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Mussel production carrying capacity : the need for an in situ and multidisciplinary approach

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Mussel Production Carrying Capacity: The need for an *in situ* and multidisciplinary approach

A Thesis submitted to Bangor University

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for the degree of

Philosophiae Doctor

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A Pedro et Luca,

et à Domi et Gérard …

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SUMMARY

Mussels (*Mytilus edulis*), are important aquaculture suspension feeder species, which can have a significant impact on coastal marine systems due to the removal of considerable amounts of suspended food particles from the water column. Understanding the production carrying capacity (stocking density of bivalves at which yields are maximised) is necessary for sustainable yields to be maintained. The goal of the present study was to gain an understanding of the feedbacks and interactions between the various components that determine production carrying capacity; Mussel feeding behaviour, grazing rate and growth were investigated together with the seston concentration and prevailing hydrodynamics using an *in situ* approach which has been lacking in previous modelling.

Mussel feeding behaviour in the Menai Strait was directly coupled to the hydrodynamics and food concentration of the water column. Mussel feeding behaviour in the subtidal area was only synchronized with the advected chlorophyll *a* (chl *a*) concentrations and not with other environmental factors measured (e.g. predators, suspended particulate matter, current velocity…). Mussel shell growth was also coupled with chl *a* concentrations. The development of the internal shell microgrowth methodology proved better than the use of traditional morphometric measurements to quantify mussel growth depending on the temporal scale used. The *in situ* defecation methodology, using chl *a* concentration as a proxy, was developed to estimate the clearance rate of *M. edulis* and then compared to other *in situ* methodologies (biodeposition and adapted suction) applied in the same natural conditions. This method was reliable and useful, if applied under certain constraints.

All these *in situ* methodologies (feeding behaviour, clearance rate, microgrowth) developed during this PhD project were combined in one study over an intertidal mussel bed of the Menai Strait. The same results were found using different instruments and methodologies following the dual approach of "ask the water, ask the animals": the amount of food from the water distributed to the mussels was equal to the amount of food filtered by the mussels. The results from this study showed the robustness of the methodologies employed but also the importance of the mussel bed patterning (presence of bare patches within a mussel bed) as a facilitator for food supply.

Most of the results in this study contribute to the growing evidence that the use of *in situ* approaches is necessary to avoid under or over-estimations of production carrying capacity due to incorrect modelling. The interactions of the three components that determine production carrying capacity were demonstrated in this project. These findings open new perspectives for improving production carrying capacity modelling for future applications in the Menai Strait with new re-laying strategy and in other systems.

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Mussel seed re-lay at Bangor Pier by Valente (Myti Mussel Ltd) 21/10/2004

Chapter I General Introduction

1.1 Mussel (Mytilus edulis L.) ecology

The blue mussel, *Mytilus edulis* (L.) is a bivalve mollusc that occurs in the intertidal and subtidal zone at temperate latitudes (Gosling, 1992). The principal physical factor influencing its distribution is temperature, which affects the survival of larvae and adults (Seed, 1976). The upper distributional limit of *M. edulis* on the shore is determined by their physiological intolerance to extreme temperature and desiccation whereas the lower limit is strongly influenced from biological factors such as predators (Paine, 1974; Seed and Suchanek, 1992). The main predators of mussels in Northern Europe are starfish (*Asterias rubens*), crabs (*Cancer pagarus* and *Carcinus maenas*) and at low tide birds such as oystercatcher *Haematopus ostralegus* (Dare, 1980). The magnitude of predation pressure and the identity of the main predators depends upon mussel size, season and height relative to the shore and location (Hamilton *et al.*, 1999).

Mussels are found in any substratum that provides a secure anchorage (Seed, 1976) such as rocks, stones, shingle, dead shells, mud and also on sand where they attach to each others. Mussel beds are often dominant in terms of biomass, and form a key component (foundation species) of many marine communities (Bruno and Bertness, 2000; Herman, 1993; Seed, 1976). These beds support their own diverse communities as the mussel matrix, composed of layers of mussels with accumulated sediments and debris, provides numerous microhabitats and an organically enriched environment (Ragnarsson and Raffaelli, 1999; Bruno and Bertness, 2000).

Marine bivalve communities often appear in open water or estuaries (Seed, 1976) where the energy of the water flow is sufficient to replenish food and to flush away faeces, pseudofaeces and inorganic material (Dame and Prins, 1998).

Feeding

Mussels use their gills to filter seawater from which they extract food particles from the seston. The feeding varies as a function of the composition and concentration of the seston, the morphology of the feeding organs of the animals,

their feeding behaviour and other factors such as water flow and mixing, temperature and seasonality. The food quality of the seston for suspension feeders depends on the fraction of living material and labile detritus in the total seston (Smaal and Haas, 1997). Nevertheless, food availability is often considered limited or diluted by the large inorganic fraction (Widdows *et al.*, 1979).

The size range of the seston particles varies from 3-200 μm and the minimum particle diameter for the mussels 100% particle retention efficiency is 4 μm (Shumway *et al.*, 1985; Møhlenberg and Riisgård, 1978). Food consumed by mussels consists of different types of suspended particles such as bacteria, phytoplankton, microzooplankton, detritus and dissolved organic material (DOM such as amino acids and sugars). Larvae of benthic animals, such as crustacean and bivalve (10-1000μm size range) can be captured and ingested by carnivorous *M. edulis* (Davenport *et al.*, 2000; Lehane and Davenport, 2004). Mussels can retain flagellates ~1-2 μm and bacteria 0.3-1.0 μm from suspension (Gosling, 2003). Moreover, *M. edulis* has a very fine filter with a mesh size of 2.7 x 0.6 μm allowing a high retention of 1-2 μm particles (Møhlenberg and Riisgård, 1979).

In estuaries, the major part of the seston is inorganic, leaving a small fraction with food value (Smaal and Haas, 1997). However, in subtidal and intertidal habitats, the quality and quantity of seston are dependent on several factors that vary temporally and spatially. These factors are the mixing of the water column by tidal flow (major factor), and/or by wind speed and direction, and/or by upwelling, sedimentation, erosion characteristics, depletion by benthic filtration (Smaal and Haas, 1997). Current speed and vertical mixing determine the food supply to dense bivalve beds (Fréchette *et al.*, 1989; Wildish and Kristmanson, 1984). Moreover, a mussel bed consumes phytoplankton (produced per day) from an area of 11 to 16 times the size of the bed (Smaal and Prins, 1993). If there is a correlation between mussel growth and annual primary production, then phytoplankton is presumed to be the main source of food, as demonstrated by Smaal and Van Stralen (1990).

Food is extracted from the inhalent current (Gosling, 2003), before passing through the exhalant siphon (Jørgensen *et al.*, 1990) (Figure 1. 1). *M. edulis* gills are homorhabhic filibranch with a w-shaped filament and have two roles: respiration and food assimilation. Defossez and Hawkins (1997) have suggested that preferential rejection of larger particles as pseudofaeces could be due to their lower nutritious value compared to smaller particles. The pseudofaeces result from the process of

particle selection. Pseudofaeces are composed of high viscosity mucous accumulates indigestible and rejected particles, whereas low-viscosity mucus is used for particles destined for ingestion of nutritive particles diluted from the environment (Hawkins *et al.*, 1996).

Figure 1. 1: Picture of mussel inhalant (fringed with cilia) and exhalant (conical and smooth) siphons. Arrows indicate the flow of water through the siphons.

The control of mussel feeding behaviour, either physiologically or automatically via the pump, has been subject to ongoing debate (Bayne, 1998; Hawkins *et al.*, 1998b; Jørgensen, 1996, Riisgård, 2001a). A second debate is the proper use of appropriate methodology to assess the clearance rate and hence grazing capacity of mussels *in situ* (Riisgard, 2001). There are clear differences in the definition of terms used in the literature to describe the rate of water processing by the mussel. For convenience, the terms used in this study are the ones defined in Riisgård, 2001a). Clearance rate is the volume of water cleared of suspended particles (100% retention efficiency) per unit of time, while filtration rate is the pumping rate or the volume flow rate per unit of time. Only in specific circumstances is clearance rate equal to filtration rate. Ingestion rate is the mass of particles filtered minus the pseudofaeces production per unit of time and the absorption rate is the ingestion rate minus the faeces production per unit of time.

Growth

Mussel growth studies are numerous (Seed, 1969b). Growth corresponds to the integration of physiological processes (energy acquisition and distribution) dependent upon environmental and endogenous factors. Growth in *M. edulis* occurs mainly during spring and summer months, with energy being allocated to gametogenesis during the winter months in the UK (Seed, 1976).

Morphological shell characteristics are widely influenced by environmental and endogenous factors. The shell consists of two valves, which are attached and articulated to one another by the hinge system which includes a ligament, teeth and other specific specializations (Figure 1. 2). Environmental factors influencing the growth include food supply, tidal air exposure, light, temperature, salinity, turbulence, type of pollutants and concentration, particle concentration in suspension, density of the mussels, habitat type and the presence of predators (Seed, 1968, 1969b; Beadman *et al.*, 2002), while endogenous modulators comprise genotype, age and size, hormonal and innate rhythms. One of the most important factors influencing the shell characteristics is food supply.

In temperate climates, during the winter months, there is little or no growth of the shell whereas somatic growth occurs at the expense of the stored food for the gametogenesis; this slow growth could be attributed more the reduction of food supply than to low temperature (Seed, 1976). During the spring and summer rapid shell growth occurs whereas the tissue development is smaller (Seed, 1969b). There is a difference in shell/body ratio throughout the year. Flesh weight varies seasonally with the life cycle and the formation of gametes. Shell length of *M. edulis* can reach up to 15 cm but is normally located in a maximum range of 5-8 cm. Some specimens of *M. edulis* have been reported to live for 18-24 years (Thiesen, 1973).

Growth is a component of the energy balance of individuals and is usually measured as an increase in shell dimensions: morphometric measurements (Shell length, width, height), micro-growth bands present in the internal structure of the shell (annual, seasonal or tidal growth rings). Growth is also used for population dynamic studies (size frequency distribution) (Gosling, 2003). In most studies, growth rates of *M. edulis* or bivalves are calculated from increases in shell length (and other shell dimensions), and from wet and/or dry weight of tissue soft parts (Jørgensen, 1976). Several methods are used to measure shell growth of bivalves

such as modal length frequency distribution, length measurement of marked animals, patterns on the shell and less commonly X-rays, radioactive Ca^{15} or C^{14} (Seed, 1969b).

The shell in *M. edulis* is produced by the mantle (Gosling, 2003). The calcareous and organic materials for shell formation are deposited in the minute space containing the pallial fluid separating the mantle from the shell (Gosling, 2003). The microstructure of *M. edulis* shell is two-layered aragonite (nacreous inner layer) and calcite (prismatic outer layer) (Morton, 1992; Lutz and Kennish, 1992). In the mussel *M. edulis*, micro-growth is characterised by bands and increments present in the shell visible internally and externally. The internal structure of the shell has been studied and marks can be created to measure growth (using markers such as notching or fluorescence, Kaehler and McQuaid, 1999; Leder *et al.*, 1996; Day *et al.*, 1995). Disturbances can occur in the two-layered microstructure of *M. edulis* shell due to seasonal variations in the physical environment. These types of disturbances have been used to establish the age of individuals in several disciplines: terrestrial botany with trees rings, in ichthyology on the scales and otoliths of fish and in bivalves in the shell or in gastropods on the operculum and in statoliths (see review Richardson, 2001 for molluscs). The disturbance rings found in bivalves appear during unfavourable external conditions, when the mantle edge is slightly withdrawn into the shell causing a cessation of accretionary growth at the shell margin (Seed, 1969b; Lutz, 1976). Seed (1969b) showed that growth rings present in the shell were produced annually, while Richardson (1989) found that the periodicity of the inner-shell bands where correlated with the tidal and daily emersions as well as an innate rhythm of shell deposition related to shell growth and independent from the light regime (Richardson, 2001).

The tidal periodicity of growth bands in certain bivalves has been widely accepted for intertidal animals, and although for subtidal animals, more experiments need to be done, as the mechanism is still not yet clearly understood (Richardson, 1990; Richardson, 2001). The bands are laid down during emersion, whereas the increment occurs while the animal is actively feeding during immersion (Richardson *et al.*, 1981). During tidal exposure, the animals cannot feed, and as such, animals in the intertidal zone exhibit reduced growth rates in comparison to the subtidal animals (Seed, 1976; Seed, 1969b; Gosling, 2003). In most bivalves, it has been noticed that if the time of air exposure during low tide is >50% growth does not occur

(Jørgensen, 1976; Seed and Suchanek, 1992). The relocation of slow-growing intertidal mussels down shore can induce a faster growth (Seed, 1968) and this knowledge has been used by mussel farmers in the Menai Strait to bank seed mussels high up the shore with slow growth rates that are later relocated to subtidal areas where faster growth occurs (Beadman *et al.*, 2003).

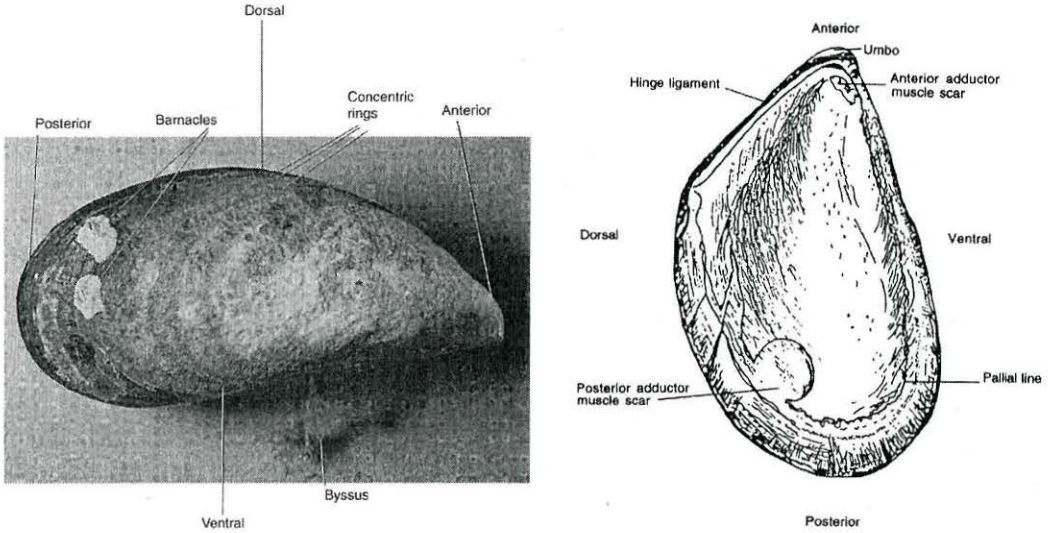


Figure 1. 2: The morphology of the shell of *Mytilus edulis* (from Gosling, 2003).

1.2 The Menai Strait

Hydrodynamic and ecology

The Menai Strait is a well-mixed channel of only 250-500 m wide and approximately 20 km long that separate the North Wales mainland from the Isle of Anglesey and connects Liverpool Bay (Northern Irish Sea) to Caernarfon Bay in the south. Due to its morphology and the high difference in tidal range between the two ends, the channel experience powerful tidal streams during spring tide (8 knots) with high turbidity and nutrient loading from land runoff (Brazier *et al.*, 1999) and strong tidal flows with velocities up to 2.5 m s^{-1} in some of the shallower areas (Rippeth *et al.*, 2002).

In the Menai Strait, shallow water depth and large tidal flow results in a net discharge in the channel varying from approximately 330 to 800 m^3 s^{-1} at neap and

spring tides respectively (Simpson et al. 1971). The association of large tidal flow and associated high levels of turbulence and Reynold stress ensures a well-mixed water column. The Menai Strait is flushed every 2-3 days due to the asymmetric tidal regime and the residual flow and transport (25 to 30 Million tonnes per tidal cycle) to the south west (Simpson *et al.*, 1971).

The Menai Strait is characterised by very turbid water that is enriched with phytoplankton and nutrients from Liverpool Bay during winter months. During spring and summer, blooms of *Phaeocystis ponchetii* (flagellate) occur in the Strait increasing water turbidity and supporting colonies of bacteria that upon breakdown are responsible for oxygen depletion (Spencer, 1981). The ebb flow orientation in the Menai Strait is from the northeast (Liverpool Bay) towards the southwest (Caernarfon bay). Ebb tide waters have higher chlorophyll concentrations than flood tide waters (see Chapter 2). The commercial lays and "wild" mussel beds lie to the West of Traeth Lafan (53°15'18"N, 04°02'31"W). This area is a Special Protection Area (SPA) under Article 4.2 of the EC Directive of the Conservation of Wild Birds (Directive 79/409). This large intertidal area is characterised by sand and mud-flats (Caldow *et al.*, 2003) (Figure 1. 3). The commercial mussel beds (northeastern end ~700 ha with only ~200 ha farmed at a density of ~100 t ha^{-1}) and rich subtidal filter feeding fauna (sponges, ascidians, bryozoans, hydroids and also bivalves on the Lavan Sand, Brazier *et al.*, 1999); coupled with large filter-feeder communities are likely to cause a substantial reduction of food concentration.

The typical seasonal pattern of chlorophyll a concentration in the Menai Strait is a first peak of ~7 $\mu g\ L^{-1}$ in mid-April (*Rhizosolenia* bloom) followed by a second peak in mid-May/June of ~10-13 $\mu g\ L^{-1}$ (*Phaeocystis* and diatom bloom) followed by a drop in concentration. Concentrations vary between 2 to 4 $\mu g\ L^{-1}$ from mid-July until late September, then a peak can occur at the beginning of October due to diatom blooms (~6 $\mu g\ L^{-1}$) (values from Beadman, unpublished data and Kratzer *et al.*, 2000).

The oystercatcher and redshank population has been increasing in the Menai Strait, possibly as a consequence of mussel cultivation, and the loss of the mud flat to mussel cultivation has not had a detrimental effect on other bird species in the area (Caldow *et al.*, 2003).

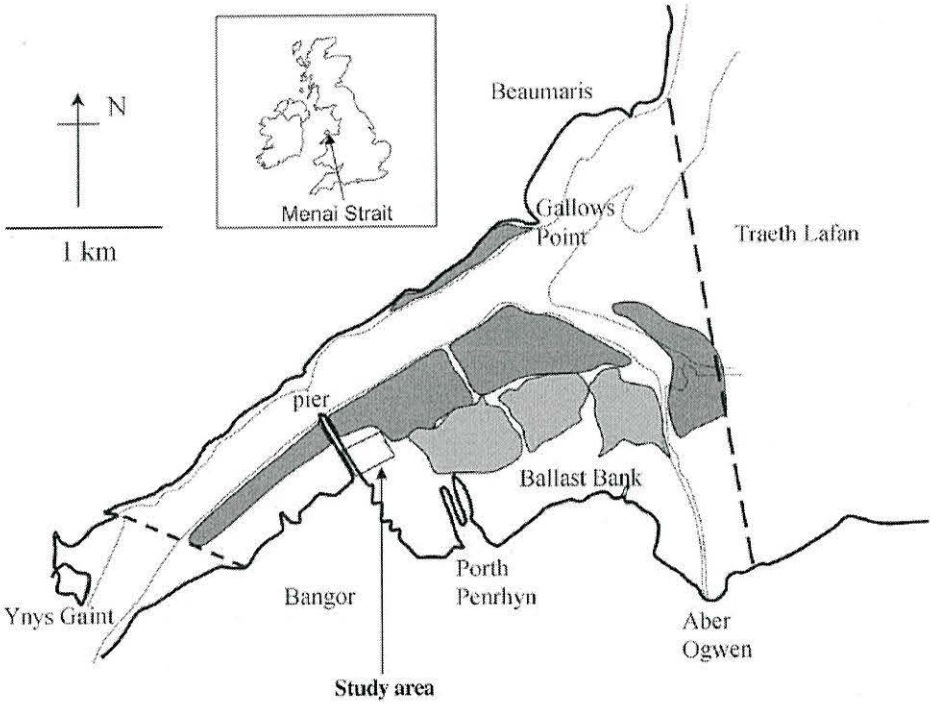


Figure 1. 3: Map of the study site showing the 3 patches of "wild" mussels *M. edulis* (light shading), the 5 principal commercial mussel lays (dark shading). From (Caldow *et al.*, 2003).

Fisheries and culture

Mussel marine farming is well developed worldwide as it can be a non-intensive system of culture relying on environmental factors such as stocks of mussel seed and food supply (Inglis *et al.*, 2000). Marine mussels are robust and because they are primary consumers the cultivator does not need to supply the stock with artificial food. In 2000 the world production of farmed mussels was 1.3 million tonnes (FAO, 2002). Mussels are the third most important marine bivalve in term of fisheries landings worldwide with 238.10^3 t in 1999 (FAO, 1999). Due to its wide distribution, the blue mussel is cultivated all around the world (China, Canada, and Europe) using on and off-bottom techniques (Hickman, 1992).

The two principal mussel species used in European fisheries are *M. edulis* (51%) and *M. galloprovinciallis* (23%). Nevertheless, approximately 80% of annual landings come from aquaculture production. Aquaculture of mussels is variable due to the fact that the intervention of humans can occur at different stage of the mussel cycle (Gosling, 2003). The seabed cultivation of mussels generates the greatest revenue of any molluscan shellfish cultivation in the UK. Production in 2001 was 14,900 t, worth £ 4,736,000 (data from DEFRA and the Scottish Executive). About

two thirds of UK production of mussels comes from Wales; much of which is from lays in the Menai Strait (11,000 t in 2003; K. Mould, Myti Mussels, pers. comm.). In UK, other areas of bottom culture include Poole Harbour, Morecambe Bay, the Wash, the River Exe and the Dornoch Firth, Scotland.

In the Menai Strait, the industry is dependent on the irregular supply of seed mussels harvested from wild subtidal stocks. The farmers dredge seed from natural seed beds from Morecambe Bay, Conwy Bay, Caernarfon Bar and Swansea Bay in South Wales. Wild seed mussels are harvested using dredges and relaid on commercial beds in sites leased from the Crown Estate via the Sea Fisheries Committees in England and Wales and via the Scottish Executive in Scotland. At present, little is known about the ecological importance of seed mussel beds or the ecological consequences of harvesting them. In the Menai Strait, the mussel seed are firstly laid in the intertidal zone for c. 18 months, until they grow large enough to reach a partial predation refuge. The mussels are then moved into subtidal lays for a final period of rapid growth. Mussels are marketable when they reach a shell length >55 mm, a process that takes approximately 2½ years from the settlement of spat to the harvesting of marketable mussels (Pillay, 1993, Kim Mould, Myti Mussels, pers. comm.).

1.3 Carrying capacity: Production model

Shellfish aquaculture is an important part of coastal fisheries and its exploitation raises fundamental questions of sustainability that can be expressed via carrying capacity, which has become an important issue in coastal zone management (Bacher *et al.*, 2003). Biologically, carrying capacity can be defined at two different levels; on a large- (ecosystem-) scale and a local-scale (exploitation of mussels). The scale is important to take into account due to the influence of the local tidal currents and the geographical position and density of the mussels which impact the mussel growth through food depletion (Grant, 1996; Pouvreau *et al.*, 2000), but also for helping the management of the sites for cultivation (Nath *et al.*, 2000).

Inglis *et al.* (2000) defined carrying capacity at four levels: Production carrying capacity (enclosing ecosystem and local scale exploitation), Ecological carrying capacity, Physical carrying capacity and Socio-economic carrying capacity. In this

study the focus is on production carrying capacity, as physical carrying capacity is defined by the fisheries orders and the economic and social carrying capacities depend on social values (McKindsey *et al.*, 2006).

Physical carrying capacity is the total area of marine farms that can be accommodated in the available physical space (Inglis *et al.*, 2000; Smaal *et al.*, 1998).

Production carrying capacity concerns the stocking density of bivalves at which yields are maximised. Hence, it takes into account the amount of food production (primary production, POM, phytoplankton), the rate of food supply (hydrodynamic transport) towards the mussel farm, as well as the food conversion to mussel biomass (mussel energetic, scope for growth). Ecosystem models have been developed to estimate the Production carrying capacity taking into account the feedback mechanisms, as for instance, the food availability for mussel depends on primary production and hydrodynamic processes (Smaal *et al.*, 1998).

Ecological carrying capacity concerns the impact of the mussel farm on adjacent surrounding environments. These issues would include mussel seed availability and the ecological footprint of dredging, the enrichment of sediments with organic material produced by the mussels (Grenz *et al.*, 1990), the effects on the benthic fauna (Beadman *et al.*, 2003) and the food-web associated with food depletion (Peterson and Black, 1991; Koseff *et al.*, 1993), the attraction of predators (Kaiser *et al.*, 1998).

Socio-economic carrying capacity is the level of farm development beyond which unacceptable social impacts occur (Inglis *et al.*, 2000) and it is linked to maximising the return on investment of the mussel exploitation (Smaal *et al.*, 1998).

Production carrying capacity is mainly limited by the renewal rate of food which in turn depends on primary production and water residence time. The identification of a 3-D space with **the water residence time** (= *physical environment*), **primary production time** or replacement time (= *primary producers*) and clearance rate or **clearance time** by the bivalve (= *grazers*) provide an indication of the fundamental parameters of highly productive and sustainable bivalve filter feeding dominated systems (Dame and Prins, 1998). Most of the important parameters controlling the carrying capacity of the ecosystems are dynamic and fluctuate markedly with the seasons and locations. Unfortunately, net fluxes and advection are not well known in

these systems (Dame and Prins, 1998). In order to evaluate the relationship between the different processes involved in production carrying capacity, the ecosystem, via simulation models is viewed as distinct compartmental or state variables (Biological and physiological) for which the flow of energy and material (Grant, 1993) and feedbacks between compartments are quantified (Prins *et al.*, 1998). Previous carrying capacity models have considered mostly biochemical (primary production and gazing) processes influencing growth of bivalves whereas physical processes (such as sediment deposition and resuspension) have been addressed more simplistically (Duarte *et al.*, 2003).

These ecosystem models with feedbacks are 1) hydrodynamic models describing the physical transport of material in the water column, the effect of tide and turbulence, residence time and current flow; 2) primary production relating to the quality and abundance of phytoplankton; 3) mussel energetics describing the growth (ingestion, assimilation) condition and reproduction of the mussels (Figure 1. 4).

Mussel and hydrodynamics interactions

Mussel beds dynamic, are controlled by feedbacks between biotic and physical processes (Dankers *et al.*, 2001; Gascoigne *et al.*, 2005). Mussels depend on water column movement for food supply, as well as to transport the mussel larvae to a suitable habitat. Mussel beds often form in highly energetic areas with high flow rates and turbulent near-bed mixing. Biogeomorphological processes, in intertidal environment, are related to the small scale interactions between the activities of benthic organisms and their environment, in return, these processes have impacts on the geomorphology of the seabed at larger scales (Murray *et al.*, 2002). The mussels produce byssus threads to fix themselves to each other and to rocks or gravel on muddy intertidal area, and this provides advantages (positive feedback) in term of avoiding wave dislodgement and predatory losses (Bertness and Grosholz, 1985; Okamura, 1986). The byssus thread matrix protects the bed from dislodgement by hydrodynamic forces and can lead to the formation of armoured bioherms or hummocks (Murray *et al.*, 2002). These armoured bioherms affect the hydrodynamics in the boundary layer transforming the pattern of sediment erosion, deposition and transport with effect on a larger scale (Murray *et al.*, 2002).

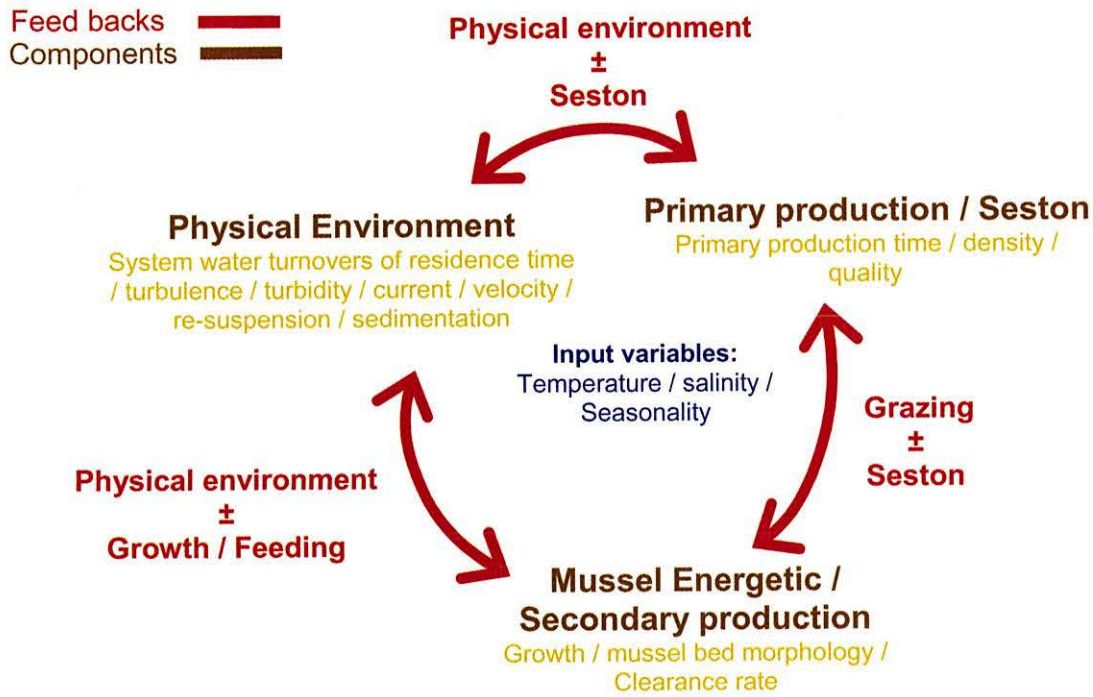


Figure 1. 4: Schematic diagram of the three main components of production carrying capacity models with feedbacks: 1) physical environment, 2) primary production and 3) mussel energetic.

Mussels also play an important role in "benthic-pelagic coupling", by transferring material (nutrients, pollutants…) from the water column to the sea bed. The large volume of water filtered by the mussels creates a continuous flux of particulate matter from the water column to the bivalve beds (Smaal and Prins, 1993). The rate of particle sedimentation in cultivated mussel beds can be 2 to 3 times higher than comparable locations without mussels (Inglis *et al.*, 2000). Consequently, mussels have a large impact on the seston flux in the water column (Dame *et al.*, 1991). Filtered inorganic material is either ingested, resulting ultimately in faeces production, or rejected prior to ingestion as pseudofaeces (Bayne *et al.*, 1976; Smaal, 1991). The deposited material is enriched in organic content.

Only a fraction of the suspended particulate matter (SPM) filtered by the mussel population is stored as deposits in the sediments (Dame *et al.*, 1991). The majority of filtered and biodeposited material is re-suspended immediately (Smaal *et al.*, 1986) due to its low density and high water content relative to non-biogenic sediment (Stuart *et al.*, 1982). The re-suspension of particles from the bottom can be another

source of food for mussels (Fréchette and Bourget, 1985a). The mussel beds increase sediment flux both from the water column to the seabed and from the bed back to water column, and mussel biodeposits may contribute significantly to the total suspended load in estuarine and coastal environments (Kautsky and Evans, 1987). Moreover, the mussel bed itself modifies the turbulence via i) the bed roughness created by emergent mussel shells (Butman *et al.*, 1994), ii) biomixing created by the exhalent current from mussel siphons in the near-bottom water (Lassen *et al.*, 2006; van Duren *et al.*, 2006) and iii) the complex bed topography created by bare area constrasting with mussel patches (Butman *et al.*, 1994).

Mussel and primary producer interactions

Mussel growth depends on food supply (Fréchette *et al.*, 1989; Larsen and Riisgård, 1997). Mussel beds can process large volume of water (Jørgensen, 1990), up to 170 m^3 m^{-2} d^{-1} (Prins *et al.*, 1996) and consequently affect the abundance and structure of phytoplankton communities. Conversely, food availability for mussel varies seasonally with strong short term variability related to phytoplankton blooms (Legendre, 1981). The uptake of phytoplankton by large bivalve beds tends to exceed the primary production per m^2 of bottom area in shallow water (Smaal and Prins, 1993). Mussels feed on seston from the benthic boundary layer (Fréchette and Bourget, 1985a) and phytoplankton biomass reduction in the water column, as result of mussel feeding, has been demonstrated in numerous studies with depletion in phytoplankton biomass ranging from 10% to 74% (e.g. the Oostercheekde Estuary, Netherland, Prins *et al.*, 1996, Roskilde Fjord, Denmark, Møhlenberg, 1995; Limfjorden Denmark, Dolmer, 2000a; Oeresund Strait, Sweden, Noren *et al.*, 1999; Wadden Sea, Germany, Asmus and Asmus, 1991). Phytoplankton depletion due to filter feeding depends on mussel density (Prins *et al.*, 1995) and water column mixing. Vertically declining profiles of phytoplankton biomass are produced in the water column (Dolmer, 2000a), and food limitation of mussel growth immediately above mussel beds has been observed (Fréchette and Bourget, 1985a, 1985b; Bertness and Grosholz, 1985; Okamura, 1986; Fréchette *et al.*, 1992; Newell, 1990; Svane and Ompi, 1993; Haamer and Rohde, 2000; Smaal *et al.*, 2001).

Several approaches have been used to model feeding in suspension-feeding bivalves, such as food concentration vs clearance time or food concentration vs

ingestion rate (Grant, 1996). The **Bivalve clearance time** is the time that is theoretically needed for the total bivalve filter feeder biomass within an ecosystem to filter particles from a volume of water equivalent to the total system volume (Smaal and Prins, 1993) and has been used to model carrying capacity (Dame and Prins, 1998). This turnover time is a function of bivalve biomass and the seasonal influences of particulate concentrations, seston quality, and temperature on the filtration rate of bivalves. An important factor to take into account while studying food depletion is the scale of measurements. Bacher *et al.* (2003) stressed that a large scale study (i.e.1000 m scale) was necessary to study food depletion arguing that it would not occur over smaller distances. However, food depletion has been observed at smaller scales (Dolmer, 2000a; Riisgård *et al.*, 2006; Saurel *et al.*, 2007).

The food depletion phenomenon could have important competitive impact for the ecosystems, e.g. competition with encrusting bryozoan (Buss, 1979). The mussel filter feeding can affect the plankton community structure, skewing it towards smaller faster growing species (Furnas, 1990; Prins *et al.*, 1995; Noren *et al.*, 1999). This can cause a shift in the population to higher proportions of diatoms (high growth rate species) and declines in relatively slow growing dinoflagellates (Prins *et al.*, 1995). Cropping of phytoplankton population by bivalve filter feeders has been suggested as a natural control of eutrophication (Officer *et al.*, 1982; Alpine and Cloern, 1992; Thompson, 2000). In certain circumstances, this could therefore result in fewer toxic algal blooms (Noren *et al.*, 1999). Mussel filter feeding may not be completely unselective, however, since some species may be unpalatable, including many toxic or noxious species.

Although mussels consume phytoplankton, they may also help regenerate phytoplankton populations by increasing nutrient availability through nutrient regeneration (Prins *et al.*, 1995). Filtered material is re-mineralised through the direct excretion by filter feeders or via bacterial processing in the underlying sediments. When phytoplankton growth is nutrient limited, this release of nutrients may promote phytoplankton growth (Asmus and Asmus, 1993). A study in the Wadden Sea indicated that induced phytoplankton production supported by ammonium released from a mussel bed could be higher than the actual phytoplankton uptake (Asmus and Asmus, 1991). The seston concentration and composition can vary in near bed and surface above bivalve bed during a number of tidal cycles (Smaal and Haas, 1997). Moreover, re-suspension of benthic microalgae can have a positive effect on the food

quality for oysters (Grant *et al.*, 1990). Frechette and Grant (1991) showed that mussels suspended 1 m above the bed grew faster than those on the ground. Nevertheless, the estimation of food availability for our mussel bed requires near-bottom seston measurement (Fegley *et al.*, 1992).

Hydrodynamic and primary production interactions

The hydrodynamic processes of transport have been described for ecological models in a simple way that consider only residuals flows, a tidally averaged situation and diffusion coefficient (Duarte *et al.*, 2003). The system studied is influenced by the residence time of water in the system (turnover time), turbulence, current velocity, sedimentation and re-suspension influencing both primary and secondary production; the latter in turn influences the physical environment. Tidal advection and vertical turbulent flux are the major factors involved in the supply of food in the benthic boundary layer. Nevertheless, the turbulence, mixing and food supply down towards the mussel bed are not only derived from current driven by tidal advection, wave action or upwelling action, but are also created by the mussel bed itself. The mussel bed growth and individual morphology coupled with filtration actions (expulsing and filtering) affect the turbulence in the boundary layer and contribute to the re-suspension of deposited material (Lassen *et al.*, 2006; van Duren *et al.*, 2006).

The turbulence that occurs above mussel beds can lead to re-suspension of nutrients that induce growth of phytoplankton in the water column. However, the effect of nutrient release on phytoplankton productivity will be dependent on various environmental conditions, and the nutrients will also be available to other primary producers such as benthic algae and microalgae (Asmus and Asmus, 1993). Moreover, the turbulence associated with re-suspension induces silt suspension and higher turbidity with an associated reduction of light penetration that can lead to a reduction of photosynthesis and primary production.

1.4 Scope and Rationale

There is a growing awareness of the ecological role of filter-feeders in terms of their impact upon coastal and estuarine environments, but also on the biotic and abiotic factors that influence communities. Despite our increasing understanding of the role of filter-feeders, their response to environmental and ecological factors remains a subject of ongoing debate in the scientific community. For instance, the factors that control mussel feeding behaviour are still the subject of disagreement or different interpretations (physiological vs automatic pump; Bayne, 1998; Hawkins *et al.*, 1998; Jørgensen, 1996, Riisgard, 2001). Further debate is focussed on the proper use of methodologies to assess the clearance rate and hence grazing capacity of mussel in laboratory or *in situ* studies (Riisgard, 2001). The feeding behaviour and clearance rate of mussels are key measurements to understand and model carrying capacity of coastal systems dominated by mussel beds.

The main aim of this study is to investigate the individual problems of the mussel energetic/secondary production component from the production carrying capacity (PCC) components (Figures 1.4 & 1.5) under a new *in situ* insight for a better modelling of the production carrying capacity. This study attempts to answer key questions concerning the factors that influence mussel feeding behaviour, and investigates further the use and development of adequate *in situ* methodologies to assess the grazing rate of mussels, or to assess the influence of food on mussel microgrowth. Finally, the study targets the interactions between the 3 main components of production carrying capacity over an intertidal mussel bed using *in situ* measurements.

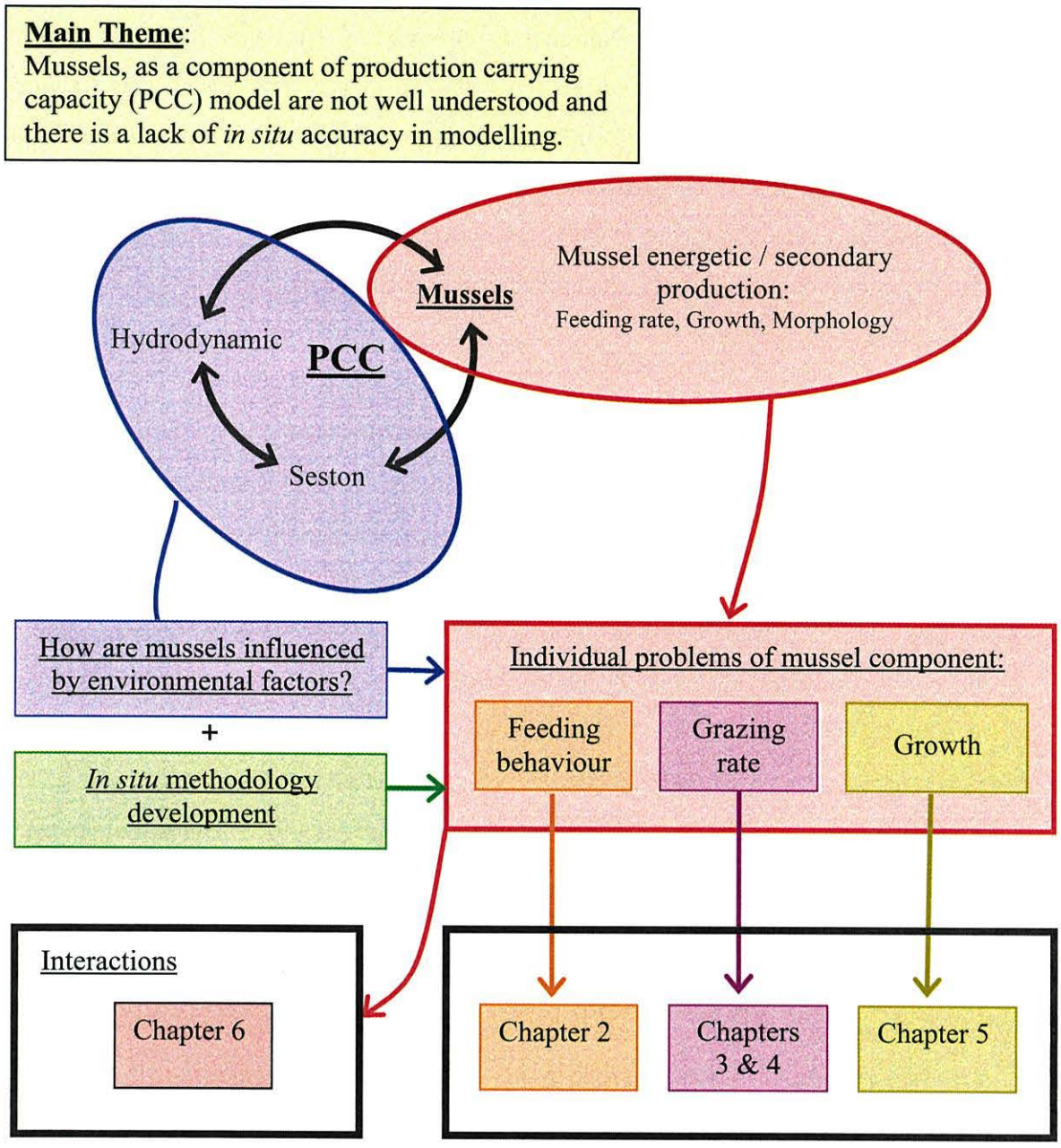


Figure 1. 5: Flow diagram showing the approach taken to study the influence of the environmental factors on the individual elements from the mussel component of the production carrying capacity (PCC).

The majority of research undertaken in this PhD occurred in the School of Ocean Sciences (SOS), University of Wales, Bangor. Nevertheless, some parts of it were conducted with the collaboration of other scientists and institutes. In SOS, Chapters 2, 5 and 6 were conducted within a large multidisciplinary project focused on the carrying capacity of the Menai Strait. Chapter 6 resulted from the collaboration

between SOS and DMU (or National Environmental Research Institute - NERI, Roskilde, Denmark), and the research undertaken in North Wales; it was financed partly by BBSRC (D18866) and BBSRC SIS (award 1554). Eventually chapter 3 was conducted in DMU with CREAM European funding (HPMT-CT-2001-00265-17) and chapter 4 was conducted in DSU (Denmark) with the DMU team. Therefore, at the beginning of each chapter, the contribution from different scientists and institutes is detailed.

The first chapter provides an overview of the mussel ecology and the environment of the Menai Strait (UK), which was the primary location where the experiments took place. This chapter introduces the notion of production carrying capacity and the different components that are studied in the following chapters.

The second chapter links the hydrodynamic and mussel feeding components of the production carrying capacity model. In this chapter a multidisciplinary approach is adopted that examines the feeding behaviour of mussels measured *in situ* and their relationship with hydrodynamic forces. This chapter, due to the complexity of the hydrodynamics experienced in the Menai Strait, identifies those drivers that act upon the feeding behaviour of the mussel. This chapter contributes to the current debate about physiological vs automatic regulation of the mussel feeding behaviour.

The third chapter explores an *in situ* methodology to improve estimates of mussel grazing capacity. This chapter describes and validates the use of an *in situ* method to estimate clearance rate via mussel defecation. Using a combination of laboratory experiments and simplified modeling of the digestion factor, this method applied in the field allows a more realistic estimation of mussel clearance rate and hence its potential impact for shellfisheries management. This research also contributes useful insights into the current debate surrounding *in situ* methodologies vs laboratory methodologies to measure mussel clearance rate.

Chapter 4 extends the approach of the previous chapter through an investigation of three different *in situ* methods to measure the clearance rate of mussels and to compare the defecation method to existing *in situ* methodologies. Once again, this intercalibration contributes more to the current debate about the use of clearance rate methods.

The fifth chapter focus on techniques to measure mussel growth under different food regimes and temporal scales. The mussel micro-growth technique was used and

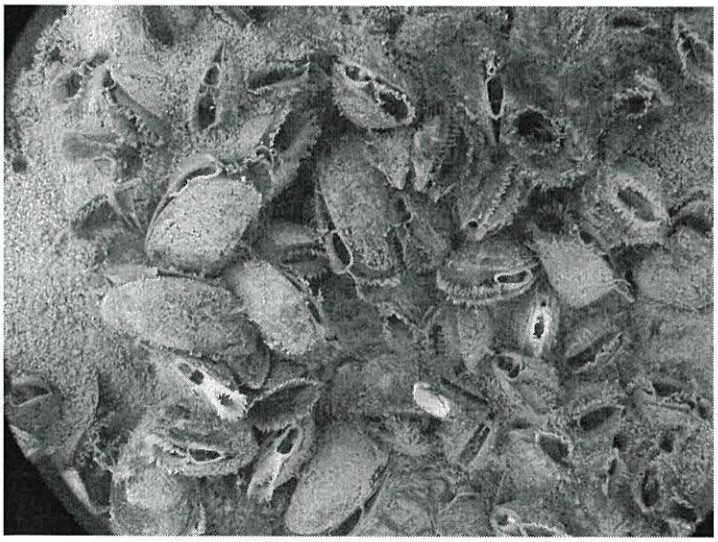
compared to traditional morphometric measurements from laboratory and field research to study mussel growth as a function of food supply in relation to a food and concentration gradient at different scales over the mussel bed: large scale (bed scale), and patch scale (<10 m).

In chapter six, another multidisciplinary approach was used to look at the three components of production carrying capacity above an intertidal mussel bed. The balance between vertical diffusion, horizontal advection, grazing capacity and seston concentration in the column were integrated to understand the consumption rate of food by the mussel bed and how this was influenced by bed topography and spatial structure.

Chapter II

Mussel feeding behaviour*

Intertidal mussel bed 7:04 25/04/2005



Intertidal mussel bed 13:34 25/04/2005

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* This chapter is published as:

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Chapter II *In situ* mussel feeding behaviour in relation to multiple environmental factors: Regulation through food concentration and tidal conditions

2.1 Contributions to this chapter

Chapter process:	Contributions
Conception	CS/MJK/JG
Design analysis	CS/MJK/JG
Data collection	CS/ JG
Data analysis/ Interpretation	CS/MP
Drafting	CS
Revising	CS/MJK/JG/MP/JKP

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MJK	M. J. Kaiser	
JG	J.Gascoigne	
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2.2 Abstract

Feeding behaviour of mussels (*Mytilus edulis*) was measured *in situ*, using a video camera and expressed as the mean percentage of valve gape aperture (VA), concomitant with environmental and biological variables over two tidal cycles. Mussel feeding behaviour and the physical parameters responded to three primary tidal components, of which semi-diurnal periodicity was dominant (12.42 h). VA was synchronized with chlorophyll *a* (chl *a*) concentration (proxy for food) with a strong positive correlation ($r = 0.72$, $p < 0.001$). Chl *a* and suspended particulate matter (SPM) were dependent on tidal advection. The combination of the reconstructed tidal constituents derived from harmonic analysis were used to successfully model mussel feeding behaviour ($r = 0.90$, $p < 0.001$). In this

concentration range (0.6 to 2.5 μg L^{-1}), chl *a*, measured at 1 m above the mussel bed, regulates mussel feeding behaviour irrespective of the presence of predators, changes in SPM, or flow velocity.

2.3 Introduction

Mussels are a ubiquitous feature of intertidal and shallow subtidal areas (Seed, 1976). They are ecologically important as they form large biogenic reefs that can enhance local community diversity and they provide a critical link between benthic and pelagic systems through their filter-feeding activities (Seed, 1976; Dame *et al.*, 1991; Herman, 1993; Beadman *et al.*, 2004). The mechanisms and physiological constraints of mussel feeding have been intensively studied in order to predict and understand the effect of mussel grazing on coastal energy flow processes (Bayne, 1998; Dame and Prins, 1998; Dame *et al.*, 2002; Duarte *et al.*, 2003). In natural systems, mussels are limited by competition, predation and physical forcing (Fréchette and Despland, 1999). In contrast, cultivated systems may have an artificially elevated biomass of mussels that are primarily constrained by food availability (Beadman *et al.*, 2004; Gascoigne *et al.*, 2005). In order to achieve sustainable cultivation it is necessary to measure the carrying capacity of coastal areas. To achieve this goal it is necessary to understand how changes in the supply and quality of food, control mussel feeding and growth at different temporal and spatial scales.

Mussel grazing rates are often derived from the maximum filtration rate of mussels determined in laboratory experiments, but these parameters can be over-estimated (Prins *et al.*, 1996; Petersen, 2004a). Several approaches have been used to quantify feeding behaviour of bivalves through the direct measurement of physiological traits mostly in laboratory studies (e.g. filtration rates, clearance rates, pseudofaeces production, absorption efficiency, selection efficiency, absorption rates, rates of oxygen consumption; Bayne *et al.*, 1993; Bougrier *et al.*, 1997; see review by Riisgård, 2001a), and in the field (Newell *et al.*, 1998; Newell *et al.*, 2005a); or using still images or video recordings in the laboratory and field (Newell

et al., 2001; Macdonald and Nodwell, 2003; Riisgård *et al.*, 2003). The latter is reported to be an appropriate tool for *in situ* measurement of feeding behaviour (Newell *et al.*, 1998; Dolmer, 2000b; Riisgård *et al.*, 2003).

The regulatory mechanism of feeding behaviour in *M. edulis* has been the subject of debate that has focused on the physiological processes as a function of the food composition and nutritional requirements (Hawkins *et al.*, 1998a) or mechanical processes in which filter-feeders are considered as an automatic pump and where the regulation is determined by their capacity to process food (Jørgensen, 1990, 1996). Riisgård *et al.* (2003) asserted that these different points of view are mainly due to inconsistencies in methodological measurement of mussel filtration and that most of the reported experiments have been conducted in laboratories with high algal concentrations. Conversely, in the field, mussels are more likely to experience lower algal concentrations, with consequent lower filtration activity. The response to algal concentration appears to be non-linear beyond a threshold concentration. Low algal concentration (< 0.5 μg chl *a* L^{-1}) can induce the mussels to stop feeding to conserve energy until better conditions occur (Wilson and Seed, 1974; Dolmer, 2000b; Riisgård *et al.*, 2003), whereas high algal concentration (> 10 μg chl *a* L^{-1}) may lead to reduced valve gape and a reduction in filtration rate (Clausen and Riisgård, 1996; Macdonald and Nodwell, 2003).

In this study the feeding behaviour of mussels was measured *in situ* using the valve gape aperture (VA) of the mussels. *In situ* measurements and observations are likely to provide a better understanding of mussel feeding behaviour than laboratory experiments, since they provide a natural physical, chemical and biological environment and avoid artifacts associated with manipulation disturbance or acclimation. The control of filtration rate in relation to variation of VA has been demonstrated in previous studies (Jørgensen *et al.*, 1988; Newell *et al.*, 1998; Riisgård *et al.*, 2003) with a regulation of the VA and filtration activity in response to the presence or absence of algae (Riisgård *et al.*, 2003, 2006). Previously, VA has been calibrated to measure filtration rates in mussels (Dolmer, 2000b).

The present study is part of a wider research programme that aims to understand the physical and ecological key processes that affect cultivated mussels beds in a tidally flushed system, and to quantify the carrying capacity of this system in order to manage the mussel fishery in a sustainable manner. A time-series over two tidal

cycles including a large set of environmental and biological variables was recorded in the Menai Strait, UK. The aim was to understand the relationships between the environmental factors and their individual effect on the feeding behaviour of mussels, with a particular emphasis on those factors that regulate variability in food supply. In mussels, the consumption of food (mainly phytoplankton and particulate organic matter), may vary with concentration, flux, and quality of food (Newell *et al.*, 2005a). Assuming that chl *a* is an indicator of the concentration of the main component of food for mussels, the study was focused on the influence of the concentration and supply rate of chl *a* on the feeding activity of mussels. In addition the influence of suspended particulate matter (as potential source of food or disturbance), flow velocity (as proxy for flux of chl *a* or disturbance), and predators (a proxy of disturbance) on mussel feeding behaviour were also examined. Ultimately, the aim was to understand if the effects of these regulatory factors are quantifiable to enable us to predict the population grazing capacity.

2.4 Material and Methods

Site

The study was undertaken in the subtidal zone of the northern part of the Menai Strait, Wales, United Kingdom (53°15.025N 04°06.575W, Figure 2. 1) between the 10th and 12th September 2004. The Menai Strait is a well-mixed tidal body of water (velocities up to 2.5 m s^{-1} in certain areas) with an asymmetrical tidal flow such that the net discharge passes over the natural and commercial mussel beds from east (Liverpool Bay) to west (Caernarfon Bay) of ~330 to 800 m^3 s^{-1} at neap and spring tides respectively, and the water has a residence time of approximately 2 to 3 days (Rippeth *et al.*, 2002) (Figure 2. 1).

Feeding behaviour

A video camera was deployed from the RV Prince Madog for 48 h on the subtidal cultivated mussel bed (Rovtech SeaCam color camera). The camera was mounted on

a metal frame at a height of 450 mm from the seabed and was connected to the vessel by an umbilical cable carrying the power for the camera and the light (2 x 20 watt, 12 volt halogen lamps mounted to either side of the camera). The video signal was recorded using a Sony digital recorder. Feeding behaviour of mussels (~30 individuals) was determined from captured videos frames (every 10 minutes) and measured with an image analysis program (analySIS©). Feeding behaviour was expressed as the mean percentage of VA. The latter was defined as the percentage of the maximum recorded distance between mussel valves measured between the two siphons. This relative measurement allowed for the fact that the mussels were i) different sizes and ii) randomly aligned and thus presented varying angles to the camera. Measurements were discarded for individual mussels that moved during the observations.

Environmental factors

During the camera deployment, CTD (conductivity, temperature, depth - SeaBird Electronics) casts were conducted every 30 minutes for 48 hours. A bottom mounted RDI 1.2-MHz Workhorse Acoustic Doppler Current Profiler (ADCP) was positioned on the seabed in close proximity to the camera frame for the duration of the experiment to measure and average across bins of 10 minutes current velocity and depth. Due to the restriction of the ADCP, the closest measurable velocity was at 84 cm (~1 m) above the mussel bed and flow velocity was calculated as the water column mean longitudinal velocity. The CTD could not be deployed during the periods of maximum flood current flow resulting in some unavoidable gaps in the dataset. Seawater from near the seabed (~1 m above the mussel bed) was collected using rosette mounted Niskin bottles for chl *a* and total SPM.

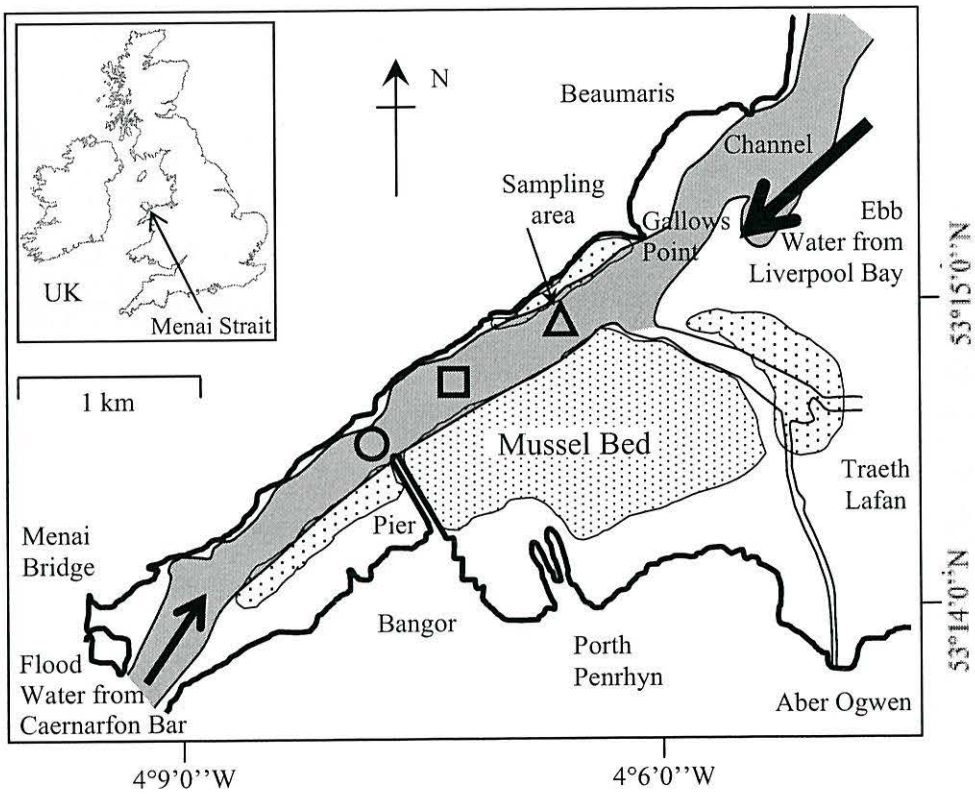

Figure 2. 1: Map of the Menai Strait, United Kingdom, the arrow indicates the direction of the current at ebb and flow regime (adapted from Caldow et al. 2003). Data collected subtidally at Gallows Point (53°15.025N 04°06.575W, triangle) between the 10 to 12 September 2004 (~1.8 km^2 farmed at density ~10 kg m^{-2} in the studied area). Commercial mussel beds are laid intertidally (dotted area) and subtidally (in the channel). Sampling August 2004 (53°14.680N 04°07.257W, square), August 2005 (53°14.432N 04°07.767W, circle).

Chl *a* was obtained after filtering 500 mL of seawater through GF/F (pore size 0.7 μm) 47 mm diameter glass filter and stored in a -70 °C freezer; chl *a* was extracted for 18 h at 4°C with 90% acetone and concentration was measured on a Turner Design 10-AU fluorometer (method adapted from Parsons et al. 1984). SPM was measured from 1.0 L of seawater, filtered on pre-weighted GF/F 47 mm diameter glass filters and dried in the oven at 90°C for 24 h. Flux of chl *a* (FC in g m^{-2} d^{-1}) was calculated according to FC = chl *a* concentration × V where V is the flow velocity. Two other sets of chl *a* concentration, velocity, and depth data were measured and treated following the same methodology as September 2004 during two campaigns conducted next to this study site in August 2004 (53°14.680N 04°07.257W) and August 2005 (53°14.432N 04°07.767W) (Figure 2. 1). These data sets were

compared in order to complete the gap in the data in relation to chl *a* concentration collected in September 2004. Air temperature data were obtained from a local meteorological station.

Predators

The number of mussel predators (the green crab, *Carcinus maenas*, and the common starfish, *Asterias rubens*) were counted from within the field of view of the camera (~550 cm^2) every 10 minutes.

Data analysis

Environmental data were re-sampled at 10 minutes intervals as for the measurements of the VA to compare the data on the same time scale. In order to clarify the interaction between feeding behaviour and environmental factors, the most significant frequencies present in each dataset were identified by spectral analysis using a Lomb normalized periodogram (Press *et al.*, 1992). Prior to spectral analysis, temperature and salinity were de-trended. The amplitudes and phases of the statistically significant constituents were calculated using harmonic analysis employing a least squares fitting technique (Emery and Thomson, 2001). The combination of the reconstructed tidal constituents derived from harmonic analyses were used to predict and model the feeding behaviour and chl *a*. Cross-correlation analysis was performed on the environmental factors, the change in abundance of predators, and the mean percentage VA. This analysis enabled us to determine which time lag provided the best correlation between two factors. The relationships among the different parameters were calculated using linear regression if the data met the assumptions of normality and homogeneity of variance. For comparisons among datasets, ANOVA was used to test for significant differences if the data met the assumptions of normality and homogeneity of variance, otherwise either a Kruskall-Wallis or Mood's Median test was used.

2.5 Results

Physical environment

CTD profiles (temperature, salinity, fluorescence, SPM) showed that the water column was well mixed. In the present study ebb tide is defined as a negative flow velocity and flood tide as a positive velocity (Figure 2. 2). All of the measured variables had significantly higher values on the ebb than on the flood, except velocity and temperature (Table 2. 1). There was a decrease in mean seawater temperature of 1.5 °C (17.4 to 15.9 °C) which was related to the decrease in mean daily air temperature (drop from 19.6 to 13.3 °C over 5 days). Water height above the mussel bed varied between 10.8 and 5.9 m (Figure 2. 2). Salinity varied only slightly from 33.0 and 33.3 with a trough of 32.8 possibly due to the input of fresh water from the River Ogwen located next to our sampling site (Tweddle et al., 2005), while chl *a* concentrations (Figure 2. 3A) ranged between 0.6 and 2.48 $\mu g\ L^{-1}$ and SPM concentrations ranged between 2.05 and 19.86 $mg\ L^{-1}$ (Figs. 2 and 3B). The mean flux of chl *a* was 38.02 $g\ m^{-2}\ d^{-1}$ and oscillated between 0.43 and 93.31 $g\ m^{-2}\ d^{-1}$.

Figures 2. 3 and 2. 4, show the current velocity decomposed into 4 phases: ebb and flow separated into phase up (increasing velocity) and down (decreasing velocity). Chl *a* concentration repeated the same pattern over the two tidal cycles (Figure 2. 3A) such that fluorescence increased during the ebb regime and reached its maximum during the ebb down due to the tidal advection of the water that originated from Liverpool Bay. The SPM data did not follow this pattern (Figure 2. 3B). The concentration of SPM was significantly higher on the ebb compared with the flood phases. After the turn of the tide, the time taken for the new water enriched in chl *a* to pass over the mussel bed was calculated to be ~90 min.

Table 2. 1: Descriptive statistics of all the variables measured; mean and standard error are indicated for ebb and flood regime. Statistical comparison with p value between ebb and flood for each factor (aperture, velocity, height above the bed, temperature, salinity, chl *a* concentration, SPM, starfish, and crabs) is indicated with TT for t-test, MT for Mood test, KW for Kruskall Wallis test, df = 1.

	Mean ± SE ebb	Mean ± SE flood	Difference ebb vs flood	
Aperture (%)	46.11 ± 0.95	39.41 ± 0.80	TT: $t_{249} = 5.40$	$p < 0.001$
Velocity (m s^{-1})	0.299 ± 0.013	0.386 ± 0.016	MT: $\chi^2 = 7.94$	$p < 0.05$
Height above bed (m)	9.89 ± 0.06	7.69 ± 0.15	MT: $\chi^2 = 147.83$	$p < 0.001$
Temperature (°C)	16.60 ± 0.04	16.84 ± 0.05	KW: $H = 13.21$	$p < 0.001$
Salinity	33.21 ± 0.01	33.17 ± 0.01	KW: $H = 9.87$	$p < 0.05$
Chl *a* (µg L^{-1})	1.63 ± 0.05	1.22 ± 0.05	KW: $H = 31.42$	$p < 0.001$
SPM (mg L^{-1})	9.23 ± 0.44	4.42 ± 0.16	MT: $\chi^2 = 46.65$	$p < 0.001$
Starfish (number)	5.8 ± 0.2	4.1 ± 0.3	MT: $\chi^2 = 18.69$	$p < 0.001$
Crab (number)	0.9 ± 0.1	0.8 ± 0.1	MT: $\chi^2 = 0.38$	$p = 0.537$

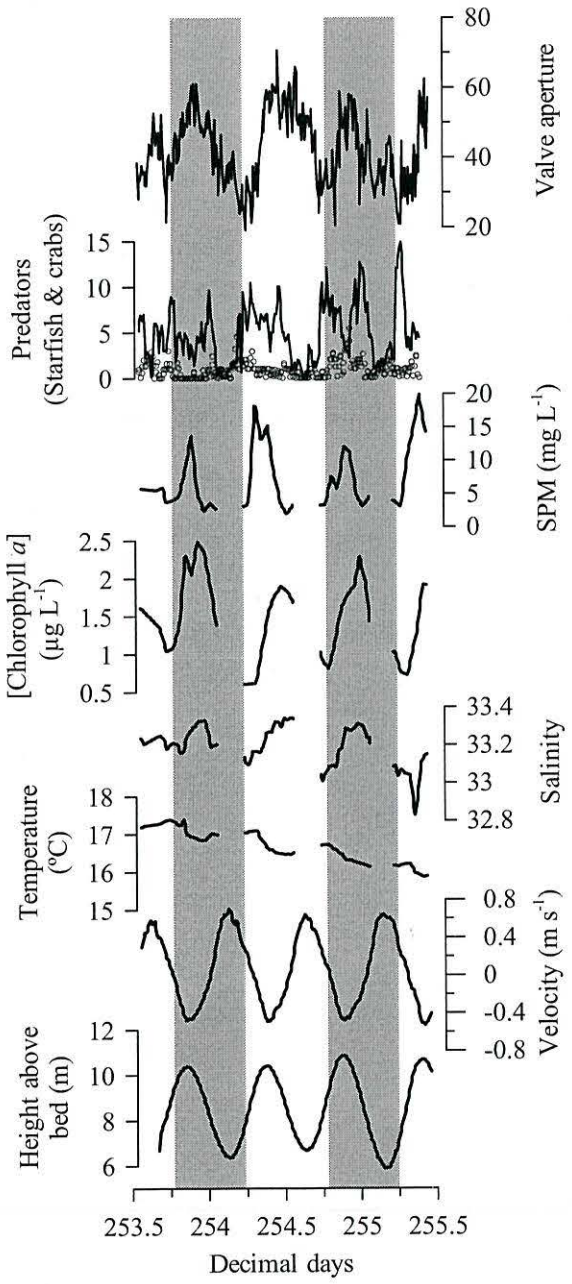

Figure 2. 2: Mean percentage valve gape aperture (valve aperture) of mussels and environmental factors: predators crab (circles) and starfish (solid line), total SPM, chl *a* concentration, salinity, temperature, velocity and height above the bed) during a 48 hour period in September 2004. Grey bands represent the night periods. Gaps in the dataset collected via CTD (SPM, chl *a*, salinity and temperature) are due to strong currents at flood regime where the CTD was not deployed.

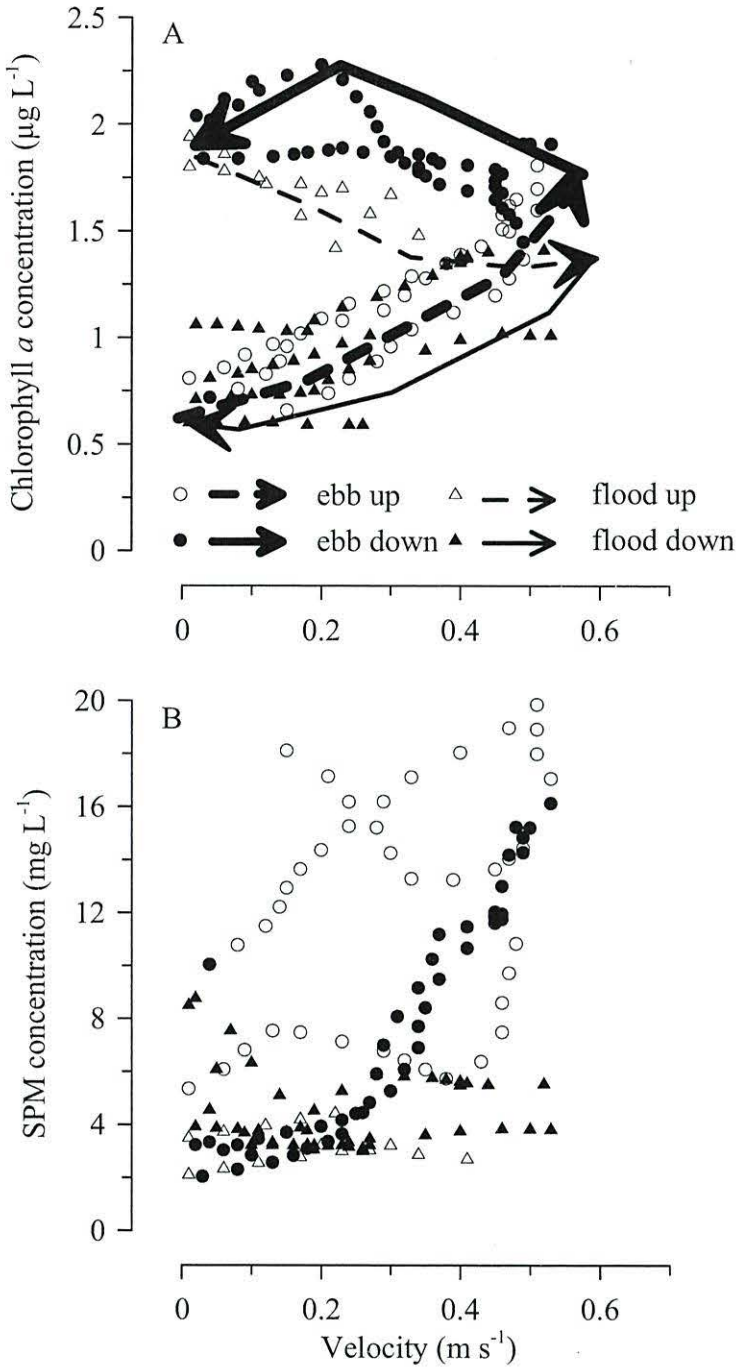

Figure 2. 3: (A) Relationship between chl *a* concentration and velocity in the Menai Strait (September 2004). (B) Relationship between SPM concentration and velocity at four different phases of the tide in the Menai Strait (September 2004). Solid and dotted (thin and thick) lines are arbitrarily drawn by eye to draw your attention to the tidal cycle movement. Phase up = increasing velocity; phase down = decreasing velocity.

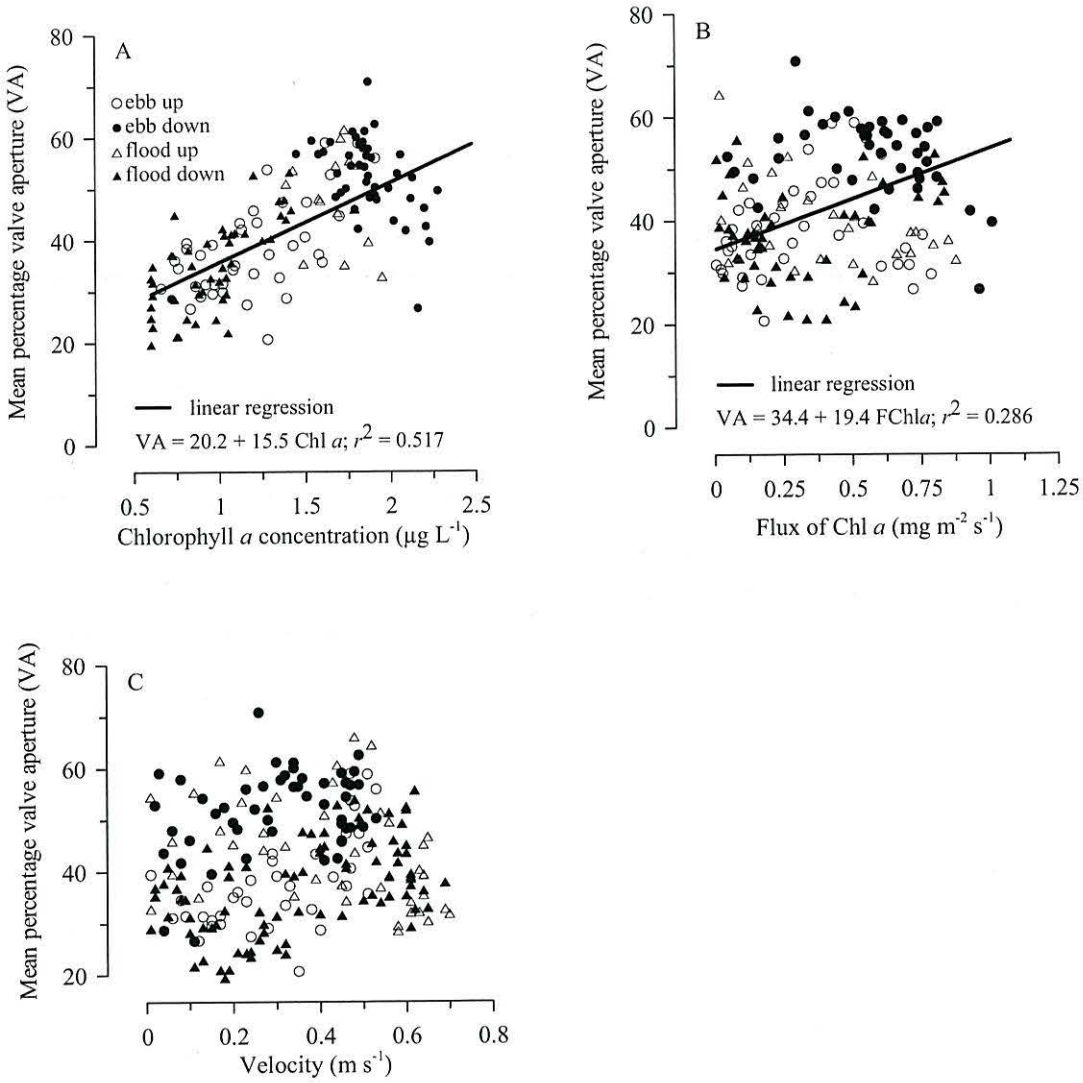

Figure 2. 4: (A) Relationship between mean percentage valve aperture of mussels (VA) and chl *a* concentration (µg L^{-1}) with linear regression (September 2004). (B) Relationship between VA and flux of chl *a* (mg m^{-2} s^{-1}) with linear regression (September 2004). (C) Relationship between VA and velocity (m s^{-1}) (September 2004). Phase up = increasing velocity; phase down = decreasing velocity.

Spectral analysis revealed the significant presence of the three primary tidal components (M_2 period =12.42 h, M_4 period = 6.21 h, and K_1 period = 23.93 h) for almost all the physical factors and VA (refer to Table 2. 2). The strongest tidal component associated with the variables measured was M_2 except for SPM for which it was M_4 (Table 2. 2).

The relationship between the environmental variables was analyzed by cross-correlation. These analyses showed that none of the environmental variables were synchronized (best correlation for time lag > 10 min) and there was great variation in

the lag phase among them (from -120 min to +340 min) (Table 2. 3). The de-synchronization of all the environmental variables facilitated investigating the effect of each separately on the feeding behaviour of the mussels.

Table 2. 2: Spectral analysis of mean percentage valve gape aperture, chl *a* concentration, SPM total, salinity, temperature, velocity, and depth for September 2004 and chl *a* concentration for August 2004, velocity and depth for August 2004. Primary tidal components are M_2, M_4, and K_1. A: when the primary tidal component was present and significant at 95% CI. NA: when no cycle was present. * De-trended data.

	Primary tidal components		
Parameters	Semi-diurnal (M_2)	M_4	Diurnal (K_1)
Aperture	A	A	A
Chl *a*	A	A	A
SPM total	A	A	NA
Salinity *	A	A	A
Temperature *	A	A	NA
Velocity	A	NA	NA
Depth	A	NA	NA
Crabs	NA	NA	NA
Starfish	A	A	NA
Day/night	NA	NA	A
Chl *a* Aug 2004	A	A	NA
Velocity Aug 2004	A	NA	A
Depth Aug 2004	A	NA	A

Table 2. 3: Cross-correlation analysis between the different factors. In each cell, the top line is the strongest correlation from the analysis (only $p < 0.05$ are shown) and the bottom line is the time lag. One time lag unit represents a value of 10 minutes. NA = non applicable. Correlation > 0.6 indicated in bold. FC = flux of chl *a* in mg m^{-2} s^{-1}. * De-trended data.

	Day/ night	Starf.	Crab	Vel.	Height	Temp.	Salinity	SPM	Chl *a*	FC
Aperture %	0.261 0 or 1	0.213 -10	NA	**-0.634** **14**	0.570 12	-0.368 3	**0.667** **0**	**0.607** **11**	**0.721** **0**	0.550 -2
Chl *a* (µg L^{-1})	-0.466 -8	-0.282 6	NA	**-0.769** **9**	**0.611** **11**	-0.316 1	**-0.767** **9**	**0.619** **13**		
SPM total (mg L^{-1})	0.261 0 or -1	0.392 16	NA	**-0.641** **4**	0.496 -4	**-0.701** **-9**	**-0.765** **11**			
Salinity*	-0.329 -7 or -8	-0.358 13	NA	-0.522 10	0.582 14	-0.216 9				
Temperature * (°C)	0.700 34	-0.327 14	NA	0.553 6	-0.195 8					
Height (m)	0.275 20	-0.365 -6	0.281 -5	**-0.674** **2**						
Velocity (m s^{-1})	0.327 -12	NA	NA							
Crabs (number)	-0.202 11	0.268 8								
Starfish (number)	-0.229 16									

Mussel feeding behaviour

The mean observed orientation of twenty-five mussel shell valves observed *in situ* were found to be randomly orientated to a hypothetical current direction (observed vs random *t*-test $t = -1.15$, df = 47, $p = 0.257$). The percentage VA of individual mussels was variable and maximum aperture from 80 to 100% occurred for an average of 1.5 h over the 46 h survey. Figure 2. 5 shows 3 mussels as an example. VA above 80% occurred only for an average period of 3 hours for these 3 mussels over the survey. The mean percentage VA of mussels during this experiment varied from 19.4 to 70.8% and there was a distinct periodic pattern of valve opening across the tidal cycle (Figs. 2 and 5).

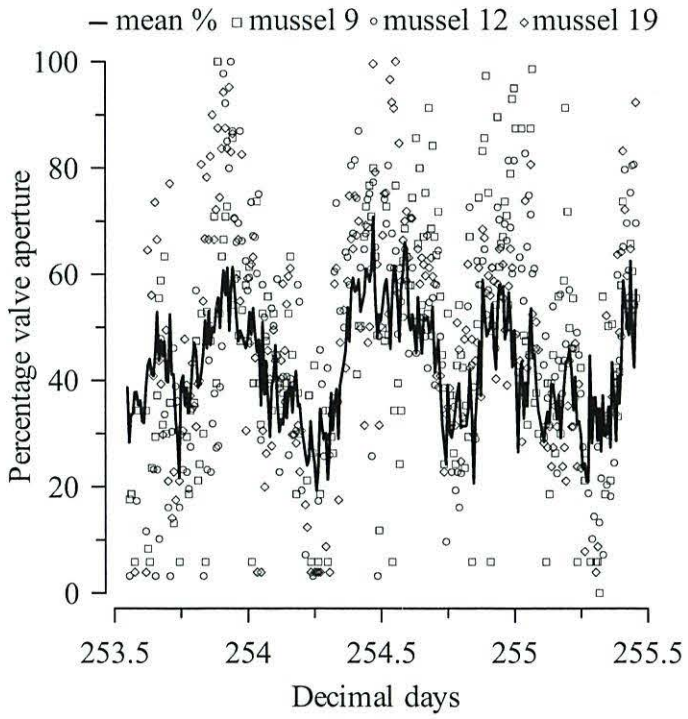

Figure 2. 5: Example of percentage valve aperture (VA) for three mussels (number 9, 12, and 19) and mean percentage VA during 48 h period in September 2004.

The comparison of feeding behaviour with the environmental factors was first done using regression analysis. Thereafter, time-series analyses were conducted firstly using spectral analyses, then cross-correlation which revealed more accurate relationships. Eventually, feeding behaviour and chl *a* concentrations were modeled via harmonic analysis. There was a strong linear relationship between feeding

behaviour and chl *a* concentration (Figure 2. 4A; Spearman $r = 0.734$; $p < 0.001$), flux of chl *a* was weaker (Figure 2. 4B; Spearman $r = 0.547$; $p < 0.001$) while a non-linear relationship was observed between feeding behaviour and the other environmental variables (e.g., Spearman SPM $r = 0.072$; $p = 0.315$). Subsequently, spectral analyses were carried on all the data. This analysis revealed three significant frequencies in the data similar to the one of M_2, M_4, and K_1 (Table 2. 2). As for the linear regression analysis, the best correlation obtained via cross-correlation occurred between feeding behaviour and chl *a* concentration ($r = 0.721$; $p < 0.001$) followed by salinity ($r = 0.667$; $p < 0.001$) with a time lag of 0 minutes, meaning that when chl *a* concentration and salinity were at a maximum, mussel VA was also at a maximum (i.e., the response to that variable is instantaneous) (Table 2. 3). Feeding behaviour was synchronized with day and night rhythms, thereby explaining the K_1 component (diurnal) with a weak correlation obtained via cross-correlation (Table 2. 3; $r = 0.226$; $p < 0.001$). Contrary to the linear regression analyses (Figure 2. 4C), there were high correlations obtained via cross-correlation between VA, SPM, and current velocity ($r = 0.607$, $r = -0.634$ respectively; $p < 0.001$) with a time lag of 110 min and 140 min respectively. No strong cross-correlation was found between feeding behaviour and the flux of chl *a*, and there was a small time delay of 20 minutes between the two variables (Table 2. 3; $r = 0.550$; $p < 0.001$).

The arbitrary periodic functions used in the harmonic analysis were principal lunar semidiurnal constituent (M_2 period =12.42 h), first overtide of M_2 constituent (M_4 period = 6.21 h) and luni-solar declinational diurnal constituent (K_1 period = 23.93 h) that had previously be identified with the spectral analysis of VA. The combination of the reconstructed tidal constituents (M_2, M_4, and K_1) were used to model and predict the feeding behaviour (Figure 2. 6). The M_2 pattern was the strongest significant individual predictor of mussel feeding behaviour (f = 1.97; spectral power = 53.4; $p < 0.05$). The prediction using the combination of the three components explained 62% of the variation of the raw data (linear regression: VA = -0.02 + 1 × (model VA); $r^2 = 0.624$; $p < 0.001$) and 81% of the block averaged VA to 1 hour (Spearman $r = 0.90$; $p < 0.001$). A paired *t*-test was used to compare the model from the raw data ($n > 100$ assume normality, assume equal variance) and revealed no significant difference ($t = -0.66$; $p = 0.512$).

Chl *a* concentration was compared with two other surveys undertaken in August 2004 and 2005, at a distance of approximately 1 km southwest of our sampling site (Figure 2. 1). The concentration in August 2004 was almost twice as high as in September 2004 or August 2005. The pattern of chl *a* concentration, velocity and flux of chl *a* was similar for the three surveys (Figure 2. 7A). The Chl *a* concentration showed two peaks at high velocity at ebb and flood with a lower peak at flood (water coming from Caernarfon Bar) for August 2004 and 2005. This flood peak is missing in the September 2004 data due to the gap in the dataset (c.f. methodology and Figure 2. 2). Spectral analysis made on the chl *a* concentration measured in August 2004 indicated similar tidal constituents as for September 2004 (Table 2. 2); the difference in the frequency number is due to the distance between the sites.

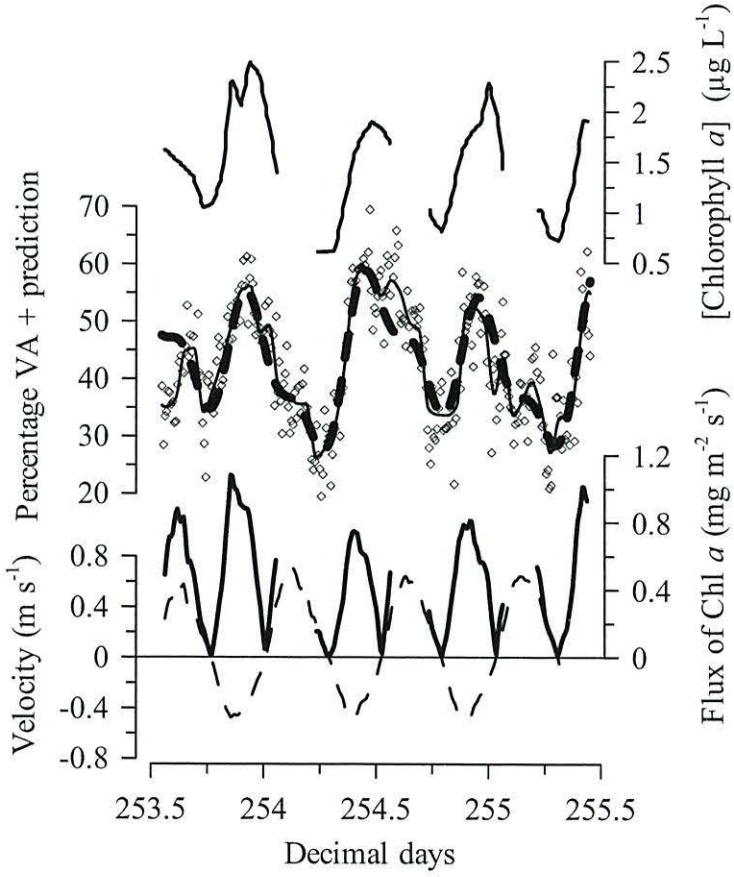

Figure 2. 6: Time series of VA model using three primary tidal components of the mussel feeding behaviour (mean percentage valve aperture VA) over a 48 h period in September 2004. Raw data = diamonds, block averaged VA 1 hour = thin solid line, fitted model = black dashed line. Changes in chl *a* concentration (µg L^{-1}), velocity (m s^{-1}), and flux of chl *a* (mg m^{-2} s^{-1}) are superimposed. Numbers 1 to 7 indicate slack water sequences, A to D maximum VA peaks.

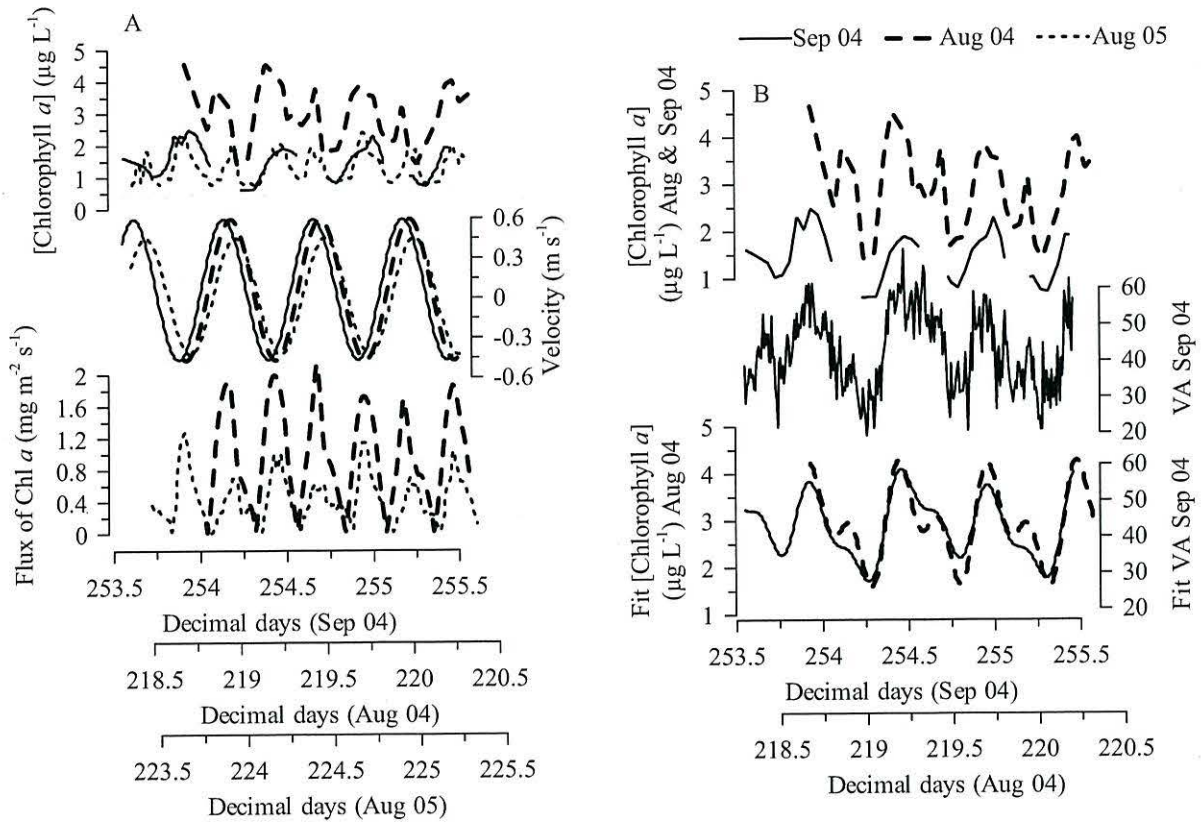

Figure 2. 7: (A) Superimposition of chl *a* concentration (µg L^{-1}), velocity (m s^{-1}), and flux of chl *a* (mg m^{-2}) from September 2004 (solid line), August 2004 (dashed line), and September 2004 (dotted line) cruises. (B) Superimposition of the mean percentage valve aperture (VA) September 2004, chl *a* concentration (µg L^{-1}) for September 2004 (solid line) and August 2004 (dashed line), and fitted chl *a* concentration from August 2004 (dashed line) and fitted mean percentage VA from September 2004 (solid line) cruises.

The combination of the reconstructed tidal constituents (M_2, M_4, and K_1) of the chl *a* concentration from August 2004 leads to a predicted line that matched strongly the one calculated for the mean percentage VA, with the presence of a second peak in the chl *a* concentration and feeding behaviour at high flood velocity (Figure 2. 7B). The Spearman correlation between VA and modeled chl *a* August 2004 is almost as high as the modeled VA (linear regression: VA = 11.5 + 10.8 × (model chl *a*); $r^2 = 0.547$; $p < 0.001$). Therefore, these additional field measurements support the conclusion that feeding behaviour is synchronized and regulated with food concentration.

Predators

The relationship between the presence or abundance of predators and changes in the feeding behaviour of mussels was either absent (for crabs) or weak (starfish: $r = 0.213$; $p < 0.001$). The spectral analysis of the crab distribution (*Carcinus maenas*) (Table 2. 2) did not follow a M_2, M_4 or diurnal cycle, but conformed to a significant 16.05 hours cycle. On the other hand, spectral analysis of starfish abundance (*Asterias rubens*) revealed two significant tidal components: M_2 and a more pronounced M_4 component (Table 2. 2). Starfish abundance was negatively correlated with current velocity (Starfish abundance = 13.10 – 20.43 x velocity + 7.96 x velocity^2; Spearman $r = -0.699$; $p < 0.001$).

2.6 Discussion

The present study provides direct evidence of a strong relationship between the valve aperture (VA) of blue mussels (feeding behaviour) and chl *a* concentration (Correlation $r = 0.72$; $p < 0.001$) and clearly separates this factor from the other physical parameters that may contribute only indirectly towards feeding activity (Table 2. 3). To date, few experiments have quantified feeding behaviour *in situ* in relation to multiple environmental parameters, but none of them were able to distinguish which among these various factors was controlling feeding behaviour (but see Dolmer, 2000a, 2000b Newell *et al.*, 1998). The parameters controlling the availability of chl *a* for the mussel bed are the chl *a* concentration in the water and the extent to which the tidal flow advects it over the mussels. The Menai Strait is dominated by a M_2 tide supplying the primary production coming from the Liverpool Bay over the mussel bed, but this phytoplankton patch partly depleted is advected back over the mussel bed by the return of the tide and this explains the M_4 constituent of the model (Figure 2. 7). Newell *et al.*, 1998) showed that feeding behaviour was correlated to the tidal cycle without specifying time delays. The authors did not record the chl *a* concentration concomitantly but noticed a difference between ebb and flood probably linked to the sedimentation of estuarine flocs. The combination of the asymmetrical tidal regime of the Menai Strait (Figure 2. 2) and

the use of appropriate analytical tools (predictions via harmonic analyses and cross-correlations) enabled us to discriminate chl *a* concentration asynchronies from all the other measured physical variables (Table 2. 3). For example, at our sampling site, high tide was not synchronized with slack water and it took 90 minutes (time lag between high chl *a* concentration and high velocity at ebb) for the water rich in chl *a* from Liverpool Bay to reach the point where it began to pass above the mussel bed when the tide changed to ebb flow due to advection (Figure 2. 3A; Tweddle et al., 2005).

Mussel feeding and growth vary with algal concentration and composition, flow and water column mixing, temperature, and seasonality. Wilson and Seed (1974) showed that mussels stop feeding at very low food concentrations (e.g., in winter) and thereby conserve energy until better conditions occur. Our results corroborate observations from laboratory and field studies in which the mean percentage VA decreased with low food concentrations (Newell *et al.*, 1998; Dolmer, 2000a; Macdonald and Nodwell, 2003; Riisgård *et al.*, 2003) until eventual closure occurred at a threshold of ~0.5 μg chl *a* L^{-1} which was very similar to the lowest concentration found during the present study of 0.6 μg chl *a* L^{-1} at ~1 m above the mussel bed (see review in Riisgård, 1991; Newell *et al.*, 2001; Riisgård *et al.*, 2003). Laboratory and field experiments have shown that mussels reduce filtration rate through a reduction of VA accompanied by retraction of the mantle edges and the exhalant siphon and by reducing the width of the inter-filament canals (Jørgensen et al. 1988; Clausen and Riisgård, 1996; Newell *et al.*, 1998). Conversely, Riisgård and Larsen, (1995) suggested that this is a mechanism to cope with sub-optimal feeding conditions. On the other hand, laboratory studies have demonstrated that above a certain algal concentration (> 10 μg chl *a* L^{-1}), a threshold is reached such that the animal's digestive capacity is fully saturated, leading to the rejection of surplus particles in pseudofaeces (Clausen and Riisgård, 1996). This maximum threshold is well outside the chl *a* concentrations reported during the current study, measured at a maximum of 2.5 μg L^{-1} which coincided with the maximum percentage VA recorded. The year-round chl *a* concentration in the Menai Strait is relatively low, mostly under 5 μg L^{-1}, varying from ~1 μg L^{-1} to 10-20 μg L^{-1} (*Phaeocystis* and diatom blooms occur only in mid-May/June). The large input of chl *a* from Liverpool Bay to the Menai Strait comes from a part of the Irish Sea where the standing stock is characterized by high

production measured to a maximum phytoplankton spring biomass of 43.9 μg L^{-1} (May 1997) and a mean summer biomass of 2.5 μg L^{-1} (0.6 to 4.2 μg L^{-1} Gowen *et al.*, 2000). Chl *a* availability for the commercial mussel bed deserves more study with a large mudflat at the Strait entrance possibly acting as a large source of primary production (microphytobenthos) or a sink (due to a high biomass of filter-feeders such as cockles, *Cerastoderma edule*).

The use of VA as a proxy of filtration activity was an appropriate tool for the purpose of the present study: it did not disturb the mussels in their natural environment and allowed a long enough survey of feeding activity during natural tidal cycles to remove potential artifacts found in the laboratory such as acclimation, disturbance, and removal of natural factors such as predators. The fairly low mean percentage aperture of the mussels during this study may be linked to a number of factors. When chl *a* concentration was close to the minimum threshold level from the literature (0.6 μg chl *a* L^{-1}), mussels reduced their feeding through VA closure (~19% maximum mean VA), whereas when chl *a* concentration increased up to 2.5 μg chl *a* L^{-1}, mussels fed at their maximum recorded capacity (~71% maximum mean VA). However, chl *a* was measured only at ~1 m above the mussel bed; the chl *a* concentrations in the boundary layer are expected to be reduced with grazing, as observed on the intertidal zone (pers. obs.;Dolmer, 2000a, 2000b; Riisgård *et al.*, 2006), or to be increased due to flocculation and sinking (Gascoigne *et al.*, in prep). Riisgård *et al.* (2006) showed that in the field, the VA response of mussel to near bed algal concentration below 1 μg chl *a* L^{-1} took on average 50 ± 19 min to change from 100% to 50% VA and 59 ± 22 min to return to 100% VA. In our study, the minimum chl *a* measured at ~1 m was close to or lower than the threshold found by Riisgård *et al.* (2006) near the bed. The mussels took some time to increase VA from ~20 to 70% because chl *a* increase gradually to maximum and vice versa. Food depletion down to a minimum chl *a* concentration (~0.5 μg L^{-1}) could only have occurred at periods of slack water (3 and 5 in Figure 2. 6) when the tide switched from flood to ebb. A maximum mean VA of 80 – 100% occurred for only c. 45 min per day, suggesting that optimal feeding conditions are rarely experienced in the Menai Strait (periods A, B, C, and D in Figure 2. 6). The variation in aperture of certain individuals is also responsible for the low mean percentage aperture. The latter might also be explained by the choice of VA as a proxy for feeding behaviour, as opposed

to another proxy using exhalant siphon area. Siphon area has been reported to reflect better the sensitivity of feeding behaviour to the effect of current speed; the siphon is oriented toward the flow direction rather than the valve gape (Newell *et al.*, 2001). The use of siphon aperture as the response variable was not possible in the field and appeared to be unnecessary as mussels *in situ* were observed to be dynamic, were able to change position markedly within the mussel bed, and were randomly orientated contrary to other species (Wildish and Kristmanson, 1997 for review). Therefore, given the constraints imposed by working *in situ*, mean percentage VA measurement was a good proxy with which to quantify feeding behaviour.

In our study, flow velocity did not influence the feeding behaviour of the mussels. At low flow velocity measured ~1 m above the bed there was no significant relationship with food concentration (varying from 0.6 μg chl *a* L^{-1} to 1.9 μg chl *a* L^{-1}; Figure 2. 3A) or mean percentage VA (Figure 2. 4C). Other studies have shown that flow velocity could reduce the filtration rate of mussels under different conditions of food concentration (Wildish and Miyares, 1990; Newell *et al.*, 2001). In the present study, flow varied from 0.007 m s^{-1} around slack water up to a maximum of 0.61 m s^{-1}. Tweddle *et al.* (2005) argued that the pattern of food depletion occurred twice in a 25 h survey at between 2.5 and 1 m above the mussel bed during slack water, when levels of Reynolds stress are negligible. The food concentration decreased at ~1 m above the mussel bed and in the whole water column at slack water (Figure 2. 6, slacks 3, 5 and 7). Therefore, at low flow speed, due to low food concentration, mussels reduced their filtration activity. Biogenic structures such as mussel beds and their filtration activity create physical roughness which introduces turbulence into the boundary layer and reduces velocity. Van Duren *et al.* (2006) measured at a high velocity of 0.35 m s^{-1} at 150 mm above the bed, a decrease in the velocity in the lower boundary layer. The values of velocity obtained at around 7 mm high in the lower boundary layer were between ~0.03 and 0.05 m s^{-1}, independent of mussel activity. The maximum flow rate velocity at 84 cm above the mussel bed was 0.61 m s^{-1} and a large decrease of the velocity could be expected in the lower boundary layer. Therefore, the measured velocities were a good indicator of velocity patterns, but were not representative of the velocity potentially affecting the mussels.

At the start of the study, the presence of predators was expected to lead to a behavioural response in the mussels (valve closure). However, this turned out not to be the case. Although the starfish and crab predators were observed in close contact with mussels, the number of predators did not appear to alter the feeding behaviour of mussels except when the predator interacted directly with individual mussels. Other studies suggest that the response of mussels to the presence of predators occurs via chemical cues and is expressed through changes in morphology, physiology or allocation of energy to different tissues rather than directly via contact (Reimer and Harms-Ringdahl, 2001).

The assessment of carrying capacity is an important goal in research and management and requires the use of appropriate models and tools. Carrying capacity models for bivalve culture are complex and hierarchical (see review by McKindsey *et al.*, 2006). This study demonstrates that it is possible to determine and quantify the effects of environmental factors on feeding behaviour in order to calculate and predict the population grazing capacity. This then enables assessment of both production and ecological carrying capacity. The data indicated that feeding activity was regulated in two ways: when food was present at an optimal level (in our study 2.5 μg chl *a* L^{-1}) mussels filtered at their maximum capacity and actively removed food from the water column; in contrast, when chl *a* concentration declined to the minimum threshold (chl $a < 1$ μg L^{-1}), mussels filtering activity declined until mussels stopped filtering and depleting the water column (valve closure, Dolmer 2000b) until the surrounding water was naturally replenished with food. Newell et al. (1998) suggested that VA is a proxy of filtration activity although some calibrations would be needed, which have been done by Dolmer (2000b). The calibration between VA and filtration rate provides another tool to estimate mussel carrying capacity in a coastal system. By monitoring variation in the feeding behaviour of mussels directly in their environment, filtration rates can be adjusted to take environmental conditions into account. Nevertheless calibrations from other studies need to be used carefully, since bivalve feeding and physiology is likely to vary in different sites (McKindsey *et al.*, 2006); this is often a source of approximation or error in modeling. Therefore these calibrations were not used for our study; this will be the next step within the project. The VA calibrations is recommended with other *in situ* filtration rate techniques (i.e. biodeposition, defecation) should be done *in situ* at the appropriate site.

In terms of production carrying capacity, it seems unlikely that the Menai Strait has reach its maximum capacity: in this study, food (chl *a*) was always available to the mussels and potential food depletion could only occur at slack periods 3 and 5 when the chl *a* concentration is very low at the point when the tide switches from flood to ebb (just above the minimum threshold, Figure 2. 6). This short duration of periods of food depletion is a feature of the Menai Strait that creates conditions that maximize the potential rate of mussel production. Moreover, the seawater in the Menai Strait has a very short residence time of 2 to 3 days, and the clearance time by mussels was calculated at ~15 days in the subtidal area (Gascoigne *et al.*, unpublished data). Nevertheless, the intertidal area is a complex part of the system and exhibits periods of food depletion (Chapter 6). The background chl *a* concentrations are quite low, but the mussels in the Menai Strait have a commercial growth cycle (4.5 cm in 2.5 to 3 years) similar to other bottom culture systems (i.e., Wadden Sea in Netherlands or Limfjord in Denmark; Dolmer and Frandsen, 2002). In terms of ecological carrying capacity, establishing the importance of mussel grazing capacity provides the basis to investigate other environmental issues related to carrying capacity such as: competition for food with other components of the ecosystem that consume similar food resources; release of nutrients from mussel faeces and pseudofaeces production; the capacity to ameliorate the effects of eutrophication.

Models used to estimate carrying capacity are rather imprecise as they are subject to large uncertainties (McKindsey *et al.*, 2006), but one aim of researchers is to make the model represent as closely as possible the functioning of the natural environment. This study provides a better understanding of blue mussel grazing and a more appropriate means of calculating grazing rate dependent upon one predominant environmental factor: food concentration. Although our study clearly shows that mussel feeding behaviour is principally food regulated, it is certain that feeding behaviour for other species is controlled by other factors (Wildish and Kristmanson, 1997). Moreover, in our system, mussels were subjected only to the lowest threshold chl *a* concentration. This study thus cannot be used to draw conclusions about feeding behaviour in bivalves generally, nor with mussels in systems that are highly eutrophic with high chl *a* concentrations. This study has demonstrated that video monitoring of bivalves *in situ* is a useful technique which should be used during studies of system carrying capacity for adjustment of grazing capacity calculations.

Chapter III

Mussel *in situ* grazing rate method*

Faeces and pseudofaeces production from mussel in a bucket left defecating

18/07/2006

* This study was presented at the 41st European Marine Biology Symposium September 4-8 – Cork Ireland, as a poster:
Saurel C., Petersen, J.K., Møhlenberg, F., Schlüter, L. and M.J. Kaiser. 2006. Grazing rate of mussel measured *in situ* via chlorophyll a as tracer.

Chapter III Mussel defecation to measure ingestion rate via chlorophyll *a* proxy.

3.1 Contributions to this chapter

Chapter process:	Contributions
Conception	CS/JKP/FM
Design analysis	CS/JKP/FM
Data collection	CS/JKP/FM
Data analysis/ Interpretation	CS/JKP/FM
Drafting	CS/JKP/FM
Revising	CS/MJK/JKP/FM

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3.2 Abstract

The defecation methodology uses chlorophyll *a* (chl *a*) concentration in the faeces as a proxy to calculate the ingestion rate (IR) of the blue mussel (*Mytilus edulis*). The defecation methodology assumes a steady-state between ingestion and excretion after correction for pigment degradation by digestion. Budget experiments using algal monocultures (*Rhodomonas* sp. and *Thalassiosira* sp.) were performed in order to model the variation in the digestion factor (DF) associated with variable environmental conditions. DF (expressed as a percentage) varied significantly with temperature (range 6.8 – 18.3°C; $F = 14.10$; $p < 0.001$) or chl *a* concentration in the water (range 2.7 to 28.1 μg L^{-1}; $F = 13.24$; $p < 0.001$), but no significant difference was found among the two monocultures of algae. *In situ*, all pigments identified in mussel faeces (> 6, except fucoxanthin) were related to the concentration of the pigments in water. The pigments breakdown was addressed via the separation of pigments in the mussel faeces using HPLC. These results indicated that the use of

total chl *a* or chl *a* equivalent (chl a_{eq}) for the *in situ* defecation method is appropriate, as it takes into account phaeopigments, which are the main chl *a* degradation product. The validity of the DF model for use *in situ* was confirmed by a budget experiment conducted using natural seston: DF modelled was not significantly different from DF calculated via the budget experiment. In view of these results, the use, under certain conditions of the defecation method together with the modelled DF is recommended in order to measure *in situ* IR of *M. edulis*.

3.3 Introduction

Benthic suspension feeders such as mussels, oysters or clams are important components of coastal and estuarine ecosystems (Herman *et al.*, 1999) and important in terms of their contribution to marine food production either from fisheries or aquaculture production (e.g. Smaal, 2002). These benthic suspension feeders rely entirely upon suspended particulate material such as phytoplankton for their source of energy. Consequently, in areas with a high biomass of suspension feeders such as mussels, their grazing may have a major impact on the water column particle concentration and thus on food web structure (Petersen, 2004a and references therein). Hence, they have the capacity to exhaust their food supply, which may have severe implications for mussel production and thus carrying capacity (Prins *et al.*, 1998). The grazing or suspension-feeding capacity is thus a key parameter in understanding and modelling the impact of populations of benthic suspension-feeders in coastal areas. Methods for the determination of grazing capacity in mussels (e.g. measured as clearance rate (CR) or ingestion rate (IR)) have been a subject of debate for decades (Riisgård, 2001a). Clearance rate is defined as the number of particles removed from a standard volume of water per hour while ingestion rate is the mass of particles filtered per hours minus pseudofaeces production. Consequently, when no pseudofaeces are produced, the clearance rate is representative of the ingestion rate.

The debate above started as a discussion of the magnitude of clearance rate but to a large extent centres upon the validity and proper use of the proposed methodologies (Riisgård, 2001a, 2001b; Cranford, 2001; Petersen *et al.*, 2004; Petersen, 2004a,

2004b; Riisgård, 2004; Bayne, 2004). In addition, it has been argued that CR and IR determined in the laboratory are not truly representative of *in situ* conditions, since constant and optimal conditions in the laboratory do not occur in the field and that CR determined in the laboratory overestimates *in situ* CR (Cranford and Hill, 1999).

Methodologies for *in situ* measurements are still not properly developed and only occasionally used. A few *in situ* or "semi-*in situ*" methods (defined as using natural seston, but with manipulations of the mussels) have been used in the field. These methods include the biodeposition method (mussels in Hawkins et al. 1996; scallops in Cranford *et al.*, 1998, Cranford and Hill, 1999), the modified suction method 'InEx', (Yahel *et al.*, 2005), and the less developed defecation method (Kotta and Møhlenberg, 2002; Kotta *et al.*, 2004). The most frequently applied method is the biodeposition method, which is based on quantification of the ingestion and defecation of inorganic material by the mussel to determine IR. This method has been applied in the field for several decades, with constant improvement of the collection devices or the sediment traps deployed (Cranford and Hargrave, 1994; Cranford and Hill, 1999). However, there are recognised weaknesses with this method; for instance mussels are manipulated and placed on sediment traps, which separates them from conspecifics and their natural habitat. These manipulations may modify the natural conditions experienced by the mussels, by altering food concentration, intra and interspecific competition and flow regime and thus will affect the measured CR. Another problem is the duration of sampling, i.e. CR from short or long time integration may vary due to fluctuation in environmental conditions that occur during the period of observation, or due to the degradation or loss of some of the collected faeces. Moreover, this method results in lower CR estimates compared to other laboratory methods performed under identical conditions (Riisgård, 2001a, Petersen, 2004a).

Defecation-based methods to measure CR were originally developed for zooplankton (Dagg and Walser, 1987; Peterson *et al.*, 1990). The principle of the method is to estimate the ingestion rate of the animal by recovering the chlorophyll related pigments from the ingested algae knowing the gut content of the animal and the gut content turnover rate (Harris *et al.*, 2000 and references therein). A modification of this method focussing on temporal variation in faeces production rather than variation in gut content has been applied to mussels from the Baltic using total chl *a* or chl *a* equivalent (chl a_{eq}) as a food proxy (Kotta and Møhlenberg, 2002;

Kotta *et al.*, 2004). The standard procedure behind the defecation method is to collect mussels from the field and to allow them to defecate for a short period of time (0.5 - 1 h), then, knowing the amount of chl *a* in the water, the chl *a* excreted and the chl *a* digested, the food ingested can be calculated. The loss of chl *a* during the gut passage due to digestion and assimilation is calculated as the digestion factor (DF) via an additional budget experiment. In the budget experiment, CR is measured using standard CR/IR methods in the laboratory, such as the indirect method (Riisgård, 2001a). The digestion rate (converted to DF) is then calculated as the difference between the CR and defecation rate measured on the same mussels from the field. The DF calculated via the budget method is then applied in the defecation method to calculate the CR of other mussels taken from the field. The equations associated with this method are dealt with in the methodology section.

Mussel gut retention time has been calculated to vary between ~1.30 h to 2 h (Hawkins *et al.*, 1990; Kiørboe *et al.*, 1980; pers. obs.). The gut retention time has implications for the estimated IR (using the defecation method) as it reflects the IR experienced by the mussel ~1.5 h to 2 h prior to the start of the experiment. Therefore, the temporal variation of chl *a* concentration in the environment is an important factor to take into account and chl *a* concentration from the water needs to be sampled around 2 h prior to the beginning of the experiment.

The defecation method has the potential to become a unique method for the estimation of IR for benthic suspension feeders, as it has been for zooplankton, because animals can be collected directly from any type of environment (e.g. long line cultures, natural or commercial mussel beds, rocky shores) and the IR estimated reflects the condition experienced by the undisturbed animals prior to collection. This makes it by definition an appropriate *in situ* method. Nevertheless, the defecation methodology has not been used widely due to the fact that some questions, such as variation in DF or the recovery of measured pigments need to be addressed. The present study considers the variations of DF in relation to chl *a* concentration, temperature, and phytoplankton composition which are factors that control the gut clearance time (in copepods: Kiørboe *et al.*, 1982; Dagg and Walser, 1987; Head and Harris, 1994); Moreover, this study aims to model the DF in relation to these environmental conditions, in order to make the defecation method easier to apply and more accurate for *in situ* application. This model was calculated in a

controlled environment in the laboratory and tested and validated using natural seston in a budget experiment. Another issue addressed during this study is the influence of pigment composition which varies according to identify of the phytoplankton taxa, and selectivity by the animal. chl *a* is a "biological marker" and the assessment of its transformation products during gut passage needs to be addressed. The High Performance Liquid Chromatography (HPLC) technique has been successfully used to separate pigments in order to address the problem of phytoplankton selectivity in copepod studies (Harris *et al.*, 2000 and references therein) and in the mussel *M. edulis* (Hawkins *et al.*, 1986; Szymczak-Zyla *et al.*, 2006). Thus the same technique was used in order to measure the different pigments recovered from the mussel faeces, in order to validate the use of chl *a* and its degradation products (phaeopigments).

3.4 Material and Methods

Digestion factor

Estimation of the digestion factor (DF) as a function of environmental conditions (chl *a* concentration, temperature, algae species) via a budget experiment, was carried out at the NERI laboratory, Roskilde, Denmark. DF was calculated as the relative difference between the clearance rate (CR) and the defecation rate (DR) of chl *a* measured on the same mussels. Mussels, *Mytilus edulis* L., were collected from the Isefjorden at a salinity ranging from 16 to 20 psu. The mean shell length was 45.2 ± 2.8 mm. Mussels were transported to the laboratory and kept at 15.6°C and salinity 20 and allowed to acclimatise for at least several days and up to some weeks.

Gut retention time was checked in a separate experiment in which mussels where fed *Rhodomonas* algae mixed with diluted carmine powder. Once the faeces of the mussels kept in the tank were coloured red/pink, indicating that the mixed solution had passed through the gut, mussels were transferred to a separate aquarium tank with no addition of carmine and left to defecate. Faeces were collected every ~10 min. After ~1 h 40 the faeces were no longer coloured by the carmine powder and in

the remainder of the experiments a gut passage time of 1.5 – 2 h (Hawkins *et al.*, 1990; Kiørboe *et al.*, 1980) was assumed.

Three sets of experiments were carried out to enable the determination of DF under different conditions (see Table 3. 1):

1) The effect of four different food concentrations on mussel feeding when maintained at a constant temperature (15.6°C)

2) The effect of four different temperatures on mussel feeding when maintained at constant algae concentration (*Rhodomomas* sp.; 3.5 ± 0.3 µg chl *a* L^{-1}).

3) The effect of two different algae species on the mussel feeding: the flagellate *Rhodomonas* sp. (mean eqv. spherical diam. 7.08 ± 0.08 µm), and the diatom *Thalassiosira weissflogii* (mean Ø 11.73 ± 0.07 µm) at 15.6°C.

For each experiment, 10 mussels were placed in 18 L tanks supplied with aeration and a water pump for water mixing (Aqual Qmax ~250 L h^{-1}). Mussels were fed overnight before the experiment from an algal culture administered via a peristaltic pump. Each experiment was replicated 3 times, making sure that all the mussels were wide open and filtering during the experiment. Food concentration in the tank was measured as chl *a* and particle concentration using an particle counter Elzone 5380 (mounted with a 95 µm orifice tube and a lower threshold set at Ø 4 µm; each samples was counted at least 3 times). Samples for tank concentration were collected from the middle of the tank using a 50 mL syringe.

Clearance rate (CR) of the mussels in the tank was determined using the indirect method (Riisgård, 2001a). With the indirect method, CR was determined from the exponential decline in particle concentration in the tanks over time, by taking water samples at regular time intervals and determining the particle concentration using the particle counter (particles > 4 µm in diameter). Algae were added to the tank after each CR determination in order to maintain a constant mean concentration and the experiment was repeated. Particles were counted 2 – 3 times for each water sample. Clearance rate (L ind^{-1} h^{-1}) was defined as the volume of water cleared of all 100% efficiently retained particles per unit of time and was calculated according to (Riisgård, 2001a):

$$\mathbf{CR = V/(nt)*\ln(C_0/C_t)} \qquad \mathbf{(1)}$$

where V is the volume of the tank, n is the number of individuals in the tank, C_0 and C_t are the algal concentration at time 0 and time t respectively.

After the calculation of CR, defecation rate (DR) was determined. Mussels were kept in the tanks for a minimum of 3 h. Individual mussels were transferred to 500 mL beakers with seawater for defecation to occur whereupon faeces were collected with a plastic pipette 0.5 and 1 h after transfer (these time intervals were selected from several experiments in which faeces were repeatidily sampled). In most cases, no significant difference was found between the DR at 30 min and 1 h, therefore 1 h was chosen for all the analysis. The sampled faeces were transferred to a GC-MS filter paper to remove excess water, and left for extraction in 10 ml of 96% ethanol in a dark place at room temperature for more than 10 h. Concentration of chl *a* and phaeopigments (Phaeo) were determined using a Turner Design 10-AU fluorometer before and after acidification with 0.1N HCl (method adapted from Parsons et al., 1984). Total chl *a* or chl *a* equivalent (chl a_{eq}, µg L^{-1} or µg unit $faeces^{-1}$) was calculated according to Kotta and Møhlenberg (2002):

$$\textbf{chl } a_{eq} = 1.52 \times \textbf{Phaeo} + \textbf{chl } a \qquad (2)$$

DR (µg chl a_{eq} $ind.^{-1}$ h^{-1}) of individual mussels were calculated as:

$$DR = (F_{chl}/(t_1 - t_0))/n \quad (3)$$

where F_{chl} is the chl a_{eq} of faecal material sampled, t_0 and t_1 are start and end of sampling period in hours and n is the number of individual mussels (= 1 in these experiments).

The DF was then calculated using the DR and the CR measured using the indirect method under identical conditions. The DF was then multiplied by the mean of the concentration of chl a_{eq} measured in the aquaria over a period > 3 h prior to transfer of mussels for the study of faeces production, which gave the ingestion rate. Using this estimate of ingestion rate (IR), DF was calculated as:

$$DF = (IR - DR) / IR \text{ as a } \% \quad (4)$$

Following these experiments, DF was modelled in function of chl *a* concentration, seawater temperature, and algal species composition.

Defecation method

After the estimation of the DF via the budget experiment, IR and CR were calculated for mussels obtained from the same batch by measuring the DR and the chl a_{eq} in the water. Ingestion rate (µg chl a_{eq} ind.$^{-1}$ h^{-1}) can thus be calculated as:

IR = DR * 100/(100 - DF) (5)

Knowing the DF, CR (l ind.$^{-1}$ h^{-1}) can be calculated as:

CR = IR/chl a_{eq} (6)

Validation of the digestion factor model

A budget experiment together with the defecation method using the modelled DF was undertaken at the Danish Shellfish Centre in Nykøbing Mors, Denmark, in order to validate the DF model. Mussels from the field were transported to the laboratory and kept in a 1.5 m^3 tank supplied with running seawater pumped directly from Limfjorden through a filter (100 µm mesh size). The mean shell length of the mussels was 60.16 ± 1.91 mm. CR was estimated using the indirect method as described above, but modified according to Petersen *et al.* (2004). Four mussels were individually placed in 1.1 L buckets supplied with running seawater and aeration to maintain mixing. Three extra buckets without mussel were used as controls. Particle concentrations in the buckets were measured with an Elzone 5380. During the experiment for determination of CR, the running seawater was turned off. This experiment was replicated 7 times with different mussels. DR was subsequently determined from faecal production in mussels used for CR experiments. Water samples for chl *a* determination were taken in the control buckets according to the procedure described above at the start of the experiment. Two budget experiments were performed by measuring the chl *a* concentration in control chambers and in the faeces of mussels from which CR had previously been calculated. Temperature, suspended particulate matter and chl *a* concentration was found to be constant during the experiment.

Pigment recovery

Corresponding samples of water and mussels were collected regularly during a field campaign in Knebel Vig, Denmark. Mussels were incubated in groups of three individuals in filtered sea water (400 ml) for 60-80 min at 17°C in the laboratory. Faeces were collected by a pipette, which was emptied in dim light onto GF/F filters placed on absorbing paper, immediately frozen in liquid nitrogen and stored until analysis. Water sampled along with mussels was filtered on to GF/F filters in subdued light and immediately frozen in liquid nitrogen. The filters were thawed, placed wet in 3 ml 100% acetone, resulting in a nominal concentration of 90% acetone (Bidigare 1991), sonicated on ice for 10 minutes, and extracted for 24 h at 4°C. The extracts were filtered using disposable syringes and 0.2 μm teflon syringe filters. One mL extract and 0.3 mL water (HPLC-grade) were transferred to HPLC vials that were then placed in a cooling rack of the HPLC. The samples were injected into a Shimadzu LC-10A HPLC system according to the method described by Wright et al. (1991), with the solvent system as follows: solvent A: 80:20 methanol:0.5 M ammonium acetate (aq.; pH 7.2 v/v), solvent B: 90:10 acetonitrile:water (v/v), solvent C: ethyl acetate. The linear gradient was modified slightly: 0 min: 100% A, 2 min: 100% B, 2.6 min: 90% B/10% C, 13.6 min: 65% B/35% C, 20 min: 31% B/69% C, 28 min: 100% B, 31 min: 100% A. Carotenoids and non degraded chloropigments were detected at 436 nm, while phaeopigments were detected at 405 nm. The HPLC system was calibrated with pigment standards from The International Agency for ^{14}C Determination, DHI water environment health, Hørsholm, Denmark. Peak identities were determined by diode array, and by comparing the retention time of the pigments in the samples with the retention time of a mixture of pigments of the authentic standards.

Data analysis

Prior to analysis, the data sets were checked to see if they met the assumptions of normal distribution and homogeneity of variance in order to apply ANOVA. Appropriate data transformations were applied to adjust the data such that they met these assumptions (Table 3. 2). Crossed ANOVA analyses were completed for

experiment 1 in the determination of DF (effect of chl *a*) with date and treatment as factors. For all the treatments, the mussel shell length was not significantly different (Table 3. 2). The three experiments were replicated 3 times, and no significant difference was found between the dates, except for experiment 1 where one chl *a* concentration was higher in the tanks at one time in comparison to the other days. The chl *a* concentration was used as a covariate in the analysis of experiments 2 and 3 (effect of temperature and algal species), in order to remove the potential effect of the difference between the dates. Therefore the 3 repeated experiments were considered as replicates for the 3 experiments.

3.5 Results

Digestion factor and model

An increase in the concentration of chl *a* induced an increase in IR and DR that eventually reached a plateau, while CR decreased with increasing algal concentration (Figure 3. 1; Table 3. 2). DF decreased proportionally to the increase of pigment in the water (Figures 3. 1 & 3. 2; Pearson correlation: r = -0.587, p < 0.001). Temperature also affected CR, IR and DF leading to higher rates of CR and IR, and a higher digestion factor at higher temperatures (Tables 3. 1 & 3. 2, Figure 3. 2; Pearson correlation: r = 0.291, p = 0.002). In contrast, experiments comparing 2 different algae species (diatom and flagellate) provided to mussels, showed no significant effect on DF when chl a_{eq} in the water was set as the covariate (ANOVA F = 3.51, p = 0.061; Figure 3. 3). Indeed, the chl *a* concentration in the tank was significantly different between algae species (*Rhodomonas sp.* ANOVA $F_{1,17}$ = 18.82, p < 0.001) affecting CR (ANOVA $F_{1,17}$ = 13.47, p = 0.003) and IR (ANOVA $F_{1,17}$ = 5.27, p = 0.039) in a similar manner as described above (Tables 3. 1 & 3. 2).

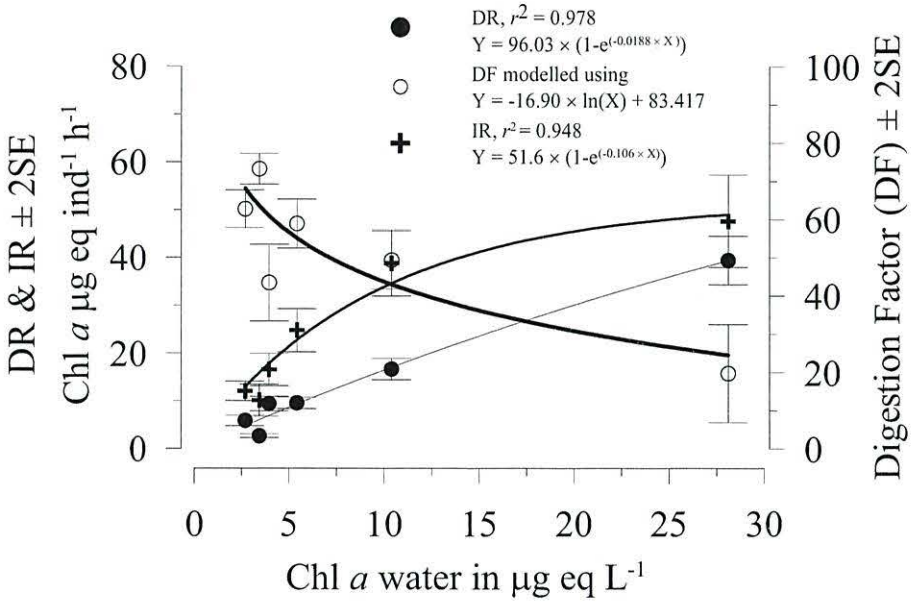


Figure 3. 1: Ingestion rates (IR), defecation rates (DR), and defecation factor (DF) ± 2SE in function of the water mean fluorescence with logarithmic and Ivlev's regression lines, at 15°C using *Rhodomonas* sp. feeding algae. Each value is the mean ± 2SE from 30 incubations each containing 1 individual.

Table 3. 1: Mean tank chl *a* concentration ± standard error, shell length, clearance rate, ingestion rate, defecation rate and digestion factor calculated from the budget experiment and calculated from the model for the 3 experiments at different treatments. R is rate.

	N	Mean tank chl *a* µg L^{-1} ± 2SE	Shell Length mm ± 2SE	Clearance R L $ind.^{-1}$ h^{-1}	Ingestion R µg chl a_{eq} $ind.^{-1}$ h^{-1}	Defecation R µg chl a_{eq} $ind.^{-1}$ h^{-1}	DF mean Calculated	DF mean Modelled♠
Exp. 1 : Algae concentration								N = 87
1	30	28.11 ± 4.67		1.58 ± 0.18	47.65 ± 9.66	39.45 ± 5.1	19.77 ±12.86	27.48
2	30	10.40 ± 1.33	45.92 ± 0.42♣	3.44 ± 0.47	38.88 ± 6.92	19.69 ± 2.26	49.40 ±7.54	44.04
3	30	5.43 ± 0.97		4.58 ± 0.69	24.77 ± 4.52	9.57 ± 1.18	58.92 ± 6.42	54.87
4	30	2.70 ± 0.67		4.57 ± 0.32	12.06 ± 2.04	5.89 ± 1.11	♦62.8 ± 4.92	66.51
Exp. 2 : Temperature								N = 116
18.3 °C	30			3.56 ± 0.93	12.22 ± 3.02	1.81 ± 0.40	85.41 ± 2.82	85.43
15.6 °C	30	3.45 ± 1.17 ♣	43.45 ± 0.52♣	3.69 ± 0.48	10.14 ± 3.36	2.66 ± 0.38	73.27 ± 4.04	73.24
11.9 °C	30			3.19 ± 0.35	10.53 ± 3.23	3.41 ± 0.48	65.90 ± 6.00	65.91
6.8 °C	30			2.36 ± 0.11	9.40 ± 2.05	2.42 ± 0.53	73.62 ± 4.90	73.61
Exp. 3: Algae species								
Rhodomonas	30	3.97 ± 0.14	46.91 ± 0.64♣	4.15 ± 0.27	16.56 ± 3.40	9.41 ± 1.53	43.49 ±10.04	NA
Thalassiossira	30	2.17 ± 0.08		5.11± 0.30	11.06 ± 1.02	6.74 ± 1.52	44.74 ± 9.44	NA

♣ Anova, no significant difference between the 4 treatments
♦ removed in the mean the value from 18/12/05, mean = 48.52 ± 9.52
♠ model Exp.: $r^2 = 0.313$ $p < 0.001$; Exp.2: $r^2 = 0.281$, $p < 0.001$

Table 3. 2: ANOVA analysis with covariate summary for experiments 1 and 3 and crossed ANOVA for experiment 2 for the tank fluorescence, shell length, clearance rate, ingestion rate, defecation rate and Median for percentage degradation of the pigments and percentage loss by digestion. df is Degree of Freedom, Date the replicas of the experiment, treatment the different concentration (Experiment 1), temperature (experiment 2) or algae (experiment 3). Covariate is different algae concentration for experiment 1 and 2. Values in bold indicates significant difference $p < 0.05$. Transformation is the transformation applied on the set of data to fulfil assumptions for an ANOVA analysis.

	Mean chl *a* concentration	Shell Length	Mean Clearance R	Mean Ingestion R	Defecation R	% Digestion
Experiment 1 : Algae concentration						
Transformation	$Y' = \log_{10}Y$	No	No	No	$Y' = \ln Y$	$Y' = \arcsin Y$
df	51	117	11	11	118	116
Date	**F=3.79 *p*=0.031**	F=0.25 *p*=0.777	F=2.37 *p*=0.174	F = 8.09 *p* = 0.020	F=2.34 *p*=0.101	**F=4.99 *p*=0.008**
treatment	**F=116.93 *p*<0.001**	F=0.92 *p*=0.435	**F=51.28 *p*<0.001**	**F = 65.57 *p* < 0.001**	**F=113.91 *p*<0.001**	**F=13.24 *p*<0.001**
Crossed	F = 0.54 *p* = 0.776	F= 0.18 *p*=0.981	NA AdjMS error = 0	NA AdjMS error = 0	**F=4.47 *p*<0.001**	**F = 4.67 *p*<0.001**
result	[1] > [2] > [3] > [4] No difference in date for 1, 2, 3, 4 separately	No difference	[1] < [2] < ([3] = [4])	[1] > [2] > [3] > [4]	$[4]_{1812} > ([4]_{1912})$ [1] > [2] > [3] > [4]	$[4]_{1812} > ([4]_{1912=2012})$ [1] < ([2] = [3] = [4])
Experiment 2 : Temperature						
Transformation	No	No	No	No	No	$Y' = \arcsin Y$
df	11	118	11	11	118	114
Date	**F = 16.77 *p* = 0.003**	F = 0.33 *p* = 0.721	F = 2.52 *p* = 0.175	F = 4.74 *p* = 0.070	F = 0.19 *p* = 0.824	F = 0.65 *p* = 0.526
treatment	F = 3.48 *p* = 0.091	F = 0.17 *p* = 0.919	**F = 7.49 *p* = 0.027**	**F = 17.11 *p* = 0.005**	**F = 8.10 *p* < 0.001**	**F = 14.10 *p* < 0.001**
Covariate chl *a*	Non applicable	Not applicable	**F= 7.06 *p* = 0.049**	**F = 10.61 *p* = 0.022**	F= 0.12 *p* = 0.732	F = 0.01 *p* = 0.924
result	1711 > 0112 No difference treat	No difference	$T_{18.3} > T_{6.8}$	$T_{18.3} > T_{6.8}$	$T_{6.8} < T_{11.9}$ $T_{18.3} > T_{11.9}$	$T_{18.3}$ > all
Experiment 3: Algae						
Transformation	No	No	No	No	$Y' = \ln Y$	$Y' = \arcsin Y$
df	17	59	17	17	46	55
Date	F=0.13 *p*=0.879	F=1.49 *p*=0.234	F=0.41 *p*=0.672	F=0.08 *p*=0.924	**F = 5.70 *p*<0.001**	**F=5.61 *p*=0.006**
treatment	**F=18.82 *p*<0.001**	F=1.15 *p*=0.288	**F=13.47 *p*=0.003**	**F=5.27 *p*=0.039**	F = 1.55 *p* =0.218	F=3.51 *p*=0.061
Covariate chl *a*	Non Applicable	F= 1.05 *p*=0.356	F= 0.02 *p*=0.884	F= 0.44 *p*=0.517	F= 0.23 *p*=0.634	F= 3.61 *p*=0.063
result	**R > T**	No difference	**T > R**	**R > T**	R = T; **12=13a<13b**	**R = T**

Following the experiments in the laboratory, models of the DF in relation to temperature and concentration of chl *a* fitted within the ranges ascertained from the experiments. Equation 7 models DF as a function of chl *a* concentration in the range from 2-30 µg chl a_{eq} L^{-1} at a temperature of 15.6°C ($n = 87$, $r^2 = 0.314$, $p < 0.001$, residuals normally distributed $a^2 = 0.664$, $p = 0.080$).

$$\mathbf{DF = -16.90 \times \ln(chl\ a_{eq}) + 84.417} \qquad \mathbf{(7)}$$

Equation 8 models DF as a function of temperature in the range from 6-15°C and at a mean chl *a* concentration of 3.5 ± 0.3 µg L^{-1} ($n = 116$, $r^2 = 0.25$, $p < 0.001$, residuals normally distributed $a^2 = 0.589$, $p = 0.122$).

$$\mathbf{DF = 115.92 - 8.962 \times T + 0.3962 \times T^2} \qquad \mathbf{(8)}$$

Combination of the two equations assuming a cumulative effect of chl *a* concentration and temperature generates a single model for DF based on only one size of mussels (45.17 ± 0.33 mm), hence the effect of size is not accounted for in this equation:

$$\mathbf{DF = DF_{chl15} + DF_{temp} - DF_{temp15}}$$

$$\mathbf{DF = -16.90 \times \ln(chl\ a_{eq}) - 8.962 \times T + 0.3962 \times T^2 + 129.70} \qquad \mathbf{(9)}$$

where DF_{chl15} is model of digestion factor for the recorded chl *a* concentration at 15°C, DF_{temp} is the model of digestion factor at the recorded temperature, and DF_{temp15} is the digestion factor at 15°C = 70.64%

Validation of the DF model *in situ*

The model of DF derived in the laboratory was tested with natural seston in the Limfjorden. The mean chl *a* concentration was 5.5 µg L^{-1} (range 2.1 – 7.4), TPM was 16.3 ± 1.1 mg L^{-1} and the water temperature was 19°C. The budget experiment carried out on two sets of 4 mussels had a mean CR calculated from measurements using the indirect method of 2.09 and 3.25 L ind^{-1} h^{-1} while when calculated from DR via the modelled DF, mean CRs were 2.1 and 3.12 respectively (Table 3. 3). No significant difference between the predicted and measured DF was found (log transformed data, Mann-Whitney test: W = 73, $p = 0.424$). These results support the validity of the DF model.

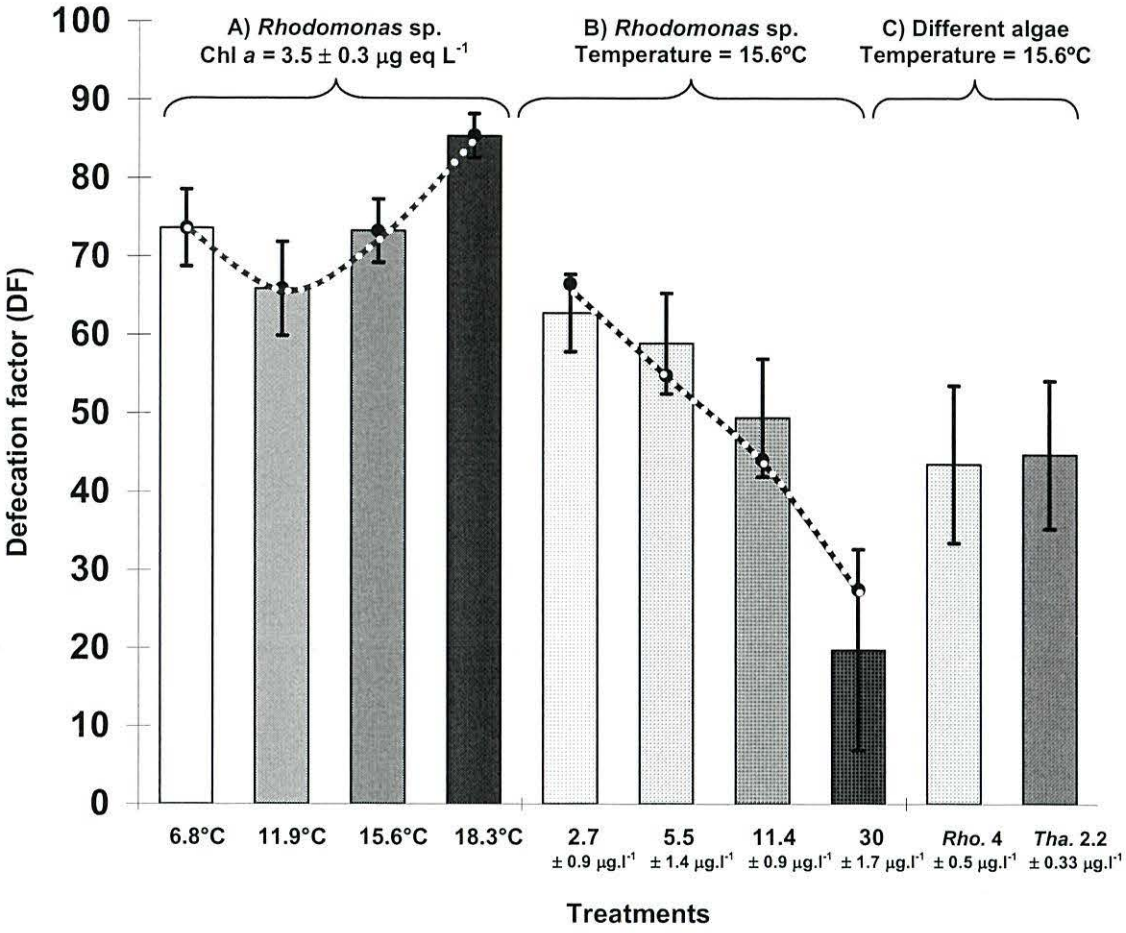


Figure 3. 2: Percentage digestion ± 2SE for 3 different treatments: temperature, algae concentration and algae species. Model for temperature and algae concentration treatments is represented with a black dot connected by dotted line.

Pigment recovery

HPLC analysis of the water samples taken in front of the mussel bed in Knebel Vig showed that chl *a* was relatively high, > 16 µg chl *a* L^{-1}, in the beginning of the field study but declining the following days to 1 – 4.5 µg chl *a* L^{-1}. Fucoxanthin and peridinin were the dominant diagnostic pigments, indicating the presence of diatoms and dinoflagellates, respectively. Furthermore, chlorophyll b (chl b), alloxanthin and zeaxanthin were detected in relatively small amounts (along with non-diagnostic pigments, i.e., chlorophyll c's, diadinoxanthin, diatoxanthin, neoxanthin, violaxanthin, lutein, a- and b-carotene, not shown), indicating sporadic presence of cryptophytes and cyanobacteria. Of phaeopigments, the following were detected; phaeophytin a, phaeophorbide a, and pyrophaeophytin a and three phaeo a-like pigments. These pigments were, however, co-eluting with other carotenoids (deduced from the scans by the diode-array), and since they were relatively low

concentrations, they were ignored. For all pigments identified, except fucoxanthin, pigments defecated were related to the concentration of the pigment in water (Figure 3. 3). There was a significant relationship ($r^2 = 0.523$; $p < 0.001$) between chl *a* in the faeces and in the water.

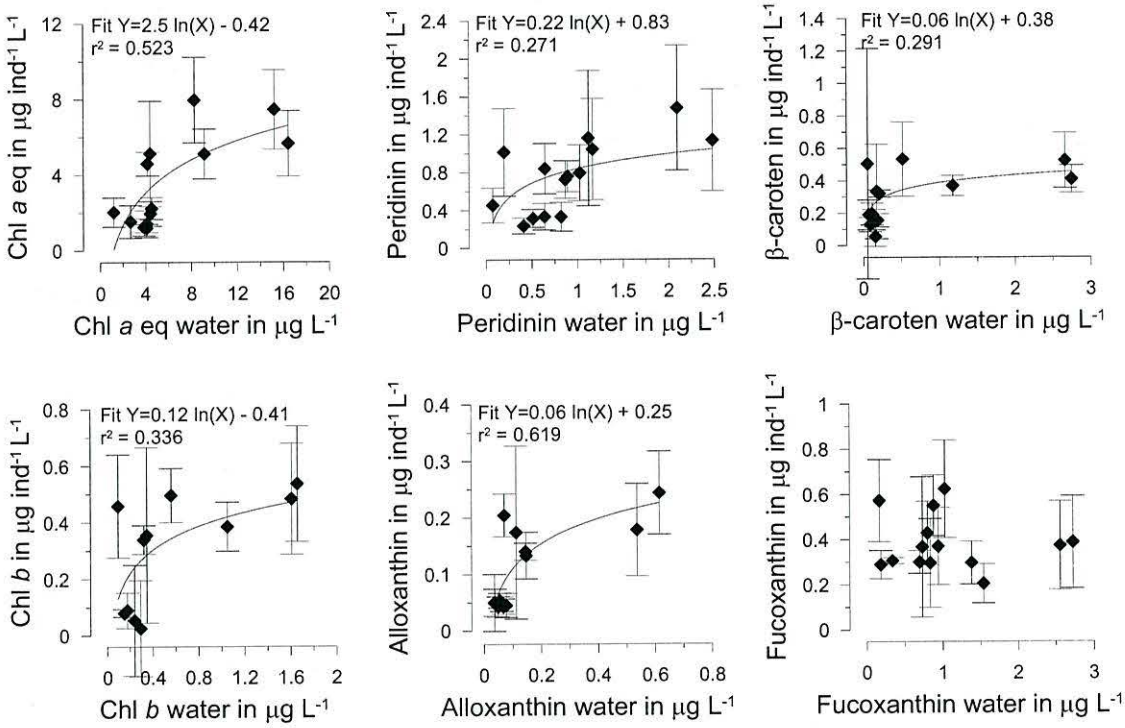


Figure 3. 3: Relation between rate of faeces production of algal pigments (y) and corresponding pigments in water (x) collected up-stream of mussel bed, measured with HPLC. Each value are the mean ± 2SE from 3-4 incubations each containing 1-3 individuals.

Table 3. 3: Comparison of the digestion factor (DF) calculated via a budget experiment and modelled with natural seston for 2 experiments (4 & 7, September 2006). CR = Clearance rate, IR = Ingestion rate, DR = Defecation rate, Dig = digestion rate.

	CR L ind^{-1} h^{-1}	IR µg eq ind^{-1} h^{-1}	DR µg eq ind^{-1} h^{-1}	Dig µg eq ind^{-1} h^{-1}	DF (%)	Water µg chl a_{eq} L^{-1}	Notes
Experiment 4							
Mussel 1	2.12	16.53	9.68	6.85	41.42	7.79	open
Mussel 2	0.77	6.03	1.83	4.20	69.65	7.79	Partly closed
Mussel 3	2.14	16.66	2.89	13.77	82.64	7.79	open
Mussel 4	3.34	26.02	6.73	19.28	74.12	7.79	open
Mean ± 2SE	**2.09 ± 1.05**	**16.31 ± 8.17**	5.28 ± 3.61	11.02 ± 6.82	**66.96 ± 17.86**	7.79	eq.3
Min	0.77	6.03	1.83	4.20	41.42	7.79	
Max	3.34	26.02	9.68	19.28	82.64	7.79	
Model 15.6°C	4.04	31.5			48.67	7.79	eq.11
Model 19°C	3.5				88.69		eq.12
Model exp 4	**2.10 ± 1.44**	**16.35 ± 11.18**	5.28 ± 3.61		**67.71**	7.79	eq.13
Experiment 7							
Mussel 1	2.95	18.93	6.05	12.88	68.02	6.42	open
Mussel 2	3.90	25.06	6.25	18.80	75.04	6.42	open
Mussel 3	3.04	19.54	7.27	12.26	62.77	6.42	open
Mussel 4	3.10	19.91	4.41	15.50	77.86	6.42	open
Mean ± 2SE	**3.25 ± 0.44**	**20.86 ± 2.83**	6.00 ± 1.18	14.86 ± 2.98	**70.92 ± 6.83**	6.42	eq.3
Min	2.95	18.93	4.41	12.26	62.77	6.42	
Max	3.90	25.06	7.27	18.80	77.86	6.42	
Model 15.6°C	4.21	27.0			51.99	6.42	eq.11
Model 19°C	3.5				88.69		eq.12
Model exp 7	**3.12 ± 0.62**	**20.02 ± 3.95**	6.00 ± 1.18		**70.03**	6.42	eq.13

3.6 Discussion

The defecation method as used in this study appears to be an appropriate and promising *in situ* method to measure mussel ingestion or clearance rate. The modelling of the digestion factor (DF) as a function of chl *a* concentration and temperature makes the application of the defecation method faster, easier and more accurate. This model is an important step in the use of the defecation method and once applied, it enables one to omit the budget experiment from the method, in contrast to previous applications of the method on macro invertebrates (Kotta and Møhlenberg, 2002; Kotta *et al.*, 2004). In the laboratory DF and IR changed with ambient chl *a* concentration (Figure 3. 1; Kotta and Møhlenberg, 2002) and also with temperature (Jørgensen *et al.*, 1990). These results concur with the findings of previous copepod studies that describe the gut clearance coefficient, where temperature could explain ~70% of the variability if food concentration was not a limiting factor (Dam and Peterson, 1988).

The DF model was calculated for temperatures ranging from 6-19°C and phytoplankton concentration ranging from 2-30 μg chl a_{eq} L^{-1} implying a limited use of its application. The DF model was simple and assumed a cumulative effect between chl *a* concentration and temperature variation. The model can be improved in the future by extending the ranges of temperature and phytoplankton and by including additional factors such as total suspended material and analysing their mixed effects. The model also provides a single DF, though DF may vary substantially, as shown in the validation experiment. Here the DF varied with a standard deviation of up to approx. 20%. In the same experiment, measured CR also varied substantially and was in general low, probably due to high levels of TPM and highly variable chl *a* concentrations that ranged from 2.1 – 7.4 μg L^{-1}. The latter concentration can be classified as high and valve gape closure did occur and reduction of CR due to gut satiation can be expected (Clausen and Riisgård, 1996; Riisgard, 2001b). Nevertheless, the model was validated with the *in situ* measurements. Finally, the size of the mussels was not incorporated in the model of DF. Although size naturally influences CR and IR and hence DR, there is not obvious reason why size should affect the DF. Gut morphology or nutritional state of

the animal such as starvation (Abele-Oeschger and Theede, 1991) may, however, vary with size and thereby affect gut residence time and digestion/absorption of algal pigments in the digestive system, but our experiments within an albeit limited size range of shell length did not support such an assumption. Further studies are encouraged to verify the importance of mussel body-size for the DF.

Kotta and Møhlenberg (2002) suggested that the high variation in DR from individual mussels, as found in our study, was related to the variation in chl *a* degradation and its storage in the digestive system. The pigment loss in the digestive tube has been found in copepods to be highly variable (Penry and Frost, 1991). Pigment breakdown was addressed in this study, via the separation of pigments in the mussel faeces using HPLC. The most abundant degradation product of chl *a* in the faeces of *M. edulis* was phaeophorbide a-like pigments, which is consistent with the findings of Szymczak-Zyla *et al.* (2006) and Hawkins *et al.* (1986), who in addition to allomerized chl *a*, phaeophobide *a*, phaeophytin *a* and pyrophaeophytin *a* also found chlorophyllide *a* (not found in this study) in faeces of *M. edulis*, fed an algal culture of *Phaeodactylum tricornutum* (Bohlin). Furthermore pyrophaeophytin *a* was found in our study, but was not detected by Hawkins *et al.* (1986). The chl *a* degradation products found in our study was more in agreement with the findings of Pastoureaud *et al.*, 1996) in oysters (*Crassostrea gigas,* Thurnberg) fed on a natural diet of phytoplankton, indicating that a diet of an algal mono culture can produce different chl *a* degradation products than a natural diet of phytoplankton. The results of the study indicate that the use of chl a_{eq} for measuring CR with the defecation method is appropriate, as it takes into account, *in situ*, the main chl *a* degradation products (phaeopigments).

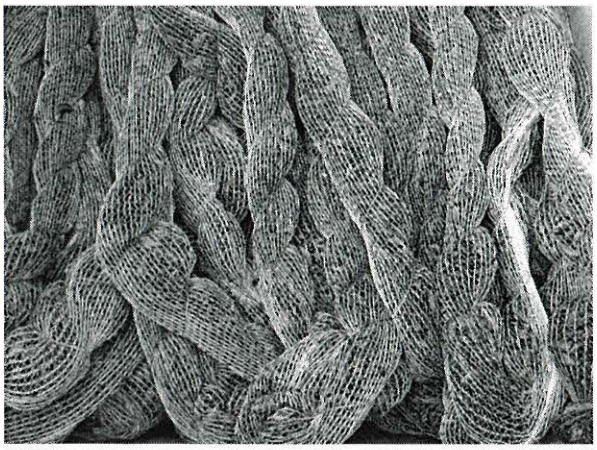
There are temporal and spatial resolution issues that need to be taken into consideration when applying the defecation method. An important point is that this method depends on the measurement of chl *a* concentration in the field. The latter requires to be measured very close to the mussels in order to be representative of the exact conditions experienced by the mussels. It has been shown that chl *a* depletion can occur very close to the mussel bed (Fréchette *et al.*, 1989; Dolmer, 2000a, Petersen, 2004a) and sampling at ~1 m above the mussel bed, or at the water surface can overestimate the chl *a* concentration ingested by the mussel. Consequently the

CR estimated via the defecation methodology will be underestimated. In terms of temporal resolution, the DR method does not integrate mussel CR for long period of time as governing factors like temperature and phytoplankton concentration may vary dynamically in the coastal zone, and the method thus represents a point measurement integrating only a short time period 1.5 – 2 h prior to the start of the experiment.

In summary, the defecation method is a non-disturbing *in situ* technique allowing point measurement of IR and CR directly from the field that with the use of a DF model does not have to be supplemented with budget experiments. Another advantage of the defecation method is that the CR estimated can be applied to mussels sampled from any type of environment, i.e. its natural environment, long line cultures, bottom cultures, etc. Estimating CR with the defecation method is simple to use once the requirements for its use is fulfilled: chl *a* needs to be sampled close to the mussels, the modelled DF can only be used within a certain range of chl *a* concentrations and temperatures, and gut residence time needs to be taken into account.

Chapter IV

In situ mussel clearance rate methodologies



Mussel socking in the Limfjorden, 09/2006

Chapter IV Assessment of methodological approaches for determining *in situ* clearance rate of mussel (*Mytilus edulis* L.).

4.1 Contributions to this chapter

Chapter process:	Contributions
Conception	JKP/ JG/ PC
Design analysis	CS/JKP/ JG/ PC
Data collection	CS/JKP/ JG/ PC
Data analysis/ Interpretation	CS/JKP/ JG/ PC
Drafting	CS/JKP/ JG/ PC
Revising	CS/MJK/JKP/ JG/ PC

Initial	Name	Institutions
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MJK	M. J. Kaiser	
JKP	J. K. Petersen	NERI, Roskilde, Denmark
JG	J. Grant	Dalhousie University, Halifax , Canada
PC	P. Cranford	Bedford Institute of Oceanography, Dartmouth, Canada

4.2 Abstract

Clearance rate of the adult blue mussel (*Mytilus edulis* L.) from Limfjorden (Denmark) was calculated using three different *in situ* methods: biodeposition, defecation and suction. The latter were also combined together with the indirect method using natural seston in the laboratory. The biodeposition method gave the lowest clearance rates with least variation, while the defecation data were similar but higher and with more variations. The suction method data was significantly higher *in situ* than the two other methods, but similar to the defecation method in the laboratory. The mussel clearance rate was relatively low for the three methods (range: 0.16 – 1.67 L h^{-1}) due to the high seston concentration. Indeed, the mean

surrounding chlorophyll *a* (chl *a*) and TPM concentrations during the experiments were high and varied greatly both *in situ* and in the laboratory respectively (chl *a*:16.36 and 5.49 μg L^{-1}; TPM: 20.4 and 6.3 mg L^{-1}).These data indicate that the possible explanations for the low values found with the biodeposition method *in situ* are: the technical modifications of the method, the mechanical and food disturbances, the time-scale for faeces integration and environmental sampling and the possible errors stemming from differences in lower retention limit of glass fibre filters compared to the mussel filter. These explanations can however, not account for all of the observed discrepancy.

4.3 Introduction

Macroinvertebrate suspension-feeders are a conspicuous and often dominant part of the coastal benthic community (Seed, 1976). The water processing capacity of benthic suspension-feeders may in shallow areas be up to several fold the total volume of these areas (Petersen, 2004a; Dame and Prins, 1998) and appear to exert a controlling influence on the biological structure of some coastal ecosystems (Cloern, 1982; Newell, 2004). Benthic suspension-feeders are also an important source of food and income for humans. Mussels, clams and oysters are produced in large quantities in various forms of on-bottom or off-bottom cultures or dredged directly as a part of fisheries for wild stocks. Knowledge on their feeding capacity is thus important both in an ecological context, when modelling their impact on the ecosystem, but also from a point of view of understanding carrying capacity for shellfish production.

The determination of water processing capacity, clearance rate or ingestion rate of bivalves has been the subject of considerable discussion in the scientific literature (Jørgensen, 1990, 1996; Bayne, 2004; Riisgård, 2001a, 2001b; Cranford and Hill, 1999; Cranford, 2001; Petersen *et al.*, 2004; Petersen, 2004b; Riisgård, 2004) and it has been difficult to find agreement on both the appropriate use of methods and the accuracy of clearance rates measured for various suspension-feeders under different conditions. Clearance rate may vary due to several internal and external variables

such as size, reproductive state, temperature, and the concentration, composition, and packaging of suspended particles (e.g. Jørgensen, 1990; Smaal and Haas, 1997; Bayne, 2004). Some of the discussions on clearance rates partly have been blurred by invalid comparisons stemming from responses to different experimental conditions.

Prerequisites for any comparison of clearance rate measurements include the standardization of ambient environmental conditions and ensuring that each method is applied in a valid manner (Riisgård, 2001a). An intercalibration exercise that took all of these factors into account demonstrated that results were inconsistent among different methods (Petersen *et al.*, 2004). In a laboratory comparison of the indirect methods, the flow-through method and the biodeposition method, the latter gave significantly lower clearance rates than the former (Petersen *et al.*, 2004). In contrast, other direct comparisons of the biodeposition method with the flow-through method provided equivalent clearance rate results (Iglesias *et al.*, 1998; Bayne, 2004). Several explanations were offered for the discrepancy reported by Petersen *et al.* (2004), including the fact that the biodeposition method gives an integrated value over a longer time period compared with methods that provide snapshot measurements of clearance rates of mussels. Food concentration varied over short time-scales and this may have been reflected in methodological variation in feeding activity (Petersen *et al.*, 2004). Another explanation for the estimated lower clearance rate using the biodeposition method is the production and the loss during the collection of some pseudofaeces that results in an underestimation of clearance rate (Bayne, 2004).

Clearance rates measured in the laboratory under optimal conditions, using only algal cell diets provided at constant concentrations, are likely to result in very different results from those obtained using field measurements of clearance rate and the latter should be prioritised in order to better understand the complexity of mussel feeding behaviour. In an indirect comparison, clearance rates obtained in the laboratory have been reported to be higher than clearance rates measured *in situ* using the biodeposition method (Cranford and Hill, 1999), although some field results have also given comparable rates (Cranford et al., 1998, 2005). So far, only a few methods exist to measure clearance rate *in situ* and they always required mussel manipulations. Apart from the biodeposition method (Hawkins *et al.*, 1996; Iglesias

et al., 1998; Cranford and Hargrave, 1994; Cranford and Hill, 1999), alternatives include the defecation method (Kotta and Møhlenberg, 2002; Kotta *et al.*, 2004), and the less developed modified suction method 'InEx' (on filter-feeders, Yahel *et al.*, 2005). The manipulation in the biodeposition method involves the removal of mussels from their natural environment and placement in traps *in situ* for acclimation (Cranford and Hill, 1999). The conditions experienced by the mussels on top of the trap do not strictly reflect the environmental conditions formerly experienced by the mussels (new intra/inter-specific competitions and modified flow regime). Defecation is a well known method for assessing zooplankton clearance rates (Dagg and Walser, 1987; Peterson *et al.*, 1990), but this method has been applied infrequently to macroinvertebrates, using temporal variation in faeces production rather than gut content variation (Kotta and Møhlenberg, 2002; Kotta *et al.*, 2004; Chapter 3). Using this method, the disturbance consists of removing the mussels from their environment and allowing them to defecate in beakers. The ingestion, thus assessed, reflects clearance rate prior to the manipulation due to delay in the passage of food through the gut. The 'InEx' method requires divers to collect water samples from animal inhalant and exhalant siphons and is based on a principle similar to the laboratory suction method (Møhlenberg and Riisgård, 1979). Using this method, disturbance may be induced by the divers' activities around the filter-feeders. Unfortunately, from the few methods developed for conducting *in situ* clearance rate measurements, there have been no intercalibrations with other techniques and there are no comparisons under conditions comparable with laboratory measurements.

The aim of this study was to compare measurements of the clearance rate of mussesls feeding upon natural seston using three different *in situ* methods on mussels (*Mytilus edulis*) under identical conditions. The methods tested were: i) biodeposition, ii) defecation and iii) suction. To date, the suction method (Møhlenberg and Riisgård, 1979) has only been used in the laboratory. Furthermore, the methods were applied under laboratory conditions, by using natural food supplies, and under these conditions added the iv) indirect method (Riisgård, 2001a) to the comparison.

4.4 Material and Methods

Experiments were carried out in the harbour adjacent to the Danish Shellfish Centre (DSC) in Nykøbing Mors in the middle of the Limfjorden, Denmark, from 21 to 27 September 2006 (Table 4. 1). Mussels, *Mytilus edulis* L., from nearby mussel culture long-lines were transported and suspended in "socks" from a pier at DSC ~1 mo prior to experiments. *In situ* experiments were performed from a platform attached to the pier. In the laboratory, mussels from the same socks were simultaneously placed in a 1.5 m^3 tank with unfiltered running seawater (6 – 10 L min^{-1}) pumped from a depth of 4 – 5 m in the Limfjorden close to the experimental site. After the experiments, the mussels were measured for shell length (L ± 0.01 mm) and dry weight (DW ± 0.001 g) of body parts was obtained by drying the excised tissue at 80°C until constant weight was obtained (Table 4. 2).

Table 4. 1: Summary of experiments carried with dates and sites. X denotes the method used. September 2006.

Date	Site	Biodeposition	Defecation	Suction	indirect	Notes
22	Pier	X				Trap vs bucket
23	Pier		X			Trap vs bucket
24	Lab.	X	X			
25	Pier	X	X	X		Time series
26	Pier	X	X	X		Time series
27	Lab.	X	X	X	X	Time series

A Meerestechnik GMBH ME-profiler CTD and an *in vivo* fluorometer (SCUFA, Turner Design) were deployed close to the platform (approx. 1 m) 1 m below the surface and sampled every 5 min. Fluorescence data from the SCUFA were converted to chlorophyll *a* (chl *a*) concentrations using a regression between fluorescence and chl *a* measured in water samples (r^2 = 0.55, p = 0.002). Water samples for ambient chl *a* and total suspended matter (TPM) concentrations were collected next to the experimental setup using a Ruttner design 5 L water sampler and processed as described below.

Table 4. 2: Shell length (L), dry weight of body parts (DW) and Condition Index (CI = DW/L^3) for experimental mussels.

	L (mm)	DW (mg)	CI (DW/L^3)
Average	58.21	1.62	8.95
Minimum	51.18	0.80	5.72
Maximum	64.90	2.67	15.53
St. Dev.	2.80	0.48	2.27

Biodeposition method:

Determination of clearance rate was based on the egestion of inorganic material using a simplified version of the *in situ* method described by Cranford and Hill (1999), which was designed to provide data on cohort feeding responses using time-series sediment traps for automated faeces collection at prefixed intervals. Instead, individual mussels, randomly collected from the available stock, were placed over small funnels that contained a 50 mL screw top glass bottle attached to the base of the funnel to collect the faecal pellets (Figure 4. 1a). Initial experiments (22 to 24 September) had the mussels placed on a coarse plastic mesh (10 mm), but faeces were observed to become trapped in the mesh, so it was replaced by a thin metal wire loop that the mussel was placed on over the top of a funnel. During field experiments, the traps were hung from the platform at 1 m below the sea surface. Mussels were left to acclimate on the trap at least 1 h prior the start of the experiment. After the acclimation period, the traps were raised to near the surface and the faeces collection bottle was replaced with a clean one filled with ambient water. Fifteen traps were loaded with a single mussel and five were empty to serve as controls for natural particle sedimentation. For the experiments in the laboratory, the same traps were suspended in a 1.5 m^3 tank with continuous renewal of seawater. The seawater pumped into the lab from nearby the experimental site, was either unfiltered seawater (24 September) or seawater filtered through a 100 μm screen (27 September). Faeces were separated from pseudofaeces when present, but pseudofaeces was generally not found or very scarce. On the 22nd September, some non-faecal sedimentation occurred in the control and sampling bottles from the traps. Both sediments and faeces in the sampling traps were collected separately, as well as the sediments in the control trap in order to check if they were pseudofaces associated with sediments. No pseudofaeces were significantly present in the

sampling trap when compared to the sediment present in the control traps (Table 4. 3).

Table 4. 3: a) Mean dry weigh and ash weight for non-faecal sedimentation in control trap, faeces and non-faecal material in the sampling trap (22nd September). Analysis of variance (ANOVA) for dry log transformed weight and ash weight. b) Multiple comparisons between log transformed dry weight and ash weight in bold using Bonferroni's method. All differences given in log mg minimum difference required for significance at 5% level = 0.16 log mg for dry weight and 0.28 log mg for ash weight. Columns means are subtracted from rows mean. * = significant at 5%, ** = significant at 1%.

a)

	Non faecal control	Non faecal sample	Faeces sample	ANOVA
Dry weight	14.97 ±8.09	14.23±5.77	7.64±3.21	$F_{2,30} = 10.36$ **
Ash weight	11.90±6.87	11.54±5.11	5.79±2.38	$F_{2,30} = 11.32$ **

b)

	Control	Non-faecal material	faeces
Control		0.0084	0.2853*
Non faecal material	**0.0026**		0.2769 **
Faeces	**-0.2982 ***	**-0.3008 ** **	

In order to check the validity of the traps using the mesh and to be sure that no faeces were lost during the manipulation, an experiment was conducted on the 22 September where the faeces were collected either by the traps or by letting the mussel defecate outside their natural environment in buckets filled up with water, according to the defecation method and described below (see design in Table 4. 4). Two groups of 7-8 mussels from the same sock were place on the traps ($T1 + T2_1$) for a period of time A, at the end of the period, the vials with faeces were collected, the mussels from group 1 were placed in buckets (B1) on shore, while the mussels from lot 2 were left in the traps ($T2_2$) for a period B. At the end of period B, faeces from B1 and $T2_2$ were collected and mussels from lot 2 ($T2_2$) were placed in the bucket and left to defecate for the period C (for clarification refer to Table 4. 4).

The experiment using the biodeposition method was carried out five times: three times *in situ* and twice in the laboratory. On the 22nd September, the method was carried out using traps or a bucket to collect the faeces and check if the faeces collected by the two methods gave similar results. All the experiments carried out using the biodeposition methods are summarized (Table 4. 1).

Table 4. 4: Sampling design for faeces collection in traps vs. buckets on the 22nd and 23rd September. For each mussel lot, 7-8 animals were individually tested with either the biodeposition or the defecation method. * indicate faecal material sampling.

Time (h)	A	B	C
Trap (in water)	Mussels lots 1 + 2 ($T1 + T2_1$) *	Mussels lot 2 ($T2_2$) *	
Bucket (on shore)		Mussels lot 1 (B1) *	Mussels lot 2 (B2) *

TPM (mg L^{-1}), particulate inorganic matter (PIM) and particulate organic matter (POM = TPM - PIM) were determined from seawater samples. Water samples were filtered onto Whatman® GF/C filters, dried (80°C for 24 h), weighed and burnt to ash (450°C for 4 h) before final weighing. At the end of each experiment, faeces were pipetted from the glass bottles and treated as the water samples. Clearance rate (l g^{-1} DW h^{-1}) was calculated according to Cranford and Hargrave (1994):

$$\mathbf{CR = (E_{ash}/F_{ash})/(TPM)} \qquad \mathbf{(1)}$$

where E_{ash} is the egestion rate of ash and F_{ash} is the proportion ash in the food (PIM).

Defecation method:

Clearance rate determination was based on the ingestion and egestion of chl *a*. The defecation method follows the methodology detailed in Chapter 3. Mussels (*in situ* or in the lab) were collected from the funnels at the end of the exposure period (described above) along with water samples from their surrounding environment from two hours prior to the experiment. Mussels were then placed individually in 1 L buckets filled with ambient seawater and left to defecate for 1 h (Figure 4. 1b). Faecal pellets were collected with a plastic pipette after 0.5 and 1 h and placed on a Advantec ® GC50 filter to remove excess water. The filters were extracted in 96% ethanol and analysed on a Turner Design 770 fluorometer, calibrated against a chl *a* standard, before and after acidification (method adapted from Parsons *et al.*, 1984). Clearance rate (L g^{-1} DW h^{-1}) was calculated according to Chapter 3:

$$\mathbf{CR = (DR * 100 / (100 - DF))/chl\ a_{eq}} \qquad \mathbf{(2)}$$

where DR is the defecation rate (µg chl a_{eq} h^{-1}), chl a_{eq} is the chl *a* equivalent concentration (chl a_{eq} = chl *a* +1.52Phaeo, µg L^{-1}) in the surrounding water 2 hours

prior to experiment (to take into account the gut residence time) and DF is the digestion factor (%):

$$\mathbf{DF = -16.90 \times \ln(chl\ a_{eq}) - 8.962 \times T_w + 0.3962 \times T_w^{\ 2} + 129.70} \quad \mathbf{(3)}$$

where T_w is the mean seawater temperature during the experiment. The DF was adjusted firstly for chl a_{eq} and temperature assuming a cumulative effect of both. The DF was calculated for each experiment with temperature and chl a_{eq}, it varied from 33.4 to 63.5% depending on the conditions in the field and laboratory.

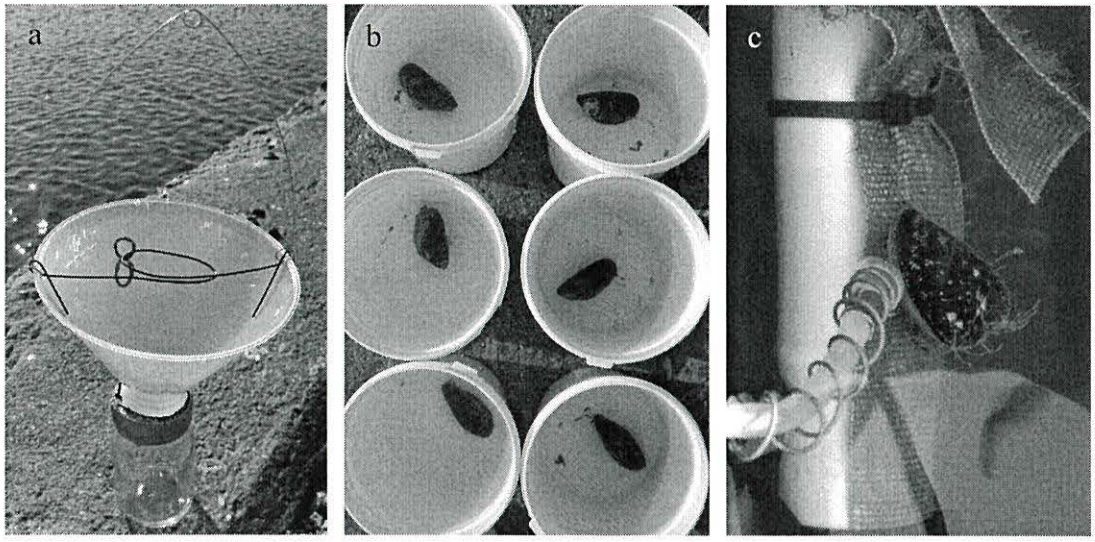


Figure 4. 1: Experimental setup pictures, 1a is the sediment trap used for biodeposition, 1b is the buckets used for defecation, 1c is the suction method setup *in situ* .

In order to be sure that the differences found between the biodeposition and defecation methods were not due to the method of faecal pellet collection (use of trap *in situ* or free standing bucket filled with seawater), two experiments were conducted using a trap and the bucket technique simultaneously for the two methodologies, see design in Table 4. 4 and description in the biodeposition method. This test was carried out on the 23rd of September for the defecation method in the field. All the experiments carried out with the defecation method are summarized in the Table 4. 1.

Suction method:

Clearance rate was determined from the difference in particle concentration between inhaled and exhaled water by the mussel. This method was applied in the field on mussels from socks attached to a platform close to the one with the traps,

using a peristaltic pump at a flow rate higher than mussel filtration and a lower diameter tube than the exhalant siphon. Different flow rates were tested with the peristaltic pump, in order to determine if an adequate pump rate was obtained (Møhlenberg and Riisgård, 1979). This method was performed using a different batch of mussels than for the methods used in the field (25th and 26th September), while mussels used in the laboratory (27th September only) were exactly the same as those used for the two other methods. In the laboratory, clearance rate was measured at the same time as the other methods, making a direct comparison possible. Samples of inhaled water were collected via plastic tubes placed 2 to 4 mm from the siphon opening of mussels deployed *in situ* and in the laboratory (Figure 4. 1c). Particle concentration from the samples was counted using an Elzone® 5380 particle counter mounted with a 95 µm orifice tube, with the lower threshold set to 4 µm; each sample was counted at least 3 times. Some samples were also collected for chl *a* determination as described above. Clearance rates were estimated for a total of 8 mussels, on 2 – 4 occasions for each mussel and making 3 consecutive replicates on each occasion. The mean of the clearance rate measurements estimated for each mussel was used for comparison with the other methods. Clearance rate (L g^{-1} DW h^{-1}) was calculated according to Møhlenberg & Riisgård (1979):

$$\mathbf{CR = F\,(1 - C_e/C_i)} \quad \mathbf{(4)}$$

where F is the suction flow rate through the plastic tubes and C_i and C_e the concentrations of 100% retained algal cells in water collected simultaneously from inhalant and exhalant currents, respectively.

Indirect method

Mussels acclimatized in the laboratory with seawater filtered through a 100 µm screen, pumped into the lab from nearby the experimental site were used to determine clearance using the indirect method (27th September only): The method is based on the exponential decline in particle concentration over time in closed containers, but was here modified as described by Petersen *et al.* (2004). Mussels were placed individually in four 1.1 L cylindrical jars (and three additional jars were used as controls) supplied with the same running natural seawater as used for the semi *in situ* experiments with the other methods. Water was mixed through gentle aeration. The water supply was stopped, and after for 30 to 45 min, water samples

were collected and particle concentrations were measured using the particle counter as described above. A series of three experiments were carried out with three sets each composed of four mussels, where clearance rate was determined 2 to 3 times per individual. Clearance rate (l g^{-1} DW h^{-1}) was calculated according to Riisgård (2001):

$$\mathbf{CR = V/t \times \ln(C_0/C_t)} \qquad \mathbf{(5)}$$

where V is the volume of the jar, t is time and C_0, and C_t are particle concentrations at times 0 and t, respectively. An exponential line was fitted to the decline in algal cell concentration over time and only regressions with an r^2 value > 0.90 were considered.

Data analysis:

All clearance rate measurements were standardized to a 1 g mussel using an allometric factor of 0.67 according to the following equation: $Y_s = (W_c/W_s)^{0.67}\ Y_c$ where Y_s is the standardised parameter, W_s is the standardised weight (1g), W_c is the weight of the experimental animal, Y_c is the uncorrected parameter (Hawkins *et al.*, 1996). For comparisons among datasets, paired t-test, 2 samples t-test and ANOVA were used to test for significant differences after checking that the data met the assumptions of normality and homogeneity of variance. In some cases, data were transformed in order to meet the assumptions. Kruskal-Wallis, Mann-Whitney or Mood Median tests were carried when the data failed to meet the assumptions.

4.5 Results

Concentrations of chl *a* and suspended particulate matter were high but highly variable during the experimental period, but were lower in the laboratory (Table 4. 5). The average water temperature was higher in the laboratory (19°C) than *in situ* (17.2°C).

Table 4. 5: Ambient conditions during the experiments.

	Field				Laboratory		
	Temp. °C	Chl *a* µg L^{-1}	TPM mg L^{-1}	POM mg L^{-1}	Chl *a* µg L^{-1}	TPM mg L^{-1}	POM mg L^{-1}
Average	17.2	17.5	20.4	16.3	5.4	16.3	4.5
Minimum	16.4	8.0	9.0	14.8	2.2	14.8	3.0
Maximum	17.8	45.2	32.0	19.5	7.4	19.5	6.8
St Dev	0.3	6.2	4.7	1.1	1.5	1.1	1.2

On the 23rd and 24th of September, there was no significant effect of sampling in buckets next to the platform on shore, or using traps in the water and attached to the platform, for both the biodeposition and the defecation methods when they were carried out simultaneously (Table 4. 6). Following the design (Table 4. 4), when sampling in the trap at the same time (A) for the 2 lots of mussels T1 (lot 1) and $T2_1$ (lot 2) there was thus no significant difference (*t*-test: biodeposition: $T_{10} = 0.41$, $p = 0.692$; defecation: $T_{13} = 1.07$, $p = 0.305$). Similarly, there was no significant difference during the time interval B between lot 2 left in the trap $T2_2$ and lot 1 moved to the bucket B1 (*t*-test: biodeposition: $T_{11} = 2.31$, $p = 0.069$; defecation: $T_{16} = -0.34$, $p = 0.738$). Comparing T1 vs. B1 and $T2_2$ vs. B2 using the different collection methods and 1 h delay also demonstrated no significant difference (paired *t*-test: biodeposition: $T_7 = 2.40$, $p = 0.053$; and $T_4 = 1.57$, $p = 0.215$ respectively; defecation: $T_9 = -0.57$, $p = 0.587$; and $T_7 = -1.93$, $p = 0.102$ respectively).

***In situ* experiments:**

Clearance rates varied during the sampling period for each method and between methods (Figure 4. 2).

Estimates of clearance rate using the biodeposition method were not significantly different between sampling occasions on the same day, but varied significantly between days (ANOVA, $F_{2,45} = 57.91$, $p < 0.001$) and a Bonferroni's post-hoc analysis showed that all daily estimates were significantly different at the 5% level. Mean daily clearance rates (22nd September: 0.13 ± 0.05; 25th September: 0.36 ± 0.10; 26/9: 0.22 ± 0.06) for each sampling period did not correlate significantly to mean chl *a* concentration measured from 2 – 1 h prior to sampling, corresponding to a gut passage time of 1 – 2 h (Pearson $r = 0.604$, $p = 0.15$). A similar picture could be shown for clearance rate measurements derived with the defecation method,

rendering significant differences only between 1 and 2 sampling days (23rd and 25th September) (Log 10 transformation applied to data, ANOVA, $F_{2,56} = 3.88$, $p = 0.027$ followed by a Bonferroni's post hoc analysis; 23rd September: 0.42 ± 0.13; 25th September: 0.61 ± 0.25; 26th September: 0.51 ± 0.22). There was however, no significant difference between sampling occasions on the same day (23rd September *t*-test, $T_{14} = 1.33$, $p = 0.204$; 25th September ANOVA, $F_{2,26} = 0.27$, $p = 0.763$; 26th September ANOVA, $F_{2,12} = 1.90$, $p = 0.200$). Mean clearance rates estimated from defecation were not significantly correlated to the chl *a* in the water 2 – 1 h prior to the start of the experiment (Pearson r = -0.725, $p = 0.065$). Using the suction method, the mean clearance rate for individual mussels varied from 0.5 – 2.88 L h^{-1} g^{-1} and was correlated negatively with mean chl *a* concentration from 2 – 1 h or 2 – 0 h prior to sampling (Pearson $r = -0.646$ and $r = 0.78$ respectively, both $p < 0.001$, Figure 4. 3). For both the biodeposition and defecation methods mean estimated clearance rates were negatively correlated with TPM and chl *a* equivalent respectively (Pearson: biodeposition: $r = -0.97$, $p < 0.001$; defecation t: $r = -0.87$, $p = 0.005$).

Table 4. 6: *In situ* collection methodology for biodeposition (mean TPM: 21.7 ± 9.8 mg L^{-1}) and defecation method (mean chl *a*: 14.35 ± 3.6 µg L^{-1}). Mean clearance rate ± SD of standard 1 g mussel (Ll g^{-1} h^{-1}) for 2 mussel lots for biodeposition (n = 7) and for defecation (n = 8), using trap or bucket collection.

Biodeposition	Time interval	(A) 0 – 2 h	(B) 2 – 3 h	(C) 3 – 4 h
22nd September	Trap	T1: 0.14 ± 0.06 $T2_1$: 0.13 ± 0.04	$T2_2$: 0.09 ± 0.07	
	Bucket		B1: 0.08 ± 0.02	B2: 0.10 ± 0.03

Defecation	Time interval	(A) 0 – 1 h	(B) 1 – 2 h	(C) 2 – 3 h
23rd September	Trap	T1: 0.39 ± 0.30 $T2_1$: 0.29 ± 0.20	$T2_2$: 0.35 ± 0.18	
	Bucket		B1: 0.38 ± 0.15	B2 0.46 ± 0.11

Since there were no differences in clearance rate estimates between sampling occasions on the same day for both the biodeposition method and the defecation method and since they were sampled more or less simultaneously, a comparison of estimated clearance rates could be made. For both 25th September (Mann Whitney: w = 271.5, $p < 0.001$) and 26th September (*t*-test: $T_{20} = -4.31$, $p < 0.001$) the clearance rates estimated with the two methods were significantly different (

Table 4. 7).

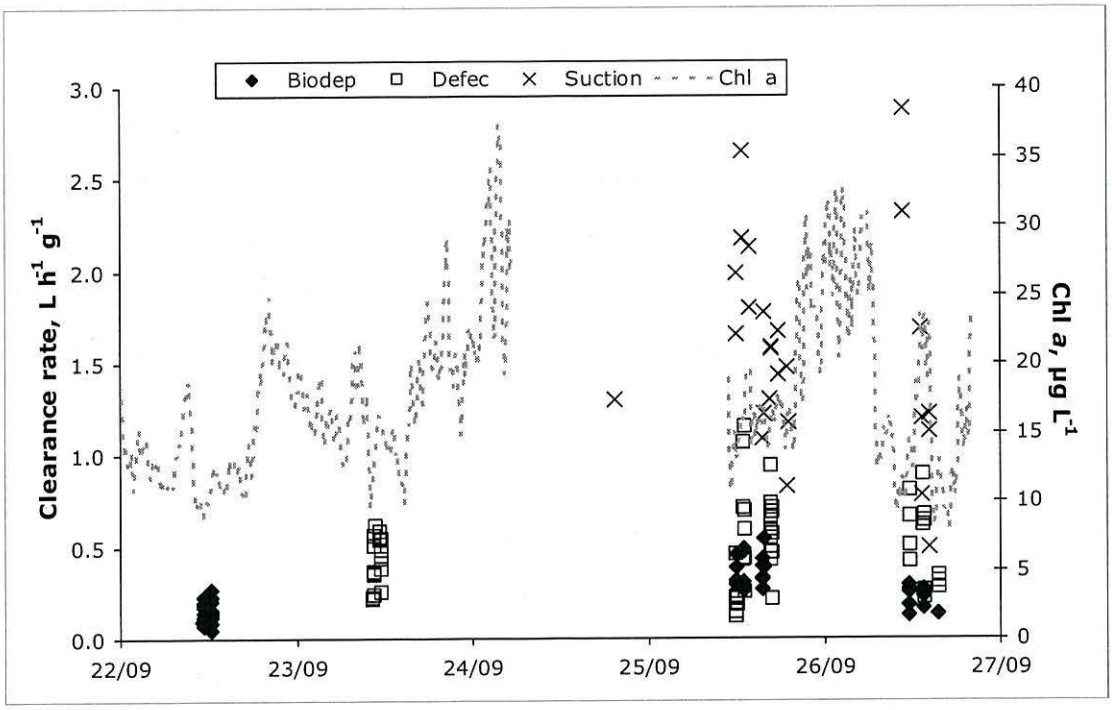


Figure 4. 2: Concentration of chl *a* in the ambient water and *M. edulis* clearance rates, measured using different methods during the experimental campaign period. Rates determined using the biodeposition and defecation method are shown for individual animals. Clearance rates using the suction method are given as a means of 2 – 3 consecutive measurements for each sampling occasion.

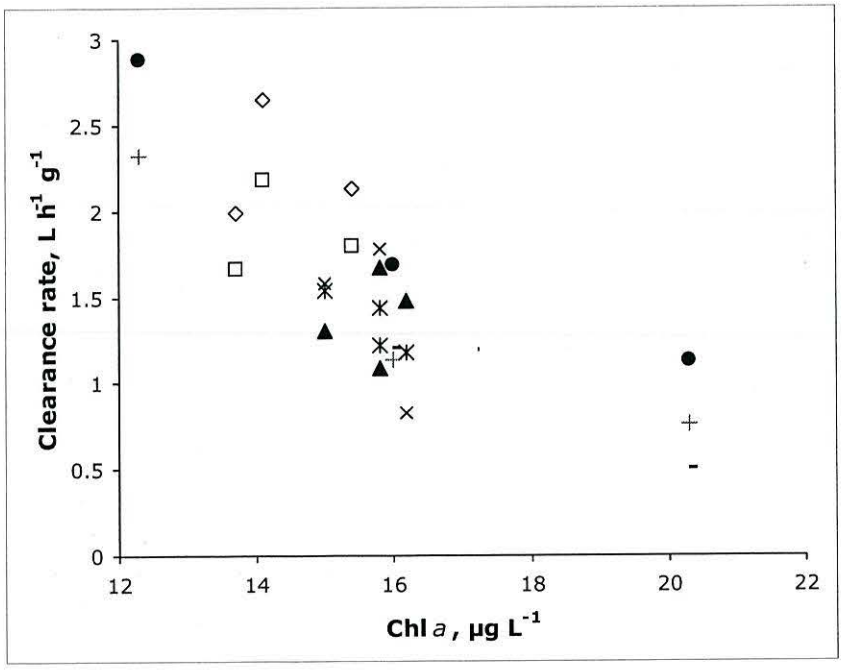


Figure 4. 3: Clearance rates (standardised per 1 g of mussel dry flesh) in 8 individual *M. edulis* as a function of the mean concentration of chl *a* 2 h prior to measurement of the clearance rate using the suction method. Each point represents a mean of 2 – 3 consecutive measurements and symbols denote the different individual mussels used.

Comparison with the suction method was less straightforward. On 25th September there was little variance in ambient chl *a* prior to sampling with the suction method (13.7-16.2 μg L^{-1}) and five different animals were tested that day. Mean clearance rate for these was 1.66 L h^{-1} g^{-1} (range: 1.35-2.26 L h^{-1} g^{-1}) and this was significantly higher than estimates using the other methods (Kruskal Wallis: H_2 = 26.40, $p < 0.001$). On 26th September ambient chl *a* varied substantially and only three animals were used, thus a meaningful comparison is not possible (Table 4. 7).

Table 4. 7: *In situ* measurements of clearance rate (mean clearance rate ± SD of standard 1 g mussel, L g^{-1} h^{-1}) on 2 occasions using different methods. Sampling of the different methods was simultaneous.

	25th September	26th September
Biodeposition	0.36 ± 0.08	0.22 ± 0.05
Defecation	0.61 ± 0.25	0.51 ± 0.22
Suction	1.61 ± 0.45	1.45 ± 0.80

Laboratory experiments:

Two comparisons were made in the laboratory. On 24th September the biodeposition and defecation methods were compared at an average chl *a* concentration of 6.5 ± 0.7 μg L^{-1} and a TPM concentration of 15.9 ± 0.8 mg L^{-1}. The estimate of clearance rate using the defecation method was significantly higher (log 10 transformation, *t*-test: T_8 = -5.03, $p < 0.001$) than the estimate using the biodeposition method (Table 4. 8). On 27/9, mean chl *a* concentration was 4.5 ± 1.5 μg L^{-1} and TPM concentration was 17.0 ± 1.3 mg L^{-1}. There was a significant difference between the four methods (Mood median test: Chi-square = 17.24, df = 3, $p < 0.001$) and only clearance rate calculated from biodeposition method was significantly lower than all the other methods (log 10 transformation, *t*-test with defecation, suction and indirect methods respectively: T_7 = -7.20; T_{13} = -10.07; T_{14} = -19.85; for all tests $p < 0.001$) that did not differ between each other (Mood median test: Chi square = 1.65, p = 0.439, df = 2; Table 4. 8).

Table 4. 8: Measurements of clearance rate (mean clearance rate ± SD of standard 1 g mussel, L g^{-1} h^{-1}) on 2 occasions using different methods in the laboratory using natural seston. Time interval represents the starting and ending time for which the methodology was applied.

	25th September	Time interval (h)	27th September	Time interval (h)
Biodeposition	0.21 ± 0.07	0 – 1	0.16 ± 0.03	0 – 4
Defecation	0.86 ± 0.51	0 – 1	1.37 ± 0.80	4 – 5
Suction	-		1.60 ± 0.26	2 – 4
Indirect	-		1.67 ± 0.77	2 – 4

4.6 Discussion

There was a significant difference between the results of estimated clearance rate obtained for the three methods applied *in situ* under identical conditions: the biodeposition technique gave the lowest values, but with least variation; the defecation method gave slightly higher values that the biodeposition method although these were more variable; and the suction method gave substantially higher values than either of the other two methods. *In situ* measurements varied with ambient particle concentration for all methods, whether measured as TPM or chl *a* concentrations. Under identical conditions in the laboratory, the biodeposition method resulted in significantly lower clearance rates than the other methods. The generally low clearance rates in the laboratory study can be explained by the high ambient TPM and chl *a* concentrations.

The lower estimates of clearance rate obtained with the biodeposition method can partly be attributed to the sampling technique: biodeposition and defecation give integrated values, whereas suction and indirect methods provide snapshot measurements. However, this does not explain all the variation, as defecation and biodeposition methods gave different results, despite the fact that they rely on the same principle and only differ in the material sampled: chl *a* or TPM. The suitability of the *in situ* biodeposition method for estimating clearance rates requires that several critical criteria are met (Cranford and Hargrave, 1994; Cranford *et al.*, 1998; Cranford and Hill, 1999). First, the faeces must be collected quantitatively. In the present study, some of the faecal material may have been lost due to the design of the

funnel and this problem has not been observed in previous studies using a different design (Cranford *et al.*, 1998; Cranford and Hill, 1999). Nevertheless, the results of the first experiment (22nd September) showed no quantitative difference in the number of faeces collected between the buckets (onshore) and traps. Further, faeces collection in the relatively static conditions in the laboratory can be assumed to be quantitative and still resulted in lower estimates of clearance rate than the other methods. The second criterion is that the animals should remain undisturbed. The simplified approach utilized in this study required that sample bottles are placed under funnels after the acclimation period; an obvious disturbance with unknown consequences. However the defecation method that was subjected to the same disturbances gave a higher estimate for clearance rate than the biodeposition method. The time-series trap approach permits a non intrusive and automated period of at least 1 h prior to sampling for the animals to resume normal feeding behaviour. A similar time-series approach was used in the laboratory for a period of < 4 h and the corresponding biodeposition results were lower than with the other methods by a factor of ten (Table 4. 8). The third criterion is that the mussels do not selectively ingest food particles for ingestion, as evidenced by a lack of significant pseudofaeces production. This has by definition significant implications for the clearance rate calculation and the relatively high TPM levels measured in this study generally result in the production of large amounts of pseudofaeces by mussels (e.g. Hawkins *et al.*, 1998a). Pseudofaeces production in the present study would result in an overestimation of both the inorganic egestion rates (E_{ash}; trap samples include rejected inorganic matter) and the ash content of ingested food (F_{ash}; some organic matter was selectively ingested). These errors have opposing effects on clearance rate estimates based on Equation 1. However, the net result of particle selection is likely to be an underestimation of true clearance rates if any pseudofaeces material is lost, and only on one occasion, when ambient food supply was high (22nd September), could sedimentation be recognized in the traps; but, no significant amounts of pseudofaeces were produced or identified within this sedimentation, which discard pseudofaeces production as underestimating the clearance rate. Another reason for the underestimation of clearance rate by the biodeposition method is the use of microfibre filter with a retention efficiency of ~1.2 μm; this may overestimate the particle concentration available for the mussels of which the filtering apparatus efficiency is only estimated at ~4 μm (Møhlenberg and Riisgård,

1978) and hence underestimate the clearance rate according to Equation 1 (Petersen *et al.*, 2004).

The time-scale is important in understanding the estimated clearance rates and mussel gut dynamics, since it is the environmental food concentrations that are used to calculate the clearance rate of the food ingested. *In situ* measurements are subjected to variable environmental conditions (temperature, chl *a* and TPM concentrations) and therefore, the integration time of faecal material sampling has repercussions on clearance rate estimations. This contrasts sharply with laboratory conditions where environmental conditions are held constant. The biodeposition method usually involves sampling and integrating faecal material over a long period of time (e.g. one sample per day for 40 days, Cranford and Hill, 1999) in comparison to the defecation method (sampling for a 1h period only). Therefore, the environmental conditions and the faecal material collected by the biodeposition method do not take into account the small time-scale environmental variations (hourly or tidally) and only provides an estimate of an averaged clearance rate. On the other hand, the defecation method gives precise estimation of clearance rate for short periods of time. It is for this reason, that the defecation method requires collection of regular chl *a* samples from the surrounding water especially for the period ~2 h prior the start of experimental measurements, so that clearance rate is estimated with a known food concentration prior to defecation. This routine permits the appropriate use of the defecation method and provides an accurate estimate of clearance rate. By using a simplified version of the biodeposition method with a short integration period (~1 h), the short time variations in TPM concentrations from the field were averaged and therefore not used in the estimation of the clearance rate. In this case, one should expect that these variations in environmental conditions should give either higher or lower estimates of clearance rate in comparison to the other methods (from Equation 1). This was not the case during this sampling period, where biodeposition was always lower than the other methods and with the lowest variations. There was also no variation in the calculated clearance rate within the same day (Table 4. 6). Furthermore, the lack of correlation between chl *a* prior to the experiment (integrated from 2 – 1 h) and the clearance rate calculated via biodeposition and defecation method (in contrast to the suction method (Figure 4.

3)), indicated that the gut and ingestion dynamics were possibly more complex in the variable environment (i.e. high TPM and chl *a* concentrations Table 4. 5).

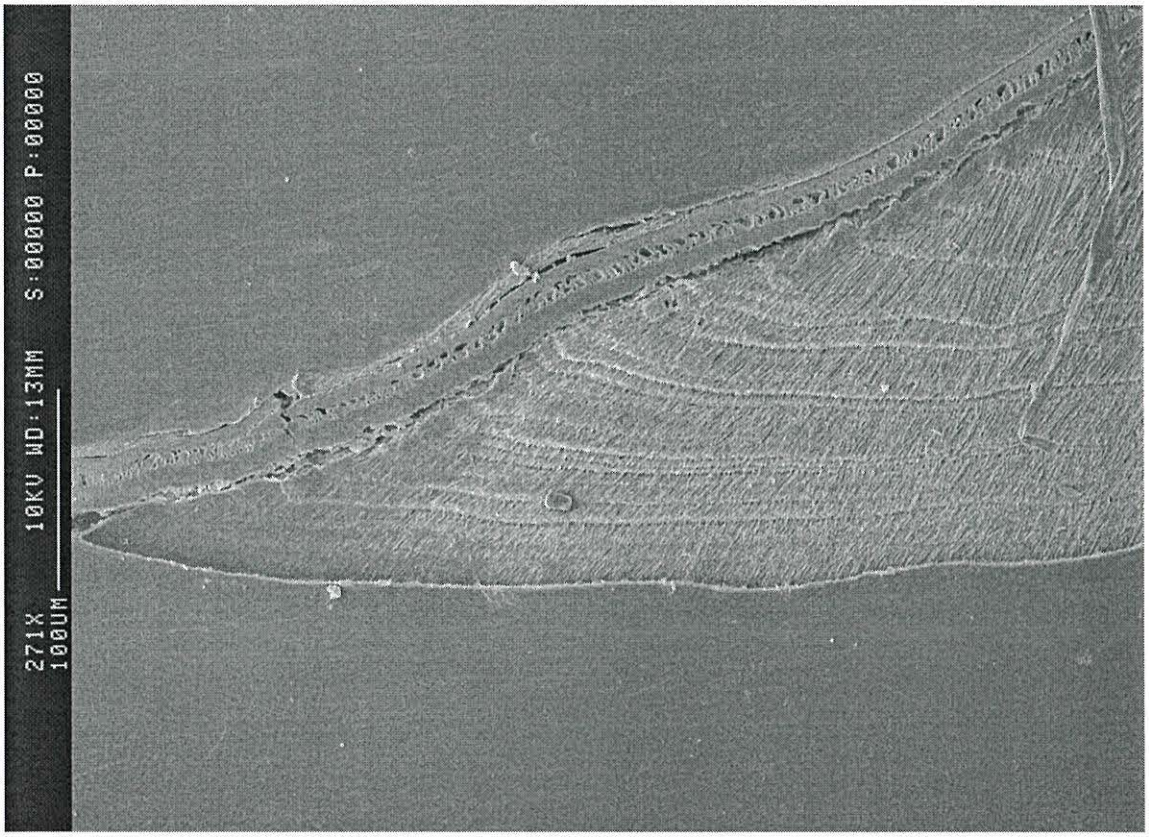
Comparing clearance rates from the present study (range: 0.3 – 1.7 g^{-1} h^{-1}) with various clearance rates calculated from model predictions determined either during laboratory studies (using optimal algae concentration under optimal conditions), or using seston from the natural environment is appealing but can lead to false comparisons. Indeed, the differences (lower clearance rates in this study) are due to the use of adequate *in situ* methods and the environmental conditions. The comparison with laboratory models using optimal algal concentration (CR = 6.05 to 6.54 L g^{-1} h^{-1}; Møhlenberg and Riisgård, 1979; Riisgård and Møhlenberg, 1979) is without taking the ambient conditions into consideration; the unusually high TPM and chl *a* concentrations and potential manipulation disturbances from our study (Table 4. 5) can account for most of this discrepancy. On the other hand, studies using seawater from the natural environment, although conducted at lower temperatures, found clearance rates (1.24 to 2.0 L g^{-1}) more comparable to the ones measured in the present study (Smaal *et al.*, 1997; Fréchette and Bacher, 1998; Thompson, 1984).

The suction method is difficult to implement in the field and is only included as an alternative to estimating *in situ* clearance and a reference value for comparing with the two other *in situ* methods. While the suction or the indirect methods measure at a single point in time, whereas the defecation and biodeposition methods integrate the clearance rate over a period of ≥1 h. Interestingly, the suction data and defecation data were different in the field, while they were equivalent in the laboratory. This could be due to the suction method being applied to open mussels rather than the randomly opened mussels used for other methods; but is unlikely to be true in practice as at least some of the clearance rate estimated with the defecation method should have been similar to the suction method and the overlap between the two methods is actually very small (Figure 4. 2). A more realistic explanation could be the effect of the higher frequency of manipulation disturbance associated with the replacement of glass bottles that impacted upon the biodeposition/defecation.

In summary, all the methods used in this study gave a lower estimate of individual clearance rate *in situ* and in laboratory (0.16 to 1.67 L g^{-1} h^{-1}) when compared to filtration rate predictions from the literature using optimal condition and algae concentration (6.0 to 6.5 L g^{-1} h^{-1}), but were very similar to other estimates from literature using natural seston (1.24 to 2.0 L g^{-1} h^{-1}). As expected, the biodeposition method gave lower estimates than the suction and indirect methods. It also gave a lower estimate than the defecation method, despite both techniques being based on similar principals. This could be due to several reasons: 1) the environmental conditions, chl *a* and TPM concentrations, being exceptionally high (Table 4. 5) with unusual observations of non significant pseudofaeces produced; 2) the level of disturbance *in situ* was higher for the biodeposition and defecation methods in comparison with the suction method; 3) the time-scale and method adaptations were unfavourable to the biodeposition method due to their unknown consequences. Nevertheless, there were no explanations to the lower clearance rate estimation of the biodeposition method (by ~10 folds) when it was compared to the clearance rate estimations from the three other methods in laboratory with natural seston (27th September). The defecation method appeared to be reliable and useful if applied properly; the biodeposition method might underestimate the *in situ* clearance rate and would need to be applied under its original use; the suction method is not practical and not recommended for use in the field due to the inability to obtain adequate numbers of replicates but it provides an individual clearance rate baseline for methods comparisons.

Chapter V

Mussel microgrowth



SEM picture of a thin section of a mussel shell grown under low food concentration.

Chapter V Environmental factors influence on macro- and micro-shell growth rate of the mussel *Mytilus edulis* L measured at different temporal and spatial scales.

5.1 Contributions to this chapter

Chapter process:	Contributions
Conception	CS/MJK/CR/JG
Design analysis	CS/MJK/CR/JG
Data collection	CS/CR/JG/ ED/VH/PF
Data analysis/ Interpretation	CS/CR
Drafting	CS/CR
Revising	CS/CR/MJK/JKP

Initial	Name	Institutions
CS	C. Saurel	SOS, UWB, Menai Bridge, Wales, UK
MJK	M. J. Kaiser	
CR	C. Richardson	
JG	J.Gascoigne	
ED	E. Donald	
PF	P. Freitas	
VH	V. Hernandez	
JKP	J. K. Petersen	NERI, Roskilde, Denmark

5.2 Abstract

This study focused on food concentration as an environmental factor controlling the microgrowth increments in mussel shells at different temporal scales (from a few days to years) and spatial scales (mussel bed or mussel patch). Microgrowth measurements were compared to traditional macrogrowth technique on the same mussels for future applications in the growth monitoring of farmed mussel areas. The internal growth band from mussels placed at mean low water spring (MLWS) in the field or under constant emersion in the laboratory showed a tidal periodicity and no

influence of the spring/neap cycles. In contrary, mussels in the laboratory under constant immersion and mechanical emersion once a day presented only one band day^{-1}. Multi-marking using Calcein was performed on the same individuals and was not reliable (successful in < 15% of mussels). The microgrowth bands were also only clear enough to be measured repeatedly in 48% of the resin-embedded and sectioned mussel shells. Internal bands widths were correlated to food concentrations, no band was visible with low food concentration. The microgrowth technique revealed variations in growth over short periods of time (week) that macrogrowth could not detect on small spatial scale (edge and middle of a mussel patch). This study indicated that the use of microgrowth technique, due to its temporal scale resolution, allows the estimation of the growth variation due to environmental change and also the monitoring of mussel population dynamics during the mussel bed formation.

5.3 Introduction

Bivalve molluscs, such as mussels (*Mytilus edulis*) are an important source of food and income for humans. As part of their production carrying capacity in a mussel bed, the component of mussel growth need to be clearly understood and optimised in order to maximise flesh yields. Mussel shell and tissue growth vary seasonally in a predictable manner. Flesh growth fluctuates mainly in response to the reproductive cycle (gametogenesis and spawning, Seed, 1969a), however, in a similar way to growth of the shell, tissue growth depends mainly on food supply (Fréchette *et al.*, 1989; Grant, 1996; Larsen and Riisgård, 1997) and varies with environmental factors such as seawater temperature (Almada-Villela *et al.*, 1982; Grant, 1996), population density (Seed and Suchanek, 1992; Bacher *et al.*, 1998; Smaal *et al.*, 2001), water flow (Wildish and Kristmanson, 1988), pollution (Seed, 1968, 1969b), and endogeneous factors such as genotype, age (ontogenetic effects), size, hormonal and innate rhythms (Hawkins and Bayne, 1992). As a result, morphometric relationships between shell length and flesh dry weight or body mass vary seasonally during the year.

Shellfish farmers generally assess mussel *M. edulis*, growth rates using morphometric techniques such as measuring variations in soft tissue weight, in shell dimensions and conditions indices (Jørgensen, 1976; Davenport and Chen, 1987; Smaal and Haas, 1997). Mark-recapture experiments have been used to measure the temporal changes in growth rates over periods of time (e.g. month, years). Due to the scale of measurements (mm, g), daily mussel growth rates calculated during those periods have conventionally been averaged and extrapolated. However, knowledge of short-term changes in mussel growth rates can be important for shellfish farmers in managing their mussel beds, e.g. mussel stocks may have to be thinned out because of changes in density dependent growth, mussel bed might have to be moved to more favourable growing areas. In this chapter the aim was to investigate the use of microgrowth patterns in the mussel shell as a means of reconstructing temporal changes in shell growth and for examining the effect of environmental factors on shell growth rate over short temporal scales.

The study of growth series in bivalve shells (sclerochronology) has traditionally focused on their use in ecology in order to study the age and population dynamics of these molluscs (e.g. Craig and Hallam, 1963; Kennish, 1980; Lutz and Rhoads, 1980; Richardson and Walker, 1991; Richardson *et al.*, 1995; Cerrato, 2000; Richardson, 2001; Schöne *et al.*, 2005a; Peharda *et al.*, 2007) and in paleoecology in which mainly geochemical proxies have been used to reconstruct the environmental and climatic conditions changes pertaining at the time of shell deposition (e.g. Klein *et al.*, 1996; Freitas *et al.*, 2005; Gillikin *et al.*, 2005). More recently, Miyaji *et al.* (2007) examined the effect of seawater temperature on the control of microgrowth line deposition in the shells of the bivalve *Phacosoma japonicum* as a tool for potential paleoenvironmental reconstruction. A few studies (e.g. *Cerastoderma edule,* Richardson *et al.*, 1980; *Arctica islandica,* Schöne *et al.*, 2005b) have related environmental variables to internal microgrowth line deposition and shown that they can be used as a proxy for environmental changes such as seawater temperature and food supply. By contrast, no relationships have been found between external shell surface microgrowth ridges and environmental factors (Bérard *et al.*, 1992). Growth lines/bands are deposited in bivalve shells for different reasons e.g. as a result of seasonal changes in growth rates (Richardson and Walker, 1991; Peterson *et al.*,

1983; Richardson, 2001), as a result of spawning (Kennish, 1980; Gilkinson *et al.*, 1986; in Richardson 2001), damage due to storms (Cunliffe and Kennish, 1974; Kennish, 1980), mussel handling and heat shocks (pers. Observation; Richardson, 1989; Kennish, 1980; Thompson *et al.*, 2000), parasite infestation or predation (Richardson, 2001).

Deciding which environmental perturbation is responsible for a change in growth increment/line width or for the production of a growth disturbance is not possible from an examination of the incremental growth record. Growth marks or changes in increment width are morphological identities which represent events that can be linked to physiological or environmental factors (Richardson, 2001). The use of growth lines/bands and growth marks as indicators of environmental change relies on the fact that their periodicity has first been validated. The periodicity varies depending on the bivalve species (tidally, daily, lunar spring-neap tidal cycle, and annually see review in Richardson 2001).

Intertidal bivalve species such as mussels and cockles produce tidal and daily growth bands in their shell which are formed during tidal or daily emersion and result in spring-neap lunar tidal growth band patterns in their shells (see review in Richardson, 2001). In the intertidal mussel *Mytilus edulis* (L.), shells, narrow growth bands are produced during emersion whilst wider growth increments are formed when the animal is actively feeding during immersion (see Richardson *et al.*, 1981; Richardson, 1989). Richardson (1989) also suggested that mussels held subtidally had an innate rhythm of shell deposition related to the rate of shell growth since mussels can feed and deposit their shell throughout the entire tidal cycle (Seed, 1976; Seed, 1969b; Gosling, 2003). However, this work was not entirely conclusive and more experiments are required to understand growth band formation in mussels growing subtidally since the mechanism of band formation is still not yet clearly understood (see Richardson, 1990; Richardson, 2001). In comparison to other common bivalve species (e.g. cockles, *Cerastoderma edule*, oysters, *Ostrea edulis* or ocean quahogs *Arctica islandica*, pers. obs.) the internal growth bands and lines are relatively difficult to read in mussel *Mytilus edulis* shells (see also Richardson, 1989).

The aim of this chapter was to assess the coupling between the internal microgrowth increments and food concentration in order to develop the use of the microgrowth methodology as a routine tool for growth measurements in mussels and for reconstructing the effects of environmental factors e.g. seawater temperature and food supply on shell growth rather than using traditional morphometric measures for investigating changes in shell growth. In the study mussel shells were initially marked either using emersion and air exposure for several hours (see Richardson, 1989), and through the incorporation of a fluorescent Calcein mark into the shell (see Kaehler and McQuaid, 1999). These disturbance marks were the used as a temporal starting point with which to compare all subsequent microgrowth during a known period of time. Following shell marking mussels were either grown in the laboratory under constant conditions of seawater temperature and light regime and supplied with different food concentrations or grown in the natural environment. In the natural environment, marked, transplanted and subsequently recaptured mussels or natural unmarked mussels were sampled over large and small spatial and temporal scales from a large commercial mussel bed in the Menai Strait. Over a large temporal scale, the food supply advected over the Menai Strait mussel bed is bi-directional as a result of the tide (see Simpson *et al.*, 2007; Saurel *et al.*, 2007). The effects of the diurnal tidal reversal in the Menai Strait on mussel growth were investigated given that differences in current direction are thought to induce differential growth in mussels exposed to either fresh or recycled water from conspecific mussel filtration (Fréchette *et al.*, 1989). Over smaller scales a food gradient has been observed between the edge and middle of mussel patches within a mussel bed, due to intra-specific competition between the mussels for the available food (Dolmer, 2000a, Chapter 6), where mussels grow faster and larger on the edge rather than the middle of the patch (Okamura, 1986; Svane and Ompi, 1993). An analysis of the microgrowth patterns in mussel shells, as a tool for reconstructing the effects of environmental changes (food concentrations) on mussel growth both over the long term (months) or short term (1 tidal cycle) in mussel beds, has not been yet used and is a promising tool for shellfisheries managements and ecology.

5.4 Material and methods

Five experiments were conducted in the laboratory and in the natural environment (see summary in Table 5. 1 and detail of the five experiments in part 5.3.3) in order to clarify the macro and microgrowth techniques of *Mytilus edulis* shell growth by i) determining a suitable shell marking procedure, ii) investigating the periodicity of the microgrowth increments, iii) investigating short-term and long-term shell growth rates and eventually in order to test the hypothesis that mussel shell growth in terms of macrogrowth and micro-incremental growth varies with food concentration.

The hypotheses that were tested for the validity of the microgrowth technique were:

1) that the periodicity of the shell deposition with continuous emersion is twice a day following an innate rhythm while the mussel shell grows continuously (experiment 1)., 2) that emersion and Calcein marking are valid methods to internally mark the mussel shells and thus producing a mark to which all subsequent shell growth could be related (experiments 2 and 3), 3) that the microgrowth technique allows reconstructing the past daily growth of the mussels collected or placed in the field over long period of time (up to the mussel age) (all experiments) and 4) that in the field, spring and neap cycles can be observed in the deposited band, e.g. longer emersion at neap tide induce larger bands, while spring bands are more marked due to the long time to air-exposure (experiment 3).

The hypotheses that were tested to investigate the influence of food on macro and microgrowth were:

1) That growth band periodicity does not vary as a function of food concentration (experiments 1 and 2), 2) that macro- and micro- shell growth vary as a function of food concentration under constant laboratory conditions (experiment 2), 3) that there is a difference in the growth of mussels along a transect across the mussel bed i.e. mussels from Bangor Pier grow more slowly than those at Gallows point due to the flow direction of the water which has a higher food concentration in the main channel coming from the Liverpool Bay (see Chapter 2, experiments from 2004) (experiment 4) and 4) that macro- and micro- shell growth are density dependent due to intra-specific competition for food amongst patches of mussels on the commercial

mussel bed i.e. there is a food gradient between the edge and the middle of the patch which affects shell growth (experiment 5)

Table 5. 1: Summary of the experiments carried out in laboratory or in the field for the microgrowth methodology and the comparison between macro- and microgrowth. Some experiments were used for both parts. NA is non applicable.

	Experimental conditions	Time scale	Microgrowth technique / marking	Influence of food supply on macro and micro-shell growth
	Laboratory Tanks			
1	Constant food Continuous immersion Constant seawater temperature	Daily/ Weekly (3 weeks)	Growth band periodicity Emersion marking	Constant food supply
2	3 food concentrations Constant seawater temperature Daily emersion	Daily/ Weekly (~2 months)	Growth band periodicity Calcein marking	Food supply, different concentrations
	Natural environment – Large spatial scale			
3	Cages (2 stations)	Monthly (1 month)	Calcein marking Growth band periodicity Spring vs neap	Food gradient
4	Transect (13 stations)	1 day	NA	Food gradient
	Natural environment – Small spatial scale			
5	Patch (3 patches)	1 day	NA	Food gradient Density dependence

5.4.1 Macrogrowth measurements: morphometric analysis

Mussels (range 10 – 45 mm) from the five experiments (either sampled in the field or on completion of the laboratory experiment) were frozen as soon as possible after the sampling. Once the mussels were thawed, shell length (L) shell height (H) and shell width (SW) were measured with vernier callipers to the nearest 0.1mm. Flesh dry weight (DW) was weighed to the nearest 0.0001g following drying of the excised tissue at 90°C until constant weight was obtained. Condition Index: CI = DW/SL and increase in shell growth (GI) obtained from measurement of the distance between the internal shell mark and the shell margin: Growth Increment: $GI = SL_t - SL_0$ where SL_t and SL_0 are the shell lengths at the end and beginning of the experiment, were calculated.

5.4.2 Analysis of the internal microgrowth patterns in the shell

Acetate peels: Mussel shells were embedded in metaset resin (Buehler U.K. Ltd.) before cutting the shell along the axis A-B (Figure 5. 1a). The cut surfaces were grounded on progressively finer wet and dry trimite paper, washed and finally polished with diamond paste and thoroughly washed with detergent before etching. Different etching treatments were applied to the shells in order to investigate the best method for revealing the growth bands and growth increments. Two etching reagents were applied to the polished shell sections: 1) Hydrochloric acid solution (HCl 0.01M) for 25 – 30 minutes (Richardson, 1989) and 2) a solution 1:1 of 1% acetic acid and 25% glutaraldehyde (method modified from Schöne *et al.*, 2005a) for 35 to 50 min. depending on the size of the shell section. Once the shell sections were etched, they were rinsed with tap water and left to dry overnight. Acetate-peel replicas were prepared (see Richardson, 1989) and then mounted between a glass slide and cover slip. The slides were viewed under a light microscope and the patterns of growth increments and bands observed at the margin of the shell. The peels were photographed under a light microscope and then digitally imaged and the distance between adjacent growth bands and between the internal mark and the shell margin measured using the AnalySIS© software package. In order to further study the growth increments and growth bands thin (100 μm) shell sections were prepared. The polished surfaces of the resin embedded shell blocks were glued, using rapid setting araldite to the surface of a glass slide, the glue allowed to harden and then excess shell and resin sawn off of the slide to leave a thin (1 mm) shell section. The shell section was gradually ground down until the desired thickness (100 μm) was achieved. The section was polished with diamond paste and viewed in the light microscope

SEM: The shell margin of the shells from polished etched shell sections or thin sections were etched with acetic acid and gluteraldehyde and mounted on aluminium stubs and coated with a thin layer of gold (~250 Å) and the microstructure observed in a Scanning Electron Microscope (SEM Hitachi S520).

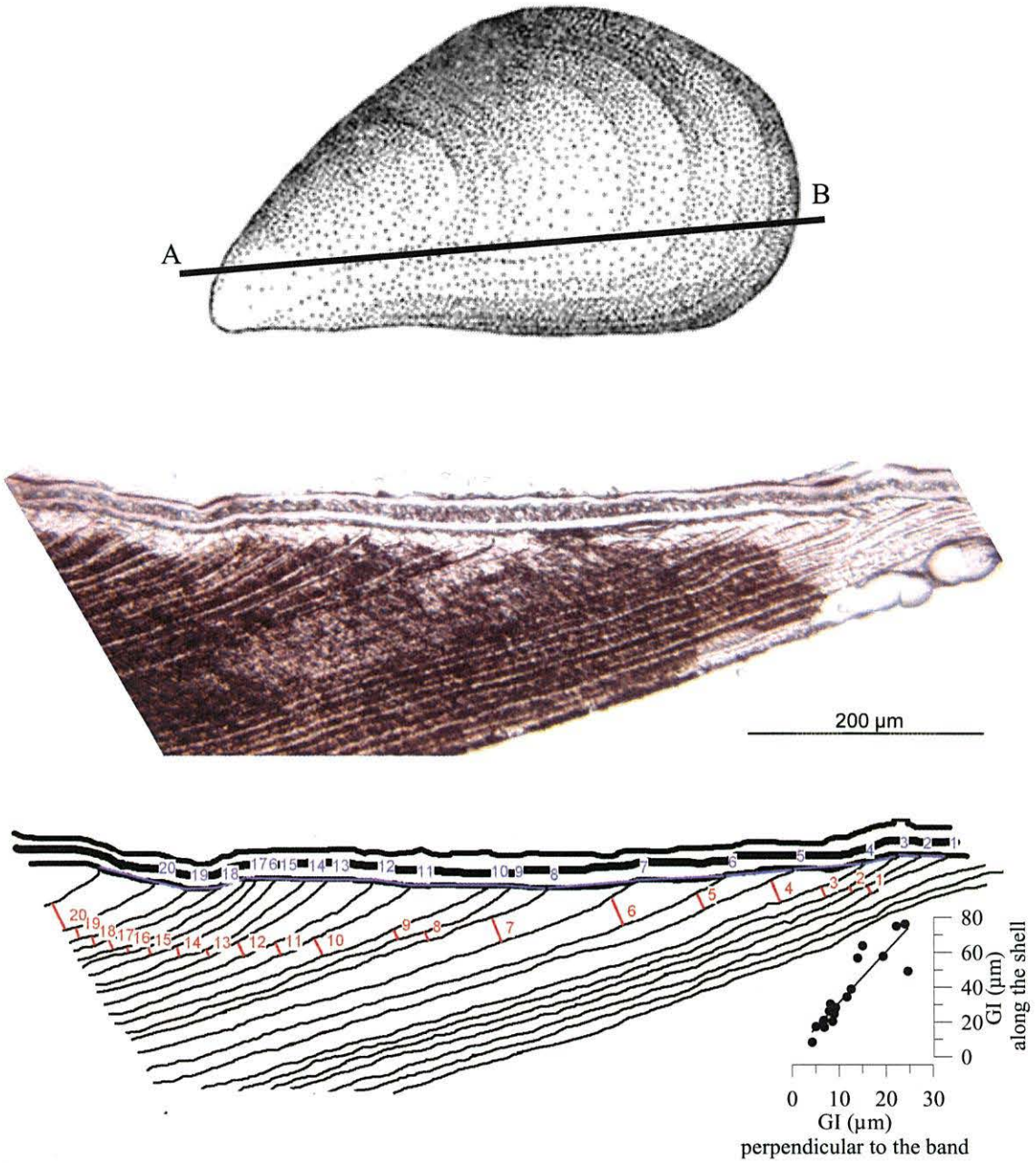


Figure 5. 1: a) Diagrammatic representation of the shell of *Mytilus edulis*. The shell was sectioned along axis A-B. b) Photomicrograph of an acetate peel replicas of a shell section and its diagrammatic drawing representing some of the growth bands present in the peel. Measurement of growth increment were realised perpendicular to the bands (in red) rather than along the edge of the shell (in blue). Relationship between growth increment (GI) measured perpendicular to the bands and along the shell is presented as a graph with fitted linear regression: $y = 2.9061x + 1.9675$ $r^2 = 0.8237$, $p < 0.001$.

<u>*Calcein marking*</u>: Some of the mussels were marked using fluorochrome Calcein (Kaehler and McQuaid, 1999). A solution of filtered seawater with Calcein 125 mg L^{-1} (CAS 1461-15-0) was introduced into the mussel cavity via a syringe on the ventral portion of the mussel at the same level as the byssal gape. Once the mussel

cavity was flooded with the Calcein, mussels were left out of the water for a period of 6 h and then immersed back into a seawater tank. The fluorescent dye Calcein is incorporated into the mineralising shell and forms a thin growth line that could be observed in thin polished sections of the mussel shell viewed microscopically under Ultra Violet light. The position of the fluorescent line was photographed and compared with photographs of the acetate peel in order to identify the position of the Calcein line in the peel and the beginning of the growth experiment.

Band counting and measuring: Each growth increment was measured from the acetate peel replicas of the shell section perpendicularly to each adjacent band to ensure that measurements were standard for all the shells (see Figure 5. 1b)

5.4.3 Experimental design

Laboratory experiments

- Continuous immersion – one food regime (experiment 1)

Mussels were collected in December 2003, from a naturally occurring mussel bed comprising settled spat (1 cm < size < 2 cm; age < one year) from Cable Bay, a site on the south west coast of Anglesey, northwest Wales. Mussels were acclimated to a constant seawater temperature of 20°C in the laboratory over 6 months and kept in individual plastic mesh cages in a tank (~650 L), under constant light.

Mussels were fed daily with a mixture of 3 algae: *Pavlova lutheri*, *Rhinomonas reticulata* and *Tetraselmis chui* daily at ~10:00 from stock cultures, split into equal volumes. Mussels were fed daily with the same mix of algae described above, from containers with a drip-tap, throughout the day at rates of ~330 mL h^{-1}. Algal mixing in the tank was ensured via a large pump (2100 L h^{-1}). Experiment duration was between 17th May and 10th June. Mussels were removed from the water three times at weekly intervals (17th May, 24th May and 31st May) during the experiment and air exposed for a duration of 5 to 6 hours in order to create a disturbance mark within the shell. At the end of the experiment mussels were frozen, the shell removed and prepared for microgrowth analysis, using glutaraldehyde and acetic acid etching and SEM examination.

- Discontinuous immersion – three food regimes (experiment 2)

Mussels *Mytilus edulis* were collected from the intertidal commercial mussel bed in the Menai Strait (March 2006 and 30 mussels ranging in shell length from 30 to 45 mm) were placed in each of three ~650 L tanks under constant illumination, constant seawater temperature (15°C), constant flow and microalgal food. The mean water flow was low (<10 L h^{-1}), slow enough so that the microalgal food was not flushed out of the tank before it was consumed by the mussels and the aerated algal food supply distributed to each tank from a stock container using a peristaltic pump (500 mL h^{-1} flow) in each tank. Algal mixing in the tank was ensured via a small pump (800 L h^{-1}). The food concentration varied between the 3 tanks, from full concentration, 0.2 and 0.1 of the original concentration of a mix of three cultured algae: *P. lutheri*, *R. reticulata* and *T. chui* collected every morning from stock cultures, split into equal volumes.

The three tanks were labelled as high, medium and low food concentration (TH, TM, and TL). The concentration of algae in each tank was monitored with a Scufa *in vivo* fluorometer (Turner instrument) rotating each day from 1 tank to the other tanks, and 50 mL water samples collected from the middle of the tank to calibrate the Scufa. Water samples were filtered through Whatman® GF/F filters. The chlorophyll *a* (chl *a*) from the filters was extracted for 18 h at 4°C with 90% acetone and the concentration was measured on a Turner Design 10-AU fluorometer (method adapted from Parsons *et al.*, 1984). All the mussels were left in their respective tanks for 13 days to acclimate to the new feeding conditions before the experiment commenced.

The experiment was separated in two phases of 28 and 24 days duration. During the first phase (7th April to 4th May 2006), each group of mussels supplied with H, M, L concentrations of food were emersed daily for 2 hours (between 10:00 & 12:00) by lifting the cages containing the mussels out of the water, the food supply was stopped during this period and the mussels were then lowered back into the tank. During this first phase, individual mussels were marked 4 times with Calcein (7th April, 14th April, 21st April and 28th April 2006), and left out of the water for at a least 6 h period to allow the mussel to incorporate the Calcein into the shell. In the second phase mussels were marked only once with Calcein (5th May 2006) and the mussels killed 24 days later on the 29th May 2006. The same procedure was adapted as in the first phase, except that daily, mussel groups H and L were swapped between the two

tanks (tank H and tank L), while mussel group M stayed in tank M: i.e. day 1: H in tank H, M in tank M, L in tank L, day 2: H in tank L, M in tank M, L in tank H, day 3: H in tank H, M in tank M, L in tank L, etc.. (see Table 5. 2).

Table 5. 2: Sampling design for the laboratory experiment 2, during the acclimation period (13 days), Phase 1 (28 days) and phase 2 (24 days). Three groups of mussels: High (H), medium (M) and Low (L) were placed in tanks (T) with different food concentration TH, TM and TH.

Tank T	Acclimation	Phase 1	Phase 2 Day 1	Day 2	Day 3	Day 4	D etc…
TH	H	H	H	L	H	L	…
TM	M	M	M	M	M	M	…
TL	L	L	L	H	L	H	…

The purpose of this experiment was to expose the mussels to alternating daily periods of high food and low food concentrations. At the end of the experiment, mussels were frozen, and the shell length and dry flesh tissue weight measured. Shells were prepared for microgrowth increment analysis as before using glutaraldehyde and acetic acid etching and SEM observations performed to study the growth patterns.

Large spatial scale experiments (mussel bed size > 1 km)

The effect of the spring vs neap lunar cycle and different food regimes on microgrowth increment width (experiment 3) .Three replicate cages, each containing 80 mussels were positioned at five different stations (i.e. 15 cages) along an east-west transect between Gallows point and Bangor Pier in the Menai Strait between May 2005 and September 2005 (Figure 5. 2). At the start of the experiment, the shell length of the mussels (mean: 30.8 ± 2.33 mm) was not significantly different amongst the mussels from the 15 cages (ANOVA: $F_{14,1199} = 1.29$ $p = 0.203$). Before the first cage deployment, a number from 1 to 80 was engraved with a hand drill through the periostracum onto the surface of each shell, hence, each month, measurement of an individuals shell length and incremental shell growth could be determined when the cages where retrieved. Furthermore, each month, all the shells were marked via air-exposure for 6 hours and those shells numbered 41 to 80 were also Calcein marked.

Unfortunately due to accidental dredging by fishermen, nine out of the fifteen cages were lost, so that the cage design was subsequently modified and adapted to

the circumstances; hence, the six remaining cages were re-located within the stations; three cages were placed directly at Bangor Pier and three at Gallows Point. With the marking of the shells, re-location was not an issue in the design, as the mussel's growth each month could be recognized within the shell section, although mussel shell length in the cages was significantly different after a few months due to the different locations of the six remaining cages. Shell length was used as a covariate in the analysis of the data. Therefore, mussel growth was studied only at two stations within the Menai Strait: Bangor Pier southwest part (cages 1, 2, 3) and Gallows Point northwest part (cages 7, 8, 9) and on the mussel growth between August and September 2005, the last month of the experiment. Acetate peels were produced after shell sectioning, polishing and glutaraldehyde and acetic acid etching and HCl etching.

- Snapshot sampling along a transect (experiment 4)

Snap-shot sampling of 30 mussels collected from the main commercially laid mussel beds at the original 13 stations (150 to 250 m distance between each station) along the Menai Strait channel was undertaken during mean low water of spring tides (MLWS) on the 25th April 2006, within a 30 min time interval (Figure 5. 2). Mussel shell length, biomass, condition index (CI) and the width of the last 14 growth increments representing the last seven days of growth were measured.

Small spatial scale experiments (patch size) in the natural environment (Experiment 5)

In July 2006, samples of mussels were collected, using 15 cm diameter cores pushed into the mussels beds, from existing mussel patches at the edge and middle of the mussel patches in from the middle, front and rear edges of a new mussel bed re-laid in the intertidal area on month prior to the experiment (mean shell length: 28.4 – 34.7 cm) (Figure 5. 2). Mussel density, shell length, biomass, CI and shell microgrowth of the last seven days (14 last bands of growth increment) were measured.

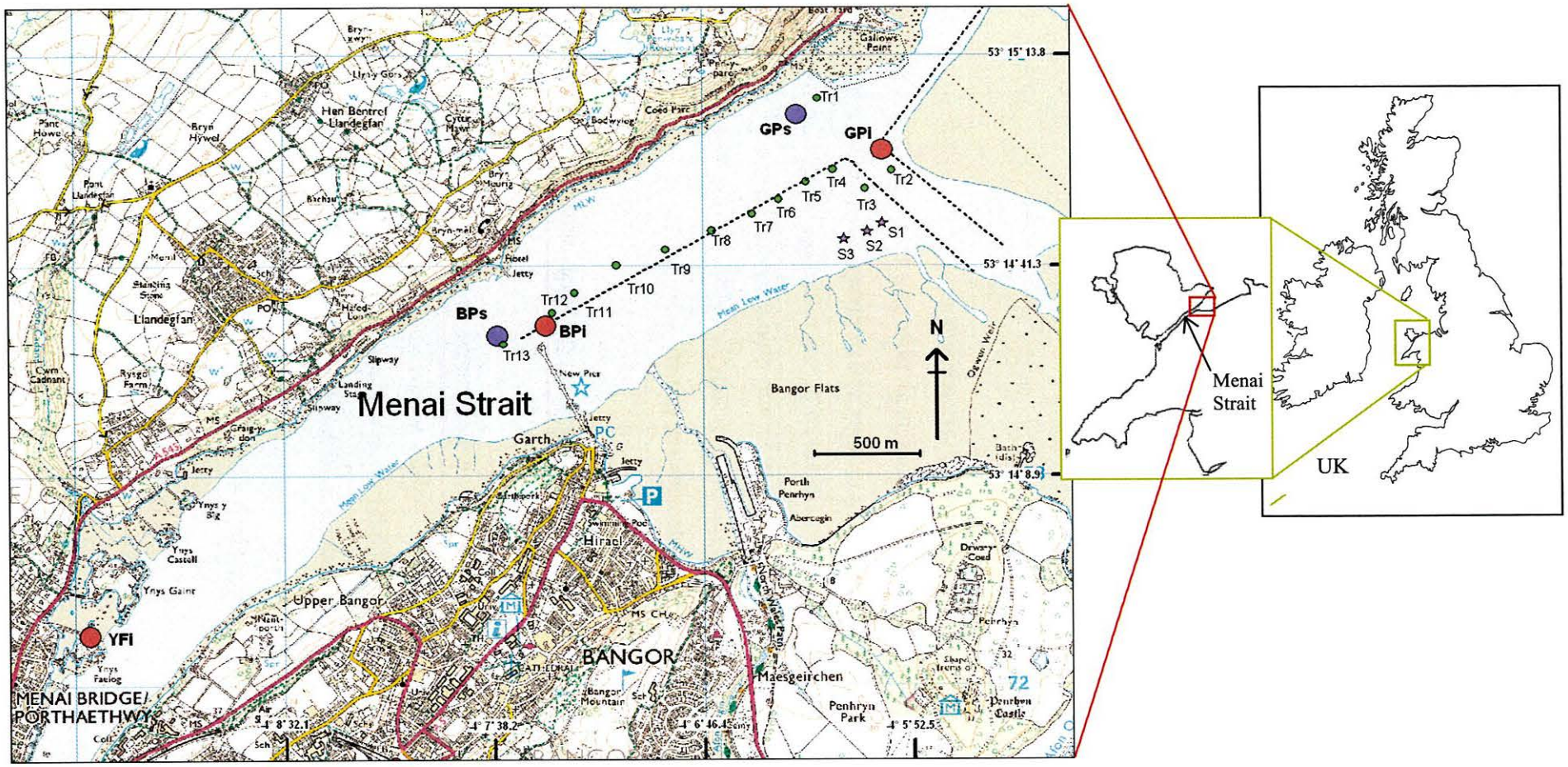


Figure 5. 2: Map of the Menai Strait with the 13 stations marked along a transect (tr1 to tr13, green circles) from which mussel were collected simultaneously from MLWS on 25/04/06. Five stations (blue circles subtidal, red circles intertidal) in the Menai Strait where 3 replicate cages containing 80 mussels half of which half were Calcein marked were deployed between May and September 2005 (adapted from © Crown copyright 2007). One day patch sampling (purple stars S1, S2, S3) BP = Bangor Pier, GP = Gallows Point, YF = Ynys Faelog, i = intertidal, s = subtidal. The dotted line represents mean low water of spring tides

5.4.4 Data analysis

Prior to analysis, the data were checked to assess if they met the assumptions of normal distribution and homogeneity of variance in order to apply analysis of variance (ANOVA). Appropriate data transformations were applied to adjust the data such that they met these assumptions. If the data did not meet the assumptions of normality and homogeneity of variance, either a Kruskall-Wallis or Mood's Median test was used to analyse the data.

5.5 Results

5.5.1 Microgrowth patterns and marking

Comparison of the techniques used to examine micro-growth patterns

Figure 5. 3a shows the appearance of an acetate peel replica from a shell section etched with HCl, while in Figure 5. 3b the shell has been etched using 1:1 1% acetic acid and 25% glutaraldehyde (modified Mutvei's solution). The latter technique gave better visualisation of the bands which were more easily counted than those etched using the conventional HCl solution; the modified Mutvei's solution was the etching agent of choice. Figure 5. 3c shows a scanning electron micrograph of a shell section treated with the Mutvei's solution without alcian blue; the arrow indicates an area of the shell where a supposedly organic layer was preserved by the glutaraldehyde making the bands unreadable. This phenomenon can also be seen in the scanning electron micrograph (Figure 5. 4a), where there is a zone in the shell covered by a visible, possibly organic, layer and in Figure 5. 4b, on the acetate peel replica of the same area of shell, the part covered by the organic layer was not replicated in the peel and hence no bands were readable.

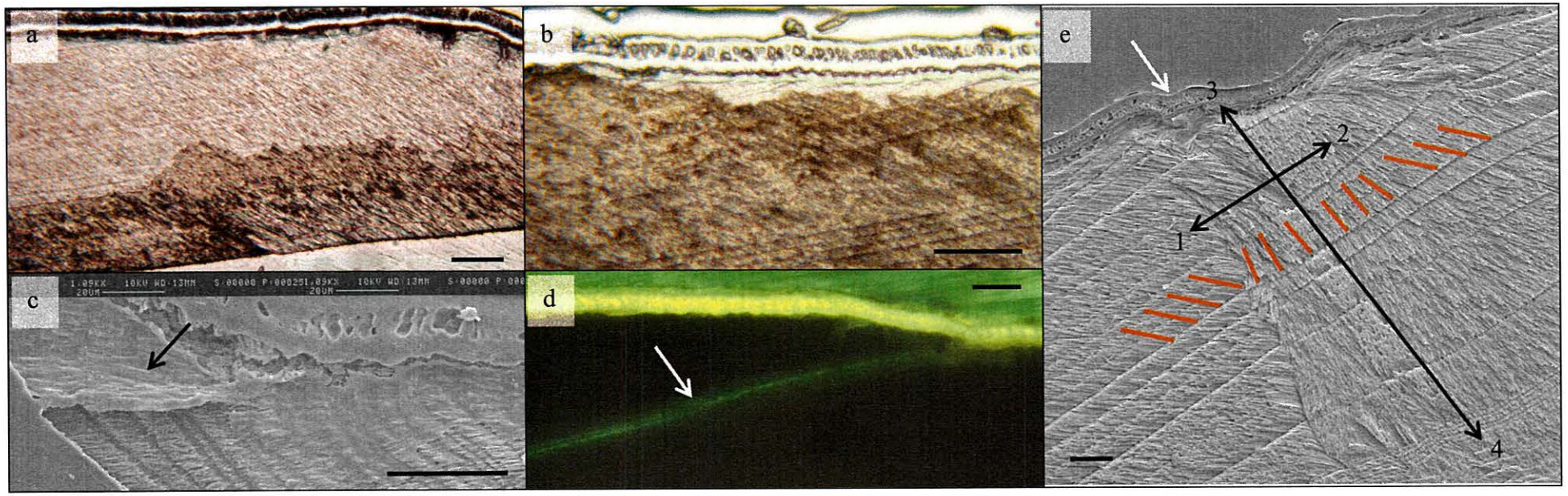


Figure 5. 3: Photomicrographs of *Mytilus edulis* (a, b and d), SEM photographs (c and e). a) Acetate peel replica of a polished and etched section using HCL 0.01M of a mussel shell from Gallows point. b) Acetate peel replica of a polished and etched section using 1:1 of 1% acetic acid and 25% glutaraldehyde of a mussel shell from Gallows point. c) Polished radial shell section treated with gluteraldehyde and acetic acid solution (J06_M4), the arrow indicates a layer obscuring the growth bands. d) Thin section of a mussel shell under a UV light, the arrow indicates the growth band marked by the calcein. e) Polished radial shell section treated with gluteraldehyde and acetic acid solution, the arrow shows the disturbance mark created in the structure of the shell with reorganization of the crystals, direction indicated by orange bars. Section 1-2 represents the reorganization of the crystals in the shell growth direction from umbo to tip of the shell while section 3-4 represents the reorganization of the crystals still visible in the accumulated layers on the thickness of the shell. Scale bar represents 50 µm.

Figure 5. 3d, clearly shows the appearance of the Calcein mark in the shell section under UV light. Marking using Calcein was highly successful and allowed an emersion band to be marked and its position then viewed in the acetate peel replica (e.g. Figure 5. 5a & b). In this example, the air marking associated with the calcein marking did not produce a clear visible disturbance in the shell section. Air (emersion) marking or traumatic conditions such as a temperature shock or transport from the field to the laboratory also created a disturbance mark which was visible even without the Calcein marking. For instance, Figure 5. 3e shows a disturbance in the calcite crystals created by emersion shock. Once the shock occurs in the shell, the arrangement of the crystals is modified for a short period of time around the growth band (section 1 – 2 in the Figure 5. 3e) in the direction of the umbo to the tip of the shell, but as the new layers of shell are created, they follow the new direction of the disturbed crystal for a longer period of time (section 3 – 4 in Figure 5. 3e) in the thickness of the shell direction. Figure 5. 6a shows a scanning electron micrograph of a section of a mussel shell that had been etched with glutaraldehyde and acetic acid. The mussel had been removed three times from the water and left in air for marking ~5 h. Three disturbance marks are clearly visible without the Calcein marking.

Band periodicity in mussels reared in the laboratory and natural environment

Mussels growing under continuous immersion in the laboratory (experiment 1) and mussels placed in cages at the position of lowest spring low tide deposited two growth bands day^{-1}, whereas mussels kept continuously immersed in the laboratory (experiment 2), but emersed only once a day (2 h each day), displayed only 1 band day^{-1}. In experiment 1 under constant food concentration and 20°C the measured increment width between two bands for period 1 was 18.73 ± 7.02 μm and for period 2: 16.56 ± 8.65 μm.

Figure 5. 6a shows an example of a shell section from a mussel immersed continuously for 3 weeks with weekly emersion of 6 h under constant light, temperature and food concentration conditions; three disturbance marks created during air exposure of the mussel are visible on the scanning electron microscope (SEM) photograph. Figures 5. 6b & c show an SEM micrograph where the bands can be counted between each air exposure mark, and Figure 5. 6d is the cumulative width

of the increments for the 2 time periods. Between marks 1 and 2, only 11 bands were visible, whereas between marks 2 and 3, 12 bands were counted. For those two periods of time under similar conditions, the growth was similar with a total of ~200 µm in 6 days. These results indicate that without air exposure disturbance, and with constant food delivery, the mussels were producing 2 bands per day due to an endogeneous rhythm. The irregularities in the band width are potentially due to a variation in the daily quality of the microalgal food supplied to the mussels.

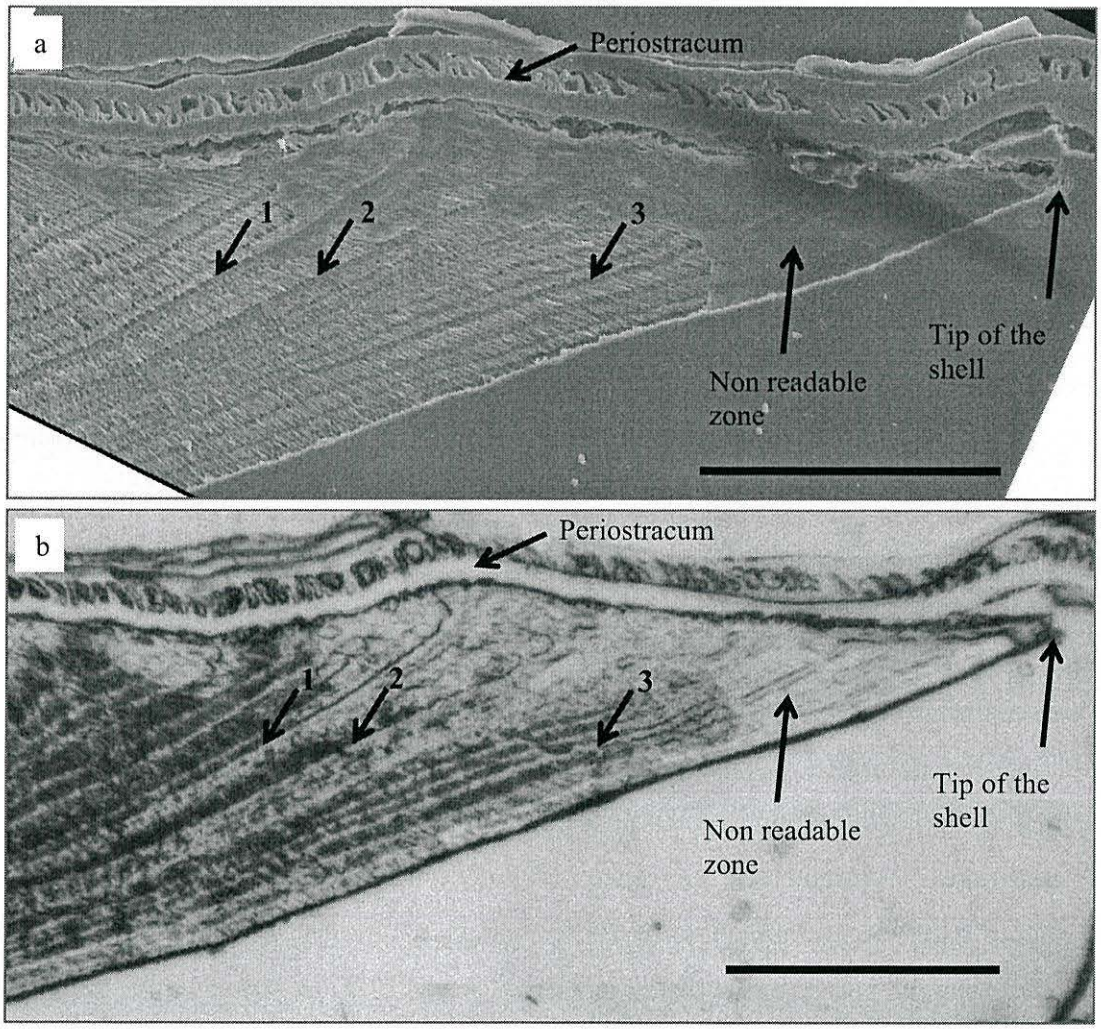


Figure 5. 4: Comparison of two photomicrographs of a section from the same mussel shell: a) a scanning electron micrograph of a shell section etched with gluteraldehyde and acetic acid solution and b) a photomicrograph of the acetate peel of the same shell. An example of 3 bands is indicated by numbers 1 to 3.

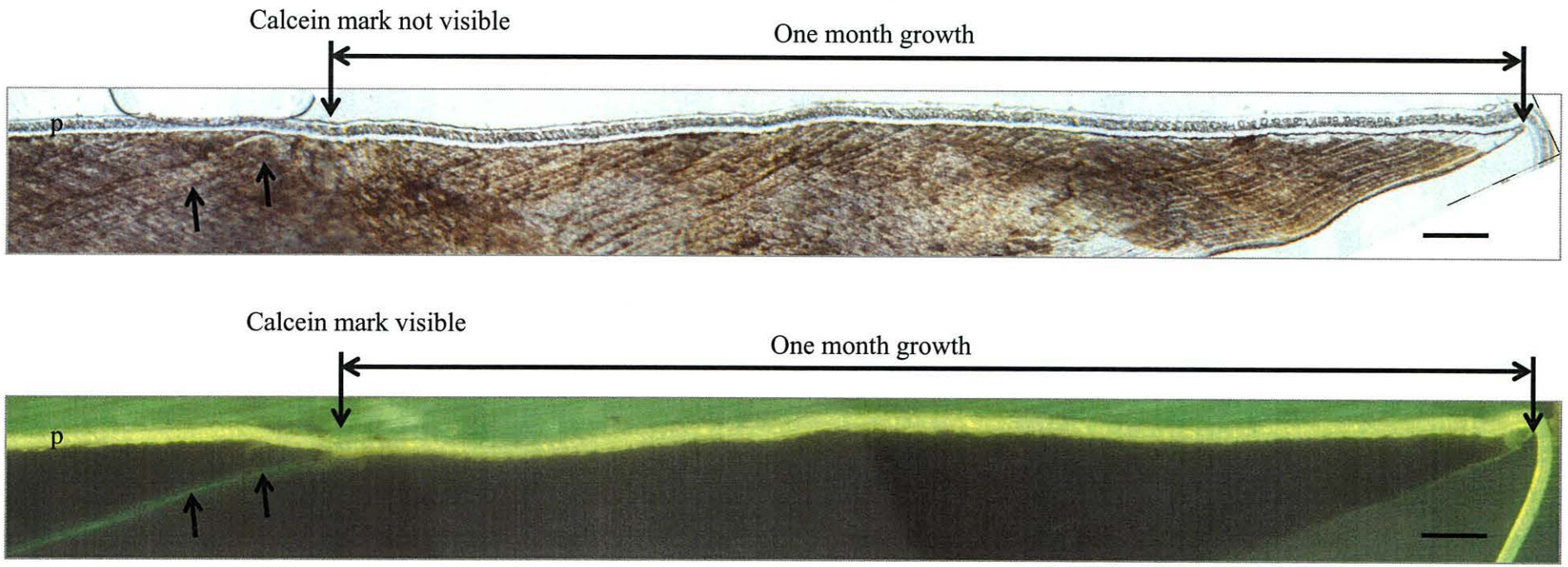


Figure 5. 5: a) Photomicrograph of an acetate peel from the shell of a mussel (GP9_61) marked with Calcein and allowed to grow for 1 month in the Menai Strait. The position of the Calcein mark is difficult to see and b) photomicrograph of a thin section of same shell, observed in UV light, the Calcein band (arrows) is visible. p is periostracum.

The mussels placed in cages deployed at Bangor Pier and Gallows Point during August to September 2005 were located at the same height on the shore and were emersed during spring low tides. Approximately 60 bands were counted during the 1 month time interval corresponding to 2 tidal bands day^{-1} (Figure 5. 7a). Figure 5. 7a show the individual mean increment widths in the shells of mussels sampled from Gallows Point and Bangor Pier with the semi-diurnal tidal cycle superimposed. The position of the cages above chart datum is also indicated on the graph. There is no apparent correlation between increment width and immersion amplitude, or with the period of emersion. By contrast, there was a significant correlation between the increment widths measured in the mussels from the two stations indicating that mussel growth is synchronised with the environmental factors present at the two stations ($r = 0.547$, $p < 0.001$, Figure 5. 7b). Individual increment widths showed less variation closer to the shell margin than near the position of the Calcein mark (Figure 5. 7c).

In the laboratory (experiment 2), mussels were grown under constant food concentration, temperature and light and were emersed daily for 2 hours out of the tank produced a growth band each time they were emersed. Over phase 2 of the experiment, in which mussels were moved daily from a tank with high food concentration to a tank with low food concentration, only 1 mark was observed day^{-1}. These results show that the emersion bands caused by air exposure were more distinct in the mussel shells given daily emersion and appeared to override the innate banding produced during the constant immersed laboratory conditions.

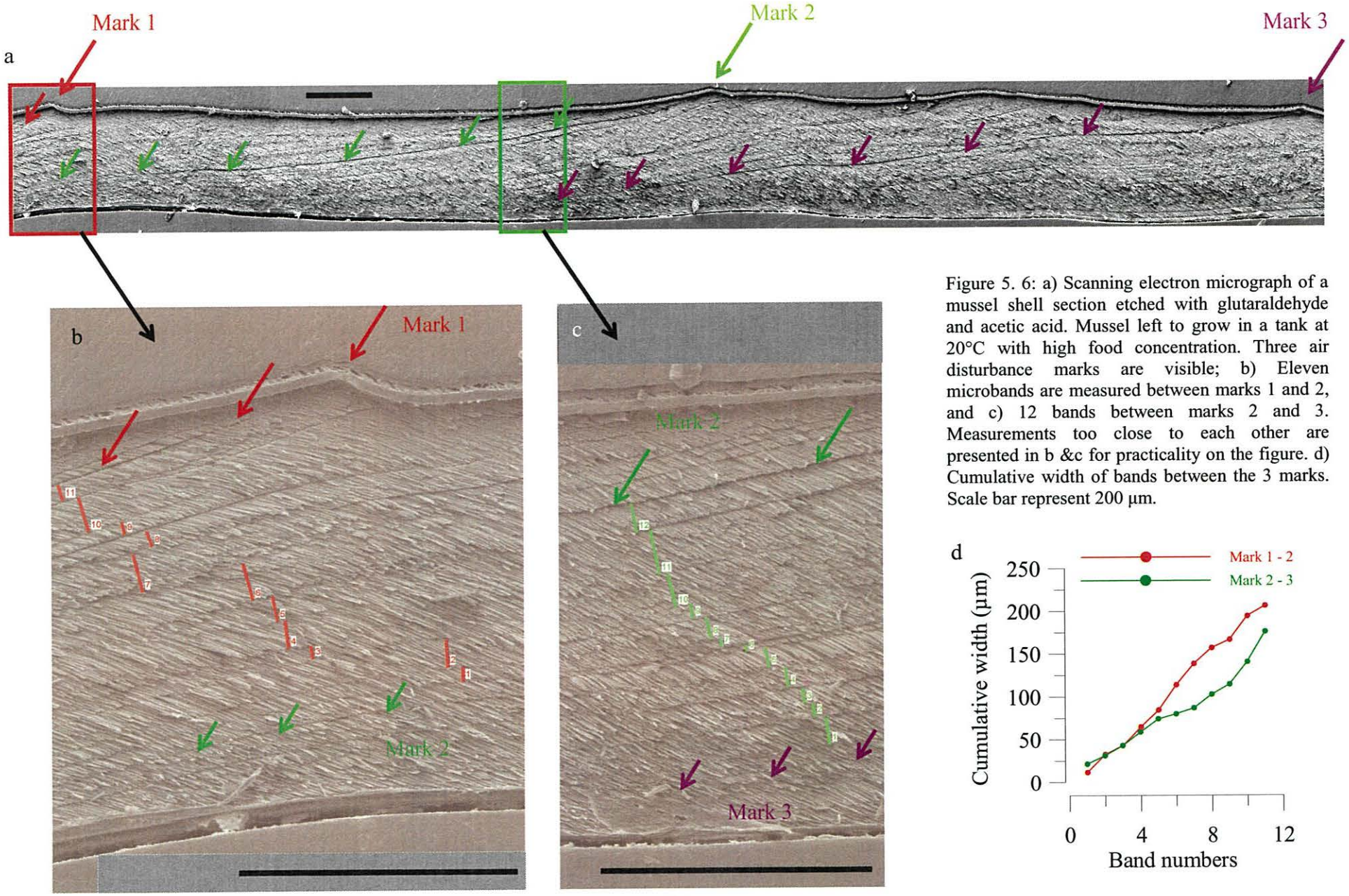


Figure 5. 6: a) Scanning electron micrograph of a mussel shell section etched with glutaraldehyde and acetic acid. Mussel left to grow in a tank at 20°C with high food concentration. Three air disturbance marks are visible; b) Eleven microbands are measured between marks 1 and 2, and c) 12 bands between marks 2 and 3. Measurements too close to each other are presented in b &c for practicality on the figure. d) Cumulative width of bands between the 3 marks. Scale bar represent 200 µm.

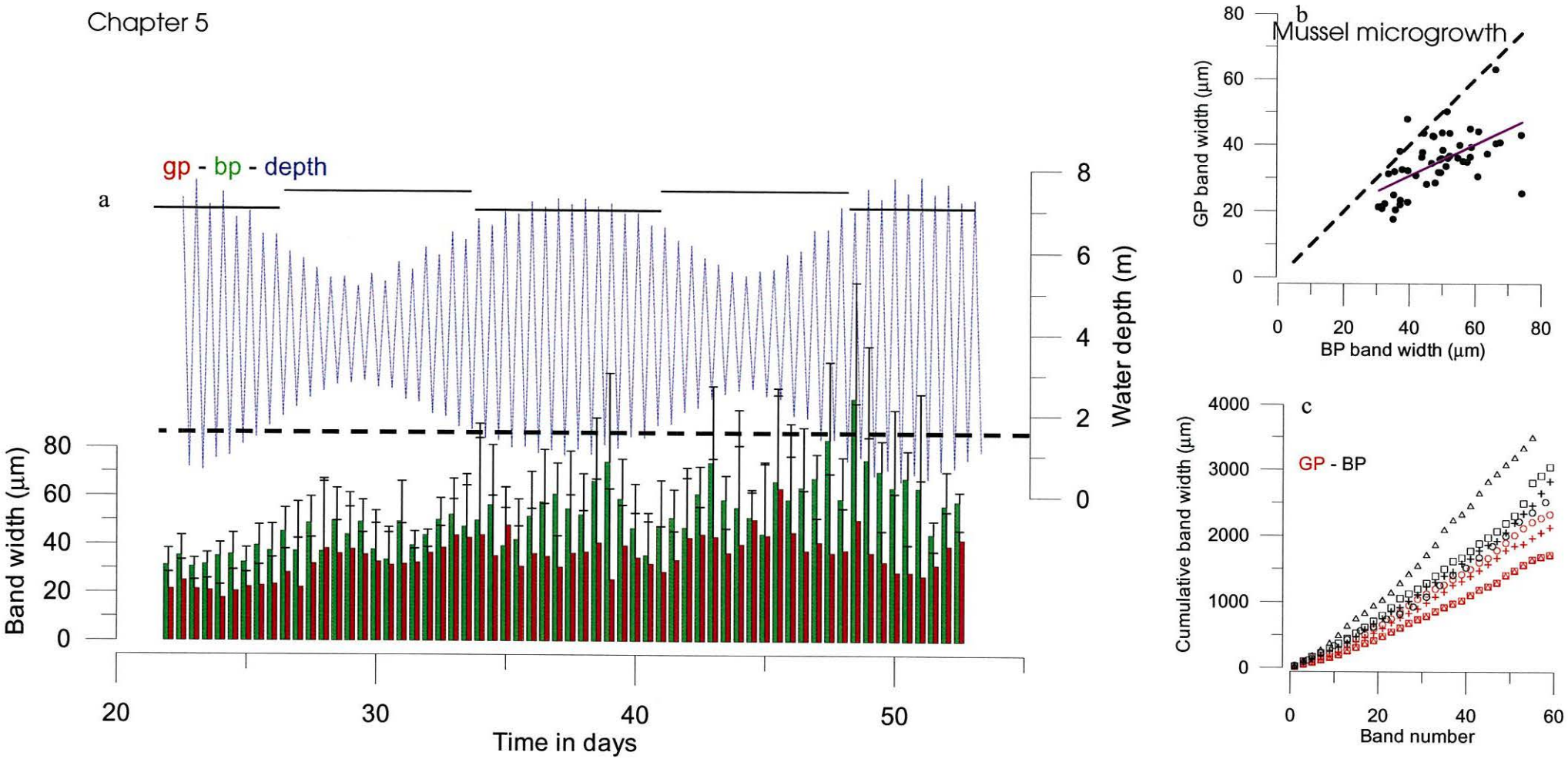


Figure 5. 7: a) Comparison of the temporal variation in growth increment width (+ standard deviation) in mussels from Gallows Point (GP) and Bangor Pier (BP) stations, ~2 bands measured daily, with the tidal amplitude for Menai Bridge during the 1 month period. The depth for which the mussels are air exposed is ~1.5m marked on the graph by discontinuous line. b) Relationship between the band width of the 2 stations with linear regression (GP = 11.8 + 0.477 x BP; $r^2 = 0.33$, residuals normally distributed), dotted line represent linear regression for GP = BP. c) Cumulative Microgrowth increment of mussel shells at GP (red symbols) and BP (black symbols).

Limitations of the Calcein marking and microgrowth band methodologies

The success in marking the shells with the fluorescent Calcein marker was variable. The technique involved intrusive manipulation that could have seriously damaged the shell margin and mantle epithelium if the needle had not been inserted at the right position. Nevertheless, marked mussels from the cage experiment from both stations showed no significant difference in shell macrogrowth whether they were marked with calcein or left unmarked indicating that the Calcein marking over a period of one month did not disturb shell deposition: Bangor Pier (replicas 1-3) 2 samples *t*-test: T = -0.21; p = 0.837; df = 27; GP (replicates 7-9) 2 samples *t*-test: T = 2.03; p = 0.054; df = 24). The location of the Calcein mark in thin mussel shell sections under UV light was a time consuming activity which only allowed a small number of shells to be inspected. Of those shell sections examined (N = 52) under UV light only 6% of the shells showed exact correspondence between the number of calcein marks and the number of Calcein injections administered to the mussels (maximum injections between 4 and 8). Fifteen percent of shells showed a discrepancy of one Calcein mark between the observed and predicted number of marks and 63% of shells displayed < 50% of the number of injections. Therefore overall these data suggest that the method is unreliable when more than 1 injection are administered to the mussels

Difficulties were encountered in reading the microgrowth increments in the acetate peels of some of the mussel shells due to poor preservation of the growth bands in some of the acetate peel replicas. From the observations of 275 peels of mussel sections, a large number were discarded due to poor reading: 48% of the slides were readable, 35% were potentially readable but not very good and 17% were discarded as unreadable.

5.5.2 Comparison of macro and microgrowth in response to variations in food concentration

Macro- and microgrowth varied according to variations in the food concentrations provided to the mussels in the experiments or supplied to the mussels in the natural environment and the results from two laboratory experiments and three field experiments are summarized in Table 5. 3 and detailed in the following section. In all

these experiments where there was a difference in mussel macrogrowth as a result of differences in food (chl *a*) concentration there was also a difference in the shell microgrowth of the same mussel (see Table 5. 3). By contrast, in the intertidal experiment (experiment 5), measurements of macrogrowth integrated too long periods of growth to detect small changes in shell growth which the microgrowth methodology detected (Table 5. 3, see paragraph in section 5.4.2.3).

Table 5. 3: Summary of the five experiments in which measurements of macrogrowth and microgrowth were conducted over different scales and under different food concentrations. Shell length (SL mm), shell length microgrowth bands (SL_b), growth increments (GI, mm or µm), flesh dry weight (DW mg), condition index (CI). High (H) or low (L) food concentrations, Bangor Pier (BP), Gallows Point (GP), stations along a large transect (1km long) from 1 to 13 (Tr1 to Tr13), edge (e) and the middle (m) of the patch. * indicates statistically tested. For the snapshot experiments (4 and 5) GI, DW and CI are not measurable using macrogrowth as the mussels were not marked and then recaptured. NA: Not applicable. N is the number of shells used for the microgrowth analysis

Experiment	Factor	Macrogrowth	Microgrowth	$Sample_N$	Mean SL (mm) Mean increment width (GI in µm)
1- Laboratory 05/04 *(Fig. 5 . 6)*	H food constant 20°C	NA	**2 bands day^{-1}**	X_1	SL 29.32 **GI: 17.60 ± 7.82**
2- Laboratory 03-06/06 *(Tables 5 . 4,5; Fig. 5 . 8,9,10)*	H food vs L food 15°C	SL: H = L* GI: H > L* DW: H > L* CI: H > L*	GI: H > L* **No visible band if no food.** **1 band day^{-1}**	H_3 Phase 2 L_3 Phase 2	SL: 42.67 ± 2.58 **GI: 4.89 ± 2.62** SL: 34.35 ± 2.80 **GI: NA**
3- Large scale 08/05 – 09/05 *(Tables 5.- 6,7; Fig. 5 . 11)*	Food gradient, 2 sites	SL: GP > BP* GI: BP > GP* DW: BP = GP* CI: BP = GP*	SL_b: GP > BP* GI: BP > GP* **2 bands day^{-1}**	GP_4 BP_4	SL: 45.5 ± 2.0 **GI: 25.15 ± 6.16** SL: 44.1 ± 2.1 **GI: 37.60 ± 6.20**
4- Large scale 25/04/06 *(Table 5. 7 ; Fig. 5 . 12,13)*	Food gradient, 13 sites	SL: all equal*	SL_b: No ≠* GI: No ≠**	Tr 2_4 Tr 10_5	SL: 48.9 ± 4.8 **GI: 11.02 ± 2.66** SL: 50.4 ± 8.0 **GI: 10.87 ± 2.09**
5- Small scale 17/06/06 *(Tables 5.. 8,9; Fig. 5 . 14)*	Edge vs middle	SL: e > m* in two patches **Density dependent**	SL_b: e > m * in all patches **Not only density dependent**	e_9 m_9	SL: 33.64 ± 3.53 **GI: 9.82 ± 1.92** SL: 30.86 ± 3.90 **GI: 6.59 ± 0.98**

Laboratory experiment 2: constant food concentration

In the laboratory, mussel morphometric growth was coupled to food concentration (chl *a*). The high and low food concentrations in the tanks were significantly different during the 3 phases of the experiment i.e. the acclimation, phase 1 (constant food) and phase 2 (alternation H and L food concentrations) (Table 5. 4). No significant differences were found between the Medium and Low food concentrations, therefore the data from the Medium tank were not used.

Table 5. 4: Summary of chlorophyll *a* (chl *a*) concentrations (μg L^{-1}) in the middle of the tanks during the three parts of the experiment (a = acclimation, part 1 and part 2) in the tanks supplied with High and Low concentrations of microalgae. Mean, median and standard deviation of chlorophyll *a* concentration and *t*-test are indicated. * indicates significant *p* values < 0.001.

Chl *a* (μg L^{-1})	High a	Low a	High 1	Low 1	High 2	Low 2
Mean	11.89	4.63	6.03	2.55	4.43	1.83
Median	10.30	4.60	6.04	2.48	4.47	1.83
SD	3.80	1.02	1.04	0.37	0.21	0.28
t-test	$T_6 = 5.83$*		$T_{14} = 13.44$*		$T_{16} = 23.31$*	

No significant difference in initial mussel size (between 30 and 45 mm) was found between the mussels placed in the three experimental tanks. By contrast, upon completion of the experiments there were significant differences in shell growth increment width, flesh dry weight and condition index between the mussels fed the three different food regimes (Table 5. 5a). Mussels grown in the highest food concentration had significantly wider increments, dry flesh weight and CI's than those reared in the low food regime (Table 5. 5). However only a significant difference in the GI was found between the mussels grown in the Medium and Low food concentrations (Table 5. 5b). In view of these findings only the shells of mussels grown in High and Low food concentrations were further investigated using microgrowth analysis.

The effect of food concentration was visible at the microgrowth scale. Photomicrographs of thin sections of mussel shells periodically marked with Calcein throughout the experiment and observed under UV light revealed clear fluorescent

lines which were widely spaced in the mussels from the high food concentrations, while the Calcein lines in the shells of mussels grown under low food concentrations were closely spaced to each other (Figure 5. 8a & b). During phases 1 and 2, the mussels were emersed daily for 2 hours in order to create an emersion disturbance mark. Daily emersion bands were observed between the Calcein bands for the mussels under the high food regime (Figure 5. 8a), but not easily in the shells of mussels grown under low food concentrations (Figure 5. 8b) as the growth increments were too close together. It can be seen that in mussels grown under a high food regime (Figure 5. 9a), the number of growth increments deposited in the shells corresponded to the number of days of the experiment. Whereas mussels grown in the low food regime did not grow sufficiently enough each day for the increments to be clearly discernible (Figure 5. 9b).

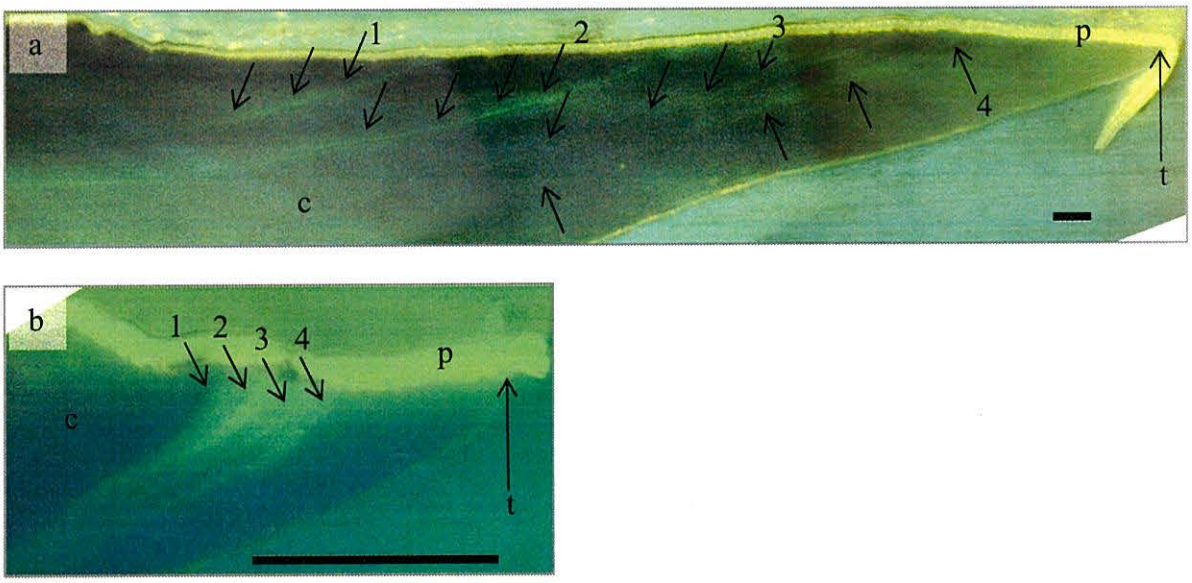


Figure 5. 8: Photomicrograph of a thin section of a shell viewed under UV light a) from the tank with high food concentration b) from the tank with low food concentration. The four Calcein bands are visible (arrows). The increments are well spaced in the mussel provided with a high food concentration whereas they are close and less visible in the mussel provided with a low food concentration. P: periostracum, t: tip of shell and c: calcite matrix.

During phase 2 increment width measured from the SEM pictures (shell H6) showed a strong correlation with food concentration (Figure 5. 9a; Pearson $r = 0.779$, $p < 0.001$ in those mussels grown under a high food regime. By contrast the growth bands were more difficult to distinguish in the acetate peel replicas, and there was no

correlation with food concentrations in shell numbers H5: Pearson r = 0.396, p = 0.076 and H8 Pearson r = 0.244, p = 0.274.

Table 5. 5: Summary of the analysis of the morphometric parameters: shell length (sl mm), flesh dry weight (dw g), condition index (ci) and growth increment width (gi mm) for three groups of mussels fed at three different food regimes: high (H), medium (M) and low (L) concentrations under laboratory conditions between the 07/04 and 29/05/2006

a) Means, median, and standard deviation (SD) of morphometric parameters.

	N	Mean	Median	SD		N	Mean	Median	SD
sl	91	38.5	38.2	3.6	dw	91	0.20	0.17	0.10
gi	89	1.54	1.39	0.70	ci	91	3.28	3.00	1.02
sl H	29	39.1	38.4	3.6	dw H	29	0.26	0.24	0.10
sl M	32	38.8	38.0	3.6	dw M	32	0.17	0.15	0.09
sl L	30	38.0	37.7	3.6	dw L	30	0.16	0.14	0.08
gi H	29	2.32	2.28	0.48	ci H	29	4.3	4.31	0.81
gi M	29	1.33	1.32	0.39	ci M	32	2.90	2.60	0.73
gi L	30	1.32	1.04	0.38	ci L	30	2.70	2.59	0.69

b) Analysis of variance table and multiple comparisons of morphometric parameters between the 3 food regimes using Bonferroni's method. * = significant at 5%, ** = significant at 1%.

Source	df	Seq SS	Adj SS	Adj MS	F	p	Comparison	Difference mean
sl (mm)	2	20.12	20.12	10.06	0.79	0.46		
Error	88	1124.14	1124.14	12.77				
total	90	1144.26						
gi (mm)	2	27.72	27.72	13.86	79.44	0.001	h vs l	-0.32**
Error	86	15.01	15.01	0.17			h vs m	-1.00**
total	88	42.73					m vs l	0.32*
dw (log)	2	1.03	1.03	0.51	15.82	0.001	h vs l	-0.21**
Error	88	2.86	2.86	0.03			h vs m	-0.25**
total	90	3.89					m vs l	**0.04**
ci (log)	2	0.74	0.74	0.37	36.50	0.001	h vs l	-0.18**
Error	88	0.89	0.89	0.01			h vs m	-0.21**
total	90	1.64					m vs l	**0.03**

During phase 1 and phase 2, the individual increment widths (µm) were not significantly different between the three shells (ANOVA log transformed data: Phase 1: $F_{2,83}$= 0.34, p = 0.713 Phase 2: $F_{2,64}$= 3.13, p = 0.051). By contrast, individual increment widths were significantly smaller during phase 2 than phase 1 (two

samples *t*-test, log transformed data: $T_{137} = -4.32$, $p < 0.001$). Analysis of variance using chlorophyll *a* concentration as a covariate showed no significant difference in increment width between the phases 1 and 2 (ANOVA log transformed data: $F_{1,148} = 0.55$, $p = 0.460$) indicating that food concentration was responsible for the differences in increment width (Figure 5. 10).

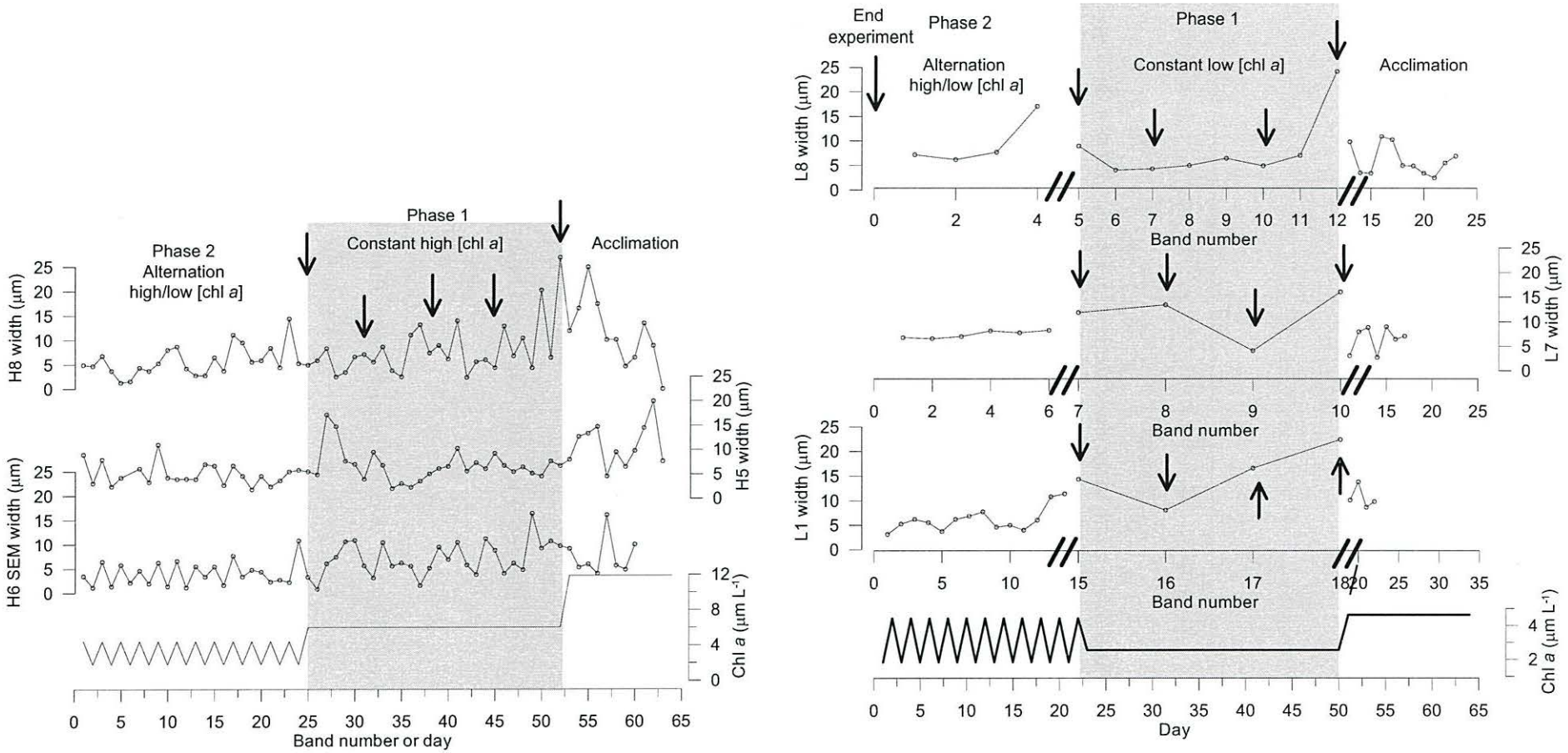


Figure 5. 9. Daily mean chlorophyll *a* concentrations (chl *a*) in the tank and individual increment widths in mussel shells held at a) high food concentration: H5 and H6 measured from replicas peel and H6 from SEM thin section, the band number corresponded to the number of days and b) Low food concentration: L8 and L7 measured from peels and L1 from SEM thin section, the band number < number of days; during the 3 phases of the experiment: acclimation (Phase 0), constant high chl *a* (Phase 1) and alternation high/low chl *a* (Phase 2).

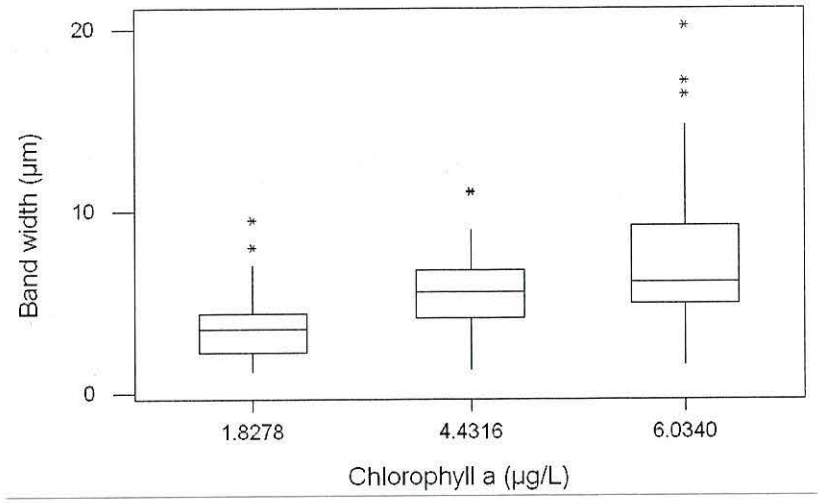


Figure 5. 10: Boxplot of increment width in the three shells (H5, H6 and H8) as a function of the mean chlorophyll *a* concentration in the experimental tank during acclimation (6.03 µg L^{-1}), phase 1 (4.43 µg L^{-1}) and phase 2 (1.83 µg L^{-1}).

Large scale experiment: no food gradient?

Analysis of the shell growth of mussels over a large spatial scale along a gradient from the eastern to the western end of the Menai Strait commercial mussel bed indicated that mussels living at the western end of the mussel bed (Bangor Pier) grew significantly faster in terms of both their macro- and microgrowth than mussels exposed at the eastern end of the Menai Strait (Gallows Point) to the inflow of fresh sea water (August 2005 experiment 3). However, by contrast, there was no significant difference in shell growth determined from the microgrowth increments in the shells of mussels collected at 13 stations along the gradient between the two ends of the mussel bed (Bangor Pier and Gallows Point) during April 2006 (experiment 4) .

Mussel growth rates from the two stations, Bangor Pier (BP, Cages 1,2,3) and Gallows Point (GP, Cages 7,8,9), separated by ~ 4.5 km at the two extremities of the mussel bed were significantly different during August 2005 (Table 5. 6). However condition index and dry flesh weight were not statistically significantly different between the mussels from the two sites (Table 5. 6). Prior to the deployment in August 2005, the mussels in GP were significantly longer than in BP (due to experimental re- design, BP = 39.65 ± 2.42 mm, GP = 43.57 ± 2.94 mm; two samples *t*-test: $T_{262} = -11.82$; $p < 0.001$). When the cages were retrieved in September

2005, the mussels from BP were significantly smaller in length than those from GP but grew significantly faster (i.e. they displayed wider growth increments) than mussels from Gallows Point (Table 5. 6 & ANOVA, square root transformed data, covariate shell length: $F_{1,133} = 9.67; p < 0.001$).

Table 5. 6: T Summary of the result of the analyses of the morphometric measurements of the mussels and their statistical analyses: Means, median, and standard deviation (SD) of shell length (sl mm), flesh dry weight (dw g), condition index (ci) and growth increments (gi mm) determined from 3 replicate samples of two groups of mussels from cages positioned at Bangor Pier (BP) and Gallows Point (GP) in the Menai Strait for a 1 month period between 22nd August and 22nd September 2005).

	Mean	Median	SD	Statistical Test
sl bp (n=30)	43.6	43.5	2.3	sl: *t*-test : $F_{56} = -2.1, p = 0.04$
sl gp (n=29)	44.9	44.9	2.5	
gi bp (n=29)	3.62	3.50	1.23	gi: *t*-test : $F_{52} = -4.08, p < 0.001$
gi gp (n=29)	2.45	2.50	0.94	
dw bp (n=30)	0.61	0.63	0.15	dw: *t*-test : $F_{50} = -1.14, p = 0.259$
dw gp (n=30)	0.65	0.66	0.10	
ci bp (n=30)	16.9	16.7	4.34	ci: Mann Whitney, $W = 953, p = 0.426$
ci gp (n=30)	16.8	15.7	2.84	

The cage mussels placed at the two stations, Bangor Pier (BP) and Gallows Point (GP) for one month were located at the same height on the shore and experienced similar levels of emersion during spring low tides. In Figure 5. 5a and b an acetate peel of a shell section is compared with the same shells appearance under UV light to reveal the position of the monthly Calcein lines in the peel. When the tidal bands were counted between the Calcein mark and the tip of the shell in 4 mussel shells collected from both GP and BP, ~60 bands were observed to have been deposited during the 1 month time interval corresponding to 2 tidal bands day^{-1} (BP: N = 4: mean number of bands = 60.75 ± 3.3. GP, N = 4: mean number of bands = 59.0 ± 5.59 bands). Figure 5. 7a shows the relationship between the mean increment widths measured from the mussels shells from GP and BP with the semi-diurnal tidal cycle superimposed. Estimates of a monthly shell growth rate from the peels, determined from the increment of growth measured between the Calcein line and the shell margin (~60 bands, N = 4, BP: 2.04 ± 0.39 mm; GP: 3.13 ± 0.36 mm), were

similar to the estimates obtained from macrogrowth shell growth i.e. (BP: N = 72: 2.36 ± 0.89; GP: N = 51: 3.38 ± 1.18 mm). Mean microgrowth increment width in the mussels shells from GP was significantly smaller than those in the mussel shells from BP (2 samples t-test: GP > BP; T_{92} = -6.44, $p < 0.001$; mean GP = 34.5 ± 9.1 μm, mean BP = 47.6 ± 10.9 μm). Similarly when measurements of the last 14 increments (corresponding to ~7 days) were compared, the mean increment width between the 2 stations was also significantly different (Table 5. 7). These observations contradict the findings (see below) from experiment 4 in which there was no difference in April in the growth of mussels along a gradient from Gallows Point in the east to Bangor Pier in the west; the results from the cage experiment conducted in August indicate that the GP mussels were growing significantly slower than those at BP.

Growth rates of the cage mussels (GP and BP) were compared with the growth rates of mussels (experiment 4) sampled at similar positions along the transect (i.e. locations Tr2 and Tr10). Mussels collected in April from the two locations along the transect from the mussel bed had a significantly lower mean increment width than mussels held in August 2005 in cages at the same locations (see Table 5. 7). Whilst the growth of the cage mussels was significantly different (Kruskal-Wallis H_1 = 12.84 $p < 0.001$) growth of the transect (Tr2 and Tr10) mussels in April were not statistically significant from each other (see Table 5. 7).

Table 5. 7: Summary of the average growth increment widths determined from measurement of the last fourteen microgrowth increments in mussels obtained from four stations, Gallows Point (GP) and Bangor Pier (BP) from the cage experiment and stations at similar locations Tr2 and Tr10 respectively from the transect experiment. ♦ data were transformed according to Y' = 1/Y. The shell length (SL in mm) and mean increment width (in μm) ± standard deviation are indicated, N is the number of shell used for the analysis.

	N	SL ± (mm)	Mean increment width (μm)	Tests:	Mood's median test ♦	Date
GP	4	45.5 ± 2.0	25.15 ± 6.16	Kruskal-Wallis ♦		Aug. 05
BP	4	44.1 ± 2.1	37.60 ± 6.20	H_1 = 12.84 $p < 0.001$	Chi^2 = 56.00	Aug. 05
Tr 2	4	48.9 ± 4.8	11.02 ± 2.66	Two samples *t*-test:	df = 3	April 06
Tr 10	5	50.4 ± 8.0	10.87 ± 2.09	T_{24} = 0.17, p = 0.87	P < 0.001	April 06

Mean mussel shell length varied at the 13 different locations along the transect and ranged from 39.1 to 57.7 mm (Figure 5. 11). Four stations (1, 5, 7 and 8) had significantly lower mean lengths (ANOVA: $F_{12, 129} = 12.59$; $p < 0.001$) than at the other locations and were subsequently removed from any further microgrowth comparisons. Measurement of the 14 last deposited microgrowth increments from at least 3 mussel shells from each station (2, 3, 4, 6, 10, 11 and 13) showed small differences in cumulative growth increment width (Figure 5. 12). The measurements from the mussels at stations 9 & 12 were removed as the microgrowth increment width measurements were only readable in 2 shells and there was unacceptably high variability. Variances in the growth data from the shells from stations at the extremities of the mussel bed (Tr 2, 10, 11 and 13) were smaller than the variances from stations inside the mussel bed (Tr 3, 4 and 6). The data were cubed transformed to meet the Kruskall wallis test assumptions and following transformation no significant differences were found between the mean increment widths of the mussel shells from the seven stations (N = 98, H = 7.46, $p = 0.28$, df = 6)

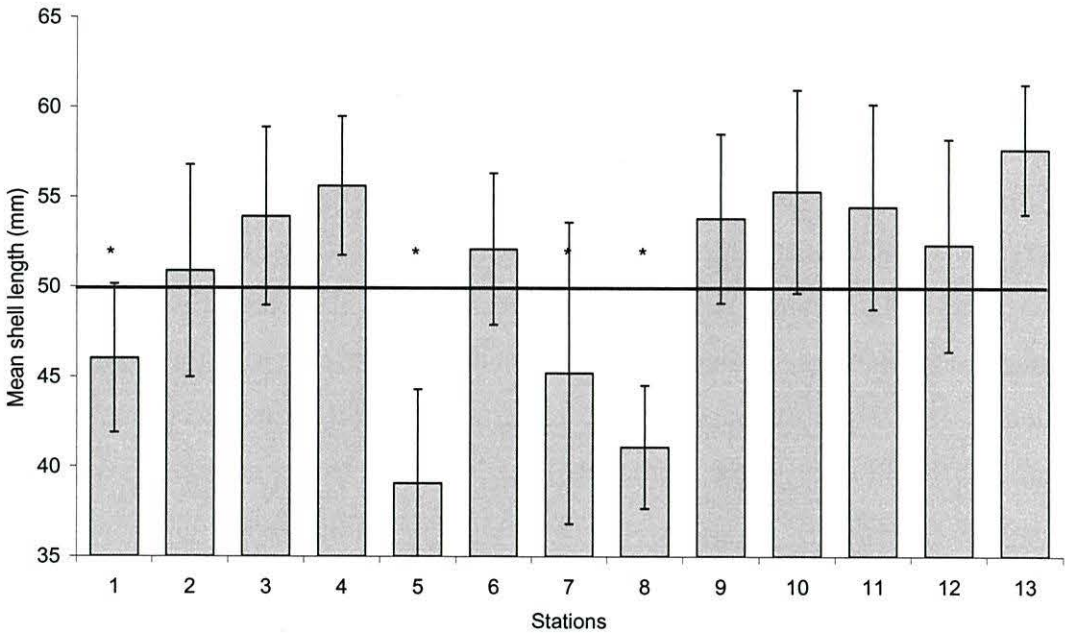


Figure 5. 11: Mean shell length (mm) ± standard deviation of *M. edulis* at 13 stations along the Menai Strait. * represents stations not included in the statistical analyses.

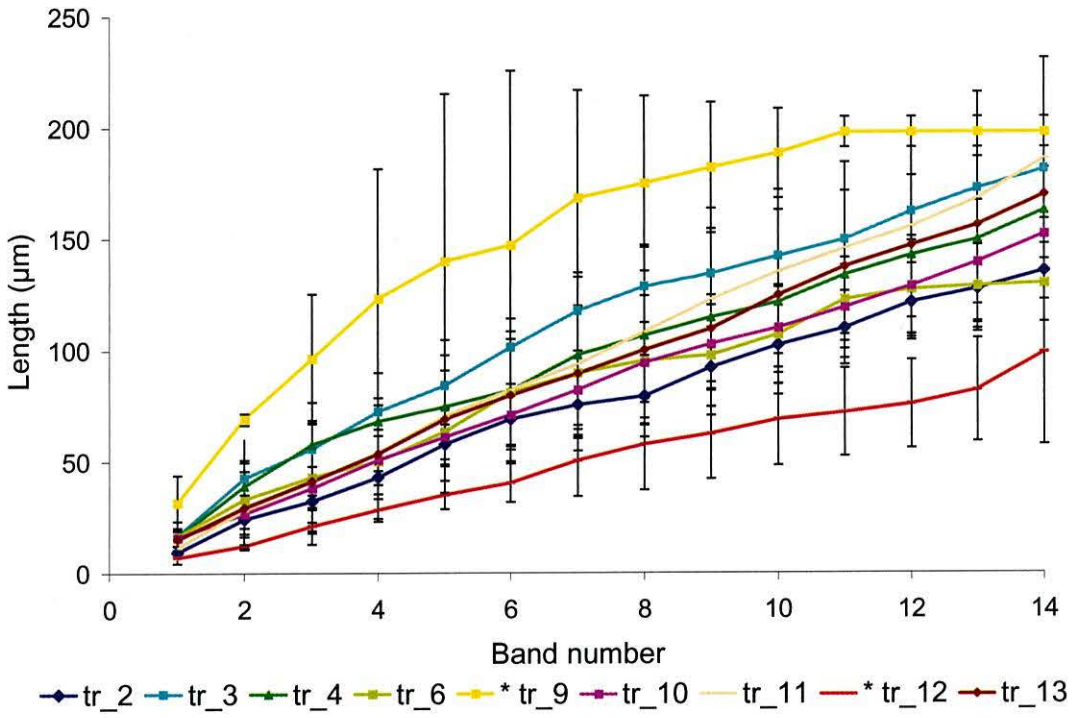


Figure 5. 12: Cumulative increase in growth increment length (µm) ± standard deviation measured from acetate peels of shell sections of *M. edulis* from each of nine stations along the Menai Strait. * represents stations not included in the statistical analyses

Small scale experiment: density dependent growth

Investigations into changes in mussel morphology and shell growth over small spatial scales i.e. the edge vs the middle of a mussel patch within a mussel bed showed using both morphometric analyses and measurement of the microgrowth increments that density dependent growth occurred. In addition measurement of the microgrowth increments revealed differences in the most recent shell growth in groups of mussels of similar density sampled from the edge and the middle of the patches. The length of mussels measured from a collection made on one occasion from 3 different areas of the mussel bed (rear and front edges and in the middle) in a mussel bed re-laid one month prior to sampling showed significant differences in two cases (2 samples *t*-test for each patch) with mussel shell length at the edge being greater than mussel length in the middle of the patch (Table 5. 8a). Mussel shell length was negatively correlated to mussel density (Figure 5. 13, Pearson: $r = -0.423$; $p < 0.001$) and no significant differences (ANOVA, using density as covariate) were found between mussel shell length on the edge and the middle of the patch (Table 5. 8b).

Table 5. 8: Summary and statistical analysis of the mussel shell length (mm) data collected from the edge (e) and middle (m) of three patches of a recently (1 month-July 2006) relaid mussel bed.

a) Means, median, and standard deviation (SD) of shell length measured by vernier calliper, density (animal m^{-2}) and two samples *t*-test are presented for shell length and density.

Sample	Mean	Median	SD	Density ± SD	Statistical Test
1 e	33.3	32.9	3.3	905 ± 284	1: *t*-test : $F_{57} = -0.23, p = 0.0818$
1 m	33.1	33.0	3.3	901 ± 91	d: *t*-test : $F_2 = -0.03, p = 0.981$
2 e	34.7	35.0	3.5	910 ± 151	2: *t*-test : $F_{51} = -5.02, p < 0.001$
2 m	30.7	32.7	2.5	1462 ± 120	d: *t*-test : $F_2 = 4.94, p = 0.016$
3 e	32.9	32.6	3.9	1080 ± 332	3: *t*-test : $F_{51} = -3.84, p < 0.001$
3 m	28.6	27.1	4.4	1966 ± 110	d: *t*-test : $F_2 = 4.39, p = 0.048$

b) Analysis of variance table, density used as a covariate

Source	df	Seq SS	Adj SS	Adj MS	F	*p*
Density	1	258.5	0.1	0.09	0.01	0.93
Station	5	72.91	73	14.58	1.14	0.34
Error	105	1343	1343.22	12.79		
Total	111	1675				

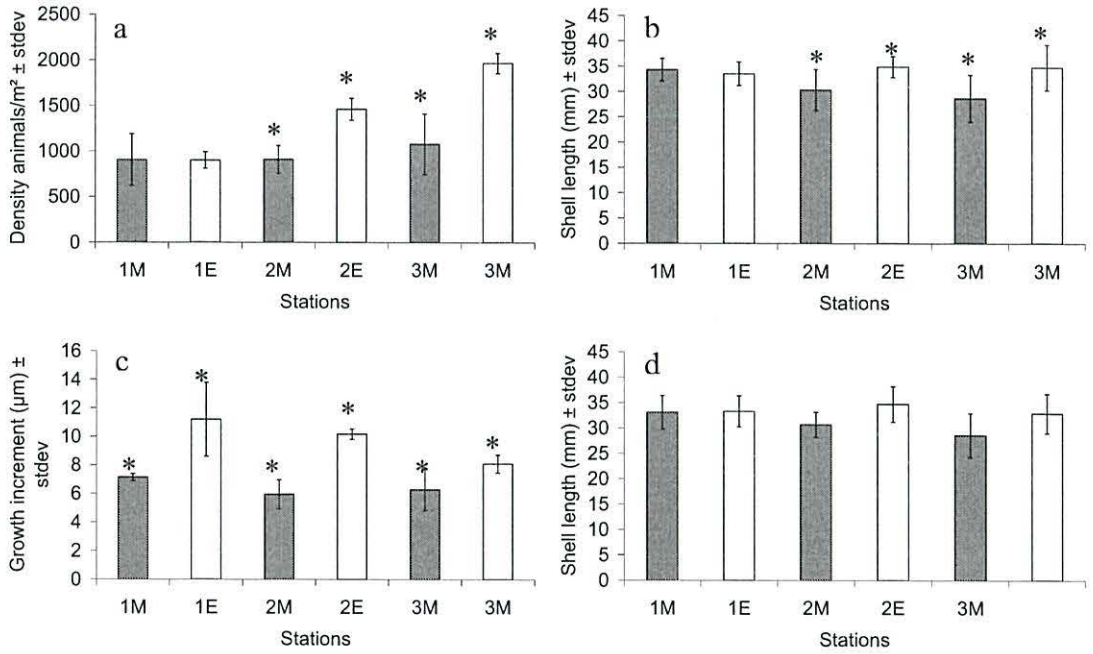


Figure 5. 13: a) Mussel density (mussels m^{-2}) during June 2007 at the edge (E) and middle (M) of three different mussel patches, b) Shell length (mm) of selected mussels used to analyse the shell microgrowth from the same patches and c) mean growth increment of the 14 most recently deposited internal growth increments for the mussel in b). * indicates significant different between E and M for the same patch at $p < 0.05$.

Further smaller (N = 4) sub-samples of mussels used in the morphometric analyses were also those mussels whose shells were sectioned and the microgrowth patterns investigated. No significant difference in shell length was found between the mussels from the edge and middle of the three patches (1, 2 & 3) (Two samples *t*-tests, 1: $T_4 = -0.44$, $p = 0.680$; 2: $T_1 = 1.46$, $p = 0.382$; 3: $T_3 = 1.64$, $p = 0.20$; Figure 5. 13). These data contrast with the earlier morphometric analyses where a larger (N = 30) sample of shells were measured and these data showed that there were significant differences in the shell length of mussels from patches 2 and 3, (see Table 5. 9 and Figure 5. 13).

Table 5. 9: Summary of the mean microgrowth increment length determined from measurements of 14 increments and statistical analysis of the mussel data collected at the edge (e) and middle (m) of three mussel patches from a 1 month relaid mussel bed in July 2006.

a) Means, median, and standard deviation (SD) of the microgrowth increment length (μm), density (animal. m^{-2}) and 2 samples *t*-test are presented for shell length.

Sample	Mean	Median	SD	Density ± SD	Statistical Test
1 e	11.2	10.6	3.2	905 ± 284	1: *t*-test : $F_{24} = 8{,}74$, $p < 0.001$
1 m	7.1	7.0	1.9	901 ± 91	
2 e	10.2	10.2	3.1	910 ± 151	2: *t*-test : $F_{66} = 7.25$, $p < 0.001$
2 m	5.9	5.7	1.8	1462 ± 120	
3 e	8.1	7.9	1.7	1080 ± 332	3: *t*-test : $F_{75} = 4.09$, $p < 0.001$
3 m	6.8	6.8	2.3	1966 ± 110	

b) Analysis of variance table, density used as a covariate

Source	df	Seq SS	Adj SS	Adj MS	F	*p*
Density	1	0.25	0.25	0.25	12.36	0.001
Station	1	1.99	0.88	0.88	44.43	< 0.001
Band	13	0.28	0.28	0.02	1.10	0.360
Error	236	4.67	4.67	0.02		
Total	251	7.18				

Using the microgrowth technique, for all the patches, there was a significant difference in average microgrowth increment width (measured from the last 14 growth increments) in mussels collected between the edge and the middle of the patch (Table 5. 9). When increment number was used as a covariate, no significant difference in shell growth between the edge and the middle of the patch was found in mussels from patch 3 ($F_{1, 83} = 0.002$, $p = 0.89$). Overall, there was a significant

difference in mussel growth between the edge and the middle of the mussel patches and this was negatively correlated to mussel density (Pearson correlation: r = -0.526, $p < 0.001$); the significant difference was also independent of mussel density when this was used as covariate (Table 5. 9b).

5.6 Discussion

This study revealed that there was close correspondence between food concentration and microgrowth increment width in laboratory reared mussels and in mussels grown in the natural environment. In the laboratory under constant conditions of seawater temperature and light but with variable food supply mussels provided with high concentrations of microalgae deposited shell whilst mussels growing in a regime of low food supply showed very little incremental shell growth. In the natural environment, mussels from the same cohort of mussels residing in a commercial mussel bed showed significant differences in shell growth in relation to their position within the whole mussel bed (large scale) and within small patches of mussels (edge and middle of a mussel patch) within the mussel bed (small scale) Comparing the measurements of both macro- and micro- shell growth, the data generated via the micro-shell growth measurement, have allowed new insights into the importance of a mussel's position within the mussel bed and food supply to be investigated over small temporal and spatial scales; data which are important for the successful management of mussels in the Menai Strait commercial fishery.

Periodicity of growth increment deposition in *M. edulis*

In this study, all the mussels from the subtidal (continuous immersion) conditions produced growth bands with an apparent tidal periodicity (12.14h cycle) i.e. two bands were deposited in the shell each day. Mussels from MLWS in the Menai Strait experienced tidal emersion during spring low tides but remained continuously immersed during neap low waters. These mussels were not exposed twice a day to the air, yet over a monthly period still exhibited ~2 bands day^{-1}. The formation of growth bands in the shells of intertidal mussels *Mytilus edulis*, has been shown in the literature to be controlled by emersion at low tide when the mussels remain closed

(Richardson 1989), while under conditions of subtidal continuous immersion it is still unclear what the periodicity of the growth bands represent (Richardson 2001). Richardson (1989) demonstrated in mussels held in constant laboratory conditions and in the natural environment that the band deposition was under the control of an endogenous rhythm and that they were related to the rate of shell deposition. The proposed explanations for subtidal shell deposition in this study are i) an innate rhythm as proposed by Richardson, 1988; 2001) and/or ii) mussel shell valve closure twice a day due to low food concentration in the subtidal area of the Menai Strait (Chapter 2, Saurel *et al.*, 2007). The same semi-diurnal periodicity was found in the shells of Calcein-marked mussels held in the laboratory under continuous immersion, constant high food supply and constant illumination. It is suggested that the semi-diurnal band deposition is controlled by an innate endogenous rhythm. Interestingly these data are in direct contrast to the data that Richardson (1989) obtained from mussels grown in the laboratory under constant conditions of immersion, constant food supply and constant darkness. The author counted between 2.69 and 3.40 bands day^{-1} in experimentally maintained mussels. However Richardson (1989) also suggested that under certain conditions the rate of band deposition in continuously immersed mussel shells was related to the rate of shell growth rather than to an innate rhythm, i.e. over the same period of time, more bands were deposited by faster growing mussels than slow growing individuals. This study showed different results, with < 2 bands day^{-1} or no bands were deposited in the shells of mussels grown under a low food regime while one (if 2h emersion disturbance day^{-1}) or 2 bands were deposited at high food regime. However, never more than 2 bands day^{-1} were deposited in the mussel shells. The mechanical disturbance in the laboratory of emersing the mussels for 2h each day coupled with a low food concentration appeared to overwrite the innate rhythm.

Comparison of the microgrowth vs macrogrowth methods in growth variation

In some circumstances the microgrowth method was better than the traditional macrogrowth technique used to measure mussel growth variation (Table 5. 10). Both techniques have advantages and disadvantages: the traditional macrogrowth method has the advantage of practicality, low cost, and is a standard procedure whereas the microgrowth technique is not a standard method, it is time consuming, more expensive and subject to visual interpretations of the band reading (Bérard *et al.*,

1992). Nevertheless, the microgrowth method provides a measure of the immediate response of growth in mussels to environmental changes without prior monitoring contrary to the need for lengthly monitoring for the macrogrowth method. This property has important applications for successful shellfisheries management, which is due to the accuracy of measurement (μm) and to the growth recording property within the shell (e.g. the last day to 15 days of mussel growth can be measured and compared between different sites). This technique could be used to measure variations in growth due to short-term environmental events/fluctuations (storms, pollution, phytoplankton blooms) and how this influences the population dynamics of mussel bed formation (mussel bed patterning and self-organisation). Experiment 5 in the current chapter demonstrated how the microgrowth technique enabled differentiation of mussel shell growth variations at the edge and the middle of a mussel patch, a phenomenon that remained undiscernable using the macrogrowth techniques. This is explained by the relatively short response time of microgrowth to food availability as compared with the longer timescale necessary for the density dependent mussel re-organisation.

Over periods of > 1 month of monitoring the microgrowth technique did not provide any advantages over applying macrogrowth techniques. There was an expectation that microgrowth would enable the determination of short term variations in mussel growth from the past, i.e. few months to years before sampling. However, the fluorescent marker Calcein was unreliable when used for multiple marking of the same internal shell such that the disturbance mark could be used as a temporal starting point against which to compare subsequent microgrowth. Calcein has been used with success for single time-event marking in other mussel species, i.e. *Perna perna* (Kaehler and McQuaid, 1999) *Modiolus barbatus* (Peharda *et al.*, 2007).

Table 5. 10: Comparison between macro- and microgrowth methodologies, their temporal scale of use and their applications as indicator of food concentration.

	Macrogrowth	Microgrowth
Methodology		
Animal number	Large	Small
Cost	Low	High
Practicality	Yes	No
Accuracy	No	Yes
Condition Index	Yes	No
Mark/recapture	Yes	Needs improvement
Growth temporal scales		
Long period	Yes	No
Short period	No	Yes
Event (pollution/bloom)	No	Yes
Applications		
Proxy for food concentration	Yes	Yes
Mussel bed growth state	Yes	No
Mussel bed reorganization	No	Yes

Increment width

This study has measured a wide range of growth increments depending on the food concentration: from quasi not visible (at low food concentration) to 37.6 μm in the field (August 2005), but higher mean increments have been measured previously (53.4 μm, Richardson, 1989). Growth increment was higher at Bangor Pier (BP) than at Gallows Point (GP) in August 2005 (BP mean: 37.6 μm, GP mean: 25.15), whereas the growth increment was smaller and no difference was measured between those two stations in April 2006 (~11 μm for both). This was linked to the growth cycle of *M. edulis* and the food supply in the Menai Strait. The shell growth in mussel is more rapid after the spring bloom of late April in the Menai Strait, which is itself dependent upon seawater temperature rise (Blight *et al.*, 1995) (Table 5. 3).

No spring/neap cycles were evident in the growth increments sampled in the shells of mussels placed in cages at Gallows Point and Bangor Pier (in contrast to Richardson, 1989 and Miyaji *et al.*, 2007), which suggest that the variation in food concentration during these periods is not strong enough to affect mussel growth. Somewhat surprisingly, the mussels located closer to the source of food advected from Conwy Bay and Liverpool Bay (GP) grew more slowly than mussels located closer to the recycled water (BP). This hypothesis was based on the hydrodynamic

properties studied in the main channel of the Menai Strait (Chapter 2). In the main channel, the current is bidirectional and there is a strong food gradient with a higher concentration of suspended food originating from the high primary production area of the Liverpool Bay at the north east end of the Menai Strait (Saurel *et al.*, 2007, Simpson *et al.*, 2007, Tweddle *et al.*, 2005). In contrast, over the intertidal areas (Chapter 6), the current was tri-directional and some re-suspension of microphytoplankton occurred at the beginning of the flood tide. These hydrodynamical properties over the intertidal and subtidal areas could explain the observed variation in mussel growth between the two stations of Gallows Point and Bangor Pier (separated by 4.5 km distance).

Microgrowth rate as food proxy

In order to use the growth rate measured via the microgrowth method as a proxy for food concentration, some further calibrations would be required. It seems difficult to separate all the environmental factors that influence growth over an annual cycle. In this study mussels were kept under constant temperature in the laboratory or with limited temperature fluctuation in the field. Moreover, this study focused only on a single cohort of mussels that permitted inference regarding the influence of food concentration on growth rate. In contrast, over a longer period of time, growth rate would vary seasonally due to the joint effects of food concentration and seawater temperature, that interact with other factors such as ontogenic and metabolic effects (Hawkins and Bayne, 1992; Sukhotin and Pörtner, 2001).

Microgrowth technique for shellfisheries management and population dynamic

The microgrowth technique is more expensive and less practical to use than the macrogrowth method. It is also difficult to obtain a large numbers of readable replicate shell sections. Consequently the microgrowth method seems quite unlikely to be chosen by mussel farmers for monitoring mussel growth if it is compared to the practicality of the macrogrowth method. Moreover, while the macro and microgrowths of the shell indicated differences between mussels at Gallows Point and Bangor Pier, the condition index and the dry flesh weight did not. Although, in

mussel, shell and somatic tissue growth are not necessarily coupled and can exhibit seasonal differences (Hilbish, 1986; Dare and Edwards, 1975; Beadman *et al.*, 2003). This depends on the main energy source for reproduction: stored energy or external food (Bricelj and Shumway, 1991).

The microgrowth technique offers a useful alternative tool for a short-term assessment of shell growth, particularly if the technique is applied comparatively at a variety of spatial scales (e.g. within mussel beds, within estuaries, between estuaries).

Chapter VI

Production carrying capacity*

Sampling over the intertidal mussel bed in the Menai Strait, 17/07/2006
Photo courtesy Melanie Bergmann

* This study was presented at the 10th International Conference on Shellfish Restoration (ICSR) November 12-16 Vlissingen, The Netherlands as an oral presentation.
Saurel C., Petersen, J.K., Wiles, P. and M.J. Kaiser. 2007. The importance of mussel bed patchiness for food supply: balance between grazing, tidal advection, vertical turbulent flux and seston concentration.

Chapter VI Food supply, turbulence and patchiness: how mussels feed copiously in the Menai Strait.

6.1 Contributions to this chapter

Chapter process:	Contributions
Conception	CS/MJK/JKP
Design analysis	CS/JKP/PW
Data collection	CS/JKP
Data analysis/ Interpretation	CS/JKP/PW
Drafting	CS/JKP
Revising	CS/MJK/JKP/PW

Initial	Name	Institutions
CS	C. Saurel	
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JKP	J. K. Petersen	NERI, Roskilde, Denmark

6.2 Abstract

An *In situ* approach was used to study three components of the mussel production carrying capacity: the mussel feeding activity and growth, the food concentration and the hydrodynamic conditions. In an intertidal mussel bed of the Menai Strait (U.K.), mussel growth was density dependent and the mussels at the edge of the patches grew bigger than mussels in the middle of the patch according to a measured food gradient. The mussels were wide open, actively filtering (mean valve gape aperture > 70%) and their grazing rate was high (*in situ* clearance rate = 3.1 L ind^{-1} h^{-1}). Low chlorophyll *a* (chl *a*) concentrations (< 1 μg L^{-1}) were measured next to the mussel bed (< 6 cm) due to mussel filtration. Furthermore, horizontal food depletion in the water column above the mussel bed was observed on a small scale (5 m) between mussel and adjacent bare patches.

In this system, the explanation for the high mussel feeding activity, despite the food depletion near the mussel bed, is the supply of an adequate amount of food to the mussel by a succession and/or combinations of three hydrodynamic processes (resuspension, vertical mixing and advection). These processes are themselves altered by the mussel bed morphology (i.e., the alternation between mussel and circular bare patches). The resuspension of microphytobenthos (measured as chl *a* and phaeopigments) from the bare patches present in the middle of the mussel bed, occurred only at the beginning of the flood tide when the current velocity was high. During the re-suspension period, the food depletion only occurred at 5 cm above the mussel bed, while higher food concentrations was measured at 1 and 3 cm. After the flood, the vertical turbulent flux and advection supplied the food closer to the mussel bed but also replenished the water column with food even closer to the seabed over the bare patches where no mussel filtration occurs. The water column refilled with more food in the bare patch was then advected to the adjacent mussel patch allowing the mussel to filtrate at high rates.

The vertical turbulent flux of chl *a* to the mussels, determined by the gradient of chl *a* and the diffusion near the bed measured with an ADCP (Acoustic Doppler Current Profiler) was equal to the actual flux of chl *a* measured from mussel grazing, and thus indicates a steady-state system. Such findings provide strong support to the *in situ* methodology used and it opens new perspectives for their use in modelling carrying capacity. Moreover, by optimising the mussel density, percentage cover and patch size, a new management of mussel re-laying could increase the mussel productivity.

Keywords: Mussel, *Mytilus edulis*, production carrying capacity, *in situ* methodologies, feeding behaviour, growth, grazing rate, seston concentration, food depletion, vertical turbulent mixing, advection, resuspension.

6.3 Introduction

Mussels are known as ecosystems engineers due to their ability to modify their habitat and environment (Bruno and Bertness, 2000; Petersen, 2004a). Mussels are a foundation species that control primary production via filtration and can be responsible for important particle depletion next to the bed (Cloern, 1982; Smaal *et al.*, 1986; Fréchette and Bourget, 1985a, 1985b; Dolmer, 2000a). Mussels are cultivated on soft bottom substrata in UK and northern Europe, using mussel seeds that are generally collected from subtidal ephemeral mussel beds and then re-laid intertidally and/or subtidally (Beadman *et al.*, 2004; Saurel *et al.*, 2004; Saurel *et al.*, 2007). The mussel production carrying capacity (the stocking density of bivalves at which harvest is maximised; Inglis *et al.*, 2000), depends on the quantity and availability of natural resources and ecosystem functioning (Bacher *et al.*, 1998). Thus, understanding practices in relation to the ecosystem (hydrodynamic processes, primary production) is important for sustainable use of coastal water (McKindsey *et al.*, 2006; Dame and Prins, 1998).

In confined waters, high bivalve grazing pressure can reduce phytoplankton concentration in the water column by more than a factor of 3 (Cloern, 1982; Smaal *et al.*, 1986; Fréchette *et al.*, 1989; Fréchette and Bourget, 1985a, 1985b; Dolmer, 2000a) and can influence the food availability downstream both within and beyond mussel beds (Butman *et al.*, 1994; Kamermans, 1994). Filtration activity is also moderated by the food availability (Clausen and Riisgård, 1996; Riisgård, 2001a, 2001b; Riisgård *et al.*, 2003; Lassen *et al.*, 2006). When food depletion occurs in the water column and/or in the boundary layer, the intra and inter-specific competition for food results in a reduction in mussel filtration activity (Riisgård, 1991; Riisgård *et al.*, 2003; Newell *et al.*, 2001; Widdows *et al.*, 2002; Saurel *et al.*, 2007) and an accompanying reduction in mussel growth that varies according to the position of the mussel in the bed (Newell, 1990; Fréchette and Bourget, 1985a; Svane and Ompi, 1993; Dolmer, 2000a; Wildish and Kristmanson, 1984; Widdows *et al.*, 2002; Fréchette *et al.*, 1989; Larsen and Riisgård, 1997; Okamura, 1986). Consequently, currents that are sufficiently strong to maintain an adequate supply of food may be a prerequisite for the formation of dense and extended mussel beds (Larsen and Riisgård, 1997).

Tidal advection and vertical turbulent flux are the major factors that affect the supply of food to the benthic boundary layer. Mussels in dense beds can filter up to 170 m^3 m^{-2} d^{-1} (Jørgensen *et al.*, 1990; Prins *et al.*, 1996), but their grazing impact is highly modified by hydrodynamic processes (Peterson and Black, 1988; Peterson and Black, 1987; Fréchette *et al.*, 1989; Prins *et al.*, 1996; Lassen *et al.*, 2006). Moreover, mussel beds modify the flow on a smaller scale by increasing roughness: i) via mussel shell shape and dense and extended mussel aggregations (Butman *et al.*, 1994) ii) via biomixing created by the flow of water from the mussels siphons (Lassen *et al.*, 2006; van Duren *et al.*, 2006) and iii) via the topographic variation of the mussel bed (alternation between mussel and bare patches) at larger spatial scales (10s m) (Butman *et al.*, 1994).

In the Menai Strait mussel fishery, annual mussel production can attain 11 000 t y^{-1} from a growing area ~700 ha. In this system, food concentration is quite low for most of the year, ca. 1 μg L^{-1} chlorophyll *a* (chl *a*), but can vary from 0.5 up to 5 μg L^{-1} during the spring/summer season. In the Menai Strait, as opposed to systems with natural settlement (i.e. Wadden Sea, van de Koppel et al., 2005; Maine USA, Snover and Commito 1998), the mussels laid on the substratum undergo a succession of organisational changes: i) initially loose mussels are re-laid in circular bands from farmers' boats on the intertidal soft sediment bed. Once on the ground, the loose mussels are washed away by the tidal current and pile up in the scars left by previous mussel-dredging events. ii) Self-organisation then occurs, driven by hydrodynamic processes, instraspecific competition and predatory pressure. After a few weeks, dense hummocks and bare patches appear. iii) After one year, mussels from the intertidal are dredged and relocated to the subtidal areas where they are large enough to resist predations by crabs (Beadman *et al.*, 2003). Bare patches within the resulting mussel beds are created in part by mussel producers during the seed re-lay (pers. obs) and through a variety of natural process (erosion, Bertness and Grosholz, 1985; Murray *et al.*, 2002; intraspecific facilitation, van de Koppel et al., 2005; mortality; predation, Okamura, 1986).

In this study, the mussel bed patterning (presence of bare patches of sediment within the mussel bed) together with the hydrodynamic processes were studied for

understanding their role in the food supply to the dense mussel patches in a commercially re-laid intertidal mussel bed. For this, the important components of production carrying capacity were considered separately: mussels, food concentration, hydrodynamics. Then the interactions between the mussel component (*in situ* feeding behaviour, *in situ* clearance rate, growth at different scales), and the temporal and spatial variation in food resources (vertical and horizontal chlorophyll *a* profiles above the mussel and bare patch within a mussel bed) were looked together with the hydrodynamic conditions (tidal advection, turbulence, vertical diffusion). Consequently, it was possible to model empirically food supply dynamics and to relate them to mussel bed patchiness.

6.4 Material and methods

Experiments were undertaken in the Menai Strait, UK during July 2006. Three sites were chosen: site 1 was a control site in a sandy area (N53°14'54.26" W004°05'46.56"), and site 2 had been re-laid with young mussels 1 month prior to the experiment (N53°14'43.40" - W004°06'12.06") from 15th to 18th of July 2006, whereas site 3 had been re-laid one year prior to the experiment (N53°14.757'-W004°05.607') from 19th to 20th of July 2006 (Figure 6. 1). Mussels from both sites were composed of a single cohort. All times in this study are in GMT.

Hydrodynamics:

The vertical velocity profile on the inter-tidal mussel bed was measured using a bottom mounted RDI 1.2-MHz Workhorse Acoustic Doppler Current Profiler (ADCP) positioned consecutively on sites 2 and 3. The ADCP sampled current velocities at 1.25 Hz in vertical bin sizes of 25 cm and the ambiguity velocity was set to 100 cm s^{-1}. Along beam velocities were recorded so that turbulence parameters could be estimated. The variance method (Stacey *et al.*, 1999) was used to estimate Reynolds stresses from the ADCP data. In order to reduce noise in the velocities and Reynolds stresses, the data was split into 24.84 hour (M_1) segments. An M_1 period was chosen as the two 12.42 h (M_2) tides during an M_1 tide were not identical (see Figure 6. 2 – tidal heights). The M_1 segments were averaged together to create one

M_1 time series of velocity and Reynolds stress data for each of the deployments. The data were rotated into along and across current components, which varied throughout the tide (see Figure 6. 2).

The vertical diffusivity (diffusion coefficient K_z) was estimated for the bottom 4 bins (at heights 0.49, 0.74, 0.99 and 1.24 m above the bed) of the ADCP using the Reynolds stress and velocities (Rippeth *et al.*, 2002). In this near bed region, both the Reynolds stresses and vertical velocity shear were above the ADCP noise threshold. A straight line was then fitted (forced to go through 0) to these values in order to obtain a value for K_z at 0.05 m above the bed. K_z was estimated from the ADCP according to:

$K_z \sim N_z = u'w'/(du/dz)$

where N_z is the viscosity, u' w' is the Reynolds' stress, du/dz is the variation of velocity with height. The eddy diffusivity K_z is assumed to be equal to the eddy viscosity (Fréchette *et al.*, 1989).

K_z was calculated from the animal and seston concentration according to:

$K_z(dc/dz) = \text{grazing}$

where dc/dz is the variation of chl *a* concentration with height.

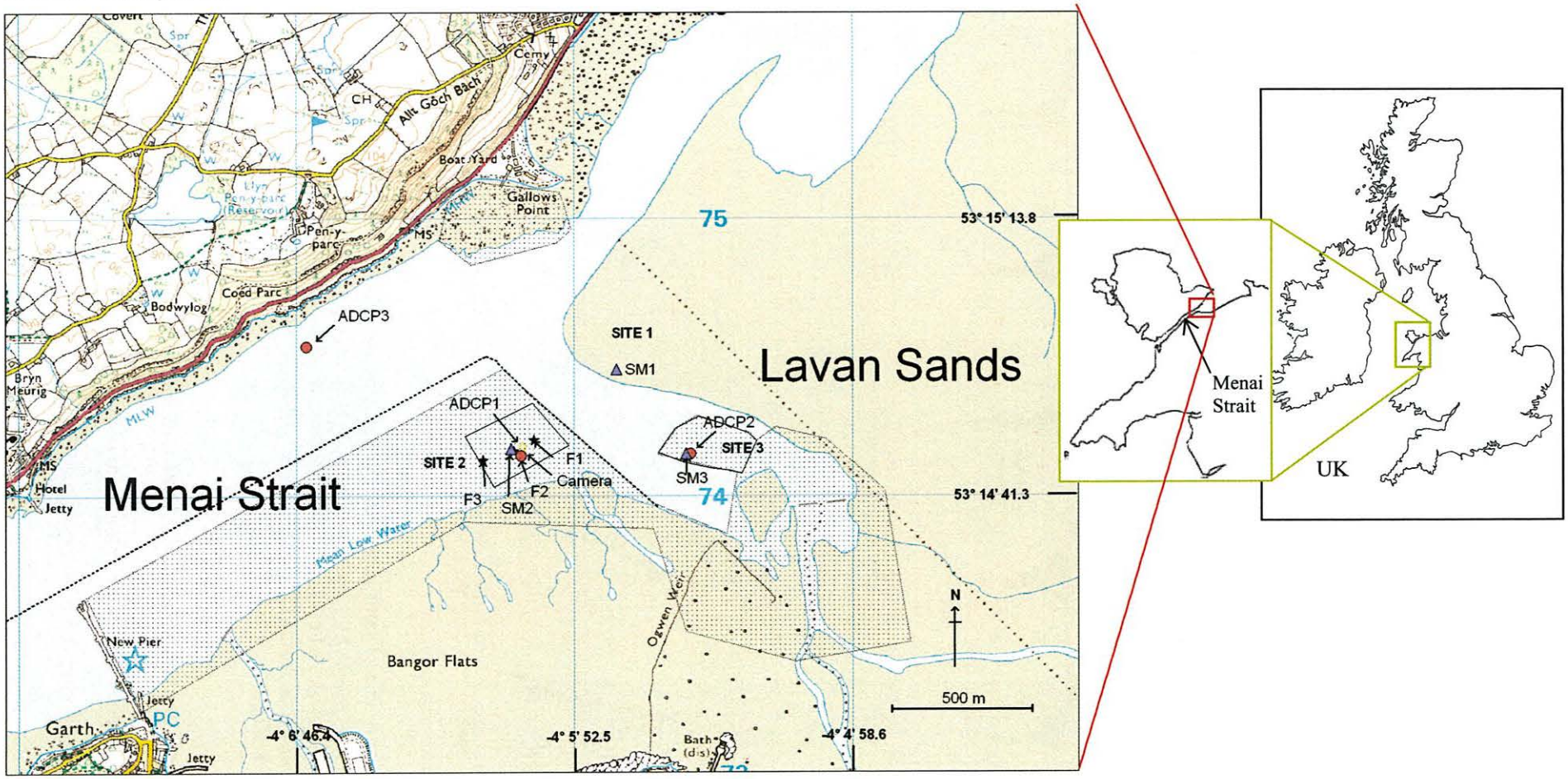
The time for vertical mixing (t_v) was estimated following this equation (Lewis, 1997):

$t_v = \sigma^2_z/2K_z = (0.8h)^2/ 2K_z = 0.32h^2/K_z$

Vertical mixing is assumed to be complete when the standard deviation (σ) equals 0.8h, where h is the total depth (Lewis, 1997).

The spatial pattern of horizontal velocities over the intertidal commercial mussel bed was mapped with a roving RDI 1.2 MHz Workhorse ADCP during a survey on the 30th of June 2006. The ADCP was attached to the side of a 5 m long boat using a Global Positioning System (GPS) and bottom tracking to reference the water velocities in relation to time and space.

Figure 6. 1: Map of the experimental sites and the position of the instruments (adapted from © Crown copyright 2007). Sites 1: sand (N53°14'54.26" - W004°05'46.56"), Site 2: mussel bed (N53°14'43.40" - W004°06'12.06") from 15 to 18th of July and Site 3: mussel bed (N53°14.757'-W004°05.607') from 19 to 20th of July 2006.



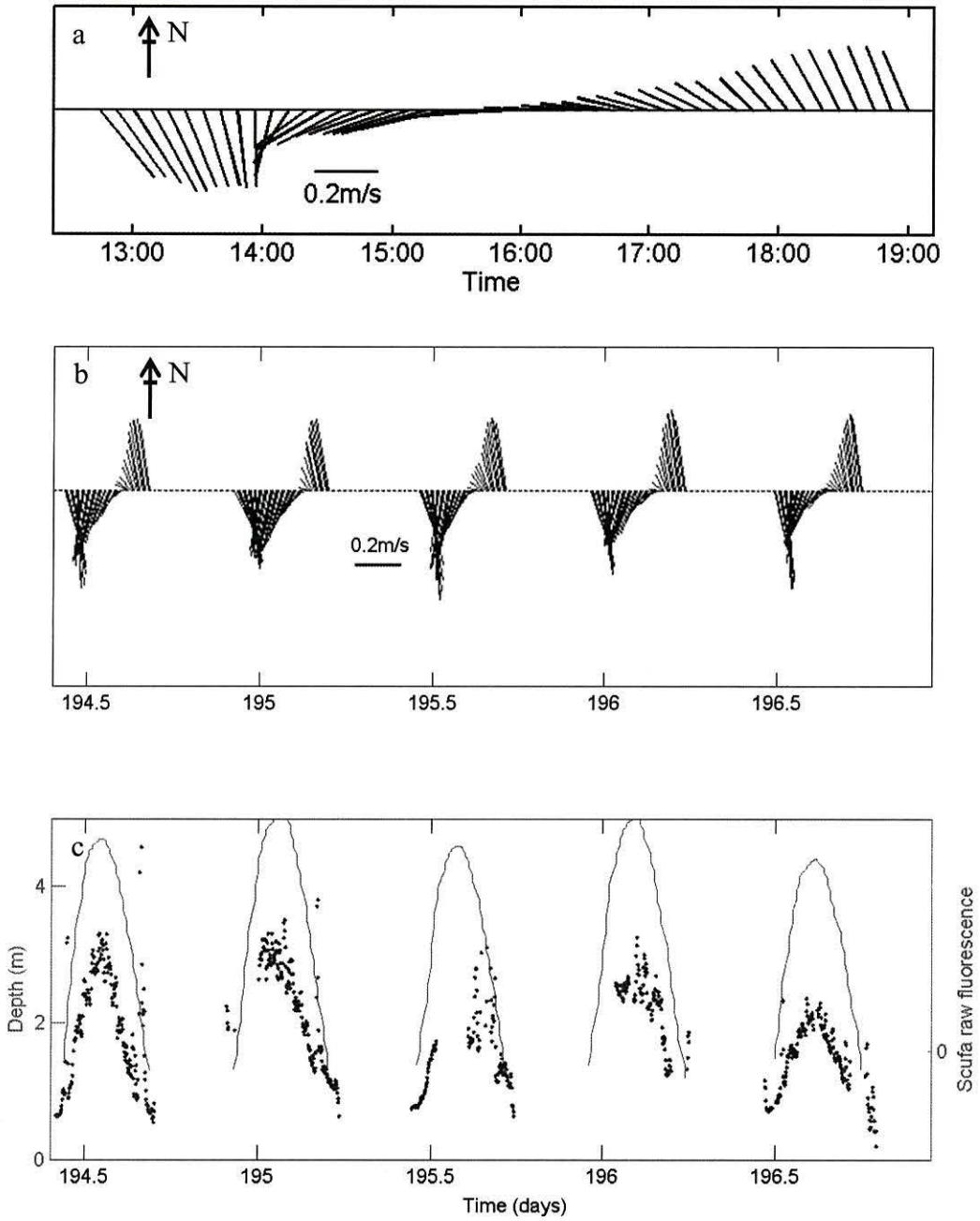


Figure 6. 2: a) Depth mean current velocity and direction (m s^{-1}) from ADCP1 represented by vectors over Site 2 on the 17th July. The origin of the vector is on the horizontal line, b) the time-series of current velocity represented by vectors over site 2 from the ADCP. c) Raw fluorescence from scufa fluorometer (dots) and the water depth (solid line) above the mussel bed in site 2.

Seston concentration: Vertical and horizontal chl a profiles

Three sets of experiments were carried out on the intertidal area. Water samples were collected by pumping water at a flow speed of 12 L h^{-1} through artificial siphon (siphon mimics SM) with an open diameter of 2 mm and a flow speed which are

bigger than the inhalant opening and filtration rate of *M. edulis*. Each siphon (polyethylene tubes length < 20 m) was buried in the sediment for ~5 m length to avoid interference with current flow and resuspension of material. The free end of the siphon mimic tubes were attached to a buoy to enable collection of samples via a peristaltic pump mounted aboard the boat.

For experiments 1 and 2 (Figure 6. 3), two sets of six tubes were positioned at 0.5, 3, 6, 11, 25 and 80 cm above the surface of the seabed and triplicate sample of water from the two sets were collected simultaneously from two small boats for ~5 minutes every 30 minutes. For experiment 3 (Figure 6. 3), two sets of siphons were positioned at 0.5, 3, 5, 11 and 25 cm above the surface of the seabed located at the middle of the mussel patch and the adjacent bare patch; three additional siphons at 5 cm height above the seabed were positioned in between the two sets of siphon: at the edge of the mussel patch, at the interface between the mussel and bare patch and at the edge of the mussel patch (~50 cm distance). A horizontal transect with five SM at 5 cm height was therefore established between the two sets of vertical profiles.

Chl *a* and phaeopigments from the water samples were measured on a Turner Design 10-AU fluorometer, after filtering 100 mL sample through GF/C filter (Advantix), soaked in 10mL of 90% acetone and placing them in a cool, dark room for extraction for a duration of 18h (method adapted from Parsons et al. 1984).

Mussels:

-*Feeding behaviour:* A waterproof digital camera (NIKON 4500) together with a timer (Digisnap) were deployed on the seabed next to the ADCP on site 2 for the whole period of the experiment. Mussel feeding behaviour (~30 individuals) was determined from still images (every 10 minutes) and measured with an image analysis program (analySIS©). Feeding behaviour was expressed as the mean percentage valve gape aperture as described in Saurel et al. (2007) (Chapter 2).

Figure 6. 3: Sites 2 (a & b) and site 3 (b & c) a & c) Aerial pictures courtesy of J. van de Koppel, NIOO Holland; b & d) siphon mimic sites

-*Mussel growth:* Mussel samples were taken using a cylindrical core (15 cm in diameter), at a patch scale, at the middle and edge of patches next to the siphon mimics. The mussels were measured for shell length (L mm), dry weight (DW g) of body parts was obtained by drying the excised parts for at least 48 h at 90 °C or until constant weight was obtained (Table 6. 1). The density and condition index (CI = DW/SL^3) of the mussels were then calculated (Table 6. 1).

The percentage of mussel cover at each of the different sites was calculated from aerial photographs (© J. van de Koppel, NIOO Holland) taken at low tide during the experiment (Figure 6. 3) where fourteen pictures from sites 2 and 3 were analysed with ImageJ 1.37v freeware: images were RGB colour split, and mussel percentage cover were calculated automatically.

-*Clearance rate*: Mussel *in situ* clearance rate was estimated using the defecation method (Saurel et al., in prep. Chapter 3). Mussels from the covered intertidal area in site 2 were collected from the small boat with a rake after the siphon mimic sampling. They were then placed individually in 1 L buckets filled with seawater

from the start and allowed to defecate for 1 h. Faecal pellets were collected with a plastic pipette after 0.5 and 1 h and put onto a GC-MS filter to remove excess water. Chl *a* was extracted following the same method as described above. Clearance rate (L g^{-1} DW h^{-1}) was calculated according to Chapter 3:

$CR = (DR * 100 / (100 - DF))/\text{chl } a_{eq}$ (1)

$DF = -16.90 \times \ln(\text{chl } a_{eq}) - 8.962 \times T_w + 0.3962 \times T_w^{\ 2} + 129.70$ (2)

where DR is the defecation rate (µg chl a_{eq} h^{-1}), chl a_{eq} is the chl *a* equivalent concentration (chl a_{eq} = chl *a* +1.52 Phaeo, µg L^{-1}) in the surrounding water 2 hours prior to experiment (to take into account the gut residence time) and DF is the digestion factor (%). T_w is the mean seawater temperature during the experiment. Clearance rate measurements were standardized to a 1 g mussel using an allometric factor of 0.67 (Hawkins et al., 1996).

Data analysis

For comparisons among datasets, paired t-tests, two-samples t-test and ANOVA were used to test for significant differences after checking that the data met the assumptions of normality and homogeneity of variance, applying the appropriate transformation when necessary. When significant differences were detected, a post-hoc Bonferroni or Tukey multiple comparison test was performed. When the data did not conform to the assumption for parametric tests, either Kruskal-Wallis, Mann-Whitney or Mood Median tests were performed.

6.5 Results

6.5.1 Mussels component

Growth

Site 2 was a re-laid self-organised mussel bed of small mussels ~3.3 ± 0.4 cm, and a maximum density of ~2000 ± 100 ind. m^{-2} for a total area estimated of 5.8 ha (Table 6. 1). In comparison, site 3 was an older mussel bed with larger mussels ~4.3

± 0.4 cm, a maximum density of ~1000 ± 200 ind. m^{-2} and a total area estimated at ~4.6 ha (Table 6. 1). Shell length was negatively correlated with mussel density of the patch on site 2 (Pearson site 2 $r = -0.559$, $p < 0.001$) but not on site 3 (Pearson $r = 0.013$, $p = 0.936$).

The difference in mussel size between the edge and the middle of the patch at site 2 was density-dependent (ANOVA $F_{1,37} = 5.04$; $p = 0.031$ Table 6. 1b) and therefore no significant difference was found when using density as covariate (ANOVA $F_{1,37} = 0.62$, $p = 0.437$ Table 6. 1b). On the other hand, there was no significant difference in density (two samples *t*-test: $T_3 = -0.91$, $p = 0.428$) and shell length between the edge and the middle of the patch on site 3 (two samples *t*-test: $T_{34} = 0.08$, $p = 0.936$).

Table 6. 1: Results of morphometric measurements at the different sites and positions within the mussel bed. a). Shell length (L), dry weight of body parts (DW), Condition Index ($CI = DW/L^3$) at the edge and middle of the patch for mussels, *M. edulis* from sites 2 and 3.

Average	Site 2				Site 3			
	Density (ind m^{-2})	L (mm)	DW (g)	CI	Density (ind m^{-2})	L (mm)	DW (g)	CI
Average	1187	32.77	0.210	5.44	947	42.96	0.268	3.34
St. Dev.	326	3.78	0.011	1.21	330	3.58	0.062	0.51
Edge								
Average	911	34.46	0.274	6.38	869	42.91	0.270	3.40
St. Dev.	151	4.02	0.108	0.74	287	2.99	0.061	0.58
Middle								
Average	1463	30.89	0.139	4.48	817	43.01	0.266	3.28
St. Dev.	120	2.44	0.046	5.52	183	4.16	0.064	0.43

b. Analysis of variance of the shell length at different position (edge or middle) on site 2 with density as covariate.

Source	df	Seq SS	Adj SS	Adj MS	F	*p*
Density (covariate)	1	165.40	51.38	51.38	5.04	0.031
Position	1	6.30	6.30	6.30	0.62	0.437
Error	35	357.16	357.16	10.20		
Total	37	528.86				

Feeding behaviour

The mean percentage valve gape aperture showed a similar pattern for each day at site 2, where mussels were filter-feeding at a valve aperture of > 60% for 90% of the time (e.g. 17th July in Figure 6. 4). The general pattern was a rapid opening (from 40 to 80% in less than 1 h) of the mussel valve aperture as soon as the water flooded onto the intertidal bed. Then, a constant aperture of between 60 to 80% maximum aperture width occurred during the remainder of the tide until one hour before low tide. In the last hour, there was a reduction of the valve gape until total closure when the mussels were exposed to the air.

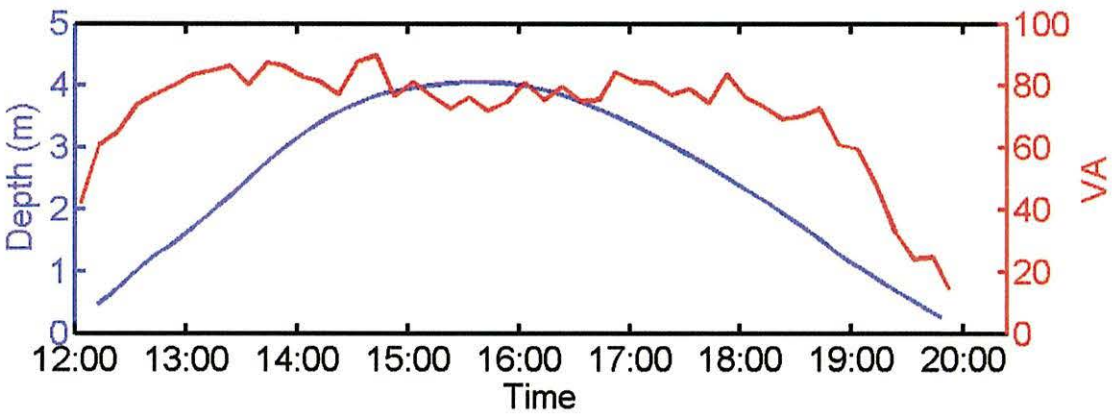


Figure 6. 4: Mean percentage valve gape aperture (red line) and the water depth (blue line) on the 17th July.

Clearance rates

The mean clearance rate calculated from the defecation method on mussels from site 2 was high: 3.1 ± 1.7 L ind. h^{-1}, or 7.8 ± 3.7 L g^{-1} dry weight h^{-1}.

The potential population clearance rate (CR) was calculated using the CR obtained from the defecation method (Table 6. 2). The percentage of the substratum covered by mussels on site 2 was 63 ± 6% while on site 3 it was much lower at 40 ± 12%. Therefore, the adjusted volume of water filtered from the actual mussel population (63 and 40 %, in site 2 and 3 respectively) was from 28.39 to 55.90 m^3 m^{-2} d^{-1} (Table 6. 2).

Table 6. 2: Estimated volume of water filtered by the mussel populations in site 2 and 3 expressed as a function of population size (assuming same clearance rate for both sites) or population dry weight.

	Site 2	Site 3
Density (ind. m^{-2}) 100% mussel cover	1187	947
Percentage cover by mussels	63.3	40.3
Density (ind. m^{-2}) mussel bed	751.4	381.6
Area bed (m^2)	58000	46000
Area covered by mussels (m^2)	36714	18538
Dry weight (g)	0.210	0.268
Population (number of individuals)	$43.580\ 10^6$	$17.555\ 10^6$
Population dry weight (g)	$9.152\ 10^6$	$4.705\ 10^6$
CR (L ind^{-1} h^{-1})	3.1	3.1
CR (L g^{-1} dw h^{-1})	7.8	7.8
Mean volume filtered (m^3 m^{-2} d^{-1})	55.90	28.39
Volume filtered (m^3 m^{-2} d^{-1}) 100% cover	88.31	70.45

6.5.2 Seston concentration in the water column

In all the experiments that used the siphon mimics to measure seston concentration (chl *a*) close to the bed, the presence of mussels was found to induce a significant food (chl *a*) depletion in the water column near the mussel bed (< 11 cm) in comparison to the bare sandy substratum or the bare patches within a mussel bed (Table 6. 3). Above the bare patches and sandy substratum, the water column had a statistically significant homogeneous chl *a* concentration from the bottom up to the measured height above the seabed (Table 6. 3). The horizontal chl *a* profile (5 stations) measured from the middle of a mussel patch to the middle of an adjacent bare patch gave a statistically significant chl *a* gradient such that chl *a* from the mussel patch was lower than the chl *a* from the bare patch of sediments (Table 6. 3). These results are detailed in the following section.

Table 6. 3: Summary of the results from the siphon mimic measurements for vertical and horizontal (horiz.) profiles above 3 sites (1: control, 2: young mussel bed, 3: old mussel bed). The stations of the horizontal profile were separated by ~0.5 m; Mm: middle of mussel patch, Me: edge of mussel patch, Im/e: interface between mussel and bare patch, Be: edge of bare patch, Bm: middle of bare patch. Chl *a* refers to the Table where the chl *a* concentration within the water column is detailed. N: no depletion, Y: depletion.

Experiment, scale & profile	Sites & stations	Depletion Height (cm)	Chl *a* table
Large scale vertical	Site 1 control sand	N	Table 6. 4a
~500 m	vs Site 2 mussel	Y (0.5-11) < (25-80)	Table 6. 4a
Small scale vertical	Site 2 bare patch	N	Table 6. 5
~5 m Site 2	vs Site 2 mussel	Y (0.5-6) < (11-80)	Table 6. 5
Small scale vertical	Site 3 bare patch	N	Table 6. 7a
~5 m Site 3	Vs Site 3 mussel	Y (0.5-5) < (11-25)	Table 6. 7a
Small scale horiz. ~0.5 m	3: Mm, Me, Im/e, Be, Bm	(Mm,e,Im/e) < (Bm,e)	Table 6. 7b

Large scale vertical processes

Vertical chl *a* profiles were measured above a mussel patch (site 2) and compared with the vertical profiles measured above a sandy control area with no mussels. Figure 6. 5 shows the chl *a* concentration obtained from the siphon mmimics measured at six heights above sandy site 1 and mussel site 2 on the 15th July and 17th July. The mean vertical chl *a* concentration above both the sandy and mussel sites followed the general pattern seen in Figure 6. 2 with low concentrations of chl *a* on the rising tide increasing with depth and later decreasing when the tide decreased (Figure 6. 5). On both days the mean chl *a* concentrations were 2.9 ± 0.9 and 2.5 ± 0.6 μg L^{-1} over the sandy area while on the mussel bed the concentrations were 1.6 ± 0.5 and 1.7 ± 0.6 μg L^{-1} respectively. All means were similar to the estimated medians (Table 6. 4a). The standardized data to residuals from their respective means were approximated to a normal distribution for the 15th July (a^2 = 0.385, p = 0.390) but not for the 17/ 7 (a^2 = 01.386, p < 0.001), nevertheless, more than 75% of the data were normally distributed. There was a significant difference between the chl *a* concentrations above the sandy site (site 1) and the mussel bed (site 2). The measured error variance was small (SD = 0.1 μg L^{-1} on 15th July and SD = 0.2 μg L^{-1}

on 17th July for any mean chl *a* concentrations) (Table 6. 4b). The greatest source of variability for both days was site (15th July $F_{1,201} = 1448.94$, $p < 0.001$ and 17th July $F_{1,182} = 776.11$, $p < 0.001$). On the 15th July, over the mussel bed, the vertical profile showed that the mean chl *a* at 0.5 cm above the seabed was significantly lower than above 11 cm (Table 6. 4a) and 80 cm was significantly higher compared to all the other heights except for 25 cm (Table 6. 5a). In contrast, over the sandy bed the vertical profile had a homogenous mean chl *a* concentration, there was no significant difference between the mean concentration with height except for 3 cm and 80 cm (Table 6. 5a). In terms of timing, for both the sandy site and mussel site, the profiles were similar for three consecutive sampling occasions (13:14, 14:02, 14:49; $p > 0.05$) then, once the current direction changed, there was a significant decrease in chl *a* concentration (15:30 to 16:40, $p < 0.001$) when the water depth decreased at the end of the tide (Table 6. 5b and Figure 6. 5).

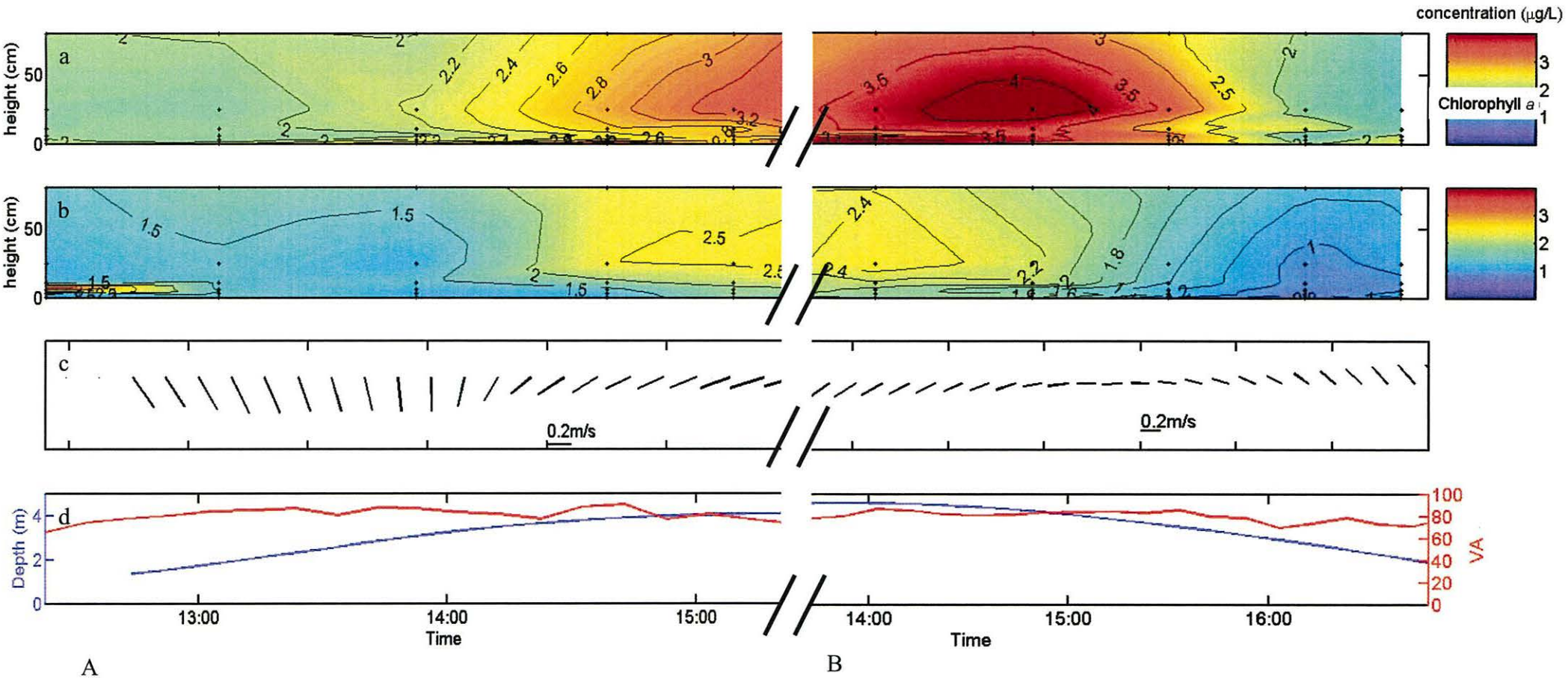


Figure 6. 5: Vertical profile of chlorophyll *a* concentration (µg L^{-1}) variation with time and height above the sandy area site 1 (a) and the mussel bed patch site 2 (b). c) Depth mean current velocity and direction (m s^{-1}) from ADCP1 represented by vectors over Site 2. d) Mean percentage valve gape aperture (red line) and water depth (blue line). The data on the left (A) were measured on rising tide on the 17th July while the data on the right (B) were measured on falling tide on the 15th July.

Similar features were observed on the 17th July, when measurements were obtained as the tide rose over the intertidal area. Chl *a* over the mussel bed was lower next to the bed and higher on the top of the water column, while the reverse was found for the sandy site (possibly due to sedimentation and sinking of phytoplankton) (Table 6. 4a & b, Table 6. 5a and Figure 6. 5). On the 17th July, the chl *a* concentration in the water column increased with the rising tide and the tide turning from northwest-southeast to northeast-southwest (Figure 6. 5). Chl *a* concentration was significantly lower at the beginning of the flood tide (12:23 – 13:52) and then during the rising tide (14:39 – 15:34) on both the mussel bed and sandy sites (Table 6. 5b).

Table 6. 4: The results and analysis of variance of chl *a* concentration (μg L^{-1}) obtained using the siphon mimics positioned on either the sandy area (site 1) or the mussel bed (site 2) measured at 6 different heights above the seabed on the 15th July and 17th July 2006.

a) Means, median (Med.), and standard deviations (SD) of chl *a* concentrations.

Height	Sand 15th July			Mussel 15th July			Sand 17th July			Mussel 17th July		
(cm)	Mean	Med.	SD	Mean	Med.	SD	Mean	Med.	SD	Mean	Med.	SD
0.5	3.1	3.0	0.9	1.4	1.6	0.5	2.7	2.5	0.7	1.4	1.4	0.3
3	3.1	3.0	1.0	1.5	1.5	0.4	2.5	2.5	0.6	1.4	1.5	0.4
6	2.8	3.0	0.7	1.4	1.4	0.5	2.3	2.1	0.4	1.9	1.7	0.8
11	2.7	2.7	0.7	1.5	1.5	0.5	2.5	2.2	0.5	1.7	1.5	0.5
25	3.0	3.2	1.0	1.7	1.5	0.6	2.6	2.5	0.7	1.9	1.5	0.7
80	2.6	2.7	0.6	1.8	1.7	0.5	2.4	2.1	0.6	2.0	2.1	0.5
mean	2.9	3.0	0.9	1.6	1.5	0.5	2.5	2.3	0.6	1.7	1.5	0.6
n	93			109			84			101		

b) Analysis of variance table, ** $p < 0.001$.

	15th July					17th July				
Source	df	Seq SS	Adj SS	Adj MS	F	df	Seq SS	Adj SS	Adj MS	F
Time (T)	6	3.25	2.98	0.50	198.36**	5	28.20	28.10	5.62	163.97**
Height (H)	5	0.08	0.07	0.01	5.70**	5	2.32	1.84	0.37	10.73**
Site (S)	1	3.81	3.62	3.62	1448.94**	1	28.79	26.60	26.60	776.11**
T*H	30	0.25	0.25	0.01	3.27**	25	8.75	10.65	0.43	12.43**
T*S	6	0.14	0.14	0.02	9.22**	5	3.67	4.21	0.84	24.55**
H*S	5	0.18	0.18	0.04	14.02**	5	5.01	4.70	0.94	27.43**
T*H*S	30	0.21	0.21	0.01	2.78**	25	9.22	9.22	0.37	10.76**
Error	119	0.30	0.30	0.00		112	3.84	3.84	0.03	163.97**
Total	202	8.23				183	89.80			

Table 6. 5: a) Multiple comparisons between mean chl *a* concentration using Bonferroni's method. All differences given in µg L^{-1} minimum difference required for significance at a) in function of height 5% level = 0.05 µg L^{-1} on 15th July and =0.28 µg L^{-1} on17th July (95% CI of the differences). b) in function of time 5% level = 0.06 µg L^{-1} on 15th July and = 0.26 µg L^{-1} on 17th July (95% CI of the differences). Bold data are for the 15th July, normal font data are for 17th

a) Height

cm	0.5	3	6	11	25	80
0.5 s		**0.01**	**-0.03**	**-0.01**	**0.01**	**-0.06**
0.5 m		**0.04**	**0.02**	**0.06***	**0.08****	**0.13****
3 s	-0.24		**-0.04**	**-0.02**	**-0.01**	**-0.06***
3 m	-0.01		**-0.02**	**0.01**	**0.04**	**0.09****
6 s	-0.46**	-0.23		**0.02**	**0.03**	**-0.02**
6 m	0.48**	0.49**		**0.04**	**0.07****	**0.12****
11 s	-0.29**	-0.05	0.17		**0.01**	**-0.04**
11 m	0.30**	0.31**	-0.18		**0.03**	**0.07***
25 s	-0.18	0.06	0.29*	0.11		**-0.05**
25 m	0.49**	0.50**	0.01	0.19		**0.05**
80 s	-0.25*	-0.01	0.21	0.04	-0.07	
80 m	0.59**	0.60**	0.10	0.29**	0.1	

b)

		13:14	**14:02**	**14:49**	**15:30**	**16:11**	**16:40**	**Time 15/7**
		-0.03	**-0.10****	**-0.10****	**-0.02**	**0.19****	**0.18****	**12:25 s**
		-0.11**	**-0.13****	**-0.11****	**0.07***	**0.25****	**0.17****	**12:25 m**
13:05 s	0.04		**-0.07***	**-0.07***	**0.01**	**0.21****	**0.21****	**13:14 s**
13:055 m	0.13		**-0.03**	**0.01**	**0.18****	**0.35****	**0.28****	**13:14 m**
13:52 s	-0.21	0.25		**0.01**	**0.08****	**0.29****	**0.28****	**14:02 s**
13:52 m	0.33**	0.19		**0.03**	**0.21****	**0.39****	**0.31****	**14:02 m**
14:39 s	-0.85**	-0.88**	-0.63**		**0.08***	**0.29****	**0.28****	**14:49 s**
14:39 m	-0.16	-0.29**	-0.49**		**0.17****	**0.35****	**0.28****	**14:49 m**
15:09 s	0.87**	-0.91**	-0.66**	-0.02		**0.20****	**0.19****	**15:30 s**
15:09 m	-0.52**	-0.65**	-0.85**	-0.36**		**0.18****	**0.10****	**15:30 m**
15:34 s	1.35**	-1.40**	-1.15**	-0.51**	-0.49**		**-0.01**	**16:11 s**
15:34 m	-0.50**	-0.63**	-0.83**	-0.34**	0.02		**-0.07***	**16:11 m**
Time 17/7	12:23	13:05	13:52	14:39	15:09			

* = significant at 5%, ** = significant at 1%. Columns means are subtracted from rows mean, s for sandy bed, m for mussel bed. July.

Small scale processes

Vertical profiles of chl *a* were measured on a small scale (< 5 m) above the mussel patch (site 2) and an adjacent bare patch (site 2). In the previous section the results demonstrated that mussels were responsible for food depletion in the boundary layer at a large scale. The following experiment tested to see if it was possible to detect the effects of mussel feeding at the scale of a patch of mussels. The mussel bed from site 2 was used for this experiment (Figure 6. 6) and such that measurements of chl *a* made over the mussel patch were compared with those obtained over an adjacent patch of bare substratum in the middle of a mussel bed. The standardized data to residuals from their respective means demonstrated that the observations approximated to normality ($a^2 = 0.663$, $p = 0.082$). The mean chl *a* concentrations above the bare patch, 1.7 ± 0.4 μg L^{-1} was similar to the concentration above the mussel patch, 1.5 ± 0.4 μg L^{-1} and all means were similar to the median (Table 6. 6).

Table 6. 6: The results of mean, median and standard deviation (SD) of chl *a* concentration (μg L^{-1}) measured using the siphon mimics positioned on a bare patch of substrateum adjacent to a mussel patch (site 2) measured at 6 different heights above the seabed on the 18th July.

	Bare patch			Mussel patch		
Height above the bed (cm)	Mean	Median	SD	Mean	Median	SD
0.5	1.8	1.6	0.4	1.2	1.3	0.4
3	1.8	1.6	0.5	1.4	1.6	0.4
6	1.8	1.7	0.5	1.5	1.3	0.4
11	1.6	1.5	0.4	1.6	1.4	0.7
25	1.7	1.6	0.4	1.7	1.4	0.7
80	1.5	1.4	0.5	1.7	1.7	0.5
Mean (n = 90)	1.7	1.6	0.4	1.5	1.4	0.6

Nevertheless, there was a significant difference between the chl a concentration above the bare patch and the mussel patch, the result was analysed by a crossed analysis of variance using time, height and site as variables (Table 6. 7a). The greatest source of variability was time (ANOVA, $F_{4,175} = 100.71$, $p < 0.001$). Above

the mussel patch the chl *a* concentration measured between 0.5 to 6 cm was significantly lower than in the upper column (11 – 80 cm) (Table 6. 6 & 6. 7b). In contrast there was no difference in chl *a* concentration in the water column above the bare patch (Table 6. 6 & 6. 7b). As observed previously, there was a general significant increase in the chl *a* concentration advected from the rising tide for both the mussel patch and bare patch substratum (Table 6. 7b, Figure 6. 6).

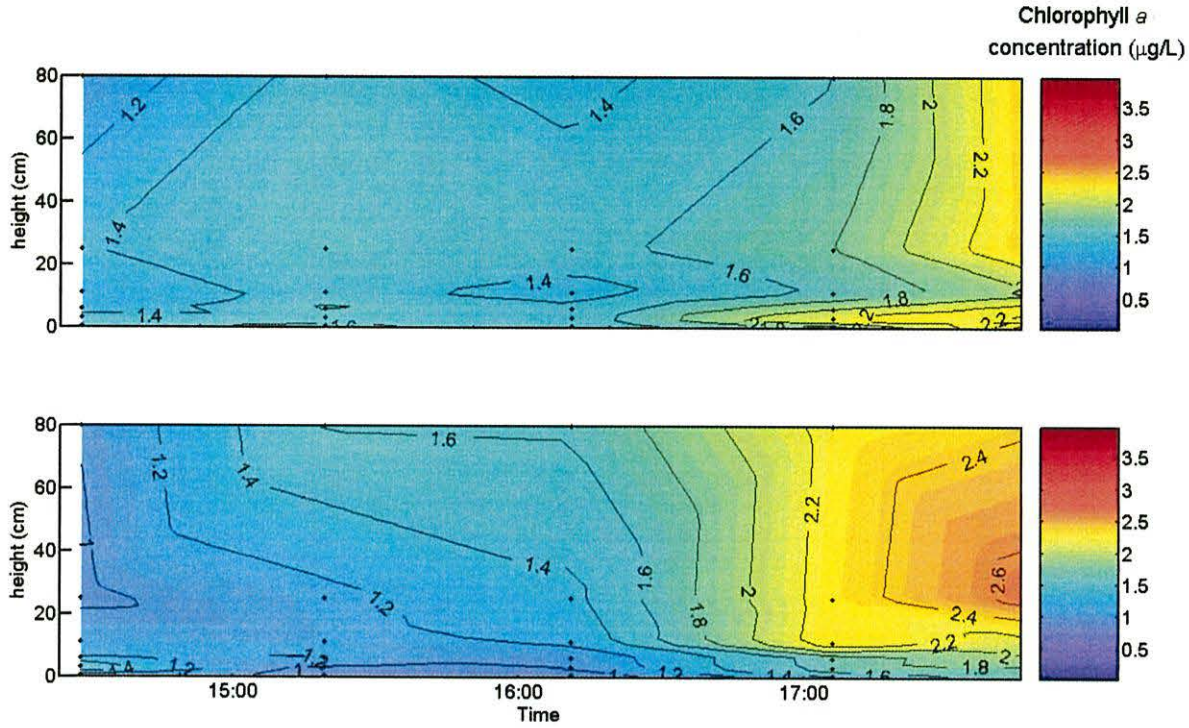


Figure 6. 6: Vertical profile of chlorophyll *a* (chl *a*) concentration (µg L^{-1}) variation with time and height above the sandy area site 1 (a) and the mussel bed patch site 2 (b).

Table 6. 7: Chl *a* concentration (μg L^{-1}) for the siphon mimics positioned on a bare patch adjacent to a mussel patch (site 2) measured at 6 different height on the 18th July.a) Analysis of variance table. b) Multiple comparisons between mean chl *a* concentration using a Tukey's multiple comparison test. All differences given in μg L^{-1} minimum difference required for significance at 5% level = 0.08 μg L^{-1} for height and = 0.07 μg L^{-1} for time (95% CI of the differences). Columns means are subtracted from rows mean, s for sandy bed, m for mussel bed. Bold font indicate MCTs the time, normal fonts are the MCTs for height.

a)	df	Seq SS	Adj SS	Adj MS	F
Time (T)	4	1.75	1.74	0.43	100.71**
Height (H)	5	0.05	0.06	0.01	2.56*
Site (S)	1	0.17	0.17	0.17	38.82**
T*H	20	0.29	0.29	0.01	3.39**
T*S	4	0.13	0.13	0.03	7.61**
H*S	5	0.24	0.23	0.05	10.47**
T*H*S	20	0.15	0.15	0.01	1.77*
Error	120	0.52	0.52	0.01	
Total	179	3.31			

* = significant at 5%, ** = significant at 1%

b)		**14:19**	**15:11**	**16:06**	**16:50**	**Time**
		0.07	**0.03**	**0.13****	**0.23**	**13:28 s**
3 s	-0.1	**-0.01**	**-0.01**	**0.22****	**0.26****	**13:28 m**
3 m	0.07		**-0.03**	**0.06**	**0.17****	**14:19 s**
6 s	-0.02	-0.01	**0.01**	**0.23****	**0.27****	**14:19 m**
6 m	0.07	0.001		**0.10****	**0.20****	**15:11 s**
11 s	-0.06	-0.05	-0.05	**0.22****	**0.26****	**15:11 m**
11 m	0.11*	0.04	0.04		**0.10****	**16:06 s**
25 s	-0.01	-0.01	0.01	0.05	**0.04**	**16:06 m**
25 m	0.12*	0.05	0.05	0.01		
80 s	-0.06	-0.05	0.04	-0.01	-0.05	
80 m	0.16**	0.09*	0.09*	0.05	0.04	
Height (cm)	0.5	3	6	11	25	

Small scale - horizontal:

Horizontal profiles of chl *a* were measured using siphon mimics positioned at 5 cm above the seabed between the middle of a mussel patch and the middle of an adjacent bare patch substratum in site 3. Five sampling positions were chosen: at the middle and edge of the mussel patch, at the interface between the mussel and bare patch substratum and at the edge and the middle of the bare patch substratum. In this experiment, these horizontal measurements were coupled to vertical measurements at the middle of the mussel and bare substratum patches to give a better definition of the food gradient between the mussel patch and adjacent bare sediment. Once again, in the vertical plane, there was a significant depletion of chl *a* in the boundary layer above the mussels when compared to the adjacent bare patch of sediment. In comparison, no significant depletion occurred over the bare sediment resulting in a statistically significantly constant chl *a* concentration in the water column (0 – 25 cm) (Figure 6. 7; Table 6. 8 & Table 6. 9a & b). From 1 to 5 cm above the surface of the seabed, food concentration was significantly lower than higher up in the water column when above the mussel patch (Table 6. 8 & Table 6. 9b). The chl *a* concentration at a height of 25 cm above the seabed was not significantly different between the bare and mussel patch (mean difference = -0.34; $p = 1$).

Table 6. 8: Mean, median (Med) and standard deviation (SD) of the chl *a* concentration ($\mu g\ L^{-1}$) sampled via siphon mimics positioned a) on two vertical transect on a bare patch adjacent to a mussel patch (site 2) measured at 5 different heights on the 19th July. b) on a horizontal transect between the middle of the mussel patch (mm) to the middle of the bare patch (bm) with three intermediate siphons at the edge of the mussel patch (me) the interface between the mussel and the bare patch (im/b) and at the edge of the bare patch (be).

a)

Height	Bare patch			Mussel patch		
(cm)	Mean	Med.	SD	Mean	Med.	SD
1	1.5	1.5	0.3	0.6	0.6	0.3
3	1.5	1.5	0.2	0.9	0.9	0.3
5	1.5	1.5	0.2	0.9	0.9	0.4
11	1.5	1.5	0.3	1.2	1.3	0.3
25	1.4	1.4	0.3	1.5	1.5	0.3
mean	1.5	1.5	0.3	1.0	1.0	0.3
n	90			90		

b)

Station	Horizontal transect		
	Mean	Med.	SD
mm	0.9	0.9	0.3
me	1.1	1.0	0.3
im/b	1.1	1.1	0.3
be	1.3	1.3	0.3
bm	1.5	1.5	0.2

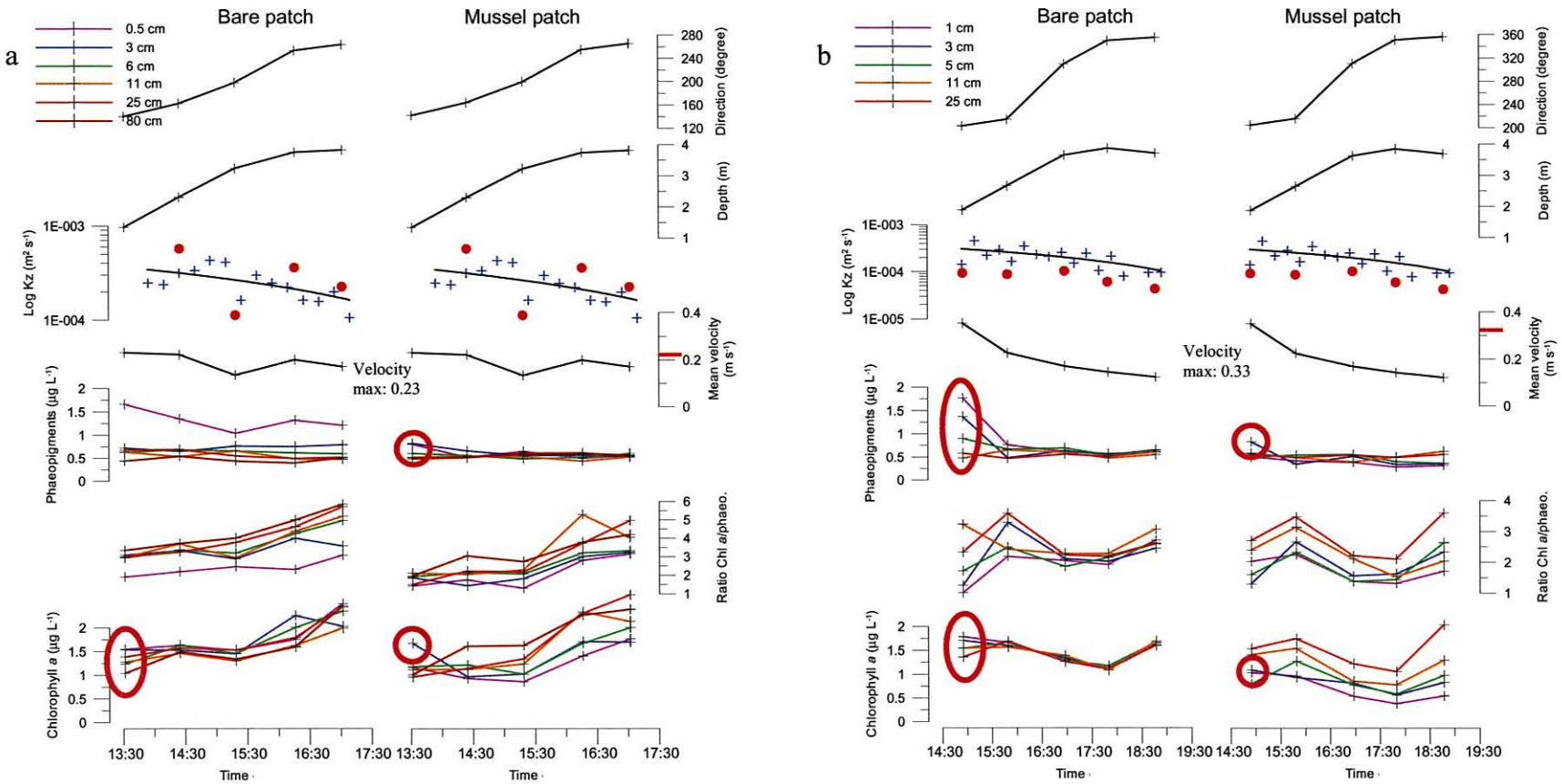


Figure 6. 7: Summary of measurements made above a bare and adjacent mussel patch on site 2 (18th July) and site 3 (19th July). Velocity direction (degree), depth (m), diffusion coefficient K_z ($m^2 s^{-1}$) on logarithmic scale estimated from the ADCP (blue cross with fitted line: site 2 $Y = -0.001456X + 0.289$ $r^2 = 0.48$; site 3 $Y = -0.001265X + 0.24$, $r^2 = 0.371$), and calculated from the animals in red solid circle, mean bin1 velocity at 49 cm above the bed ($m s^{-1}$). The phaeopigments concentration ($\mu g L^{-1}$), chl *a* concentration ($\mu g L^{-1}$) and ratio chl *a*/phaeopigments measured at five or six different heights above the bare patch and mussel patch simultaneously are represented with different colours (pink at 0.5 or 1cm, blue at 3 cm, green at 5 or 6 cm, orange at 11 cm, red at 25 cm, brown at 80 cm). Red circles indicate the re-suspension. A chl *a* gradient is visible above the mussel patch but not in the bare patch. Tidal advection is bringing fresh water after 16:06 on 18th and 17:36 on 19th July.

The data obtained from the horizontal gradient using the five siphon mimics positioned to sample water at 5 cm above the seabed showed a gradient between the middle of the mussel bed and the bare patch with an increase in concentration from 0.9 ± 0.3 μg L^{-1} to 1.5 ± 0.2 μg L^{-1} (Table 6. 8, Figure 6. 8). The standardized data to residuals from their respective means demonstrated that the observations approximated to normality (squared transformed data: $a^2 = 0.370$, $p = 0.415$) with homogeneity of variance between the mussel and bare patch chl *a* concentration (Barlett's: F = 1.50, $p = 0.827$). There was a significant difference between the chl *a* concentrations above the bare patch and the mussel patch (Table 6. 10a). The chl *a* from the middle of the mussel (*mm*) patch up to the interface between mussel and bare patch (*im/b*) were similar, and significantly different from the concentration at the edge and middle of the bare patch (*be* and *bm*), which were not significantly different (Table 6. 10b).

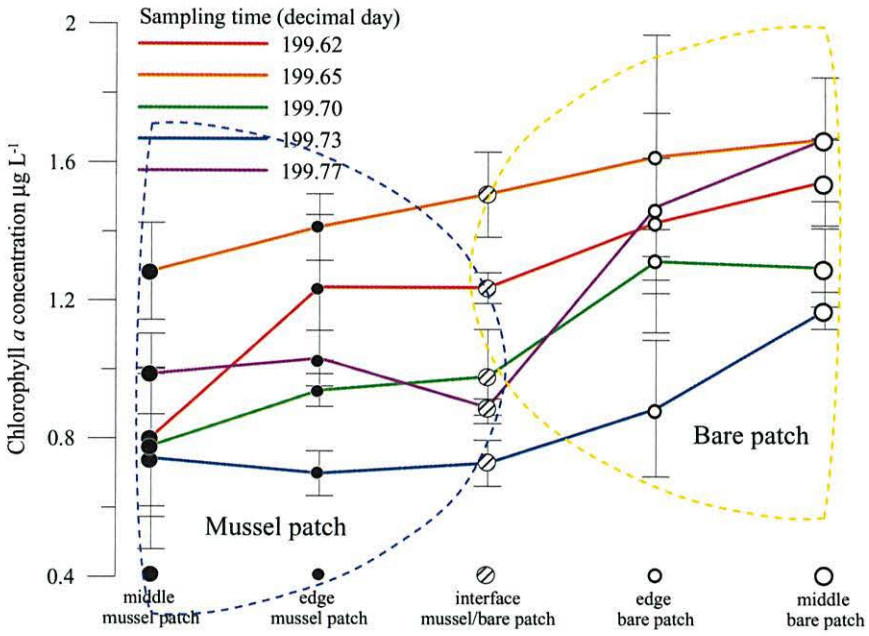


Figure 6. 8: a) Time series of horizontal chl *a* concentration (± SDev.) measured at 5 cm above the bed on 5 locations along a transect from the middle of a mussel patch to the middle of the adjacent bare patch on the 19th July, site 3.

Table 6. 9: Chl *a* concentration (μg L^{-1}) for the siphon mimics positioned on a bare patch adjacent to a mussel patch (site 3) measured at 5 different height on the 19th July. a) Analysis of variance. b) Multiple comparisons between mean chl *a* concentration using Bonferroni's method. All differences given in μg l^{-1} minimum difference required for significance at 5% level = 0.64 μg L^{-1} for (95% CI of the differences). Columns means are subtracted from rows mean, s for sandy bed, m for mussel bed.

a)

Source	df	Seq SS	Adj SS	Adj MS	F
Time (T)	4	45.13	38.94	9.74	34.94**
Height (H)	4	8.47	11.41	2.85	10.24*
Site (S)	1	29.17	30.81	30.81	110.57**
T*H	16	5.22	6.43	0.40	1.44 (p=0.141)
T*S	4	1.02	0.97	0.24	0.87 (p=0.487)
H*S	4	17.91	18.43	4.61	16.53**
T*H*S	16	5.15	5.15	0.32	1.16 (p=0.320)
Error	87	24.24	24.24	0.28	
Total	136	136.31			

b)

Height (cm)	1s	1m	3s	3m	5s	5m	11s	11m
3	-0.09	0.3	-0.12	0.36	-0.12	1.04**	-0.27	1.86**
5			-0.03	0.06	-0.03	0.73**	-0.18	1.56**
11					-0.01	0.67*	-0.15	1.50**
25							-0.14	0.82**

* = significant at 5%, ** = significant at 1%.

Table 6. 10: The result and analysis of the chl *a* concentration (μg L^{-1}) for siphon mimics (site 3) measured at 5 different horizontal distances between mussel patch and bare patch on the 19th July. a) Analysis of variance table. b) Multiple comparisons between mean chl *a* concentration using Bonferroni's method. All differences given in μg L^{-1} minimum difference required for significance at 5% level = 0.16 μg L^{-1} for (95% CI of the differences). Columns means are subtracted from rows mean, s for sandy bed, m for mussel bed.

a)

Source	df	Seq SS	Adj SS	Adj MS	F
Time	4	3.05	3.16	0.79	39.77**
Distance	4	2.87	2.79	0.70	35.03**
T*D	16	0.62	0.62	0.04	1.94*
Error	45	0.90	0.90	0.02	
Total	69	7.43			

b)

Distance (m)	0.5	1	1.5	2
1	0.14			
1.5	0.15	0.01		
2	0.42**	0.28**	0.28**	
2.5	0.55**	0.41**	0.40**	0.12

* = significant at 5%, ** = significant at 1%

6.5.3 Hydrodynamic component

The flow direction above the mussel bed was multidirectional (Figure 6. 2). The flow turned consecutively in three different directions: from the north/west – south/east direction when the tide was flooding, then with the turn of tide, the direction veered toward the south-west and eventually had a south-east/north-westerly direction (Figure 6. 2). The roving ADCP data revealed the spatial variations in the current direction over the entire intertidal bed and that similar types of current regimes occurred at the three sites chosen for the experiment.

Advection

The general pattern of chl *a* concentration pattern over the mussel bed followed water depth but more precisely the tidal advection of chl *a* depleted water derived from the Caernarfon Bay end of the Strait (at flood tide), followed by chl *a* rich water that entered from the Liverpool Bay on the ebb when the tide turned. When eventually, the water withdraws from the intertidal flats, the patch of phytoplankton that had been advected above the mussel bed, passes back across the mussel bed with chl *a* depleted water (Figures 6 .2 & 6. 5) (Saurel *et al.*, 2007). This pattern was as also discernable from the siphon mimic samples obtained over the mussel bed, the bare sediment patches and the control site (Figures 6. 6, 6. 7, 6. 8 & 6. 9).

Vertical mixing diffusion coefficient (K_z) and food replenishment

The mean vertical diffusivity (Figure 6. 9) estimated from the ADCP at 0.05 m above the mussel bed was 2.03 $10^{-4} \pm 1.03\ 10^{-4}$ $m^2\ s^{-1}$ for the first M_1 and 2.253 $10^{-4} \pm$ 8.3 10^{-5} $m^2\ s^{-1}$ for the second M_1 of the first deployment. K_z decreased with time from the low tide (Figure 6. 9).

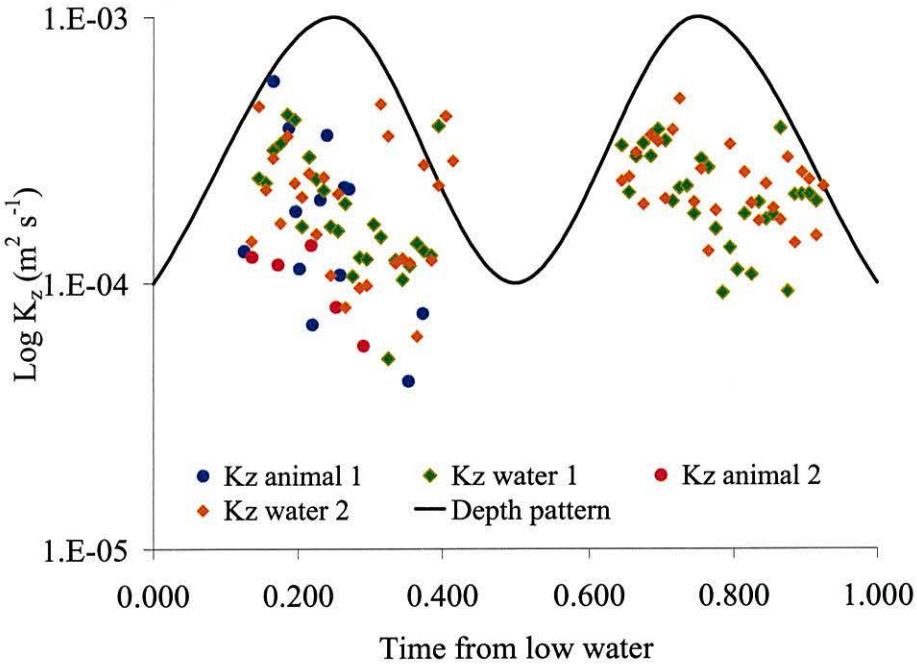


Figure 6. 9: Diffusion coefficient K_z ($m^2\ s^{-1}$) on logarithmic scale estimated from the ADCP (diamond green first deployment site 2, orange second deployment site 3) and calculated via the animals (solid circles, blue first deployment site 2 and red second deployment site 3) with time calculated from low water. Depth pattern above the bed is represented by a solid black line.

Resuspension

Vertical profiles of chl *a* and phaeopigments measured using the siphon mimics over a mussel and a bare patch suggested that resuspension occurred at the beginning of the flood when the current velocity was the strongest and only below 5 cm above the bottom. On the 18th July (13:28), there was significantly more chl *a* at a height of 3 cm above the mussel bed than at 11, 25 and 80 cm ($F_{5,17} = 5.16$, $p < 0.009$), also there was a gradient in phaeopigment close to the mussel bed with (0.5 < 3) > 6 > (11 = 25 = 80) (ANOVA $F_{5,15} = 74.33$, $p < 0.001$) (Figure 6. 7). On the 19th July (site 3) on the first sampling occasion at 14:48 GMT, which is 1.7 h after the beginning of

the flood over the intertidal area, some resuspension was measured. At this sampling time, the current velocity was the strongest (u = 0.35 m s^{-1}, direction 204°) and there was a significantly higher concentration of phaeopigments at 1 and 3 cm above the seabed in comparison to the rest of the column over the bare patch (ANOVA $F_{4,14} = 26.89$, $p < 0.001$). Similarly, at 3 cm height above the mussel patch the phaeopigment concentration was higher than on the other heights (ANOVA $F_{4,13} = 8.32$, $p < 0.001$). In contrary, chl *a* was not significantly different in the water column over the bare patch (ANOVA $F_{4,14} = 0.77$, $p = 0.567$) while it was significantly lower at 5 cm than all the other height above the mussel patch (ANOVA $F_{4,13} = 4.42$, $p = 0.030$) (Figure 6. 7). During the following sampling times (from 2.1 h after low tide), the current velocity decreased (0.12 to 0.23 m s^{-1}) and the phaeopigment profiles were homogeneous and close to the concentration measured above the mussel patch (Figure 6. 7). In contrast, two sampling time (19th July 15:40 and 18:31) exerted peaks in the ratio chl *a*/phaeopigments than the others (Figure 6. 7, Table 6. 11). This change in food composition could be attributed to i) at time 15:40 vertical mixing was taking over resuspension bringing more new food from the water column down to the bed, ii) at time 18:31, the advection of fresh water changed the water composition.

The use of phaeopigment or the ratio of chl *a*/phaeopigments obtained from the horizontal profiles did not indicate that resuspension had occurred at 5 cm above the seabed. There was no significant difference in the chl *a*/phaeopigment ratio between the stations (deployed between the bare and mussel patch) and between the stations sampled at different times (Table 6. 11).

In Figure 6. 7, the red circles indicate when resuspension of chl *a* or phaeopigments occurred; over the mussel and adjacent bare patch. The results from suggest that resuspended material provided more food in the boundary layer than could be filtered by the mussels. At a height of 1 and 3 cm above the seabed (t = 14:48) no vertical depletion was measured, only the chl *a* at 5 cm was lower than that at 25 cm (ANOVA $F_{4,13} = 4.42$, $p = 0.030$). In contrast, the vertical gradient above the mussel patch was established during the following sampling period on the 19th July at t = 15:40 and was statistically significant (ANOVA $F_{4,14} = 10.32$, $p < 0.001$, (1 cm = 3 cm = 5 cm); (1, 3) < (11, 25), (5 cm = 11 cm = 25 cm)). The

phaeopigments during the following sampling period at t = 14:48 were also significantly higher at 3 cm above the seabed than the other height ($F_{4,13} = 8.32, p < 0.001$). In the following sampling period (t = 15:40) no significant difference in phaeopigment concentrations was found between the 5 heights measured (ANOVA $F_{4,14} = 1.59, p = 0.251$).

Table 6. 11: The chl *a*/phaeopigment ratio (site 3) measured from siphon mimics at five different horizontal distances between mussel patch and bare patch on the 19th July. a) Analysis of variance table. b) Multiple comparisons between mean chl *a* concentration using Bonferroni's method. All differences are given in µg L^{-1} minimum difference required for significance at 5% level = 0.55 for (95% CI of the differences). Columns means are subtracted from rows mean.

a)

Source	df	Seq SS	Adj SS	Adj MS	F
Time (T)	4	13.05	13.76	3.44	15.38**
Distance (D)	4	2.04	1.90	0.47	2.12 (p = 0.094)
T*D	16	6.31	6.31	0.40	1.76 (p = 0.068)
Error	45	10.06	10.06	0.22	
Total	69	31.46			

b)

Time	15:40	16:46	17:36	18:31
14:48	1.2**	0.11	0.15	0.68*
15:40		-1.09**	-1.05**	-0.53
16:46			0.04	0.56*
17:36				0.52

* = significant at 5%, ** = significant at 1%

6.5.4 Interaction between the three components: hydrodynamic/seston/mussel

Combining all the results suggest that there was a succession of balanced phases during the tide that were dictated by hydrodynamic changes (Table 6. 12). When the water was flooding (Phase 1) with recycled water from the north-western end of the Menai Strait, velocity and vertical mixing were at their highest values, and resuspension was the main supplier of food with an increase in phaeopigment concentration near to the bed. In response, the mussels opened their valves rapidly and began filter-feeding (< 1 h to pass from 40 to 90%). After the flood (phase 2),

water velocity decreased and the main supply of food was through vertical mixing. Food concentration was still low, but the mussels were wide open. In phase 3, before high tide when the current direction turned and vertical mixing was still the main agent of food supply, the food concentration in the water column started to rise due to the advection of chl *a* rich water from the Liverpool Bay. At high tide (phase 4) advection input a fresh body of water, vertical diffusion was still strong, and the velocity was low. Eventually when the water level reduced, the current direction turned, recycled (depleted) water was advected above the bed and vertical mixing reduced, slowly the mussels started closing. This succession of phase is well illustrated in Figure 6. 5.

Table 6. 12: Summary of the hydrodynamics, food concentration and composition, and mussel feeding behaviour in function of the arbitrary determined phases of the tide (Tide decomposed in 5 phases). Adv. is advection, HT is high tide, VM is vertical mixing, RW is recycled water, FW is fresh water.

	Phase 1 Flooding	Phase 2 After flood	Phase 3 Before HT	Phase 4 High Tide	Phase 5 Ebbing
Hydrodynamics = Main food supplier	-Resuspension -VM high	VM high	- VM - Adv.	- Adv. - VM	- Adv. - VM reduces
Velocity	High	medium	Low	Low	medium
Current direction	NW	NW	Turn NE	NE	Turn SE
Food (chl *a*) concentration	Low (RW)	Low (RW)	Rising (FW)	High (FW)	Decreasing (RW)
Phaeopigments	High *	reduce	Low constant	Low constant	Low constant
Feeding behaviour	Fast opening	Wide open	Wide open	Wide open	Slow closing

* Only near the bed (<11 cm)

A modified model of the concentration boundary layer which develops over a mussel bed with a bare patch in the middle in a unidirectional flow (adapted from Wildish and Kristmanson, 1997) is presented in Figure 6. 10. In this model, the water advected above a mussel bed is depleted of seston in the boundary layer. Once this body of water encounters a bare patch, vertical mixing leads to food replenishment of the near-bed water column. Therefore, the same body of water is richer in food when

it encounter the next mussel patch. Simple calculations can illustrate this model: the bare patches used during the measurements taken with siphon mimics were ~5 m wide (circular shape), and the food depletion was mainly localised in the water column from the bed to a height of 11 cm. The time necessary to mix this near-bed zone is $t_v = 0.32 \times 0.11^2 / (2.03\ 10^{-4}) = 19$ s. The time necessary to advect (with unidirectional flow) the water across this bare patch, with a mean current speed of 0.25 m s^{-1}, is $t_x = 5 / 0.25 = 20$ s. Therefore, there is time for mixing and food replenishment of the < 11 cm zone during the transition cross a bare patch where no mussel filtration occurs. Reciprocally, the vertical mixing calculated from mussel filtration was equal to the one measured via the ADCP (see paragraph below), which means that with the same velocity conditions, after 5 m of mussel bed length, the minimum chlorophyll *a* threshold (Figure 6. 10) would exceed the 11 cm height and vertical mixing would not compensate for the mussel filtration.

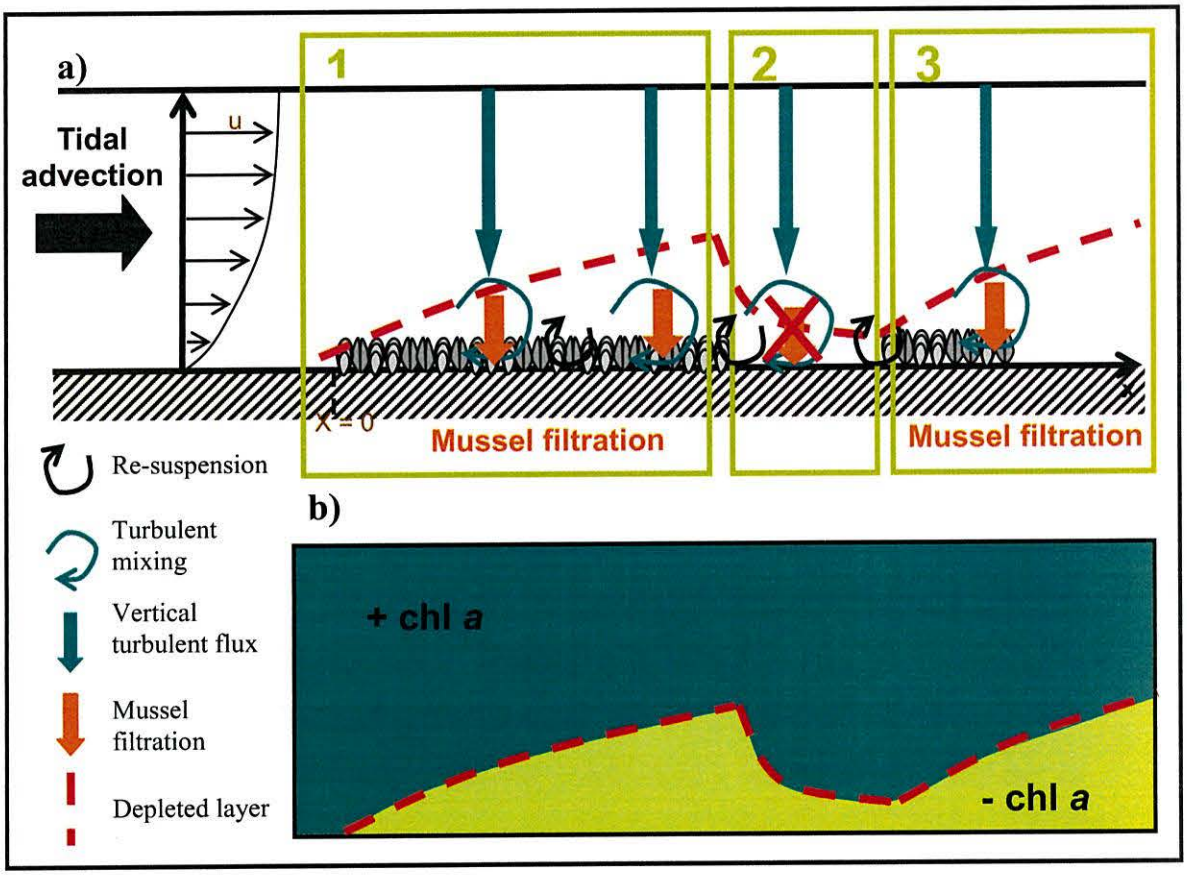


Figure 6. 10: a) The concentration boundary layer which develops over a mussel bed with a bare patch in the middle in a unidirectional flow: Z, water depth; X, mussel bed length; Co, initial seston concentration (adapted from Wildish and Kristmanson, 1997). 1) Classical model; 2) situation with bare patch within a mussel bed; 3) classical model after a bare patch. b) Simulated spatial pattern in chl *a* concentration above the seabed on both sides of the minimum chl *a* threshold line.

Following the concept of "ask the animal and ask the water" (Petersen, 2004a), the comparison of the amount of food (chl *a*) going to the bed and the amount of food filtered by the mussel was done using the coefficient of diffusion K_z estimated from the ADCP, the variation of chl *a* with height (dC/dz) and the filtration rate of the mussel (CR) (sites 2 and 3 in Figure 6. 10). Reciprocally, the diffusion coefficient K_z was calculated from the variation of chl *a* with height (dC/dz) and the grazing rate of the mussels and compared to the K_z estimated via ADCP. The results indicated that the two approaches gave similar results (for site 2 only Table 6. 13).

Table 6. 13: Comparisons of diffusion coefficient K_z and CR measured or calculated via ADCP, mussel and seston at site 2.

	Water	Animals
Measured (sampled)	From ADCP $K_z = 2.03\ 10^{-4} \pm 1.03\ 10^{-4}\ m^2\ s^{-1}$	From mussels and seston $CR = 3.10 \pm 1.65$ L ind h^{-1}
Calculated	From K_z ADCP and seston $CR = 2.62 \pm 2.09$ L ind h^{-1}	From mussels and seston $K_z = 2.08\ 10^{-4} \pm 1.52\ 10^{-4}\ m^2\ s^{-1}$

6.6 Discussion

The upper limit of carrying capacity for mussel production at the intertidal mussel bed (site 2) in the Menai Strait was reached in July 2006 as food depletion near the mussel bed occurred and mussel growth was found to be density dependent as would be expected under conditions of competition of food. This study focused on the feedback mechanisms between the three components of production carrying capacity (hydrodynamic, seston concentration and mussel growth/filtration/distribution) at a very small scale (< 10 m) and on bottom cultured mussel beds using either a recently re-laid (site 2) or a 1 year old commercial bed (site 3), produced new empirical data and a better understanding of the system. In the intertidal area of the Menai Strait, patterning within the mussel bed played an important role in the food supply to the mussel. The bare patches present within the mussel bed allowed seston replenishment due to vertical mixing and also increased the food source derived from resuspension of microphytobenthos resuspension adjacent to the mussels. At this

patch scale, a steady-state occurred with a balance between the three components (mussels/hydrodynamics/seston concentration). Obviously, this steady-state was adjusted temporally during the tide due to the succession of different food sources and hydrodynamic processes (resuspension, vertical mixing and advection Table 6. 12), but also most likely at other time scales (spring/neap cycles, seasons etc.). At a larger scale, the effect of mussel filtration on chl *a* was seen in a large a part of the Menai Strait with the creation of a food gradient along the mussel bed associated with tidal currents (Menai Strait, Tweddle *et al.*, 2005; Simpson *et al.*, 2007; Wadden Sea, van de Koppel *et al.*, 2005).

The intertidal mussel bed studied within the Menai Strait experienced food depletion due to mussel feeding on several tidal cycles. This food depletion was only present close to the mussel bed (height < 11 cm) and not in the adjacent bare patches (< 5 m distant) (Figures 6. 6, 6. 7, 6. 8). The mussels filtered at high rates during the entire tidal cycle (mean CR = 3.1 L h^{-1}). The clearance rate measured within the intertidal mussel bed were comparable to those measured in the laboratory under optimal conditions (Møhlenberg and Riisgård, 1978; Riisgård and Møhlenberg, 1979). Moreover, feeding behaviour expressed as valve aperture also indicated high filtration rates (VA > 70% Figure 6. 4) during the entire tide (Riisgård *et al.*, 2003; Riisgård *et al.*, 2006; Saurel *et al.*, 2007). These results indicate that the minimum chl *a* threshold (Figure 6. 10) at which filtration ceases was not attained over the intertidal mussel bed when covered by the tide. The total volume filtered by the population adjusted to the calculated mussel percentage cover (F_{pop}= 55.90 and 28.39 m^3 m^{-2} d^{-1} at 63% and 40% cover of mussels at sites 2 & 3 respectively) was comparable (but at the lower limit) to other systems that are micro and macro-tidally driven (Keterminde Denmark: 38 < F_{pop} < 166 m^3 m^{-2} d^{-1} Riisgård *et al.*, 2006; and Oosterchelde, Netherland 31.2 < F_{pop} < 170.4 m^3 m^{-2} d^{-1} Prins *et al.*, 1996).

The localised food depletion together with high mussel filtration rates were explained by the combination of 1) the advection of an external source of food 2) mussel bed morphology and patterning 3) hydrodynamic complexity (Figure 6. 10 and Table 6. 12). In the Menai Strait, the main source of seston is derived from the inundating of fresh sea water rich in seston from the Liverpool Bay (Gowen *et al.*, 2000, Saurel *et al.*, 2007), that is advected and flushed above the mussel beds, rather

than from a local primary production source (Saurel *et al.*, 2007; Simpson *et al.*, 2007; Tweddle *et al.*, 2005). This is due to the asymmetrical tidal flow in the Menai Strait resulting in a short water residence time (2-3 days) and a net flow from the north east (Liverpool Bay) to the south west (Simpson *et al.*, 1971, Campbell *et al.*, 1998, Rippeth *et al.*, 2002). Therefore, due to the net flow, recycled water that is depleted of food due to mussel filtration, occurred only at the beginning of flood tide, while on the ebb, fresh seawater from the Liverpool Bay rich in food flushed over the mussel bed.

The presence of bare patches in the middle of the mussel bed (Figure 6. 3) provided access to a food source close to the seabed via vertical mixing and via resuspension of microphytobenthos (Figure 6. 10). The resuspension was apparent only at the beginning of the flood tide (Figure 6. 7) when current velocity and vertical mixing were highest (Figure 6. 7). Unfortunately, data collection at the very beginning of the flood tide was not performed for logistic reasons. Nevertheless, the first seawater samplings from the siphon mimics were obtained < 1 h after the start of the flood, and the results indicated that the resuspension most likely occurred at the very beginning of the flood. These results corroborate those of Widdows *et al.*, (in prep.) who found that in the intertidal area of the Menai Strait, the current at the beginning of the flood was above the critical erosion threshold limit for resuspension of microphytobenthos to occur. Resupended benthic microphytobenthos are only likely to be important as a food source at the beginning of the flood tide, when the recycled flood-tide water arrives over the mussel bed. In some studies, a large fraction of the algal species found in mussel guts were benthic diatoms (Newell *et al.*, 1989; Newell and Shumway, 1993), while a foodweb analysis of a mudflat in France has demonstrated the importance of the primary production from the microphytobenthos (Leguerrier *et al.*, 2003). Nevertheless, as in other systems, resuspended material might not be a major food contributor for mussels overall (Fréchette and Grant, 1991) and might not be sufficient to compensate for low food concentration in the water column (Asmus and Asmus, 1993). From Figure 6. 7 it appears that resuspended material was rapidly used by the mussels and that the main supply of food was delivered via vertical mixing plus advection processes (Figures 6. 5, 6. 6, 6. 7 and 6. 8; Table 6. 12).

The occurrence of intraspecific competition for food due to food limitation and the steady-state of the system (the vertical flux of food balanced by animal filtration in the Menai Strait) as well as the robustness of the *in situ* methodologies used during the study were supported by applying the dual approach of "ask the animals, ask the water" (Petersen, 2004a) which provided similar results for the flux of food using two different techniques. The vertical profiles between mussel patches and adjacent bare patches, as well as the horizontal profiles of chl *a* measured at 5 cm above the bed, demonstrated that there was a gradient with less chl *a* in the middle of the mussel bed in comparison to the middle of an adjacent patch of bare substratum. In site 2, mussel growth was density dependent, with mussels growing bigger at the low density edges of the patches in comparison to the middle of the patch were mussels were smaller and occurred at higher density (Table 6. 1). These results are similar to other field (Svane and Ompi, 1993, Okamura, 1986) or laboratory studies (Fréchette *et al.*, 1992). Nevertheless, this study measured the food gradient at the same time as the growth gradient whereas previous studies have only inferred these relationships (Svane and Ompi, 1993; Okamura, 1986; Fréchette *et al.*, 1992). In site 3, where a food gradient occurred between the mussel and bare substratum patches, there was no mussel growth gradient with position within the patch, and the same density and size of mussels were recorded at the edge and the middle of the patch. An explanation for such a uniform density between the edge and the middle of the patch, is that the mussel percentage cover over the mussel bed was lower in site 3 (only 40%) than at site 2 (63%). Consequently, intraspecific competition for food at site 3 was reduced in comparison to site 2. Moreover, mussels at site 3 were one year older and ~10 mm larger than in site 2. Fréchette *et al.* (1992) have demonstrated that the interdependence between food limitation and space was asymmetrical with only small mussels affected by crowding.

The vertical turbulence-induced flux of chl *a* to the mussels, determined by the gradient of chl *a* and the vertical diffusion (K_z) near the bed was equal to the actual flux of chl *a* measured from mussel grazing at site 2 (Figure 6. 9, Table 6. 13). At site 3, the K_z was slightly underestimated when using the mussel clearance rate and chl *a* gradient approach in comparison to the K_z measured by the ADCP. An explanation for such observations is that the clearance rate used on site 3 was also underestimated. No direct measurements of clearance rate were performed at site 3, instead the CR measured at site 2 was used in calculations for site 3. A higher

clearance rate at site 3 would be expected relative to site 2 as more food was available to the mussels, the mussels were older and bigger and clearance rate is an allometric function of body weight (CR = *a.* Wb; Bayne et al. 1976; Prins *et al.*, 1996).

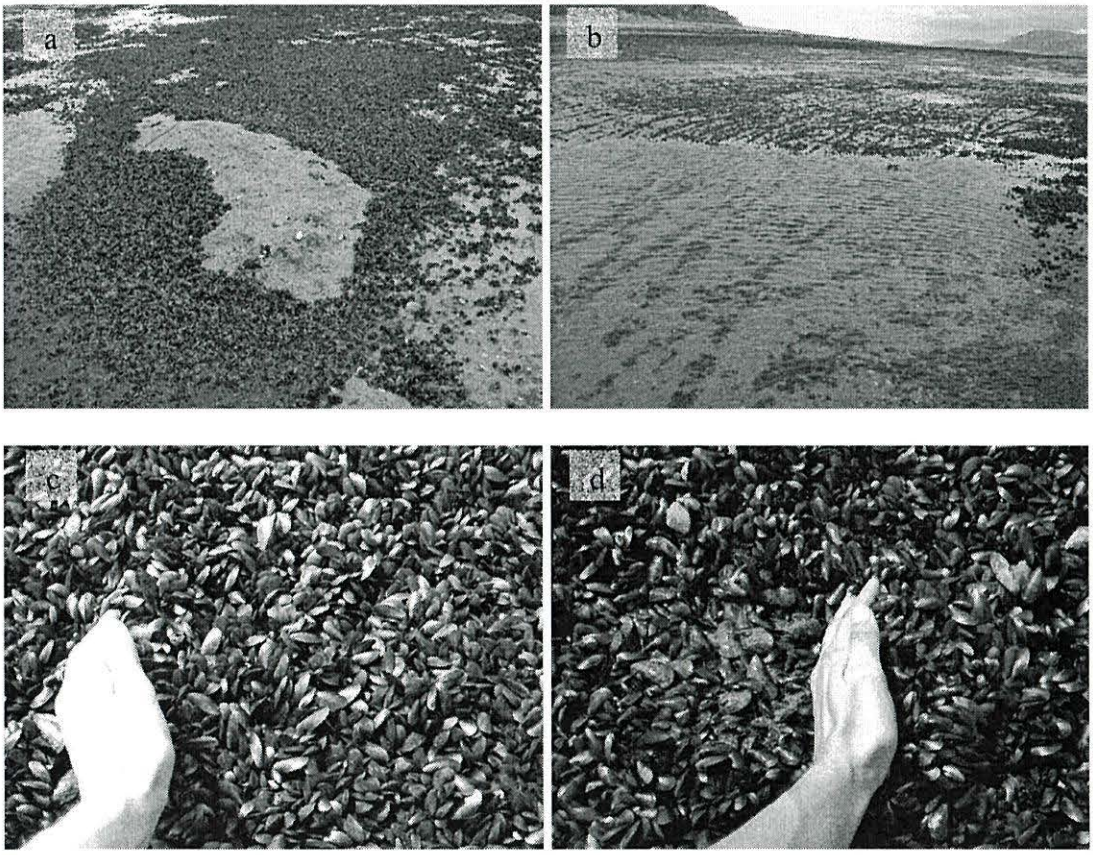
The physical and biological processes occurring in the intertidal bed were altered by bed morphology and patterning. The bed patterning in the Menai Strait evolves during the culture process and is influenced by natural forcing processes (self-organisation, van de Koppel *et al.*, 2005; dislodgement/erosion, Bertness and Grosholz, 1985, Murray *et al.*, 2002; predation, Okamura, 1986) but also due to the mechanical manoeuvring undertaken by the mussel farmers (i.e., flattening of the bed prior to the mussel relaying, circular relaying pattern from vessels, mussel redeployment from the intertidal to subtidal during ongrowing and harvesting). Within discrete mussel beds, mussel-covered and bare patches of substratum were similar in size (~5 m wide) compared with those found in other intertidal mussel beds (Wadden Sea, ~6 m, van de Koppel *et al.*, 2005; Menai Strait, 2.3 - 3.1 m, Gascoigne *et al.*, 2005). The shape of the bare patches in the Menai Strait was circular (Figure 6. 3), in comparison to the banded pattern observed in the Wadden Sea (van de Koppel *et al.*, 2005). This could be explained by the manner in which the mussels are relaid by the mussel farmers but also due to the influence of tidal currents that advect food from three main directions. In contrast, a bidirectional current occurs in the subtidal area of the Menai Strait (Saurel *et al.*, 2007; Simpson *et al.*, 2007; Tweddle *et al.*, 2005) and a similar banded pattern than in naturally settled mussel bed in the Wadden Sea would be expected, where the banded pattern is aligned perpendicularly to the flow rather than associated with sediment morphology (Figure 1 in van de Koppel *et al.*, 2005).

This study indicated the importance of the interactions between mussel feeding, growth and patterning for understanding the mussel component of the production carrying capacity in a system such as the Menai Strait. The observed mussel feeding and growth would not be possible in a homogeneous bed with the same mussel density (van de Koppel *et al.*, 2005). Due to the accuracy of the measurements made using remote sampling instruments and the robustness of the *in situ* methodologies applied in the field, these findings have important applications for the future

modelling of production carrying capacity (see Figure 6. 10). In terms of management, the optimal size for mussel and patches of bare sediment can be calculated in the Menai Strait from the empirical data collected in this study and this approach could be applied in different systems once the mechanisms are understood. Understanding the scale of appropriate patch size to maximize production would inform management decisions about stocking density and excessive initial laying of mussel seed.

Chapter VII

Conclusions



Mussel seeds re-laid at Gallows Point by Mare Gracia (Deep Dock), 07/07/2006: a) Bare patch created by erosion, b) mussels reorganised along the groves from the preliminary dredging), c) dense mussel in the patch, d) loose mussels from the same patch.

Chapter VII Conclusions

The present study focused on improving the estimation of mussel production carrying capacity in the Menai Strait (UK). More than half the cultured UK mussel production occurs in the Menai Strait (Maguire *et al.*, 2007) with an annual production > 10 000 t of mussel (> 45 mm size), most of which is derived from natural spatfall. The Menai Strait is a hydrodynamically complex system, which is macrotidally driven and exhibits high levels of turbulence (Campbell *et al.*, 1998; Simpson *et al.*, 1971; Rippeth *et al.*, 2002). In this system, local primary production is not the main source of food for the mussels due to a short residence time (2-3 days; Simpson *et al.*, 2007). The food is mainly advected from an area of high primary production in Conwy Bay and Liverpool Bay beyond. Recycled water (water that has been filtered already by mussels) occurs on the flood tide that originates from the Caernarfon end of the Menai Strait (Tweddle *et al.*, 2005; Saurel *et al.*, 2007; Simpson *et al.*, 2007). New or existing *in situ* methodologies were developed (Chapters 2, 3, 4, 5 and 6) and the interactions and feedback mechanisms between the mussels (growth, filtration rate, spatial distribution), hydrodynamics (advection, vertical mixing, resuspension) and seston concentration (Chapter 6) were integrated to understand the Menai Strait system, hence the production carrying capacity in relation to mussel cultivation.

Two main conclusions arose from this study and are discussed here: 1) A steady-state occurred between the mussels, hydrodynamics and seston concentration in the intertidal area of the Menai Strait. This state was dynamic and adjusted temporally and spatially. A mechanistic model describing the combination of these mechanisms occurred spatially over a short period of time along an intertidal mussel bed is proposed for the Menai Strait (Chapter 6); 2) The *in situ* methodologies developed were robust and appropriate if employed under specific conditions (all Chapters, but mainly Chapter 6). Moreover, chlorophyll *a* concentration (chl *a*) was found to be a valid food proxy for studying production carrying capacity in the Menai Strait (Chapters 2, 3, 4, 5 and 6).

The temporal dynamics of mussel filter-feeding informed an understanding of the mechanisms occurring in the intertidal area of the Menai Strait that could be

extrapolated to the subtidal area. In chapter 6, the tide was arbitrarily divided into four phases as a function of the variation of the hydrodynamic processes that supplied food to the mussel bed and the current direction: high current velocity with chl *a* resuspension (phase 1), followed by vertical mixing and advection of food to the mussels (phases 2, 3 and 4). The steady-state between the three components was found to adjust itself with the evolution of the tide. The response of the mussels to the resuspended material was the fast opening of their valves (< 1 h to reach 70% valve aperture in phase 1). In phase 2, chl *a* concentration supplied to the mussels via vertical mixing was low (~1.5 μg L^{-1}), but always higher than the minimum threshold at which the mussels would stop filter-feeding during the whole tide over the intertidal mussel bed (mussel valves > 70% and mean CR = 3.1 L h^{-1}). Moreover, by applying the dual approach "ask the water, ask the animals" (Petersen, 2004a), the use of different quantitative techniques gave the same results: the flux of food filtered by the mussels was equal to the flux of food supplied to the mussels via vertical turbulent mixing, thus confirming the steady-state of the system and the robustness of the methodology and measurements realised in the intertidal area (Chapter 6).

The study of the spatial variation of mussel distribution was necessary to fully understand the interactions that occurred between the three components (mussels/hydrodynamics/seston concentration) of production carrying-capacity and the steady-state of the intertidal system. Food depletion in the water column over the mussel bed (Chapter 6) was measurable vertically at a microscale (0 to 0.11 m) and horizontally at a small scale (< 5 m). These measurements informed the development of a model of the mechanisms that occurred horizontally above the mussel bed (Fig 6-10 in Chapter 6). The mechanistic model proposed included the presence of bare patches of substratum and resuspension of microphytobenthos in a modification of the diagram/model by Wildish and Kristmanson (1997). The water advected above the intertidal mussel bed was depleted of seston in the boundary layer only above the mussel patches and no food depletion occurred once this body of water moved over the adjacent bare patch. In the bare patch, vertical mixing resulted in the replenishment of food in the water column immediately above the seabed, as no significant removal of particles occurred. The same body of water is thereby enriched in food when it passes over the next mussel patch. The vertical mixing displaced the

minimum chl *a* threshold for mussel filtration closer to the mussels so that they are able to filter during the whole period of the tide. In contrast, the mussels in the subtidal area of the Menai Strait (Chapter 2) experienced a depletion of food in the water column at slack water, when the flow was reduced and vertical mixing was lowest (Tweddle *et al.*, 2005), causing the chl *a* concentration to fall below the minimum chl *a* threshold for filtration in the nearbed layer and as a result the mussels stopped filter-feeding (Chapter 2, Saurel *et al.*, 2007).

Several models have examined the food supply and gradient of food depletion over commercial or natural mussel beds in relation to the impact of mussel filtration (Wildish and Kristmanson, 1984; Fréchette and Bourget, 1985a&b; Fréchette *et al.*, 1989; O'Riordan *et al.*, 1993; Butman *et al.*, 1994; Wildish and Kristmanson, 1997; Dolmer, 2000a; Tweddle *et al.*, 2005; Simpson *et al.*, 2007). To date, the modelling of the Menai Strait has only used empirical data from measure of hydrodynamic and seston components to examine production carrying capacity (Tweddle *et al.*, 2005; Simpson *et al.*, 2007). In the latter study, the mussel beds from both intertidal and subtidal areas of the Menai Strait were represented as one box, and the mussel filtration data was an average obtained from the literature rather than data representative of measured local mussel filtration rates. The Menai Strait was modelled as a tidal channel on a very large scale (km) and thus did not take into account the smaller scale effects of mussel feeding behaviour, mussel bed patterning and the effect of chl *a* resuspension and/or sinking. The present study has demonstrated that the intertidal and subtidal areas of the commercial mussel beds have different current flow regimes that supply food to the mussels: the former had a tri-directional low current (maximum 0.35 m s^{-1} in Chapter 6), while the latter had a strong bidirectional flow velocity (up to 0.61 m s^{-1} in Chapter 2). Therefore, the two areas should be modelled separately rather than integrating them using broad assumption regarding general hydrodynamics in the Menai Strait.

The development of *in situ* methodologies, designed to obtain measurements that reflect the system studied, provided new insights and answers to previous ongoing debates in the scientific literature. For example, new insights were gained regarding which environment factor(s) controls mussel feeding behaviour, but also regarding the appropriate use of *in situ* methodology for measuring mussel clearance rate.

Three *in situ* methodologies were used and developed further to study the mussel component of production carrying capacity: 1) video recordings to measure mussel feeding behaviour from undisturbed mussel beds (Chapters 2 and 6); 2) the defecation method to measure mussel clearance rate *in situ* (Chapters 3, 4 and 6); and 3) the shell microgrowth method to measure mussel growth-variations from the field (Chapter 5). Chl *a* concentration appeared to be a key biomarker for the three methodologies deployed, despite its biodegradability or digestion depending on the nutritional state of the mussel (Chapter 3, Conover *et al.*, 1986; Abele-Oeschger and Theede, 1991; Penry and Frost, 1991). Moreover, chl *a* was a good proxy for food even though mussels also feed on other food sources (i.e. bacteria, microzooplankton, detritus and dissolved organic material, see Gosling, 2003).

Mussel feeding behaviour was influenced mainly by variation in chl *a* concentration (Chapters 2 and 6). The percentage mussel valve gape aperture methodology used for measuring mussel feeding behaviour *in situ* proved to be an useful tool as it is non-intrusive and facilitated monitoring of the mussels for long periods of time (> 24 h) (Newell *et al.*, 2001; Riisgård *et al.*, 2003; Newell *et al.*, 2005b; Riisgård *et al.*, 2006). Mussel valve gape aperture from the subtidal area of the Menai Strait was synchronised with chl *a* concentration measured at 1 m above the mussel bed (Chapter 2). In contrast to other studies (see Dolmer, 2000a, 2000b Newell *et al.*, 1998), the influence of chl *a* on mussel feeding behaviour could be distinguished from other environmental factors (i.e. velocity, predators, suspended particulate matter, temperature, water depth, light) which gave an insight into the feedback mechanisms that occurred in both systems. This distinction was possible due to the physical properties of the Menai Strait and to the analytical method used (harmonic analyses), which also enabled the mussel feeding behaviour in the subtidal area to be modelled. Mussels from the subtidal (Chapter 2) and intertidal areas (Chapter 6) exhibited different feeding behaviour, which was found to be linked to the supply of food. When attempting to develop an understanding of the carrying-capacity of new systems, it is recommended that the feeding behaviour measured via *in situ* methodology together with food concentration and supply should be applied each time a new system is studied for a complete understanding of the feedback mechanisms that operate in that system.

The model developed as part of the defecation methodology, enabled estimation of *in situ* clearance rate in two different environments i.e. the Limfjord Denmark (microtidal and wind driven system, Chapter 3) and the Menai Strait, UK (macrotidal system, Chapter 6). The defecation-based methodology used to measure bivalve clearance rate (CR) has been used previously (Kotta and Møhlenberg, 2002; Kotta *et al.*, 2004) but its development was necessary for a better application in the field. In this study, two issues were addressed: the validity of chl *a* as a food proxy and its degradability in the mussel gut via the modelling of digestion factor (DF). However, there were some limitations in the model developed: it is only applicable within a temperature range of 6.8 – 18.3 °C and it was derived by feeding a monoculture of algae in the laboratory. The biodegradability of chl *a*, was elucidated from HPLC measurements of degraded pigments in the mussel faeces (found mainly as phaeopigments), which was correlated to the same pigments that occur prior to ingestion. In terms of the nutritional state of the mussel, it was assumed that for *in situ* measurements of CR, the mussels collected were not in a starved state and adapted well to their new feeding environment. Clearance rates estimated from the developed defecation method were similar to clearance rates estimated on the same mussel population and in the same environmental conditions from other methodologies: the modified suction method (Møhlenberg and Riisgård, 1979) and the indirect methods (Riisgård, 2001a) (Chapters 3 and 4). In contrast, the biodeposition method (Hawkins *et al.*, 1996; in scallops Cranford *et al.*, 1998, Cranford and Hill, 1999) gave lower values than the other techniques (Chapter 4). The defecation method necessitates close monitoring of the chl *a* next to the mussels studied in the field, at least 2 h prior to the measurements in order to take into account the mussel gut retention time (Hawkins *et al.*, 1990; Kiørboe *et al.*, 1980; pers. obs.). This method applied together with chl *a* monitoring will provide an accurate estimation of the clearance rate of the mussel population and thus avoid using values obtained from modelling of other systems or from laboratory conditions for modelling production carrying capacity. Moreover, this method could also be developed for other commercial bivalve species.

The mussel shell microgrowth was positively correlated to food concentration (measured as chl *a*). This method (Chapter 5) provided accurate measurements of the short-term variations in mussel shell growth rate that occurred due to variation in

food (on a tidal scale). Previous studies mainly examined microgrowth rate in mussel to determine the age of the animals and in population dynamics studies (Lutz and Rhoads, 1980; see in Richardson, 2001) or in other bivalves mainly as a temperature proxy (*Phacosoma japonicum*, Miyaji *et al.*, 2007; *Cerastoderma edule,* Richardson *et al.*, 1980; *Arctica islandica,* Schöne *et al.*, 2005b). This methodology proved to be better than the traditional morphometric measurements (shell length, height, width, weight, flesh weight and condition indices) at measuring shell growth increment in certain conditions: i.e. short time scale (<15 days), single sampling with no marking, transplanting and recapture of the animals. In contrary, the traditional morphometric measurements were more robust, less expensive and more practical for monitoring over longer time-scales (≥ 1 month). The microgrowth methodology provides new expectations for understanding mussel population dynamic and the evolution of mussel bed morphology and self-organisation; but it also allows a rapid assessment of the growth state of cultivated mussel beds, essential for commercial management.

Levin, (1992) identified the importance of scale (spatial, temporal, organisational) in studying the processes and the mechanisms of observed patterns in ecology. In the current study, each component of the production carrying capacity of the Menai Strait was studied at different scales, although not exhaustively and all of them were directly reliant on the spatial and temporal scales. Fluctuations of advected/diffused chl *a* concentration can be important: temporally within a tidal cycle (Chapter 2 and 6) or seasonally (Menai Strait, Fig. 5 in Gascoigne *et al.*, 2005); and spatially within the water column (Chapter 6, Dolmer, 2000a; Tweddle *et al.*, 2005) or within the length of the mussel bed (Chapter 6; in the Wadden Sea Fig. 3 in van de Koppel *et al.*, 2005; in the Menai Strait Fig. 5 in Simpson *et al.*, 2007). Therefore, careful sampling of the chl *a* at the right spatial and temporal scales is essential to be representative of the chl *a* dynamics in the system studied. The findings of the present study should be considered in future work when using *in situ* methodologies and of a tri-component approach (hydrodynamics, mussels and seston) to study mussel population dynamics i.e. mussel bed patterning and evolution. As a fact, the mussel population dynamics has important repercussions on the mussel farmers' strategies and management of the mussel beds. Quantification of variation in mussel shell microgrowth within the mussel bed and patches in relation to food concentration and supply could lead to a better understanding of mussel bed

patterning. In the Menai Strait, the optimal patch size and mussel density to be laid in the intertidal and subtidal mussel beds could be modelled from the empirical data from Chapter 6 and a similar approach applied for modelling these same mechanisms in other systems, and thus maximise mussel yield i.e. production carrying capacity.

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