Table 1: Summary table showing control treatment, concentrations of total suspended sediment (TSS; $mg \cdot l^{-1}$) for each sediment treatment and the initial concentrations of bacteria, *Synechococcus* and *Isochrysis* ($cells \cdot ml^{-1}$).

Control treatment	Sediment Treatment: TSS ($mg \cdot l^{-1}$)	bacteria ($cells \cdot ml^{-1}$)	*Synechococcus* ($cells \cdot ml-1$)	*Isochrysis* ($cells \cdot ml-1$)
animal	High: 40	~$1.00 \cdot 10^6$	~$3.00 \cdot 10^4$	~$1.50 \cdot 10^4$
animal	Medium: 20	~$1.00 \cdot 10^6$	~$3.00 \cdot 10^4$	~$1.50 \cdot 10^4$
animal	Low: 5	~$1.00 \cdot 10^6$	~$3.00 \cdot 10^4$	~$1.50 \cdot 10^4$
no animal	High: 40	~$1.00 \cdot 10^6$	~$3.00 \cdot 10^4$	~$1.50 \cdot 10^4$
no animal	Medium: 20	~$1.00 \cdot 10^6$	~$3.00 \cdot 10^4$	~$1.50 \cdot 10^4$
no animal	Low: 5	~$1.00 \cdot 10^6$	~$3.00 \cdot 10^4$	~$1.50 \cdot 10^4$

4.2.1.2 Feeding rates and food selectivity

Water samples (100 ml) were collected immediately after adding the sediment slurry and food particles (T_0) and at the end of the experiment (T_f; 3 h after). Homogenised sub-samples were placed into sterile 1.8 mL cryovials and preserved in freshly prepared paraformaldehyde (1 % final concentration), before being frozen in liquid nitrogen and then transferred to a -80°C freezer for storage. Following the methods described in Chapter 2, flow cytometry allowed identification and enumeration of one population of heterotrophic bacteria, based on their distinct levels of green (DNA) fluorescence, using a nucleic acid stain SYBR Gold (Molecular probe), one population of autotrophic *Synechococcus* cyanobacteria on the basis of orange and red fluorescence and one population of picoeukaryotes discriminated by the red fluorescence and reduced orange

fluorescence emission (Marie et al. 1997). Measurements were carried out using a Beckman Coulter Gallios flow cytometer with 488 nm argon laser to excite ultraphytoplanktonic cells and heterotrophic prokaryotes. Data for individual samples were collected in list-mode files, analysed using Kaluza® software.

Differences between cell counts at T_0 and T_f, noted δt, were analysed to quantify the grazing on each type of food particle by the suspension feeders (Pile 2005). The uptake of picoplanktonic cell types (cells·ml^{-1}) were measured per individual, but were also standardized per gram of dry flesh weight (DW) to account for differences in mean size and between taxa. Indeed, studies on suspension feeders have shown that feeding activity (FA; pumping and retention rates) is related to body weight (*W*) according to the allometric equation:

$$FA = aW^b$$

where *a* is the rate for an animal of unit mass and *b*, the weight exponent, is the slope of log FA - log *W* plot (Bayne et al. 1985). Therefore, pumping and retention rates of suspension feeders were standardized to a 1 g dry organ weight animal using the equation (MacDonald & Ward 1994):

$$FA_s = \left(\frac{W_s}{W_o}\right)^b FA_0$$

where FA_s is the average pumping or retention rate for an organism of standard dry weight W_s (1 g), FA_o is the observed pumping or retention rate for an organism of dry weight, W_o (g), and b is the weight exponent specific to each species. Selected values for the weight exponent b were 0.67 for *Pinna bicolor* (Petersen 2007), 0.64 for *Pinna bicolor* (Bricelj & Shumaway 1991) and 1 for *Tethya* sp (Thomassen & Riisgard 1995). The mean retention efficiency (R; cells·ml^{-1}·gDW^{-1}) for each type of cell was calculated as:

$$R = \frac{(c_{T0} - c_{Tf})}{c_{T0}} \times 100,$$

where C_{T0} is the cell concentration at time 0 (cells·ml^{-1}·gDW^{-1}) and C_{Tf} is the cell concentration at the end of the experiment (3 h). Food selectivity was determined using Chesson's selectivity index (α_i):

$$\alpha_i = R_i \left(\sum_{i=1}^{m} R_i\right)^{-1}$$

where *m* is the number of food particle types and R_i is the retention efficiency for the ith food particle type. Chesson's selectivity index (α_i) can be displayed as an electivity (selection index) describing a predator's selectivity for food particle. It scales from -1 to 1, with 0 representing no preference, -1 indicating a total avoidance and + 1 a total preference for a cell type.

The electivity index was calculated as: $\epsilon_i = \frac{m\alpha_i - 1}{(m-2)\alpha_i + 1}$

4.2.1.3 Data analysis

- Initial experimental conditions

To test differences in TSS concentrations between sediment treatments (low, medium and high TSS concentrations) and the consistency of treatment between control treatments (with vs without animal) and species (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp), a three-way ANOVA was performed with each factor considered as fixed.

To determine whether the initial concentrations of the different type of picoplankton cells received by each species (*Herdmania momus*, *Pinna bicolor* and *Tethya* sp) were consistent among sediment treatments and between control treatments, two-way ANOVAs were performed with each factors considered fixed.

- Uptake of picoplankton cells by suspension feeders

To determine whether differences between picoplankton cell counts at T_0 and T_f (noted δt) resulted from suspension feeding rather than natural variations (e.g. mortality, production), two-way ANOVAs were performed to test for differences between control treatments (with vs without animal) and for each sediment treatment (low, medium and high TSS concentrations). The factors control treatment and sediment treatment were considered fixed.

Finally, the influence of sediment exposure on gross retention rates (cells·ml^{-1}·gDW^{-1}), retention efficiency (%) and selectivity for each food particle was estimated by comparing the results between each sediment treatment for each species. These comparisons were obtained using ANOVA and Tukey post-hoc tests.

4.2.2 Field transplant experiment

4.2.2.1 Study Sites

To validate laboratory experiments results on the response of suspension feeding to increased sediment exposure, a 1-month transplant experiment was conducted in the field. Two sites were used for the transplant experiment, at Woodman Point (Fig. 1): a site highly influenced by sediment plumes was selected in the vicinity (200 m) of the wash plant (site A); another site further way (>1 km) was selected as a non-impacted site, based on the findings presented in Chapter 1. Each site was located within a natural bed of *Posidonia sinuosa* in depths of 1.7 m (Site A) and 3.2 m (Site B).

Figure 1: Study sites

4.2.2.2 Transplant experiment design and set-up

The transplant experiment was performed from the 30th September to 27th October 2014. The three suspension feeder species were found naturally at both sites. At each site and for each species, 20 individuals were collected by SCUBA divers, being careful to minimize tissue damage and selecting specimens of a similar size (appendix C) in order to limit the potential effect of age on feeding behaviour. Detached animals were placed in calico bags underwater, and then transferred underwater into large containers with aerated seawater for further transportation. Ten individuals of each species were transplanted to the other site and 10 individuals were transplanted back into the site of origin, as controls. The animals were transplanted as quickly as possible, usually 2-3 h after initial collection. At the transplant sites, each individual sponge *Tethya* sp was attached by a numbered fishing line to a metal peg, which was then driven into the sediment. Each individual of *Pinna bicolor* and *Herdmania momus* was secured to the bottom by a numbered peg. The method used for the sponge transplants was unsuccessful, with all individuals drifting away. Thus, results could not be reported. The health status and morphological changes of transplanted organisms were visually checked after 1 and 2 weeks. These observations consisted on recording the number of living, dead and missing animals, but also examining their filtration behaviour. The filtration activity was estimated based on observations of faeces production and the degree of valves or siphons responsiveness to gentle tactile stimuli. At the end of the experiment all ascidians and bivalves survived. A month later, the assessment of the feeding activity, for each species, was conducted on 5 individuals naturally present and not manipulated (naturally occurring individuals), on 5 individuals re-transplanted to their site of origin (control) and 5 transplanted individuals (Table 2).

Table 2: Summary table showing each treatment of the transplant experiment.

Site	Treatment
A *(near the wash plant; high sediment exposure)*	Naturally occurring individuals Control Transplanted
B *(>1 km; low sediment exposure)*	Naturally occurring individuals Control Transplanted

Measurements of suspension feeder feeding activity were conducted following the InEx method of Yahel et al. (2005), in which paired water samples from the water inhaled and exhaled by suspension feeders were simultaneously collected by two SCUBA divers. Samples of inhaled water (In) were collected using shortened polystyrene Sterilin pipettes with the upper half of a microcentrifuge tube attached at one end and a syringe attached to the other end. Samples were taken slowly by a diver holding the tube inlet next to the animal's inhaling aperture. Simultaneously, exhaled water (Ex), was sampled using a pipette with microcentrifuge tube attached at both ends, by another diver holding inlet, from a second tube, within the exhalent jet and aligned with it, as close as possible (<2 mm) to the animal's exhaling aperture, but with no physical contact. Once sampling was accomplished, microcentrifuge tubes attached to the pipettes were simultaneously plugged. The difference in food particle concentrations between the inhaled and the exhaled water samples (InEx pair, as measured using flow cytometry, see below)) indicated particle retention rates (Yahel et al. 2005). Samples were kept cold and dark in an icebox until brought to shore, and then placed into sterile 1.8 mL cryovials and preserved in freshly prepared paraformaldehyde (1% final concentration), before being frozen in liquid nitrogen and then transferred to a -80 °C freezer for storage for further flow cytometry analysis.

4.2.2.3 Pumping rates

Ex-current flow velocity measurements were performed after each InEx sample using frame-by-frame video analysis of the speed of a small amount of fluorescein-dyed seawater (100 mg·l^{-1}) retained inside a transparent and graduated, cylindrical tube carefully positioned within the exhaling aperture. As the ex-current jet flow through the tube, the movement of the dye within the tube is videotaped, and the dye-front speed is determined using marks on the tube as a scale. The pumping rate of each suspension feeder is estimated from the instantaneous water flux (ml·s^{-1}) per ex-current aperture, which was calculated as mean dye front speed (ml·s^{-1}). This rapid measurement was replicated 5 times for each individual. Also, a set of different diameter tubes (from 0.2 to 1 cm) were used to closely match suspension feeders exhalent and inhalant apertures (Yahel et al. 2005).

4.2.2.4 Retention rates

Following the same method described for the laboratory experiment, food particles were identified and counted in InEx water samples by flow cytometry. Flow cytometric analysis allowed identification and enumeration of heterotrophic bacteria, based on their distinct levels of green (DNA) fluorescence, autotrophic *Synechococcus* cyanobacteria on the basis of orange and red fluorescence and picoeukaryotes discriminated by the red fluorescence and reduced orange fluorescence emission (Marie et al. 1997). Data for individual samples were collected in list-mode files, analysed using Kaluza® software.

The difference in food particle concentrations between the inhaled and the exhaled water samples (InEx pair) indicated uptake of picoplankton (Pile 2005). A conservative estimate of food consumption was also calculated by converting the mean number of picoplankton and bacteria cells removed to an equivalent mass of carbon. Carbon in the form of heterotrophic bacteria can be estimated using the following relationship between biovolume and carbon content per cell ($fgC \cdot cell^{-1}$) from Azam and Simon (1989):

$$Carbon\ content\ per\ cell\ = 92\left(V_{bac}^{0.598}\right)$$

where V_{bac} represents bacteria biovolume in μm^3 estimated following the method of Servais et al. (2003):

$$V_{bac} = 0.717\ (relative\ SSC) + 0.014$$

An equivalent of cellular carbon conversion of 178 $fgC.cell^{-1}$ was selected for *Synechococcus*-type cyanobacteria (Charpy & Blanchot 1998; Heldal et al. 2003). For autotrophic eukaryote cells, carbon content per cell ($fgC \cdot cell^{-1}$) was determined using the formula:

$$Carbon\ content\ per\ cell\ = 433 \times (V_{euk})^{0.866}$$

where V_{euk} represents the average eukartote biovolume of 4.57 μm^3, as determined from epifluorescence microscopy (Campbell et al. 1994).

Retention rate ($cells \cdot s^{-1}$; Yahel et al., 2005), which is defined by the number of food particles captured per unit of time, was calculated as the product of pumping rates ($ml \cdot s^{-1}$) and the uptake of picoplanktonic cell types ($cells \cdot ml^{-1}$). From the carbon equivalent conversion, Retention rate could be also defined by the amount of carbon retained per unit of time ($\mu gC \cdot s^{-1}$), was calculated as the product of pumping rates ($ml \cdot s^{-1}$) and the carbon content of picoplankton captured ($\mu gC \cdot ml^{-1}$). In order to account for the potential effect of size and weight on suspension feeding responses, pumping and retention rates were calculated per individual and standardized per gram of dry flesh weight (DW) following the same method described in the laboratory experiment.

4.2.2.5 Retention efficiencies and particle selection

The mean retention efficiency (R, expressed as %) for each type of cell was calculated as:

$$R = \frac{(cell\ count\ from\ inhalent\ water - cell\ count\ from\ exhalent\ water)}{cell\ count\ from\ inhalent\ water} \times 100$$

for the paired samples (n = 10) (Pile, Patterson, and Witman 1996). Food selectivity was determined using the electivity index previously described for the laboratory experiment.

4.2.2.6 Environmental parameters

Sediment traps were deployed at each site from the 29th of September and 30th of October 2014 to measure s edimentation rates during the transplant experiment. Following the design described by Jurg (1996), sediment traps were 30 cm long and were made of PVC tubing with an internal diameter of 5cm, the aperture positioned above the seagrass canopy and fixed to a steel rod buried in the seagrass mat. Baffles were positioned 3 cm before the top of the pipe to stop unwanted animals or objects getting into the trap. Sediment traps were collected at the end of each month, brought back to the laboratory where they were dried at 60°C for 72 hours and weighed (g). Gross sedimentation rates (g·cm^{-2}·month^{-1}) were calculated by dividing sample dry weight by the internal disc surface of the trap.

At each site, 5 l of water were collected for each monitoring period. Total suspended sediment (TSS; mg·l^{-1}) concentrations were estimated by filtering 1l of water sample through pre-combusted and pre-weighed GF/F Whatman filters (47 mm), rinsed with distilled water to dissolve sea-salt and stored frozen and then oven-dried (60 °C, 48 h), before weighing them again (mg).

4.2.2.7 Data analysis

- Initial conditions

Student's tests were performed to test for significant differences in gross sedimentation rates and TSS concentrations between the two sites. For each cell types (bacteria, *Synechoccocus* and picoeukaryote), one-way ANOVAs were used to test for differences in picoplankton concentrations and total carbon content between the two reciprocal transplant sites.

- Filtration and retention activity

Two-way ANOVA was used to test for differences in pumping rates, retention rates, retention efficiency and food particle selectivity between the two transplant sites (Fixed factor; Sites A and B) and among each treatment (Fixed; naturally occurring, control and transplant). The ANOVA were performed separately for each species.

The association between pumping rates and physical and biological environmental variables were investigated using distance-based multiple linear regression (DistLM) in PERMANOVA+ (add-on in PRIMER v6). DistLM does not assume normality of data distributions as hypotheses are tested by permutations (Anderson 2001). Draftsman plots were used to check for skewness and multi-

collinearity in the predictor variables. Variables that were highly correlated with other variables were removed in order to maximise the parsimony of models. The following 5 variables were considered in the DistLM analysis: sedimentation rates, total suspended sediment, concentrations of bacteria, concentration of picoeukaryotes, and concentration of *Synechococcus* in ambient water. Although there was a high correlation between sedimentation rates and TSS, both predictor variables were kept in the model in order to discriminate the influence of increased sediment deposition and elevation of suspended sediment. The "Best" procedure based on modified Akaike's Information Criterion (AICc) was used to determine models that best fit the data with fewest predictor variables. Particular variables or models were not necessarily causative as variables may be involved indirectly or simply acting as proxies for other underlying causes. The same procedure was performed for retention efficiency values.

4.3 RESULTS

4.3.1 Laboratory experiment

4.3.1.1 Experimental conditions

During the feeding experiment, the three levels of suspended sediment treatments (Fig. 2) were significantly different (p = 0.0001). For each treatment, TSS concentrations were similar for each separate experiments conducted on the three species (p = 0.952) and between aquaria with individual and controls without animals (p = 0.524).

Figure 2: Total Suspended Sediment at T_{3h} (TSS; mg·l^{-1}; ± standard deviation) for each treatment (High, Medium and Low concentrations; control and experiment). ★ indicates significant differences between treatments.

For the feeding experiment with *Herdmania momus*, initial concentrations of bacteria and *Synechococcus* were similar (p>0.05) for each level of sediment (low, medium and high) and between controls without animals and feeding experiment with animals (Fig.3; Table 3). However, initial concentration of *Isochrysis* were significantly different between controls and aquaria with

animals (p = 0.003) and also between levels of sediment (p = 0.020), with a significant interaction of both factors (p = 0.029). Differences were mainly explained by the concentrations of *Isochrysis* in aquaria with ascidians receiving the low TSS treatment ($1.13\pm0.03\cdot10^4$ cells·ml^{-1}). Indeed, these concentrations were significantly lower (p < 0.05) of those in control aquaria (without animals) and aquaria with ascidians experiencing highest TSS concentrations. Despite these differences, initial concentrations of *Isochrysis* were of the same order of magnitude (from 1.13 to $1.57\cdot10^4$ cells·ml^{-1}).

For the feeding experiment with *Pinna bicolor*, initial concentrations of *Isochrysis* and *Synechococcus* were similar (p>0.05) for each level of sediment and between controls without animals and feeding experiment with animals (Fig.3; Table 4). However, initial concentrations of bacteria were significantly different between sediment treatments (p = 0.003), with a significant interaction of factor experimental design (control versus experiment with animal; p = 0.003). Differences were mainly explained by the concentrations of bacteria in aquaria without ascidians receiving the high TSS treatment ($1.90\pm0.14\cdot10^6$ cells·ml^{-1}). Indeed, these concentrations were significantly higher (p < 0.05) of those in control aquaria (without animals) and aquaria with bivalves. Despite this difference, the concentrations of bacteria were of the same order of magnitude (from 0.96 to $1.90\cdot10^6$ cells·ml^{-1}).

For the feeding experiment with *Tethya* sp, initial concentrations of *Isochrysis* were similar (p>0.05) for each levels of sediment and among controls without animals and feeding experiments with animals (Fig.3; Table 4). However, initial concentration of *Synechococcus* were significantly different between controls and experiments including animal (p = 0.034). Lower concentrations recorded in control aquaria were still comparable of those in aquaria with sponges, with values ranging from 1.11 to $2.89\cdot10^4$ cells·ml^{-1}. Also, initial concentrations of bacteria were significantly different between controls and feeding experiment (p= 0.023). This difference was only explained by the significant difference in bacteria concentration in between aquaria with animal subject to the medium TSS treatment and those in control aquaria with high TSS treatment. Despite this slight difference, concentrations values remained in the same order of magnitude (from 1.07 to $1.63\cdot10^6$ cells·ml^{-1}).

Figure 3: Mean concentration of picoplankton (*Isochrysis*, *Synechococcus* and bacteria; cells·ml^{-1}) at T$_0$ for each experimental design (controls versus experiment) and for each level of sediment concentration (low, medium and high TSS concentrations).

Table 3: Two-way ANOVAs, testing for differences in initial concentrations of picoplankton cells (*Isochrysis*, *Synechococcus* and bacteria) for each experimental design (controls versus experiment), level of sediment concentration (low, medium and high TSS concentrations) and suspension feeder. P values in bold indicate significance.

		Herdmania momus		*Pinna bicolor*		*Tethya* sp	
Source	df	MS	P	MS	P	MS	P
Isochrysis initial concentrations							
Control	1	1.803×10^7	**0.003**	5.483×10^7	0.172	7.149×10^5	0.466
Sediment	2	7.419×10^6	**0.020**	3.270×10^7	0.320	3.665×10^6	0.094
CoXSe	2	6.519×10^6	**0.029**	5.389×10^7	0.169	4.056×10^5	0.732
Residuals	12	1.355×10^6		2.603×10^7		1.263×10^6	
Synechococcus initial concentrations							
Control	1	3.337×10^6	0.600	1.969×10^7	0.277	1.423×10^8	**0.034**
Sediment	2	1.302×10^7	0.355	9.869×10^5	0.937	3.043×10^7	0.331
CoXSe	2	1.698×10^7	0.267	1.899×10^6	0.883	1.702×10^7	0.526
Residuals	12	1.150×10^7		1.515×10^7		2.501×10^8	
Bacteria initial concentrations							
Control	1	5.030×10^9	**0.139**	1.245×10^{11}	0.986	2.601×10^{11}	**0.025**
Sediment	2	3.669×10^{10}	1.013	3.665×10^{11}	**0.003**	1.628×10^{11}	0.051
CoXSe	2	3.585×10^{10}	0.990	3.720×10^{11}	**0.003**	3.998×10^{10}	0.392
Residuals	12	3.621×10^{10}		3.884×10^{10}		3.950×10^{10}	

The difference (δt) in concentrations of food particles (bacteria, *Synechococcus* and *Isochrysis*) over the experimental period (3 hours) was significantly different ($p < 0.001$) between control aquaria without animal (almost no change) and aquaria with animals (Fig.4; Table 4), indicating that the changes in the treatments containing animals were due almost entirely to the presence of the animals. The difference in the concentrations of food particles (δt) was also significantly different among sediment treatments, at the exception of the feeding experiments with *Herdmania momus* regarding the concentrations of *Synechococcus* (p=0.399).

In the feeding experiment with *Herdmania momus*, δt for bacteria and *Isochrysis* were higher for the treatment with high level of TSS, while δt for *Synechococcus* remained relatively constant for each TSS level. In the feeding experiment with *Pinna bicolor*, δt for bacteria and *Synechococcus* were higher for the treatment with low level of TSS, while δt for *Isochrysis* remains relatively constant for each TSS level. In the feeding experiment with *Tethya* sp, δt for bacteria, *Synechococcus* and *Isochrysis* were higher for the treatment with high level of TSS.

Figure 4: δt (t$_0$-t$_{3h}$) picoplankton concentrations (*Isochrysis*, *Synechococcus* and bacteria; cells·ml^{-1} for each experimental design (controls versus experiment), level of sediment concentration (low, medium and high TSS concentrations) and suspension feeder.

Table 4: ANOVA, testing for differences in δt picoplankton concentrations (Isochrysis, Synechococcus and bacteria) for each experimental design (controls versus experiment), level of sediment concentration (low, medium and high TSS concentrations) and suspension feeder. P values in bold indicate significance.

		Herdmania momus		*Pinna bicolor*		*Tethya* sp	
Source	df	MS	P	MS	P	MS	P
δt Isochrysis concentrations							
Control	1	4.733×10^7	**<0.001**	4.952×10^7	**<0.001**	8.024×10^7	<0.001
Sediment	2	6.125×10^6	**<0.001**	6.460×10^5	**0.038**	8.674×10^6	<0.001
CoXSe	2	7.511×10^6	**<0.001**	1.700×10^5	**0.018**	7.678×10^6	<0.001
Residuals	12	4.110×10^5		1.479×10^5		6.802×10^5	
δt Synechococcus concentrations							
Control	1	1.986×10^8	**<0.001**	4.918×10^8	**<0.001**	4.497×10^8	<0.001
Sediment	2	1.497×10^6	0.399	4.132×10^7	**<0.001**	3.390×10^7	<0.001
CoXSe	2	7.457×10^5	0.622	4.403×10^7	**<0.001**	4.114×10^7	<0.001
Residuals	12	1.508×10^5		2.719×10^6		8.222×10^5	
δt Bacteria concentrations							
Control	1	2.152×10^{11}	**<0.001**	1.398×10^{11}	**<0.001**	4.375×10^{11}	<0.001
Sediment	2	2.578×10^{10}	**<0.001**	3.674×10^{10}	**<0.001**	5.413×10^{10}	0.035
CoXSe	2	2.507×10^{10}	**<0.001**	3.735×10^{10}	**<0.001**	4.729×10^{10}	0.049
Residuals	12	9.577×10^8		2.051×10^9		1.201×10^{10}	

4.3.1.2 Retention rates

Food particle retention rates ($cells \cdot ml^{-1} \cdot gDW^{-1}$) for the three suspension feeder species varied significantly ($p < 0.05$) among treatments (Fig. 5; Table 5). For the ascidian *Herdmania momus*, the retention rate ($3.40 \pm 0.71 \cdot 10^4$ $cells \cdot ml^{-1} \cdot gDW^{-1}$) was significantly higher at the highest concentrations of TSS, than in either the medium or low concentrations, which did not differ from each other ($1.85 \pm 0.66 \cdot 10^4$ and $1.05 \pm 0.12 \cdot 10^4$ $cells \cdot ml^{-1} \cdot gDW^{-1}$, respectively). Retention rates of *Pinna bicolor* showed the opposite pattern to *Herdmania momus*, with highest values ($3.55 \pm 0.15 \cdot 10^4 cells \cdot ml^{-1} \cdot gDW^{-1}$) recorded for the low TSS treatment (which was not significantly different to the medium TSS treatment) and minimum values for the high treatment ($5.99 \pm 0.46 \cdot 10^3 cells \cdot ml^{-1} \cdot gDW^{-1}$). Retention rates values for the sponge were not significantly different among treatments ($p = 0.2$), despite higher mean retention rates measured for high TSS treatment ($1.05 \pm 0.31 \cdot 10^4$ $cells \cdot ml^{-1} \cdot gDW^{-1}$).

Figure 5: Retention rates (cells·ml^{-1}·gDW^{-1}) for *Herdmania momus* and *Pinna bicolor* and *Tethya* sp for each treatment. ★ indicates significant differences between treatments.

Table 5: Results of ANOVAs and Tukey post-hoc tests performed on retention rates (cells·ml^{-1}·gDW^{-1}) for each suspension feeder and treatment. P values in bold indicate significance.

ANOVA							
		Herdmania momus		*Pinna bicolor*		*Tethya* sp	
Source	Df	MS	P	MS	P	MS	P
Sediment	2	4.28E+08	**0.006**	7.10E+08	**0.022**	7.45E+07	0.200
Residuals	6	3.17E+07		9.27E+07		4.71E+06	
Tukey post-hoc test							
Comparison		P		P		P	
Low, High		**0.005**		**0.022**		0.656	
Medium, High		**0.04**		**0.038**		0.060	
Medium, Low		0.250		0.658		0.616	

4.3.1.3 Retention efficiencies and Selectivity

Herdmania momus retention efficiencies (Fig.6) differed among sediment treatments (p < 0.001; Table 6) and between type of food particles (p < 0.001), with a significant interaction of both factors (p = 0.006). For the High TSS treatment, retention efficiencies for the three types of food particles were not significantly different (p>0.05; Table 7), with values around 30%, and selectivity for the three types of food particles was close to zero. For the treatment with medium TSS concentrations, retention efficiency on bacteria (15%) was significantly lower (p<0.05) than for *Synechococcus* (30%) and *Isochrysis* (25%) and selectivity for bacteria was significantly different (p<0.05) than for *Synechococcus* and *Isochrysis*. The selectivity for bacteria was negative, while those of the two other food particles were slightly positive, indicating that the ascidian was removing *Synechococcus* and *Isochrysis* more efficiently. Ascidians in the treatment

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with low level of sediment retained more *Synechococcus* (23%) than bacteria (9%) and *Isochrysis* (10%), and whereas the selectivity for bacteria and *Isochrysis* was negative, the selectivity for *Synechococcus* was highly positive.

Pinna bicolor retention efficiencies also differed among sediment treatments ($p < 0.001$) and type of food particles ($p < 0.001$), with a significant interaction of both factors ($p < 0.001$). In the high TSS treatment, retention efficiency on bacteria (5%) was significantly lower ($p<0.0001$) than on *Synechococcus* (24%) and *Isochrysis* (32%). Selectivity for bacteria was highly negative (-0.7), while values for *Synechococcus* (0.1) and *Isochrysis* (0.4) were positive. For the treatment with medium TSS concentrations, retention efficiencies on bacteria (10%), *Synechococcus* (20%) and *Isochrysis* (16%) were not significantly different ($p>0.05$). If the selectivity on *Synechococcus* (0.2) and *Isochrysis* (0.0) were comparable, the selection on bacteria was significantly lower ($p = 0.002$) and presented a negative value (-0.3). The similarity of retention efficiencies and low selectivity values indicated that the bivalves possessed very little selection among food types. At low concentrations of TSS, *Synechococcus* particles were more efficiently removed (47%), followed by bacteria (28%) and *Isochrysis* (14%), and selectivity for *Isochrysis* was strongly negative (-0.5), neutral for bacteria (0.0) and positive for *Synechococcus* (0.4), indicating that bivalves feeding in less turbid water were efficient on removing *Synechococcus* and were feeding very little on large *Isochrysis* cells.

Retention efficiencies for the sponge *Tethya* sp were significantly different among treatments ($p < 0.001$), but the retention of the three food types were similar within each treatment ($p = 0.837$). The highest retention efficiencies (mean = 40%), were recorded in the high TSS treatment, while medium and low TSS treatments were 15% and 22%, respectively. For each treatment, the sponge selectivity for the three food particles was close to zero. Overall, the sponge appeared to possess very little preferentiality on food types, independently of the sediment exposure.

Figure 6: Mean retention efficiency (%; ± standard deviation) and mean feeding electivity (± standard deviation) for each suspension feeder.

Table 6: PERMANOVA (9999 permutations), testing for differences in retention efficiency and electivity for each food particle (*Isochrysis*, *Synechococcus* and bacteria) between treatments (low. medium and high TSS concentrations) for each suspension feeder (*Herdmania momus, Pinna bicolor* and *Tethya* sp). P values in bold indicate significance.

Source	Df	Retention efficiency								
		Herdmania momus			*Pinna bicolor*			*Tethya* sp		
		MS	P	Permutations	MS	P	Permutations	MS	P	Permutations
Sediment	2	2.890	**0.0001**	9956	3.671	**0.0001**	9953	0.044	0.834	9960
Particle	2	7.673	**0.0001**	9948	2.984	**0.0001**	9955	10.646	**0.0001**	9950
SeXPr	4	0.649	**0.008**	9957	2.842	**0.0001**	9953	0.062	0.903	9953
Residuals	18	0.127			0.073			0.243		
Source	DF	Electivity								
		Herdmania momus			*Pinna bicolor*			*Tethya* sp		
		MS	P	Permutations	MS	P	Permutations	MS	P	Permutations
Sediment	2	6.520	**0.0001**	9947	5.481	**0.0001**	9954	0.567	0.646	9952
Particle	2	0.076	0.714	9959	0.047	0.615	9947	0.006	0.995	9946
SeXPr	4	2.211	**0.0001**	9963	3.305	**0.0001**	9946	0.502	0.809	9957
Residuals	18	0.220			0.096			1.269		

Table 7: Results of Tuckey post-hoc test, comparing retention efficiency and electivity for each food particle (*Isochrysis*, *Synechococcus* and bacteria) between treatments (low. medium and high TSS concentrations) and for each suspension feeder (*Herdmania momus, Pinna bicolor* and *Tethya* sp). P values in bold indicate significance.

Comparison	Retention efficiency								
	Herdmania momus			*Pinna bicolor*			*Tethya* sp		
	High	Medium	Low	High	Medium	Low	High	Medium	Low
Bacteria, Isochrysis	0.857	**0.035**	1.000	**<0.0001**	0.636	0.002	0.999	1.000	1.000
Bacteria, Synechococcus	0.925	**0.001**	**0.002**	**<0.0001**	0.061	**<0.0001**	1.000	1.000	0.987
Isochrysis, Synechococcus	1.000	0.740	0.003	0.193	0.831	**<0.0001**	1.000	1.000	0.998
Comparison	Electivity								
	Herdmania momus			*Pinna bicolor*			*Tethya* sp		
	High	Medium	Low	High	Medium	Low	High	Medium	Low
Bacteria, Isochrysis	0.985	**0.043**	1.000	**<0.0001**	0.135	0.007	1.000	1.000	1.000
Bacteria, Synechococcus	0.996	**0.002**	**<0.0001**	**<0.0001**	**0.002**	0.006	1.000	1.000	0.857
Isochrysis, Synechococcus	1.000	0.875	**<0.0001**	0.273	0.552	**<0.0001**	1.000	1.000	0.985

4.3.2 Transplant experiment

4.3.2.1 Sediment and food characteristics at the transplant sites

Sedimentation rates and TSS concentrations (Fig. 7) measured at the site in the vicinity of the impacted area (site A) were significantly higher ($p < 0.0001$) than at the site located more than 1 km away (site B). These results were consistent with long term patterns observed in Chapter 1.

Figure 7: Mean gross sedimentation Rate (g.cm^{-2}·month^{-1}; a) and mean Total Suspended Sediment (TSS; mg·l^{-1}; b) at each site in September 2013 and August 2014 (± standard deviation). ★ indicates significant differences.

The concentrations of *Synechococcus* and picoeukaryotes did not differ significantly between site A and site B (p>0.05; Table 8), averaging $4.76\pm0.34\cdot10^4$ and $3.53\pm0.12\cdot10^4$ cells·ml^{-1}, respectively. The concentrations of bacteria were significantly higher (p<0.001) than those *Synechococcus* and picoeukaryotes at both sites and were significantly higher (p = 0.0002) at site A ($1.40\pm0.14\cdot10^6$ cells·ml^{-1}) than site B ($8.01\pm7.91\cdot10^5$ cells·ml^{-1}), though of in the same order of magnitude. The total amount of carbon as *Synechococcus* and picoeukaryotes did not differ significantly between site A and site B (p>0.05; Table 9), averaging 8.46 ± 0.61 and 57.03 ± 1.99 µgC·l^{-1}, respectively Bacterial carbon was significantly higher (p = 0.0002) at site A. Total carbon as picoeukaryotes was significantly higher (p<0.0001) than those of *Synechococcus* and bacteria (22.83 ± 9.71 µgC·l^{-1}).

Figure 8: Concentration of picoplankton population in ambient water (cells·ml^{-1}; ± standard deviation) at each site. ★ indicates significant differences between each site.

Table 8: ANOVA performed on total cell concentrations (cells·ml^{-1}) for each cell type (bacteria, *Synechococcus* and picoeukaryote) at each site. P values in bold indicate significance.

Source	DF	Bacteria		Synechococcus		Eukaryote	
		MS	P	MS	P	MS	P
Site	1	5.288x10^{12}	**0.0002**	4.023x10^{7}	0.076	1.267x10^{6}	0.416
Residuals	58	1.876x10^{13}		7.142x10^{8}		1.097x10^{8}	

Figure 9: Mean carbon content of picoplankton (µgC.l^{-1}; ± standard deviation) at each site. ★ indicates significant difference.

Table 9: ANOVA performed on mean carbon content (µgC.l^{-1}) for each cell type (bacteria, *Synechococcus* and picoeukaryote) at the two reciprocal transplant sites. P values in bold indicate significance.

Source	DF	Bacteria		Synechococcus		Eukaryote	
		MS	P	MS	P	MS	P
Site	1	2.286x10^{3}	0.0002	1.275	0.076	3.301	0.416
Residuals	58	1.398x10^{2}		0.390		4.929	

4.3.2.2 Pumping rates

Mean pumping rates for both species were significantly different (p <0.001; Table 10) between sites A and B. Suspension feeders transplanted from the site with high TSS concentrations (site A) to the site with low TSS concentrations (site B) had increased pumping rates, while the reciprocal transplantation was characterised by a reduction of those rates (Fig. 10). Mean pumping rate for *Herdmania momus* transplanted to site A was 0.82 ± 0.15 l·h^{-1}·gDW^{-1} and 1.79 ± 0.20 l·h^{-1}·gDW^{-1} for ascidians transplanted to site B. Mean pumping rate for *Pinna bicolor* transplanted to site A was 5.78 ± 0.56 l·h^{-1}·gDW^{-1} and 7.83 ± 0.54 l·h^{-1}·gDW^{-1} for bivalves transplanted to site B.

At the two reciprocal transplant sites, i*n-situ* pumping rates for each suspension feeder were similar among transplanted, control and native animals (p > 0.05; Table 11). The absence of difference between controls and naturally occurring animals demonstrated that results reflected filtration activity response to changes in TSS concentrations rather than experimental artefact.

DistLM results (Table 11) for *Herdmania momus* showed that the best model for pumping rate variations contained two predictor variables explaining 87% of the total observed variations: TSS explained 84% of the total variation and the concentration of picoeukaryotes in ambient water explained an additional 3% of variation. For *Pinna bicolor*, 81% of the pumping rate variations was explained by TSS (80%) and the concentration of *Synechococcus* (1%).

Figure 10: Mean pumping rates (l.h^{-1}·gDW^{-1}) at each site for each treatment. ★ indicates significant differences.

Table 10: Two-way ANOVA, testing for differences in pumping rates (l·h⁻¹·gDW⁻¹) for *Herdmania momus* and *Pinna bicolor*) among treatments at the two reciprocal transplant sites. P values in bold indicate significance.

Source	df	Herdmania momus		Pinna bicolor	
		MS	P	MS	P
Site	1	6.977	**<0.001**	29.680	**<0.001**
Treatment	2	0.020	0.840	0.085	0.710
SixTr	2	0.004	0.934	0.008	0.969
Residuals	24	0.057		0.246	
Total	29				

Table 11: Results of best distance based linear models (DistLM), based on modified Akaike's Information Criterion (AICc), to identify the physical and biological variables that best explain variation in pumping rates.

Herdmania momus			
Selected variables	R² adjusted	AICc	Relationship
Total suspended sediment	0.84159	-49.914	Negative
+Concentrations of picoeukaryote in ambient water	0.87312	-50.995	Negative
Pinna bicolor			
Selected variables	R² adjusted	AICc	Relationship
Total suspended sediment	0.80578	-20.606	Negative
+Concentrations of Synechococcus in ambient water	0.81193	-18.27	Negative

4.3.2.3 Variation of retention efficiency and food particle selectivity

Suspension feeder retention rates (for both number of cells and quantity of carbon) were similar among transplanted, control and native animals ($p>0.05$; Table 12&13). Mean retention rates (Fig. 11&12) for *Herdmania momus* transplanted to site B with low TSS ($4.28\pm0.71\cdot10^7$ cells·h⁻¹·gDW⁻¹ or 32.34 ± 5.83 µgC·h⁻¹·gDW⁻¹) were significantly higher ($p < 0.001$) compared to those transplanted to site A with high TSS ($2.07\pm0.42\cdot10^7$ cells·h⁻¹·gDW⁻¹ or 26.27 ± 4.54 µgC·h⁻¹·gDW⁻¹). *Pinna bicolor* retention rates transplanted at the low TSS site ($2.38\pm0.19\cdot10^8$ cells·h⁻¹·gDW⁻¹ or 83.71 ± 8.45 µgC·h⁻¹·gDW⁻¹) were also significantly higher ($p < 0.001$) compared to those at the site with high TSS concentrations ($1.29\pm0.22\cdot10^8$ cells·h⁻¹·gDW⁻¹ or 114.63 ± 16.06 µgC·h⁻¹·gDW⁻¹).

As for the results on pumping rates, the absence of difference between mean retention rates for control and native animals ($p>0.05$) at the two reciprocal transplant sites reflected the response of suspension-feeding activity changes in TSS concentrations rather than experimental artefact.

Figure 11: Retention rates (cells·h⁻¹·gDW⁻¹) for *Herdmania momus* and *Pinna bicolor* and *Tethya* sp for each treatment at the two reciprocal transplant sites. ★ indicates significant differences.

Table 12: Two-way ANOVA, testing for differences in retention rates (cells·h⁻¹·gDW⁻¹) for *Herdmania momus* and *Pinna bicolor* among treatments at the two reciprocal transplant sites. P values in bold indicate significance.

Source	df	*Herdmania momus*		*Pinna bicolor*	
		MS	P	MS	P
Site	1	3.243×10^{15}	**<0.001**	8.499×10^{16}	**<0.001**
Treatment	2	4.949×10^{12}	0.933	1.538×10^{14}	0.683
SixTr	2	9.943×10^{12}	0.871	6.421×10^{12}	0.984
Residuals	24	7.162×10^{13}		3.968×10^{14}	

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Figure 12: Retention rates (µgC·h^{-1}·gDW^{-1}) for *Herdmania momus* and *Pinna bicolor* and *Tethya* sp for each treatment at the two reciprocal transplant sites. ★ indicates significant differences.

Table 13: Two-way ANOVA, testing for differences in retention rates (µgC·h^{-1}·gDW^{-1}) for *Herdmania momus*, and *Pinna bicolor* among treatments at the two reciprocal transplant sites. P values in bold indicate significance.

Source	df	*Herdmania momus*		*Pinna bicolor*	
		MS	P	MS	P
Site	1	386.215	**<0.001**	9261	**<0.001**
Treatment	2	17.705	0.740	485	0.274
SixTr	2	4.602	0.924	88	0.782
Res	24	696.011		355	

Suspension feeder retention efficiencies on the three food particles exhibited significant differences between the two reciprocal transplant sites (p<0.05) but not among transplanted, control and native animals within each site (p>0.5) (Fig. 13; Table 15). *Herdmania momus* retention efficiency on bacteria was slightly higher at site A than site B (31% v 27%, respectively, p = 0.018). Retention efficiency on picoeukaryotes was significantly higher at site A (p = 0.0001; A: ~31%; B: ~9%), while values for *Synechococcus* were significantly higher at site B (p = 0.0001; A: ~28%; B: ~42%). Retention efficiencies on picoeukaryotes and *Synechococcus* were highly correlated with ascidian pumping rates (Table14). *Pinna bicolor* retention efficiencies on bacteria (A: ~1%; B: ~23%) and *Synechococcus* (A: ~22%; B: ~57%) were significantly higher at site B (p = 0.001), while values for picoeukaryotes (A: ~34%; B: ~12%) were significantly higher at site A (p = 0.0001). Retention efficiencies on picoplankton cells were highly correlated with bivalve pumping rates.

Results for *Herdmania momus* showed that the best distance-based linear model for retention efficiency on picoeukaryotes contained two predictor variables explaining 94% of the total observed variations, concentrations of TSS (92%) and bacteria (2%; Table 16). The best model for retention efficiency of *Synechococcus* contained two predictor variables explaining 60% of the total observed variation: TSS (57%) and bacteria (3%). The best model for retention efficiency of bacteria contained only one predictor variable, concentration of bacteria explaining 10% of the total observed variation.

Results for *Pinna bicolor* showed that the best distance-based linear model for retention efficiency on picoeukaryotes contained only one predictor variable, sedimentation rates accounting for 83% of the total observed variations. The best model for retention efficiency on *Synechococcus* contained two predictor variables explaining 96% of the total observed variations, sedimentation rates (95.5%) and TSS (0.5%). The best model for retention efficiency on bacteria contained two predictor variables explaining 98 % of the total observed variations, TSS (88%) and bacteria (10%).

Figure 13: Mean retention efficiency (%; ± standard deviation) by the three suspension feeders over the three food particles at the two reciprocal transplant sites. ★ indicates significant differences.

Table 14: Correlations (R^2) between suspension feeder retention efficiency on the three picoplankton cells and pumping rates of the three suspension feeders.

Retention efficiency of:	Pumping rates	
	Herdmania momus	*Pinna bicolor*
Eukaryote	0.88	0.66
Synechococcus	0.47	0.76
Bacteria	0.05	0.73

Table 15: Two-way ANOVA, testing for differences in picoplankton (picoeukaryote, *Synechococcus* and bacteria) retention efficiency for each taxon (*Herdmania momus* and *Pinna bicolor*) at the two reciprocal transplant sites. P values in bold indicate significance.

		Herdmania momus		*Pinna bicolor*	
Source	df	MS	P	MS	P
Picoeukaryote retention efficiency					
Site	1	26.762	**<0.001**	26.044	**<0.001**
Treatment	2	0.093	0.327	0.095	0.441
SixTr	2	0.078	0.385	0.031	0.762
Residuals	24	0.079		0.113	
Synechococcus retention efficiency					
Site	1	14.396	**<0.001**	27.822	**<0.001**
Treatment	2	0.085	0.865	0.001	0.972
SixTr	2	0.203	0.710	0.018	0.690
Residuals	24	0.584		0.047	
Bacteria retention efficiency					
Site	1	5.121	**0.018**	25.732	**<0.001**
Treatment	2	1.468	0.181	0.101	0.438
SixTr	2	0.894	0.343	0.111	0.407
Residuals	24	0.798		0.119	

Table 16: Results of distance based linear models (DistLM), based on modified Akaike's Information Criterion (AICc), to identify the physical and biological variables that best explain variation in retention efficiency.

Herdmania momus					
Retention efficiency	AICc	R^2 adjusted	Predictor variables	% of variability explained	Direction
Picoeukaryote	44.62	0.92	TSS	91.946	Positive
		0.94	+Bacteria concentrations	+1.737	Negative
Synechococcus	74.71	0.57	TSS	56.818	Negative
		0.60	+Bacteria concentrations	+3.2664	Positive
Bacteria	50.36	0.10	Bacteria concentrations	10.185	Negative
Pinna bicolor					
Retention efficiency	AICc	R^2 adjusted	Predictor variables	% of variability explained	Direction
Picoeukaryote	58.89	0.83	Sedimentation rates	82.865	Negative
Synechococcus	55.56	0.96	Sedimentation rates	95.529	Negative
		0.96	+TSS	+0.422	Negative
Bacteria	25.14	0.88	TSS	87.975	Negative
		0.98	+Bacteria concentrations	+10.15	Negative

Selectivity of food types by the two suspension feeders (Fig. 14) was similar among transplanted, control and native animals within each site (p>0.05; Table 17). Selectivity by *Herdmania momus* for picoeukaryotes and *Synechococcus* was differed between the two sites (p < 0.001), but was similar for bacteria (p = 0.565). Selectivity for the three types of food particles was close to zero at site A, characterised by higher TSS concentrations and sediment deposition, indicating little preference for food types. Ascidians at site B (lower TSS and sediment deposition) presented a strong positive selectivity for *Synechococcus* (0.4), almost neutral selectivity for bacteria and a highly negative selectivity for picoeukaryotes (-0.6).

Selectivity by *Pinna bicolor* for the different food types differed between the two sites (p = 0.0001). At site A, *Pinna bicolor* selectivity for bacteria was highly negative (-0.9), while values for *Synechococcus* (0.1) and picoeukaryotes (0.5) were positive, indicating that few bacteria cells were removed but the bivalves were more efficient on removing larger cells, especially picoeukaryotes. At Site B (lower TSS and sediment deposition), the selectivity for picoeukaryotes (-0.6) and bacteria (-0.2) was negative and highly positive for *Synechococcus* (0.5), indicating that bivalves feeding in less turbid water were efficient on removing *Synechococcus* and were feeding very little on large picoeukaryote cells.

Figure 14: Mean feeding electivity (± standard deviation) by *Herdmania momus*, *Pinna bicolor* and *Tethya* sp for three types of food particles as estimated by the electivity index (ε_i), where 0 represents no preference, +1 represents high positive preference and -1 high negative preference for the different food types (Chesson 1983).

Table 17: Two-way ANOVA, testing for differences in picoplankton (picoeukaryote, synechococcus and bacteria) electivity for each taxon (*Herdmania momus* and *Pinna bicolor*) at the two reciprocal transplant sites. P values in bold indicate significance.

Source	df	*Herdmania momus* MS	*Herdmania momus* P	*Pinna bicolor* MS	*Pinna bicolor* P
Electivity for Picoeukaryote					
Sites	1	27.040	**<0.001**	28.174	**<0.001**
Treatments	2	0.051	0.515	0.005	0.850
SixTr	2	0.041	0.579	0.017	0.594
Res	24	0.074		0.033	
Electivity for Synechococcus					
Sites	1	24.653	**<0.001**	25.316	**<0.001**
Treatments	2	0.013	0.927	0.090	0.532
SixTr	2	0.154	0.411	0.090	0.532
Res	24	0.167		0.139	
Electivity for Bacteria					
Sites	1	0.373	0.565	27.619	**<0.001**
Treatments	2	0.842	0.475	0.041	0.449
SixTr	2	0.317	0.752	0.062	0.300
Res	24	1.096		0.049	

4.4 DISCUSSION

In both the laboratory and the field transplant experiment, the procedural controls confirmed that any effect of altered TSS was due to the treatment rather than an experimental artefact. Both experiments clearly demonstrated a strong effect of exposure to sediment on suspension-feeding activity (filtration, retention rates and food particle selectivity).

4.4.1 Filtration activity

Due to methodological requirements, the filtration activity of selected suspension feeders could only be measured in the field transplant experiment. The elevation of TSS concentration and sedimentation rates had a strong negative influence on both *Pinna bicolor* and *Herdmania momus* pumping rates. Other studies have demonstrated a similar inverse relationship between suspended particle concentrations and the pumping rate of ascidians and bivalves (Lohrer at al. 2006; Hewitt & Pilditch 2004; Ellis et al. 2002; Petersen & Riisgård 1992; Robbins 1984).

Ascidians generally possess mechanisms to cope with elevated sediment exposure (Petersen et al. 1995, 1999; Petersen & Riisgard 1992). The main compensatory mechanisms are squirt function, reduction of the inhalant siphon diameter and a potential decrease of lateral cilia beat frequency (Petersen 2007; Armsworthy et al. 2001; Petersen et al. 1999). Because ascidian pumping rates recorded in the study were measured apart from squirting events, this mechanism cannot be associated with the reduction of pumping rates observed. Another means of regulating water filtration is by altering siphon-opening diameter. Siphon diameter constriction associated with higher sediment concentrations has been observed in Pyurid ascidians similar to *Herdmania momus* (Armsworthy et al. 2001). Klumpp (1984) observed constrictions of the inhalant siphon for the Stolidobranch ascidian, *Pyura stolonifera* triggered by increased concentrations of non-nutritious particles (Sephadex beads). However, this mechanism often accompanied by squirting account only for a minor fraction of the overall reduction in pumping rate (Petersen et al. 1999). The main mechanism responsible for the variation in pumping rates is the modification in beat frequency of latera cilia located in the oral siphon of the ascidians (Petersen 2007). The decreased in cilia beat frequency at high particle concentration was observed for the Enterogona ascidians *Ciona intestinalis* (Petersen et al., 1999). The presence of complex sensory lateral cilia in Pyurid ascidians (Caicci et al. 2010) suggest that similar mechanism is likely to occur for *Herdmania momus*. Whereas all the above physiological mechanism considerably reduces the amount of inorganic particles ingested by ascidians, it also reduces the volume of water filtered (Mackie et al. 2006; Armsworthy et al. 2001; Petersen et al. 1999).

Several studies on suspension feeding bivalves, *Cerastoderma edule* (Widdows & Navarro 2007), *Mytilus edulis* (Petersen et al. 2004), *Crassostrea gigas* (Troost et al. 2009; Barillé et al. 1997), *Perna canaliculus* (Hawkins et al. 2001), *Mercenaria mercenaria* (Grizzle et al. 2001) and *Atrina*

zelandica (Coco et al. 2006) demonstrated reduction of pumping rates in response to elevated total suspended particulates concentrations. However, for "mussel" form bivalves, such as *Pinna bicolor*, a controversy emerges regarding the mechanisms behind reduced pumping rates at high particle concentrations. Some interpret the reduction as a result of the fullness of the gut and caused by reduction in ciliary beat frequency (Riisgård 2001) as also found in ascidians (Petersen et al. 1999), while others consider suspension feeding being controlled by physiological compensations (Bayne 1998). Despite, this controversy has not yet been solved, in the light of the results from the transplant experiment, the hypothesis of a reduction of pumping rates triggered by the satiation of the digestive system appeared unlikely. Indeed, at the site with higher TSS and sedimentation rates, not only *Pinna bicolor* exhibited lower pumping rates, but also a reduction of retention rates. From this, measured low pumping rates can be interpreted as the consequence of physiological regulation (Bayne 1998). Due to its relatively large water filtering structure and open mantle cavity, *Pinna bicolor* is able to maintain high pumping rates at low energetic cost (Bernard & Noakes 1990). However, the morphology of the pallial cavity increases the vulnerability to the deposition of sediment particles (Butler et al. 1993). To compensate this disadvantage, *Pinna bicolor* possesses specialized adaptation for cleaning the inhalant and exhalant chambers, which composed the mantle cavity (Yonge & Thompson 1976). Indeed, suspension feeding bivalves, like *Pinna bicolor*, are able to regulate the ingestion of particles by production of pseudofaeces, whereby particles are trapped in the mucus and expelled through the exhalent siphon, before they reach the mouth. As a consequence, inorganic particles are represented at a higher concentration in the pseudofaeces than in the cleared suspension (Zhang et al. 2010; Dutertre et al. 2009). Typically, bivalves pumping rates increase up to a 'threshold' concentration, above which they decrease, as energy expended on compensatory mechanisms increases (Kiorboe & Mohlenberg 1981). The threshold of sediment concentration above which filtration activity decreases varies among bivalve species (Ellis et al. 2002; Hawkins et al. 1999). Although the results here do not allow the threshold for *Pinna bicolor* to be determined, it appeared from the reduced pumping rates that sediment deposition rate (8.43 ± 1.08 g·cm^{-2}·month^{-1}) and TSS concentration (27.25 ± 1.44 mg·l^{-1}) at the site with high sediment exposure exceeded this threshold.

The influence of increased sediment exposure on pumping rates did not depend on *Pinna bicolor* and *Herdmania momus* history of exposure to such levels. Two hypotheses were considered for this study. Firstly, under stress conditions, such animals may be able to rapidly compensate for chronic and acute changes in TSS concentrations and sediment deposition rates. Alternatively, they may be preadapted to deal with higher sediment loads by, for example, increasing the size of labial palps (Kiorboe & Mohlenberg 1981). For instance, Dutertre et al. (2009) found that oysters *Crassostrea gigas* presented greater size of labial palps at site with high sediment

exposure. This adaptive characteristic allowed oysters to tolerate sediment exposure levels above the feeding cessation thresholds previously determined experimentally. Such evolutionary adaptations could occur for *Pinna bicolor* and *Herdmania momus.* Although the present study was not designed to examine differences in evolutionary adaptive traits, the long-term preadaptation hypothesis was refuted by the data from the transplant experiment. In the short term, transplanted specimens exhibited similar suspension feeding response than naturally occurring animals. Overall, the response of pumping rates to increased sediment exposure is most likely related to physiological regulations by the two suspension feeders.

The concentration of picoplankton cells explained very little of the variation in pumping rates but were nonetheless correlated, indicating a potential influence of food source concentrations on suspension feeding activity. Indeed, concentrations of picoeukaryotes accounted for 3% of the variation in pumping rates observed for *Herdmania momus* and concentrations of *Synechococcus* explained 1% of the variation observed for *Pinna bicolor*. The relation between the availability of certain food particles and pumping rates depended on the feeding ecology of the two suspension feeder species.

4.4.2 Food particles retention and selection

The laboratory experiment indicated high variations in food particle retention rates between the three suspension feeder species depending on the TSS concentrations they were exposed to.

Herdmania momus total retention rates were significantly higher for the treatment with high sediment exposure while rates for treatments with lower TSS concentrations (medium and low) were considerably reduced. These results were unusual considering ascidians' sensitivity to high concentrations of suspended particles (Petersen & Riisgard 1992; Petersen et al. 1995, 1999). Moreover, results from the transplant experiment exhibited the opposite trend, with ascidians removing 50% less particles at the site with elevated sediment exposure. The difference between feeding experiment and *in-situ* transplant experiment may have resulted from the absence of sediment deposition caused by the constant water recirculation during the laboratory experiment. In any case, interpreting the results of the field and laboratory experiments is complicated especially since many suspension feeders, such as ascidians, demonstrate the ability to maintain constant retention rates with increasing suspended sediment particle concentrations (Armsworthy et al. 2001; Petersen & Riisgård 1992; Klumpp 1984; Jørgensen 1983). Some species may even benefit from a diet supplemented with suspended sediments through the utilization of sedimentary organics, increasing absorption efficiency of smaller particles embedded in the sediment, or potentially by using sediment to grind food particles in the stomach (Armsworthy et al. 2001; Bricelj & Malouf 1984). Whereas ascidians are able to cope with increased TSS concentrations, various studies demonstrated that sediment deposition is a major environmental stressor producing significant sub-lethal effects on ascidians (Lohrer et al. 2006). Despite contradictory

results between the laboratory and transplant experiment, the opportunity given by the field experiment to estimate the amount of carbon removed, suggested that the ascidian might be able to optimise its food intake by adopting an energetically richer diet (e.g. carbon enrichment) while removing less food particles. Indeed, although the ascidians removed less carbon at the site with the highest sediment exposure, the discrepancy between the two sites (20%) was less important compared the amount of particles removed (50%). Whereas *Herdmania momus* diet in both the laboratory and field experiment was comparable of those reported from previous studies on ascidians (Lesser & Slattery 2015; Petersen 2007; Pile 2005; Armsworthy et al. 2001; Ribes et al. 1999), the results indicated a strong relationship between sediment exposure and particle selection and retention (Table 12&17). Although ascidians are often described as non-selective suspension feeders, the possibility of particle selection has been previously reported (Armsworthy et al. 2001; Seiderer & Newell 1988; Klumpp 1984). The retention efficiencies and selectivity values for the ascidian in low TSS conditions (site B and "low" treatment) indicated that it was able to select food, with *Synechococcus* cells more efficiently removed from the water column than picoeukaryotes (e.g. *Isochrysis*) and bacteria. When exposed to the highest sediment exposure, *Herdmania momus* appeared to possess very little preference among food types. The physiological mechanism behind particle selection and its relation with increased sediment exposure is unclear. However, whatever the mechanism, *Herdmania momus* appears to have some ability to extract energy from a low quality diet (increased amount of inorganic particles and decreased amount of food particles) by feeding on a broader range of food particles (e.g. picoeukaryotes and bacteria) to optimise its food intake. These findings that corroborate those found in chapter 3 could explain the presence of the ascidian in the vicinity of the wash plant, where sediment exposure is maximum (see Chapter 1).

As for its pumping rates, increased sediment exposure had a strong negative influence on *Pinna bicolor* retention rates. The linear modelling indicated that variation in the bivalve's retention efficiencies were mainly explained by sediment deposition and TSS concentrations. During the laboratory experiment, total retention rates were lower under high sediment exposure. The transplant experiment validated this finding with bivalves removing 45% less picoplankton cells at the impacted site (site A). Despite the lower pumping and retention rates under high TSS concentrations, the amount of carbon removed by *Pinna bicolor* was around 30% higher when exposed to high sediment exposure. This implies a change in the bivalve diet to compensate the reduced amount of food particles retained. If under high sediment exposure *Pinna bicolor* food particle selectivity shifted from *Synechococcus* to a predominant selection of larger picoeukaryote cells of better nutritive quality (organic carbon enrichment). Like many other suspension feeding bivalves, *Pinna bicolor* is capable of particle selection and to change is feeding depending on the quantity and quality of available food particles (Leal et al. 2008; Rossi & Herman 2004; Ropert

& Goulletquer 2000). Previous studies on bivalves reported the use of particle selection to compensate the dilution of food sources within inorganic particles (Kiorboe & Mohlenberg 1981). For instance, Safi et al. (2007) reported changes in the ability of the pinnid bivalve *Atrina zelandica* to preferentially select food particles. In this case, larger diatoms were preferentially removed or ingested, compounding the effect of increased inorganic seston concentrations. Through this compensatory mechanism, particles of higher nutritive quality are preferentially ingested over those of lower quality. Overall, *Pinna bicolor* was able to compensate the reduction of pumping rates, affecting energy allocation, by retaining food particles of higher nutritive value.

As *Tethya* sp transplants failed, food particle retention and selection were only recorded during the laboratory experiment. The sponge diet and the range of particles retention efficiencies were similar to of those reported for other demosponges (Hanson et al. 2009; Ribes & Coma 2005; Pile et al. 1997). At all sediment concentrations, retention efficiencies for *Isochrysis*, *Synechococcus* and bacteria remained relatively similar, indicating little selectivity by the sponge. Previous studies (Topçu et al. 2010; Pile & Young 2006; Duckworth & Brück 2006) also demonstrated that sponges are not able to select food particles. However gross retention efficiencies were significantly higher for the treatment with the high TSS concentrations. Thus increased TSS might have a positive effect on *Tethya* feeding activity. This positive influence might be explained by the extensive surface for the colonization of microorganisms provided by suspended sediment (Muschenheim et al. 1989) and/or the role played by "marine aggregates" comprised of picoplankton and bacteria incorporated into larger inorganic particles (Muschenheim et al. 1989). There is growing evidence that marine aggregates can contribute to suspension feeder diets (Newell et al. 2005). Also aggregates have much higher settling rates than individual picoplanktonic cells, enhancing the amount of food particles that reach the seabed (Newell et al. 2005; Waite et al. 2000; Muschenheim et al. 1989). In addition to an increased food availability, other studies reported that demosponges inhabiting highly turbid habitats could develop behavioural adaptations to expel sediment particles (Elliott & Leys 2007; Ellwanger et al. 2007; Nickel & Brümmer 2004; Reiswig 1971). However, the causal parameters explaining the benefit gained from sediment exposure observed here remained unanswered.

Although the results from the laboratory were relatively similar to those obtained from the transplant experiment and field survey (e.g. chapter 3), the decrease in food concentrations during the experiment may have introduced experimental bias. It is well-known that there is a species-influence of food concentration on suspension-feeding activity. Therefore, decreases in food concentrations in closed tanks may influenced differently the feeding activity of the 3 suspension feeder species. While only short term (3h) experiments were conducted to limit the effect of food concentration on feeding activity, its influence on retention rates and food particle selection has not been assessed. Despite many other studies have used closed systems (Ellis et al. 2002;

Riisgard 1988), flow through systems appeared to represent a better alternative to avoid variations in food particle concentration. Consequently, further investigation under flow through conditions need to be conducted to validate findings from the laboratory feeding experiment presented in this study.

4.5 CONCLUSION

This study demonstrated that increased TSS and sediment deposition influence distinctively the feeding activity of three suspension feeders. Response variations to elevated sediment exposure among species were due to differences on food particle capture mechanisms and feeding strategies. Results suggested that suspension feeding (pumping rates, food particle retention and selection) of *Herdmania momus* and *Pinna bicolor* are adaptable to changes in environmental conditions, unlike that of *Tethya* sp, which remains invariable.

At high levels of sediment exposure, the reduction *Herdmania momus* pumping rates may be the results of physiological regulations (e.g. reduction of the inhalant siphon diameter and the decrease of lateral cilia beat frequency) to diminish the number of inorganic particles ingested by ascidians. Also, *Herdmania momus* appears to have the ability to extract energy from a low quality diet, counteracting the reduction of filtration activity. Despite particle selection by ascidian has not been yet conclusively established in the literature, results suggested that *Herdmania momus* optimised its food intake by feeding on a wider range of food sources instead of preferentially selecting *Synechococcus* cells.

The reduction of pumping rates measured for *Pinna bicolor* may be also attributed to physiological compensations employed to minimize the risk of clogging the filtering apparatus while experiencing high levels of sediment exposure (Bayne 1998; Yonge & Thompson 1976). Additionally, *Pinna bicolor*, possess the faculty to sort and select particles and modify is diet depending on the quantity and quality of available food particles (Leal et al. 2008; Rossi & Herman 2004; Ropert & Goulletquer 2000). Thus, *Pinna bicolor* was able was able to increase its organic carbon intake by shifting particle selection from *Synechococcus* to picoeukaryotes, compensating the reduction of pumping rates and food particle intake.

Despite *Tethya* sp pumping rates could not be measured, results demonstrated that sediment exposure had no negative influence on its feeding ecology. Conversely, the sponge appeared to benefit from elevated sediment concentrations, as food particle retention efficiency increased, potentially related to the lack of food selectivity in this demosponge.

Overall, the feeding ecology of the three suspension feeders partly explained why a system experiencing increased sediment exposure could support an unexpectedly large numbers of suspension-feeders (cf. chapter 1). The present study corroborates the hypothesis, formulated in chapter 3, that some of the suspension feeders could cope with high sediment exposure through

physiological compensations associated with their specific feeding mechanisms and strategies. Furthermore, the data from the transplant experiment demonstrated that the influence of increased sediment exposure on the suspension feeding activity of *Pinna bicolor* and *Herdmania momus* did not depend on their history of exposure to such levels, but on their faculty to rapidly compensate for chronic and acute changes in TSS concentrations and sediment deposition rates.

Even though increased sediment exposure is unlikely to cause mortality, its influence on suspension-feeding activity may alter ecosystem-level processes. As *Herdmania momus, Pinna bicolor* and *Tethya* sp filter large volumes of water and particulate matter, decreased pumping rates could alter benthic-pelagic coupling (Ellis et al. 2002) and changes in food particle retention and selection could modify the quality and quantity of seston available for the local macrobenthic community (Lefebvre et al. 2009; Dubois et al. 2007; Safi et al. 2007).

CHAPTER 5
General discussion

The expansion of marine aggregates exploitation in recent decades placed significant pressures on shallow coastal ecosystems and their benthic assemblages (Erftemeijer et al. 2012; Cooper et al. 2011; Erftemeijer & Lewis 2006). Among other anthropogenic activities such as land reclamation, road building, logging, sand mining and port maintenance, the marine aggregates industry contribute to the discharge of a great amount of sediment in coastal waters (Ashraf et al. 2011). As sediment loads end up sooner or later on the seabed, this pressure results in major changes for sessile benthic macrofauna. Being immobile, and due to their active participation in transfers of organic and inorganic material between the water column and the seabed, sessile suspension feeders are among the organisms most likely to be negatively impacted by increased sediment exposure. Sediment exposure is defined by the concentrations of sediment particles in suspension in the water column and the amount of sediment depositing on the seabed. This study set out to evaluate the influence of increased sediment deposition and suspended sediment concentration on suspension-feeder assemblages. Despite the large body of literature describing the potential effects of sediment exposure on coastal marine ecosystem or commercial species of suspension feeders (Kach &Ward 2014; Kang et al. 2009; Carballo 2006; Ellis et al. 2002; Armsworthy et al. 2001), few have examined the impact of sediment on co-occurring species or whole assemblages.

As most of this research was conducted at a location where the suspension-feeder assemblages experience a gradient of sediment and organic matter deposition, it was possible to test the hypothesis that high sediment exposure would influence suspension feeder composition, both taxonomic and functional. The findings did not strongly support this hypothesis. To the contrary, the results revealed that suspension-feeder assemblages were strongly resistant to high sediment exposure and that some species could potentially display compensatory mechanisms. As suspension feeders extract organic particles from the water column, high concentrations of inorganic particles could directly interfere with food intake, potentially affecting their growth and survival. Thus, the degree to which increased suspended sediment and sediment deposition influence suspension feeders is likely to be species specific and depend on their feeding activity and the resilience of feeding mechanisms.

Leading on from the above, this research tested a second hypothesis; that the influence of high sediment exposure on suspension-feeding activity would vary between species with different feeding structures and strategies. Suspension-feeding activity may be regarded as a step by step process (Riisgård & Larsen 2010; Labarbera 1984; Shimeta & Jumars 1991), in which the first step consists of the organisms encountering suspended food particles generated by the activity of

filtration (Jørgensen 1983). The second step is food particle retention, which depends on various capture mechanisms, such as cirri trapping mechanisms for bivalves, collar sieving in sponges, and mucus-net suspension-feeding in ascidians (Riisgård & Larsen 2010; Ostroumov 2005; Dame et al. 1998). The third step is the ingestion of food particles (Hanson et al. 2009; Pile 2005; Armsworthy et al. 2001). This research investigated the influence of sediment exposure on filtration activity, food source and food retention of three species of suspension feeders with distinct feeding structures and strategies: the solitary ascidian *Herdmania momus*, the pteroid bivalve *Pinna bicolor* and the demosponge *Tethya* sp. *Herdmania momus* is one of the most commonly encountered species of ascidians with a pantropical and subtropical distribution (Lambert & Lambert 1987). The family Pinnidae, which included *Pinna bicolor*, is widely distributed in the Indo-Pacific region from south eastern Africa to Melanesia and New Zealand (Butler & Keough 1981), but also in Mediterranean and American waters (Butler et al. 1993; Munguia 2004; Rosewater 1961). The family Tethydae is widely distributed in the tropical and warm temperate regions, but the genus *Tethya* has also some cold temperate and one arctic representative (Sarà 2002). These widespread distributions and the variety of feeding mechanisms involved make the results of this study transferable to a wide range of suspension-feeder assemblages.

This general discussion details the key findings of the thesis by describing the relationship between increased sediment exposure and the composition of suspension-feeder assemblages (Chapter 1), the influence of suspended sediment and sediment deposition on pumping rates (Chapter 2&4), the contribution of food sources to suspension feeder diets (Chapter 3) and food particle retention and selection (Chapter 2, 3, 4).

5.1 Understanding the influence of increased sediment exposure on suspension feeder's composition

Increased concentrations of suspended sediment potentially harm suspension feeders by decreasing primary production through light attenuation and, therefore, limiting food resources (Gartner et al. 2010), but also physically damaging feeding apparatus through mechanical abrasion (Shin et al. 2002; Ayukai & Wolanski 1997). In addition to these pressures, increased sediment deposition may result in smothering and/or clogging of the feeding apparatus of certain species (Carballo 2006; Skilleter et al. 2006; Blanchet et al. 2005; Macdonald & Perry 2003). Depending on the nature of the deposited sediment, the substrate suitability for specific taxa may also be altered (Harrison et al. 2007; Skilleter et al. 2006; Carballo 2006; Blanchet et al. 2005; Macdonald & Perry 2003). From these disturbances, impacts on the health and abundance of benthic suspension feeders potentially induces a modification of assemblages composition (Szostek et al. 2013; Bolam 2011; Ellis et al. 2002; Thrush & Dayton 2002; Long et al. 1995).

In the study area, there was no strong gradient in biomass and species composition to match the local patterns of sediment deposition. It is possible that this reflected a broad impact of sediment across all sites, even at the lowest sediment loading. However, the inclusion of two additional sites (Marmion Lagoon and Garden Island) which were well-removed from any influence of anthropogenic sediment loading and are representative of local water quality conditions, provided confirmation that the suspension feeder composition was similar at these 'unaffected sites' and the study area reference sites (far from the wash plant). This results confirmed that sediment plumes at the study site did not affect the entire study area, that most distant sites could be considered as reference sites and, therefore, that the lack of difference in assemblage structure was not due to a consistent effect of sediment across all sites.

The findings indicate that suspension-feeder assemblages at this site were strongly resistant to high sediment exposure. The nature of the sediment (large dominance of the sand fraction) and the intensity of sedimentation rates near the wash plant appeared to exert no negative effect on the suspension-feeder assemblages. Despite experiencing high levels of sediment exposure, all but rare species were present, some in greater quantities. Consequently, it appeared that suspension feeders are able to maintain feeding and respiration functions to meet their basic metabolic requirements. However, some dissimilarities were still observed between sites near the most impacted area (sites located 200 and 500 m away from the wash plant) and those further away (800 and >1000 m away from the wash plant), which indicate a species-specific response to sediment load rather than a uniform whole-assemblage response. Almost 60% of the dissimilarities between sites near to and distant from the sediment source was explained by higher abundance and biomass of the ascidian *Herdmania momus*, the bivalve *Pinna bicolor* and the sponges *Coelosphaera* sp and *Tethya* sp at sites near the wash plant. It is plausible, therefore, that these suspension feeders may actually benefit from the sediment exposure. The deeding mechanisms of *Herdmania momus* and *Pinna bicolor*. (mucus-net filter-feeding species and cirri-trapping, respectively) allow active rejection of overload in inorganic particles through squirting activity, pseudofaeces, mucus production or ciliary action (Kach & Ward 2008; Armsworthy et al. 2001). Furthermore, these organisms have an erect form which may make them more resilient to sediment deposition than prostrate suspension feeders, which accumulate more sediment on their surfaces (Rogers 1990). Conversely, other species such as the ascidians *Polycarpa veridis* and *Phalusia obesa* were more abundant at sites away from the peak of sediment perturbation. Under those circumstances a trade-off can arise between negative and positive effects depending on the sensitivity of each specie (Skilleter et al. 2006 ;Kruger et al. 2005). A major finding of this study, then, is that the form and feeding mechanism of suspension feeders may dictate the response to changes in sediment loading, making it difficult to predict whole-assemblage responses.

Some of the suspension feeders within the study area clearly possess physiological and behavioural mechanisms that allow them to cope with high sediment exposure. Those mechanisms included modification of feeding behaviour (e.g. use of a different portion of the resource pool), reduction of the diameter of their feeding apparatus and/or adaptive mechanisms to expel sediment settled on them, through mucus production, ciliary action or polyp expansion (Pile 2005; Armsworthy et al. 2001; Gili & Coma 1998). It was hypothesized that physiological and behavioural mechanisms explained the presence of suspension feeders at sites highly impacted by increased sediment exposure. Because compensatory mechanisms found in the literature appeared to be closely tied to suspension feeder's pumping and retention rates, increased sediment exposure is likely to influence suspension feeder energy allocation and levels. Thus, the resilience of suspension feeders to sediment exposure is more likely to depend on regulation mechanisms associated with specific feeding structures and strategies. Based on this premise, it became necessary to test whether the influence of high sediment exposure varied between co-occurring species presenting distinct feeding ecology and compensatory mechanisms.

As there is growing evidence that species functional traits link changes in species composition and shifts in ecosystem processes (Villéger et al. 2010), this study also considers functional groups of species, based on their feeding behaviour and food-particle capture mechanism, in addition to the traditional taxonomic approach. Preliminary work on this project intended to examine the functional relationships between suspension feeder taxa and environmental characteristics. However, due to the low taxonomic diversity (15 macro suspension feeder taxa) and strong similarities of feeding habits among species, this investigation was limited by the variety of functional traits and subsequent modalities. A complete understanding on how increased sediment exposure influence suspension feeder functional diversity and explain changes in ecosystem processes would have required to broaden the amount of functional traits considered (Dolédec et al. 2006). For example, additional biological traits such as body size, life cycle, morphology, position of the structure to collect particles, method of particle transport could have been included (Gayraud et al. 2003). To supplement the variety of modalities for each trait, the study would have required to include not only macro-epibenthic suspension feeders, but also epibionts and infaunal species to broaden the diversity of functional traits. Furthermore, multiple-trait-based approach requires extensive biological measurements made across a broad range of spatial and temporal scales (Chevene et al. 1994). Limitations from the thesis data set and the spatial scale of the study area did not allow accurate assessment of the influence of sediment exposure on suspension feeders functional attributes. Consequently, the present research could be advance in the future to develop a reliable method to assess the influence of increased sediment exposure on ecosystem processes based on suspension feeder functional traits.

5.2 Understanding the influence of sediment exposure on pumping rates

The first step in suspension feeding consist of the encounter of the organisms with suspended food particles generated by the filtration of the surrounding water (Jørgensen 1983). The monitoring survey (Chapter 3) and transplant experiment (Chapter 4) investigated the influence of increased sediment exposure on the filtration activity of the solitary ascidian *Herdmania momus*, the pteroid bivalve *Pinna bicolor* and the demosponge *Tethya* sp (the latter was not included in the transplant experiment). These three suspension feeders displayed contrasting patterns in the rate of filtration at different levels of sediment exposure, but this can be reconciled with an understanding of their distinctive filtering apparatus. The solitary ascidian *Herdmania momus* actively captures seston particles through a mucus net, the Pterioid bivalve *Pinna bicolor* operates a cirri trapping mechanisms to actively gather food particles and the demosponge *Tethya* sp combines active and passive feeding using a collar sieving structure.

Both the field survey and the transplant experiment demonstrated that *Herdmania momus* had lower pumping rates at sites with higher suspended and deposited sediment concentrations. Sedimentation rates and TSS appeared to be the main factors explaining the variations of filtrations rates confirmed by the multiple linear regression models testing for the association between pumping rates and environmental variables. Linear models indicated that more than 80% of the pumping rate variations were explained by changes in levels of sediment exposure (total suspended sediment or sedimentation rates). Ascidians are generally sensitive to high sediment exposure and several studies have demonstrated an inverse relationship between their pumping rates and the concentrations of suspended sediment (Jones et al. 2011; Lohrer et al. 2006; Petersen et al. 1999; Petersen et al. 1995; Petersen & Riisgard 1992). This reduction of filtration activity may be attributed to physiological compensation (Armsworthy et al. 2001). While increased squirting and the constriction of the inhalant siphon are often associated with the reduction of pumping rates, the modification in beat frequency of latera cilia located in the oral siphon of the ascidians represents the main compensatory mechanism responsible (Petersen 2007). Whereas all the above physiological mechanisms considerably reduce the amount of inorganic particles ingested by ascidians, they also considerably reduce the volume of water filtered (Mackie et al. 2006; Armsworthy et al. 2001; Petersen et al. 1999).

Increased sedimentation rates and TSS had similar adverse effects on pumping rates of *Pinna bicolor*. This reduction of pumping rates in response to elevated sediment concentration has been previously observed in several studies on suspension feeding bivalves (Troost et al. 2009; Widdows & Navarro 2007; Coco et al. 2006; Petersen et al. 2004; Hawkins et al. 2001; Grizzle et al. 2001) and appeared triggered by physiological mechanisms of regulation (Bayne 1998). Due to its relatively large water filtering structure and open mantle cavity, *Pinna bicolor* is vulnerable

to the deposition of sediment particles (Butler et al. 1993). Pteroid bivalves, like *Pinna bicolor,* possess specialized adaptations to cope with increased suspended sediment and sediment deposition (Yonge & Thompson 1976), including fast growth to rise above the sediment, increased size of labial palps to select and sort particles, and pseudo-faeces production (Hewitt & Pilditch 2004; Kiorboe & Mohlenberg 1981). *Pinna bicolor* is able to regulate the ingestion of particles by production of pseudofaeces, whereby particles are trapped in the mucus and expelled through the exhalent siphon, before they reach the mouth. As a consequence, inorganic particles are represented at a higher concentration in the pseudofaeces than in the cleared suspension (Zhang et al. 2010; Dutertre et al. 2009), which minimize the risk of clogging of the inhalant and exhalant chambers that compose the mantle cavity. The energetic cost of those adaptations is balanced by a reduction of energy dedicated to filtration activity (Kiorboe & Mohlenberg 1981), which may explain the reduced pumping rates recorded for *Pinna bicolor* at sites with high sediment exposure. Thresholds of sediment concentration, above which filtration activity decreases, vary among bivalve species (Ellis et al. 2002; Hawkins et al. 1999). It appeared that individuals experiencing highest sediment exposure (up to 40 mg$\cdot$l^{-1} of TSS and 10 g$\cdot$cm$^{-2}\cdot$month^{-1} of sediment deposition) reached this threshold.

For *Tethya* sp, 80 % of the variation in pumping rates were explained by sedimentation rates and total suspended sediment, with generally decreasing pumping rates from sites exposed to elevated sediment exposure toward non-impacted sites. This finding contrasted with the results of studies on similar sponge species, where decreased pumping rates were observed at high sediment concentrations (Bannister et al. 2012; Tompkins-MacDonald & Leys 2008; Gerrodette & Flechsig 1979). However, Reiswig (1971) reported a relative tolerance to high turbidity in coastal waters for *Tethya crypta* and other sponges can regulate their pumping rates in response to environmental cues (Stabili et al. 2006; Simpson 1984). Indeed, some demosponges like *Tethya* may cope with high sediment exposure by developing periodic contraction of the exhalant system to effectively expel debris (Elliott & Leys 2007; Ellwanger et al. 2007; Nickel & Brümmer 2004). However, there is no evidence in the literature that demonstrate an increase of filtration activity with high sediment exposure. Data from the study suggest that sponges have a high degree of control over their filtration activity, though it is unclear whether the increased pumping rates in higher sediment load conditions are the result of adaptations or the consequence of a greater availability of food particles in the water column.

Albeit to a much lesser extent than sediment exposure (suspended and deposited sediment), the multiple linear regression models revealed that the concentrations of food particles influenced filtration activity of the three species. The relationship between food particle concentrations and filtration activity is contingent upon the distinctive filtration and particles capture mechanisms among species. Lateral cilia on gill filaments of suspension-feeding bivalves, cilia on either side

of the stigmata in ascidians and flagella in choanocytes of sponges allow suspension feeders to modulate their filtration activity in order to optimise food acquisition and maintain metabolic energy requirement (Riisgård & Larsen 2010,1995). This statement could partly explain the presence of *Herdmania momus* and *Pinna bicolor* at sites with elevated sediment exposure, despite the significant reduction of pumping rates. Based on this hypothesis, this project investigated the potential faculty for some species to optimise their food intake from a reduced pool of food particles by optimising. This work will be discussed in detail in a subsequent section of this chapter.

Overall, the three suspension feeders filtered large volumes of water. At sites exposed to natural sedimentation regimes mean pumping rates of *Herdmania momus* were 2.00 ± 0.16 $l \cdot h^{-1} \cdot gDW^{-1}$, which was similar to other solitary ascidians (Petersen 2007; Armsworthy et al, 2001). Mean pumping rates of *Pinna bicolor* were 7.26 ± 0.50 $l \cdot h^{-1} \cdot gDW^{-1}$, which was relatively similar to the mean pumping rate of the con-generic *Pinna nobilis* (5.99 $l \cdot h^{-1} \cdot gDW^{-1}$; Vafidis 2014). Mean pumping rates of *Tethya* sp were 3.31 ± 0.40 $l \cdot h^{-1} \cdot gDW^{-1}$, which is in the range of values reported for *Tethya* and other demosponges (Mentel et al. 2014; Maldonado et al. 2012; Stabili et al. 2006; Thomassen and Riisgard 1995; Reiswig 1981; Reiswig 1975; Reiswig 1974). The pumping rates of the three suspension feeders were highly influenced by increased sediment exposure near the wash plant. Pumping rates decreased by 48% for ascidians and 23% for bivalves, while those for the sponge increased by 75%. Physiological compensation mechanisms described in the literature on suspension feeders might explained the variations of pumping rates observed (Ellwanger et al. 2007; Armsworthy et al. 2001; Bayne 1998). These physiological mechanisms may considerably reduce the amount of inorganic particles ingested by suspension feeders, but, for the ascidians and bivalves, they also considerably reduce the volume of water filtered and the subsequent availability of food particles (Ellis et al. 2002; Armsworthy et al. 2001). The variations of filtering activity in response to sediment exposure fostered the need to better understand the functioning of physiological mechanisms among species presenting distinct filtering apparatus and pump designs. In an attempt to improve the interpretation of the filtering activity measurements presented in this research, endoscopic technique could provide with useful insights on specific physiological adaptations during events of increased sediment exposure. Endoscopic examination has proven to be an appropriate method to examine the functioning of specific filtering structures without surgically altering suspension feeders (Armsworthy et al. 2001; Beninger & St-Jean 1997; Ward et al. 1993). Such method would allow to identify the role of specific organs, such as latera cilia located in the oral siphon of ascidians (Petersen et al. 1999), pallial organs in bivalves (Dutertre et al. 2009) or exhalent oscula in sponges (Ellwanger et al. 2007), to adapt filtration activity in response to changes in sediment exposure. Understanding physiological mechanisms behind filtering processes is crucial because variations of pumping rates may significantly alter

benthic-pelagic coupling and the quality and quantity of seston available for the local macrobenthic community (Hewitt & Pilditch 2004; Ellis et al. 2002; Dame et al. 1998; Cloern 1982).

5.3 Understanding the influence of sediment exposure on the relative contribution of food sources to suspension feeder diets

The second step of suspension-feeding activity consists of the retention of food particles through capture mechanisms, such as cirri trapping mechanisms for bivalves, collar sieving in sponges, and mucus-net suspension-feeding in ascidians (Riisgård & Larsen 2010; Ostroumov 2005; Dame et al. 1998). This research examined the influence of increased sediment exposure on the contribution of the potential food sources to suspension feeder diets using stable isotopic analysis (Chapter 3).

At the study location, three potential food sources were identified: suspended particulate organic matter (POM), detrital organic matter and sedimentary organic matter within the seabed. $\delta13C$ for POM suggests that it largely originates from phytoplanktonic microalgae, which have $\delta13C$ values between -23 and -21‰ (Moncreiff & Sullivan 2001). While macroalgae detritus and benthic microalgae can be an important component of the suspended particulate organic matter pool (POM) present in in the region (Hyndes & Lavery 2005; Smit et al. 2005), this two organic matter sources appeared to be absent from the study area. Detrital seagrass leaves appeared to be the only source of detritus present in the study area. However, isotope ratios for seagrass detritus were significantly distinct from those for POM suggesting that detrital leaves did not contribute to the suspended particulate organic matter pool. $\delta^{13}C$ of the sedimentary organic matter was significantly lower that of seagrass detritus, but slightly higher than that that of POM. This organic matter was therefore likely originated from the organic matter in the water column rather than a benthic input. However, Smit et al. (2006) recorded low sedimentary organic matter content in seagrass beds in the vicinity of the study area, which indicated a relatively low accumulation of POM in the sediment. Since the sediment discharged from the wash plant was associated with an increase of organic matter deposition, this additional source of organic matter could potentially replenish the sediment organic matter pool within the study area. The origin and nature of the sediment dredged offshore might explained the lower $\delta13C$ and $\delta15N$ observed. Further isotopic analysis on the sediment exploited by the wash plant could help to determine the exact nature of the sedimentary organic matter.

The isotopic signatures of the three suspension-feeding species were significantly different, indicating different diets. Survival of multispecific assemblages of benthic suspension feeders depend on the faculty for each specie to maximize their food particles capture efficiency, which involve the partitioning of available food resources to limit competition on similar prey items (Gili & Coma 1998). Sharing a same habitat, the variety of feeding structures and strategies

amongst the different suspension feeders was expected to lead to dissimilarities in their diets in order to partition the food resource (Kang et al. 2009; Lefebvre et al. 2009; Pile 2005). Picoplankton populations, which account for most of the local pool of POM (Koslow et al. 2008; Hawkes 2006), were likely the main contributors to suspension feeder diets. The bivalve and sponge were also able to exploit, to varying extents, sedimentary organic matter, but there was no clear evidence of exploitation of seagrass detritus by any of the three species. Therefore, differences in the three suspension-feeder diets were potentially caused by species grazing on different types of picoplankton cells, but also the degree to which sedimentary organic matter contributed to their diets. Several studies argued that drift macrophyte might provide an alternative food source to suspension feeders (Miller & Page 2012; Smit et al. 2006; Smit et al. 2005; Lenanton 1982). Despite macroalgae detritus could not be observed in the study area, transient import of particles originated from drifting algae may occur in the study area. Therefore, future investigation could include this chronic source of organic matter to test its potential contribution to suspension feeder diets.

Increased sediment exposure had no influence on the isotopic signatures of *Herdmania momus* and *Pinna bicolor*. Presumably, the relatively low trophic plasticity associated with their specific feeding mechanisms and strategies allowed the conservation of relatively consistent diets. These findings corroborate the hypothesis that physiological and behavioural mechanisms may explain the presence of suspension feeders at sites with high sediment exposure. Conversely, spatial variations of the isotopic signatures of the sponge *Tethya sp* indicated changes in the food resource exploitation related to sediment exposure levels. Episodic organic matter inputs associated with sediment loading potentially provided an additional source of food to the sponge. This finding consolidate the hypothesis mentioned in chapter 1 about the possibility for some suspension feeder species to benefit from a diet supplemented with sediment. This hypothesis could explain the total absence of *Tethya* sp from reference sites in 2014.

Overall, the influence of sediment exposure on suspension feeder diets appeared to be to be species specific and dependent on the nature of the sediment. Feeding ecology of the three suspension-feeders partly explained why a system experiencing increased sediment exposure could support an unexpectedly large numbers of suspension-feeders. Findings from this study were subsequently implemented by the examination of the different fraction picoplankton exploited by the co-occurring suspension feeders. However, this study focusing on food resource exploitation could be implemented by further investigation on the trophic contribution of sedimentary organic matter. Muschenheim et al. (1989) demonstrated that suspended inorganic material can enhance food availability to suspension feeders by providing an extensive surface for the adsorption of dissolved organic material and microorganism. Based on this premise, future work should consider the influence of different type (e.g. organically rich versus organically

depleted) and concentrations of sediment on the trophic relationships among co-occurring suspension feeders.

5.4 Understanding the influence of sediment exposure on food particle retention and selection

The final step of suspension-feeding activity consists of the ingestion of food particles (Hanson et al. 2009; Pile 2005; Armsworthy et al. 2001). A laboratory experiment (Chapter 4), a transplant experiment (Chapter 4) and a field survey (Chapter 2) were combined to examine the influence of sediment exposure on the retention and selection of picoplanktonic cells by the three suspension feeders (Chapter 2&4).

Increased sediment exposure had an influence on the retention of food particles by the ascidian *Herdmania momus*. However, since the results from the field survey, laboratory experiment and transplant experiment differed, it was difficult to interpret the influence of sediment exposure on the ascidian retention rates. Several studies on ascidians have demonstrated that they can maintain food particle retention rates by a reduction of the filtration activity (Armsworthy et al. 2001; Klumpp 1984; Jørgensen 1983) In the field survey, *Herdmania momus* maintained relatively constant retention rates independent of sediment exposure and had reduced pumping rates. These findings were consistent with various studies on suspension feeders (Coco et al. 2006; Hawkins et al. 2001; Petersen et al. 1999). Results from the laboratory experiment indicated that total retention rates were significantly higher for the treatment with high sediment exposure while rates for treatments with lower sediment exposure were considerably reduced. These results were unusual considering ascidians are typically sensitive to high concentrations of suspended particles (Petersen et al. 1995, 1999; Petersen & Riisgard 1992). Finally, results from the transplant experiment exhibited the opposite trend with ascidians removing 50% less particles at the site with elevated sediment exposure. However, the discrepancy between the two sites was less (difference of 20%) when looking at the amount of carbon removed rather than the number of cells removed. Despite the difficulty interpreting results on food particles retention rates, the survey, the laboratory and field experiment presented similar results in regard to food particles retention efficiency (efficiency with which each type of food particles are retained) and selectivity. Differences in retention efficiency between the different groups of picoplankton indicated that *Herdmania momus* was able to select food, a capacity previously reported (Armsworthy et al. 2001; Seiderer & Newell 1988; Klumpp 1984). *Herdmania momus* preferentially selected *Synechococcus* in low sediment exposure conditions, but not at higher sediment exposure, where it exploited a wider range of food sources, including bacteria and picoeukaryotes. Thus, *Herdmania momus* might be adapted for extracting energy from food particles highly diluted within a pool of inorganic particles by optimising its food intake. By feeding on a wide range of food sources, the ascidian could gather enough energy to maintain its

metabolism despite changes in environmental conditions. Although, the physiological mechanism behind particle selection and its relation with increased sedimentation remain unclear, Armsworthy et al. (2001) described similar compensatory mechanisms for the ascidian *Halocynthia pyriformis*. This adaptation of feeding strategy could explain the presence of the ascidians in the vicinity of the wash plant, where conditions seem unfavourable.

Both laboratory and transplant experiments indicated that *Pinna bicolor* retained significantly less food particles under high sediment exposures. However, the field survey and the transplant experiment revealed that the bivalve retained similar or even higher amount of carbon at sites with increased sediment exposure. This finding implied changes in the bivalve diet to conserve its energy balance, while consuming less food particles under high sediment exposure. To cope with increased suspended sediment and sediment deposition, Pterioid bivalves, like *Pinna bicolor*, are able to increase pseudo-faeces production and the size of their labial palps to select and sort particles (Hewitt & Pilditch 2004; Kiorboe & Mohlenberg 1981; Yonge & Thompson 1976). Under low sediment exposure conditions *Synechococcus* and bacterial cells were more efficiently selected than picoeukaryotes (e.g. *Isochrysis*). When exposed to the highest levels of sediment, *Pinna bicolor* predominantly selected larger picoeukaryote cells, which contained more carbon. Previous studies reported the capacity for bivalves to sort and select particles of higher nutritive quality in order to compensate for the dilution of food sources within inorganic particles (Safi et al. 2007; Ward et al. 1998; Kiorboe & Mohlenberg 1981). The selection of larger cells of better nutritive quality (organic carbon enrichment) could explain the presence of large biomass of the bivalves at sites near the wash plant.

Both field survey and laboratory experiments revealed that *Tethya* sp was not able to select food particles, consistent with previous reports of sponges as non-selective suspension feeders (Topçu et al. 2010; Duckworth & Brück 2006; Pile & Young 2006). The rates and efficiency of food particle retention by the sponge *Tethya* sp were increased significantly at higher sediment exposure, indicating that it benefits from increased sediment exposure. This positive influence could explain the biomass and abundances of *Tethya* sp recorded in the vicinity of the wash plant and its absence from reference sites (Chapter 1). Although demosponges inhabiting highly turbid habitat can present physiological adaptations to expel sediment particles (Ellwanger et al. 2007; Elliott & Leys 2007; Nickel & Brümmer 2004; Reiswig 1971), the mechanisms behind this potential advantage from sediment exposure remain unexplained, but several hypotheses can be put forward. Organic matter inputs associated with sediment loading around the wash plant potentially provided an additional source of food for the sponge. However, this hypothesis would not explain the results from the laboratory experiment, as no additional organic matter was provided in the high sediment exposure treatments. Previous studies demonstrated that increased concentrations of suspended inorganic material could enhance food availability to suspension

feeders (Newell et al., 2005; Waite et al., 2000) by providing extensive surfaces for the microbial colonisation and formation of "marine aggregates" comprised of picoplankton and bacteria incorporated into larger inorganic particles (Muschenheim et al. 1989). There is growing evidence that marine aggregates can contribute to suspension feeder diets (Newell et al. 2005) and their higher settling rates compared to individual picoplanktonic cells enhances the amount of food particles that reach the seabed (Newell et al. 2005; Waite et al. 2000; Muschenheim et al. 1989).

A major finding of this research is that response of suspension-feeding activity to high sediment exposure is closely tied to the faculty for species to employ physiological regulations. Two hypotheses were considered for this research. Firstly, under stress conditions, the suspension feeders may be able to quickly compensate for chronic and acute changes in TSS concentrations and sediment deposition rates. Alternatively, the influence of increased sediment exposure on suspension feeding may depend on the history of exposure to elevated sediment exposure. From this, some species may be preadapted to deal with higher sediment loads by, for example, greater size of labial palps (Kiorboe & Mohlenberg 1981). Dutertre et al. (2009) studied spatial size variations of labial palps, gills and the adductor muscle of the invasive feral oyster, *Crassostrea gigas* along two gradients of suspended particulate matter. Findings from his study showed that evolutionary adaptations of pallial organ size, notably bigger labial palps at site with high sediment exposure, allow the oysters to tolerate sediment exposure levels above the thresholds previously determined experimentally. Although the present study was not designed to examine differences in evolutionary adaptive traits, the rapid physiological regulation hypothesis seems validated by the data from the transplant experiment. In the short term, transplanted specimens exhibited similar suspension feeding response than naturally occurring animals. Therefore, *Herdmania momus* and *Pinna bicolor* were able to rapidly adapt their suspension feeding to changes in environmental conditions. Because *Tethya* sp was absent from the transplant experiment, this hypothesis could not be clearly elucidated. However, variations in pumping and total retention rates during the feeding experiment suggested a similar capability of rapid adaptations. As the research only focused on suspension feeding processes (water filtration and retention activity), the influence of *long-term* exposure to high levels of sediment exposure on suspension feeder physiology remained plausible. Endoscopic examination and biometric measurement on filtering and particle capture structures could provide with valuable insights on potential evolutionary variations of certain morphological features associated with specific physiological regulations. In the same manner that Dutertre et al. (2009) observed variations of the size of labial palps on *Crassostrea gigas* along gradients of suspended particulate matter, the feeding structures of studied animals may exhibit morphological variations. Because this evolutionary aspect was not considered in the present research, further investigations should be conducted to give more precise description of the different particle-capture structure and pump

designs and to assess potential morphological variations response to changes in sediment exposure.

Overall, the present research validated the hypothesis that response of suspension-feeding activity to high sediment exposure vary among species presenting distinctive feeding structures and strategies. The modification of food particles retention, among species that best characterised suspension-feeder assemblages, could potentially have a negative influence on food resource partitioning between suspension feeders, but also limit the food available to other marine organisms.

5.5 Estimation on the influence of sediment exposure on benthic-pelagic coupling and other ecosystem-scale processes.

This study has provided a detailed investigation of the influence of sediment exposure on suspension-feeder assemblages within a temperate seagrass bed. The key findings of the four chapters help refine the understanding of suspension-feeding mechanisms and to evaluate assumptions about how these processes are influenced by increased sediment exposure (suspended sediment concentrations and sediment deposition). Overall, the findings highlight that even though increased sediment exposure (up to 40 mg·l^{-1} of TSS and 10 g·cm^{-2}·month^{-1} of sediment deposition) did not appear to reach lethal threshold levels or affect assemblage composition, it did influence suspension-feeding activity.

Suspension feeders were able to rapidly adapt to changed conditions by modifying their filtration activity and food particle retention and selection. As the three species of suspension feeders, which were studied in detail, filter large volumes of water and particulate matter, the modification of their filtration activity may influence transfers of organic matter and nutrients between the water column and the seabed. Using mean biomass of the three key suspension feeders and the differences in pumping rate and particle retention observed in the various studies, it is possible to examine the differences in pumping rates and total particles uptake, and therefore estimate the influence of increased sediment exposure on benthic-pelagic coupling and other ecosystem-scale processes.

An assemblage comprised of these species filters 19% more water under high sediment exposure (99.4 ±7.8 l·h^{-1}·m^{-2}) compared to a similar assemblage under low sediment exposure (83.8 ±3.7 l·h^{-1}·m^{-2}). On average, assemblages near the wash plant (high sediment exposure) are potentially able to filter the overlying water column (3 m water depth or 3000 l of seawter.m^{-2}) approximately daily, while assemblages at reference sites would do so in 1.5 days. These estimates agree with those obtained by Lemmens et al. (1996) in the vicinity of the study area. Such increase in pumping rates potentially enhance sediment deposition, biodeposition and the transportation of

nutrients and organic matter into the seabed (Newell 2004; Doering et al. 1986), which may contribute to its organic enrichment and to hypoxic conditions (Cugier et al. 2010).

Despite the significant difference in pumping rates (p = 0.001; α = 0.05), both assemblages consume a similar quantity of picoplanktonic cells (p =0.198; α = 0.05), retaining $2.6\pm0.7\times10^{10}$ cells$\cdot$h$^{-1}\cdot$m^{-2} near the wash plant and 3.4×10^{10} cells$\cdot$h$^{-1}\cdot$m^{-2} at reference sites. Nonetheless, the assemblage near the wash plant removes 68% more carbon from the water column ($2.3\pm0.4\times10^{3}$ µgC$\cdot$h$^{-1}\cdot$m^{-2}) compared to the assemblage at reference sites ($1.4\pm0.3\times10^{3}$ µgC$\cdot$h$^{-1}\cdot$m^{-2}). This difference in carbon removal is principally explained by changes in food particle selection. Whereas an assemblage under natural sediment exposure would retain 52% of the bacteria, 93% of the *Synechoccocus* and 33% of the picoeukaryotes, the same assemblage under high sediment exposure would retain 70% of the bacteria, 88% of the *Synechoccocus* and 98% of the picoeukaryotes. Through increased filtration activity and carbon removal, and changes in food selectivity, suspension-feeder assemblages may partially control local densities of suspended organic matter, especially in shallow oligotrophic environments. The importance of suspension-feeding organisms in controlling phytoplankton levels and influencing secondary production in coastal environments has been reported in various studies (Pile 2005; Ostroumov 2005; Dame et al. 2001; Dolmer 2000; Lemmens et al. 1996; Riera and Richard 1996; Kimmerer et al. 1994). For example, Ribes et al. (2005) demonstrated that suspension-feeding assemblages played a critical role in the removal of picoplankton within coral reef ecosystems. The fluctuations in food particle selection, especially the consumption of larger, higher in carbon content, but less abundant picoeukaryote cells, may alter food resource partitioning between suspension feeders and limit the quantity and quality of food available to other marine organisms.

CONCLUSION

A major outcome of this research is that informs shallow coastal ecosystems stakeholders of the possible consequences of anthropogenic activities that increase sediment exposure, particularly those in the order of TSS at 40 mg·l^{-1} and deposition rates in the order of 10 g·cm^{-2}·month^{-1}. This magnitude of sediment exposure had little influence on suspension feeder assemblage composition due to the physiological plasticity of individual species. However, the effects on the suspension-feeding function, including the increase of the filtration activity and transfers of carbon to the benthos, potentially influence benthic-pelagic coupling and other ecosystem-scale processes by reducing food resources to other pelagic consumers and enhancing organic enrichment of the benthos. Given the variation in sensitivity to sediment exposure among suspension feeder species, meaningful criteria to limit the effects of anthropogenic sediment loading on shallow coastal ecosystems require taking into account the whole species assemblage present at any given site.

Although, the research reveals the physiological plasticity of certain suspension feeders, levels of sediment exposure tested were bellow thresholds of feeding cessation. Hence, this research could be advanced in the future by examining the effects of sediment exposure greater than 40 mg·l^{-1} of TSS and over 10 g·cm^{-2}·month^{-1} of deposition rates. Such investigation could help to understand to which degree specific physiological mechanisms can sustain suspension feeder metabolic requirements and health conditions. It is thus evident that cost–benefit analysis on compensatory mechanisms should be also undertaken to develop ecophysiological models which seek to quantify energy budgets associated with suspension feeding and the adaptation to changes in environmental conditions.

The ever-increasing demand for coastal construction, land reclamation, beach nourishment, port maintenance and sand mining require the excavation, transportation and disposal of sediment, all of which lead to the discharge of a great amount of this sediment in coastal waters. Given recent concerns over the impacts of sediment exposure on marine ecosystems, further investigations are required to understand the magnitude of such pressure at larger spatial scales to implement these local findings. Furthermore, the present research could be advance in the future to develop a reliable model to assess the influence of increased sediment exposure on ecosystem processes based on suspension feeder composition structure, but also physiological and functional traits. Ideally, such model would allow to assess the extent of the impacts of increased sediment exposure in seagrass and other coastal ecosystems, but also provide stakeholders with indicators concerning the evolution of the trophic resource and ecosystem health.

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APPENDIX A

Photography of few suspension feeder taxa found in Woodman Point's seagrass meadow

Seagrass meadows surrounding the CCL wash plant.
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Pinna bicolor

Coelosphaera sp

Cercodemas anceps and

Herdmania momus

Tethya sp

Comatula purpurea

Pinna bicolor and colonial ascidians

Polycarpa veridis

APPENDIX B

P. sinuosa shoot densities (per 20 x 20cm quadrat) measured at each site in 2013. There was no significant difference between sites (p= 0.99; F= 0.18).

Quadrates	SITES							
	200E	200NW	500E	500NW	800E	800NW	REFE	REFNW
1	28	33	29	33	14	37	29	22
2	38	27	28	21	13	19	23	21
3	33	23	14	16	40	17	11	11
4	11	21	12	17	31	36	15	23
5	24	14	20	30	10	14	12	29
6	21	13	10	31	34	15	13	13
7	13	28	23	17	12	11	24	17
8	26	22	19	13	42	13	11	19
9	21	21	19	20	39	18	32	16
10	18	36	15	10	10	29	30	28
11	11	23	36	24	28	13	18	12
12	18	36	35	22	10	12	24	30
13	12	24	14	20	17	11	11	24
14	27	13	16	13	15	26	15	34
15	31	13	17	10	10	30	14	19
16	11	25	15	21	29	10	33	15
17	38	15	18	25	39	24	44	22
18	22	32	15	13	11	33	36	14
19	15	13	18	38	15	31	31	11
20	14	22	40	27	20	12	25	34
Means	21.6	22.7	20.65	21.05	21.95	20.55	22.55	20.7
SE	1.99	1.76	1.65	1.77	2.73	2.07	2.25	1.53

P. sinuosa shoot densities (per 20 x 20cm quadrat) measured at each site in 2014. There was no significant difference between sites (p= 0.99; F= 0.15).

Quadrates	SITES							
	200E	200NW	500E	500NW	800E	800NW	REFE	REFNW
1	12	35	26	13	15	16	20	11
2	21	11	10	20	33	19	22	42
3	25	12	21	18	24	13	21	16
4	11	40	20	17	19	17	25	13
5	16	20	10	32	14	10	10	12
6	18	37	21	12	29	17	15	16
7	15	38	31	11	20	43	19	10
8	25	12	10	23	14	32	11	32
9	14	12	39	36	18	21	25	14
10	24	18	34	31	19	10	35	29
11	41	19	22	15	29	12	22	35
12	16	39	10	18	16	35	40	20
13	14	11	21	11	31	29	10	27
14	36	39	24	12	29	11	10	19
15	16	21	11	10	14	14	45	21
16	13	13	28	30	11	12	15	24
17	37	15	17	26	12	12	17	42
18	18	18	16	23	17	27	26	11
19	16	16	25	16	28	14	11	24
20	33	22	31	45	36	26	26	18
Means	21.05	22.4	21.35	20.95	21.4	19.5	21.25	21.8
SE	1.98	2.53	1.91	1.80	1.59	2.14	2.27	2.28

APPENDIX C

Chapter 2: Dry weight DW (g) of suspension feeders collected in at each site in 2013 and 2014.

SITE	REPLICATE	2013			2014		
		Ascidian DW (g)	Bivalve DW (g)	Sponge DW (g)	Ascidian DW (g)	Bivalve DW (g)	Sponge DW (g)
200E	1	15.33	19.01	2.34	22.26	20.60	2.69
200E	2	24.23	21.63	3.09	16.03	19.52	1.96
200E	3	14.64	17.60	1.44	23.72	22.42	1.31
200E	4	18.06	18.17	1.23	16.89	15.85	2.02
200E	5	17.41	23.15	1.48	16.40	23.17	1.76
200NW	1	22.94	20.37	3.70	24.42	20.20	1.37
200NW	2	15.75	23.73	4.75	15.58	15.51	1.88
200NW	3	21.13	23.73	5.10	18.91	19.52	1.42
200NW	4	N/A	23.17	3.96	14.44	20.49	1.38
200NW	5	N/A	19.31	2.40	22.94	22.21	3.74
500E	1	23.77	17.50	1.80	24.59	15.67	1.98
500E	2	20.95	15.66	2.16	20.54	19.37	4.29
500E	3	23.21	17.72	5.11	23.81	19.48	2.33
500E	4	20.79	16.90	1.90	N/A	N/A	2.43
500E	5	25.64	22.17	2.30	N/A	N/A	1.36
500NW	1	18.60	12.28	4.24	17.93	19.95	1.49
500NW	2	20.81	18.33	4.62	17.35	22.71	1.16
500NW	3	18.58	15.32	3.52	15.13	22.57	1.56
500NW	4	19.35	16.68	5.08	17.05	18.23	1.64
500NW	5	14.44	18.78	N/A	20.95	23.35	1.19
REFE	1	21.62	20.90	1.77	19.43	N/A	N/A
REFE	2	22.31	N/A	1.29	16.02	N/A	N/A
REFE	3	24.50	N/A	2.67	15.40	N/A	N/A
REFE	4	15.94	N/A	1.57	N/A	N/A	N/A
REFE	5	N/A	N/A	2.40	N/A	N/A	N/A
REFNW	1	18.30	19.25	2.76	22.15	19.69	N/A
REFNW	2	19.19	21.09	N/A	21.28	16.38	N/A
REFNW	3	17.75	20.74	N/A	21.52	18.44	N/A
REFNW	4	22.76	18.12	N/A	27.26	20.85	N/A
REFNW	5	21.91	14.56	N/A	17.32	22.11	N/A

Chapter 3: Dry weight DW (g) of suspension feeders collected in at each site in 2013 and 2014.

SITE	REPLICATE	2013			2014		
		Ascidian DW (g)	Bivalve DW (g)	Sponge DW (g)	Ascidian DW (g)	Bivalve DW (g)	Sponge DW (g)
200E	1	19.33	20.01	3.09	15.72	20.60	1.58
200E	2	20.23	24.63	1.05	21.88	17.51	2.56
200E	3	23.64	17.60	1.44	21.39	20.41	1.51
200E	4	21.46	17.17	1.23	22.42	16.84	2.61
200E	5	16.41	25.15	1.48	24.26	20.17	1.75
200NW	1	23.94	20.37	3.70	14.57	26.19	1.97
200NW	2	19.75	18.73	1.75	25.48	21.94	1.87
200NW	3	15.12	17.73	1.69	19.91	15.51	2.01
200NW	4	N/A	15.17	2.45	20.44	20.49	2.08
200NW	5	N/A	15.31	2.40	19.94	16.21	1.43
500E	1	16.77	24.50	1.80	16.99	16.66	1.97
500E	2	15.95	15.66	2.16	19.53	17.37	1.89
500E	3	17.21	25.72	2.11	16.60	20.99	1.32
500E	4	17.79	16.90	1.90	19.09	16.52	1.42
500E	5	15.95	15.17	2.30	20.08	16.31	1.76
500NW	1	15.30	15.28	2.23	17.42	16.95	1.48
500NW	2	15.40	11.33	1.61	17.14	16.57	1.16
500NW	3	18.57	19.32	2.51	15.66	16.23	1.76
500NW	4	19.35	18.68	3.07	15.95	16.35	2.53
500NW	5	15.44	20.78	N/A	15.97	16.21	1.19
REFE	1	20.62	17.90	1.77	22.52	17.71	N/A
REFE	2	21.87	N/A	1.99	19.02	N/A	N/A
REFE	3	18.31	N/A	2.67	19.40	N/A	N/A
REFE	4	22.50	N/A	1.57	N/A	N/A	N/A
REFE	5	15.64	N/A	2.40	N/A	N/A	N/A
REFNW	1	23.29	15.25	2.75	20.15	19.68	N/A
REFNW	2	22.18	15.09	N/A	25.27	16.44	N/A
REFNW	3	23.75	16.74	N/A	19.51	20.85	N/A
REFNW	4	25.75	19.12	N/A	24.26	16.44	N/A
REFNW	5	22.91	20.56	N/A	23.32	20.11	N/A

Chapter 4: Physiological conditions (length, width, dry weight DW (g)) of each suspension feeders selected for the transplant experiment in situ.

Treatment	Site A					Site B				
	Organism	Replicate	Length	Width	DW	Organism	Replicate	Length	Width	DW
C	Ascidian	1	11.4	9.0	16.3	Ascidian	1	9.0	8.0	15.5
C	Ascidian	2	12.1	9.0	14.4	Ascidian	2	12.0	9.0	17.0
C	Ascidian	3	9.9	8.6	10.3	Ascidian	3	13.0	13.0	22.8
C	Ascidian	4	12.6	10.4	16.6	Ascidian	4	9.0	8.0	11.2
C	Ascidian	5	9.8	9.5	15.5	Ascidian	5	10.0	8.0	18.1
C	Bivalve	1	36.9	12.5	19.0	Bivalve	1	31.5	12.0	12.0
C	Bivalve	2	32.7	13.2	14.1	Bivalve	2	38.0	15.5	19.9
C	Bivalve	3	30.5	13.2	12.2	Bivalve	3	31.0	10.8	18.6
C	Bivalve	4	36.1	12.3	14.0	Bivalve	4	33.5	14.5	12.1
C	Bivalve	5	32.8	12.5	9.2	Bivalve	5	35.5	14.5	19.3
N	Ascidian	1	N/A	N/A	23.3	Ascidian	1	10.0	8.0	23.1
N	Ascidian	2	N/A	N/A	14.4	Ascidian	2	9.0	8.0	21.0
N	Ascidian	3	N/A	N/A	10.3	Ascidian	3	13.0	9.0	19.6
N	Ascidian	4	N/A	N/A	20.6	Ascidian	4	9.0	8.5	20.2
N	Ascidian	5	N/A	N/A	13.5	Ascidian	5	10.0	8.0	25.7
N	Bivalve	1	N/A	N/A	19.0	Bivalve	1	38.5	15.0	20.1
N	Bivalve	2	N/A	N/A	14.1	Bivalve	2	37.5	13.5	23.1
N	Bivalve	3	N/A	N/A	12.2	Bivalve	3	36.5	14.0	15.8
N	Bivalve	4	N/A	N/A	14.0	Bivalve	4	34.0	12.5	18.1
N	Bivalve	5	N/A	N/A	11.2	Bivalve	5	43.0	14.5	20.1
T	Ascidian	1	14.4	13.8	23.7	Ascidian	1	9.0	8.0	19.7
T	Ascidian	2	10.2	8.8	19.9	Ascidian	2	12.0	7.0	23.8
T	Ascidian	3	10.7	9.2	13.2	Ascidian	3	7.0	6.0	22.8
T	Ascidian	4	14.3	12.7	21.7	Ascidian	4	7.0	6.0	12.6
T	Ascidian	5	13.4	11.7	25.1	Ascidian	5	9.0	6.0	19.8
T	Bivalve	1	39.9	14.4	19.3	Bivalve	1	33.0	14.0	15.4
T	Bivalve	2	37.0	12.8	25.7	Bivalve	2	33.0	15.0	16.3
T	Bivalve	3	41.5	14.3	20.3	Bivalve	3	33.5	14.0	12.6
T	Bivalve	4	40.4	11.9	18.0	Bivalve	4	33.5	13.0	15.0
T	Bivalve	5	43.5	14.6	16.6	Bivalve	5	34.0	13.5	11.7

Chapter 4: Physiological conditions (length, width, dry weight DW (g)) of each suspension feeders selected for the laboratory feeding experiment.

Organism	REPLICATE	Treatment	Length	Width	DW
Ascidian	1	H	10.3	9.2	10.8729
Ascidian	3	H	12.4	10.6	15.7207
Ascidian	2	H	9.9	7.7	8.1334
Bivalve	3	H	33.2	12.4	15.426
Bivalve	1	H	31.8	12.4	13.3017
Bivalve	2	H	34.1	13.7	11.0506
Sponge (x6)	1	H	N/A	N/A	14.3728
Sponge (x6)	2	H	N/A	N/A	13.1216
Sponge (x6)	3	H	N/A	N/A	10.104
Ascidian	1	L	10.2	8.9	11.2168
Ascidian	3	L	9.2	8.3	7.3132
Ascidian	2	L	9.2	8	9.1927
Bivalve	3	L	29.3	11	8.3171
Bivalve	1	L	29.6	10.5	10.2612
Bivalve	2	L	29.7	11.8	14.8617
Sponge (x6)	2	L	N/A	N/A	14.6338
Sponge (x6)	1	L	N/A	N/A	10.7854
Sponge (x6)	3	L	N/A	N/A	12.4375
Ascidian	3	M	10.2	6.5	8.8169
Ascidian	1	M	11.8	8.5	11.365
Ascidian	2	M	12.4	9.4	13.0015
Bivalve	3	M	32.9	12.8	10.7301
Bivalve	2	M	28.1	10.8	8.9984
Bivalve	1	M	30.9	11.3	8.6679
Sponge (x6)	2	M	N/A	N/A	8.8901
Sponge (x6)	1	M	N/A	N/A	14.0336
Sponge (x6)	3	M	N/A	N/A	12.7262