Zebra mussel (*Dreissena polymorpha*) population in the newly formed Cardiff Bay

Thesis submitted for the Degree of Philosophiae Doctor

by

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A mon grand-père, Papy

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Summary

1. Zebra mussels are among the worlds most prolific invasive species, but few case studies assess occurrence and effects in artificial water bodies. Cardiff Bay was invaded soon after formation in 2001, and this thesis investigates factors affecting veliger numbers, local colonisation, adult density and age structure, as well as providing some initial data on oxygen demands. Over four years, planktonic veliger surveys, Side Scan Sonar, under-water video, colonisation samplers and mesocosm experiments were used to investigate all life stages.

2. Veligers were produced in 1-3 larval peaks from May to September, with frequency and timing apparently varying facultatively under contrasting conditions between years. Peak densities reached 8-14 ind.l^{-1}, with local variations reflecting flow, and numbers declined during high discharge. Veliger settlement as juveniles also declined at sites or times with the greatest flow, and settled densities in a dry year (2007) were 120 times greater than in a wet year (2008). Local substrate availability also affected settlement pattern.

3. While no aggregations of zebra mussels occurred in the Bay's soft sediments, hard substrates and vertical surfaces at 0.5-7 m depth have been colonised extensively at densities of 250-6 600 m^{-2}. Local density estimates alongside crude assessments of habitat available suggest that Cardiff Bay could be occupied by a population of in excess of 10- 31 million adults (excluding the Bay's aeration system), consistent with the large veliger density recorded in the water column. Most mussels are < 1 year old, but year-class structure (4 cohorts) show that the Bay must have been occupied at least as early as 2003.

4. These data are consistent with a rapidly established, large and extensive population of zebra mussels in Cardiff Bay now maintained by prolific larval production and settlement. In addition to the large risks of contamination of boats and onward dispersal, zebra mussels could have large ecological and environmental effects on Cardiff Bay. Effects on oxygen concentration are likely through direct uptake and depletion of the lake's phytoplankton, and further work is required to appraise the associated risks particularly under low summer flow and high temperature.

1. General introduction

1.1. Invasive species: a major ecological issue

Since Neolithic times, 6000 YBP, human migration has been accompanied by the transport and translocation of other organisms to geographical locations outside their normal range (Money and Cleland, 2001). However, not all translocated species establish new ranges, and not all established exotic species become invasive. Only a small proportion of species become established in new geographical ranges, although the exact reasons are unclear, and most attempts to predict which introduced species will become problematic remain theoretical (Kolar and Lodge, 2001). While it is difficult to predict the success of individual invaders, there are apparently statistical regularities to invasions (Williamson and Fitter, 1996). The 'tens rule' of Williamson (1996) states that one introduced species in ten appears in the wild, one in ten of these becomes established and one in ten of established non-indigenous species becomes invasive. However, the tens rule is controversial and some studies do not support this rule (Jeschke and Strayer, 2005). As the volume of global trade increases, the rate of establishment of alien species also increases (Mooney and Cleland, 2001). According to Colautti and MacIsaac (2004), the definition of an invasive species, is based on the concept of "propagule pressure", e.g. the number of larvae. A species become invasive in a given ecosystem when it is well established, widespread and dominant, and shows a high propagule pressure.

Invasive species are recognised as one of the leading threats to biodiversity and also cause considerable costs in agriculture, forestry, fisheries and other industries (Wittenberg and Cock, 2001). Invasions can have major consequences on the invaded ecosystem through a range of different mechanisms including: competition for food and ecological niche, predation, herbivory, the formation of mutualisms, pathogenic or parasitic effects, habitat alteration and genetic hybridization (Mooney and Cleland, 2001). Famous catastrophes include the introduction of the Nile Perch, *Lates niloticus* into Lake Victoria which led to the extinction of 200 endemic fish species (Goldschmidt *et al.*, 1993).

The key ecological questions about ecological invasions centre about factors affecting *'invasibility'*, the traits that make species invasive, invasion routes and, above all, the ecological consequences of an invasion. In general, while the causes and consequences of biotic invasions have been increasingly well described (Rejmanek and Richardson 1996; Mack *et al*,. 2000; Sakai *et al*., 2001), instances in freshwater are still comparatively poorly understood and recorded (Ricciardi and Rasmussen, 1999).

Freshwater habitats support many different organisms: macrophytes, invertebrates, fish, birds and aquatic mammals. Many freshwater invasive species in Europe are known because of their impact on native species. For example the signal crayfish (*Pacifastacus leniusculus*) which was introduced to Europe is an aggressive competitor with the white clawed crayfish (*Austropotamobius pallipes*) and a vector for the crayfish plague fungus (*Aphanomyces astaci*; Vorburger and Ribi, 1999); the rainbow trout (*Oncorhynchus mykiss*) was introduced as a game fish and is a competitor with the brown trout (*Salmo trutta*; Blanchet *et al*., 2007). The ruddy duck (*Oxyura jamaicensis*) was introduced to Europe, and hybridizes with the native species white-headed duck (*Oxyura leucocephala*; Muñoz-Fuentes *et al*., 2007). The zebra mussel, (*Dreissena polymorpha*, Pallas) is considered to be one of the most invasive freshwater species in the world because of its severe impacts, both economic and ecological. Originally native of the Caspian and Black Sea regions (Skorikov, 1903 cited by Karatayev *et al*., 2003), zebra mussels recently (c 1980) invaded North America and Western Europe causing severe impacts (Chapter 1, MacIsaac and Grigorovich, 1997; Claudi and Mackie, 1994). Zebra mussels were first recorded in the UK in 1824, and were found in other European countries, outside of their natural range, shortly afterwards. More recently, following a long stable period and, possibly even some decline, zebra mussels have spread rapidly and widely across central, eastern and southern England (Aldridge *et al*., 2003). At some point in this recent expansion, and since its closure in 2001, zebra mussels have colonised the newly formed Cardiff Bay, in Wales. The Cardiff Harbour Authority (CHA), the body responsible for the Bay's management, has taken steps to increase awareness of the risks of further dispersal from the Bay, but several general concerns necessitated the study of the population of this artificial lake.

1.2. Study area

Cardiff Bay is an artificial lake located on the south coast of Wales, UK, (51°27'18.9706"N 03°10'05.5186"W; Figure 1.1). Cardiff Bay was previously an industrial port constructed to for the trade in coal and iron, during the Industrial Revolution between the 1790's and the Second World War. Coal exports ceased in the 1960's and Cardiff Bay became a neglected marshland with derelict docks. In 1987 the Cardiff Bay Development Corporation was established to regenerate this area through the construction of a barrage across the mouth of the Bay to create a freshwater lake (Cardiff Harbour Authority, 2003). The barrage construction started in 1994 and lasted for 5 years before completion in 2001.

Today, Cardiff Bay is a shallow freshwater lake of 200 hectares, with a mean depth of 4.0m (maximum depth 13.4m), fed by the Taff and the Ely rivers. The lake received a large amount of organic matter from the two urban rivers which make an eutrophic lake with mean total phosphorous concentration 88.3μgl^{-1}, mean chlorophyll *a* concentration 6.7μgl^{-1}, and maximum chlorophyll *a* concentration 450.6μgl^{-1}. Diatom assemblages used as an indicator of water quality indicated a poor water quality in the main body of the Bay, with higher water quality in the rivers (Jüttner *et al.*, 2010). Cardiff Bay was designated as a sensitive area by the Urban Wastewater Treatment Directive (Council of the European Communities, 1991) and Cardiff Harbour Authority (CHA) is responsible to carry out continuous monitoring and management of the lake and its surroundings.

Locks in the barrage permit the passage of boats between the sea and the lake. Nevertheless, any seawater entering the lake is collected in an associated pump to maintain the area as a freshwater lake with a salinity of 0.19 ‰. A fish pass present in the barrage facilitates the migration of salmonids for spawning in rivers. To reduce the risk of a drop in dissolved oxygen, particularly during hot summers, an aeration system was installed on the lakebed to maintain an oxygen level >5 $mg.l^{-1}$ to support aquatic life, especially migratory fishes. The aeration system is composed of a series of steel reinforced rubber pipelines, laid on the lakebed and river beds connected to approximately 800 diffusers. Compressed air is pumped from cabinets located at five

sites surrounding the lake, and then directed to the pipelines. The air distributed in the entire water column prevents lake stratification. A mobile oxygenation unit can be deployed in case of the deoxygenation of the deepest waters. Regular surface-water skimming is carried out to remove vegetable debris and waste accumulated in sheltered areas.

The lakebed is mostly composed of soft sediments: mud and silt. The lake margins are mainly composed of man made hard substrates such as locks and walls from the previous harbour, and banks made of cemented pebbles. Some areas are more natural such as some banks composed of loose pebbles. A wildlife reserve, composed of wetlands, is also situated in the Northern area of the Bay. The latter is the only area with vegetation constituting good habitats for waterfowl. Fish are relatively abundant and dominated by roach (*Rutilus rutilus*), dace (*Leuciscus leuciscus*) and chub (*Leuciscus cephalus*). Just after the lake completion, chironomid larvae were in large abundance and caused nuisance to inhabitants. Monitoring and treatment with *Bti* were carried out to reduce chironomid population (Vaughan *et al.*, 2008).

1.3. Zebra mussel colonisation

In 2004, CHA discovered the presence of zebra mussels in the lake, and expert opinion suggested that occupancy began around 2003 (Aldridge D., pers. comm.). Large numbers of mussels were found at this point clinging to underwater structures on the barrage and coating the diffusers and pipes of the aeration system. Although the barrage infrastructure is coated with an antifouling agent to prevent zebra mussel colonisation, approximately four tonnes of mussels are now removed annually (CHA, pers. comm.). As one of the first stages in appraising potential risks, impacts and management responses, some assessment is required on the extent of the zebra mussel population in Cardiff Bay.

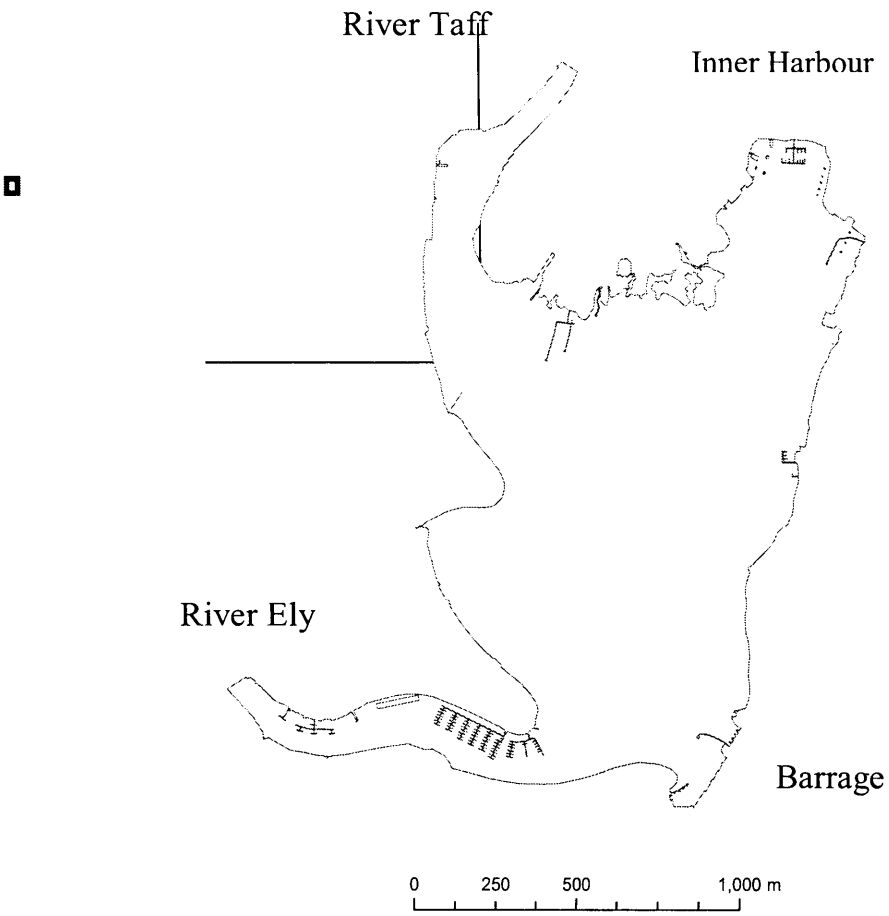


Figure 1.1: Cardiff Bay and the two rivers, the Taff and the Ely

1.4. Research aims

While many studies have been carried out on zebra mussels in lakes, there are few case studies of their occurrence and effects in newly formed artificial lakes such as Cardiff Bay (Chapter 2). The general aims and questions of this PhD were:

i. To review relevant literature on the ecology and effects of zebra mussels (Chapter 2)
ii. To assess temporal and spatial distribution of veliger larvae in Cardiff Bay, (Chapter 3)
iii. To assess the density and the distribution of juvenile settlement across the lake in contrasting years (Chapter 4)
iv. To assess the distribution and densities of adult zebra mussels across Cardiff Bay by comparing densities on different substrates, including the lakebed (Chapter 5)
v. To make initial assessments on seasonal variation in zebra mussel oxygen consumption and on the potential impacts on dissolved oxygen concentrations in Cardiff Bay (Chapter 6).

Key questions and hypotheses that follow from these aims are outlined in each chapter.

The thesis has been drafted as a series of self-contained investigations, each with their own summaries, introductions, methods, results and discussion.

2. Literature review: the ecology of the zebra mussel as an invasive species

2.1. Summary

1. Invasive species can have large effects on the species composition and ecosystem function of environments in which they become established. The resulting economic and ecological costs species have led to many studies investigating the 'invasibility' of species, i.e. those traits that cause a species to be invasive, and the ecological consequences of invasion.

2. In freshwater environments the zebra mussel, (*Dreissena polymorpha*, Pallas) is considered to be one of the most invasive species in the world causing severe ecological and economic impacts. A wide array of published articles on the biology and ecology of zebra mussels report effects across its invaded range, and reflect the substantial and growing concerns about the impacts of zebra mussels.

3. This chapter reviews the published work surrounding zebra mussel ecology and the consequences of invasion. The review examines initially the ecology of zebra mussels including physiology, life cycle (reproduction, different life stages) and habitat preferences in relation to environmental variables. The chapter continues by examining the dispersal strategies of the mussel and the range across which it has become established. Finally, the review considers the known ecological and economic impacts of zebra mussel invasion.

4. The chapter provides a foundation and context for surveys and experimental research into the assessment of the distribution and population dynamics of zebra mussels in Cardiff Bay; a newly formed water body in Great Britain. The occurrence and effects of zebra mussels in such newly formed water bodies have not been widely investigated.

2.2. Introduction

The transport and translocation of non-indigeneous species have occurred extensively with human movement and migration. Although only a small portion of non-indigenous species become invasive, the acceleration of international trade and travel enhances the rate of invasive species established (Mooney and Cleland, 2001). A non-indigenous species is considered invasive when it becomes dominant, widespread, and established with a high propagule pressure (e.g. number of larvae; Colautti and MacIsaac, 2004). The introduction of invasive species in an ecosystem has led to interactions with native species, with changes in the food web and alterations in ecosystem function (Mooney and Cleland, 2001).

Invasive species exist in a range of ecosystems and there are some well known examples in freshwater. The rainbow trout (*Oncorhynchus mykiss*), native to North America, was introduced as a game fish in European rivers and became a competitor with the native brown trout (*Salmo trutta*; Blanchet *et al.*, 2007). The signal crayfish, (*Pacifastacus leniusculus*) native to North America, was introduced as a food species, escaped into rivers and spread rapidly leading to competition with the white clawed crayfish (*Austropotamobius pallipes*; Vorburger and Ribi, 1999) and damage to river banks.

Whilst invasive species are generally of greatest interest in natural ecosystems because of their threat to biodiversity, invasive species can also have significant impacts in artificial ecosystems such as new developments and industries. Major environmental damage and considerable costs for the industry, have encouraged more proactive research on the ecology and the distribution of invasive species, on their impacts and on potential controls (Pimentel *et al.*, 2005).

The zebra mussel is an invasive species well known due to its wide range of colonisation and the considerable ecological and economic impacts caused. The highly invasive nature of zebra mussels can be explained by several characteristics presented by the species: wide environmental tolerances; high genetic variability and phylogenetic plasticity; a short life span, rapid growth and high fecundity; gregarious behaviour and an

ability to repopulate previously colonised habitats following recovery from population crashes (Lodge, 1993; Lucy, 2005). Native to the Caspian and Black Seas, zebra mussels invaded North America and Europe causing severe damage. Here, I review studies from both these regions, but special emphasis is placed on North American, where large zebra mussel populations have led to extensive research activity (Mackie and Schloesser, 1996). After describing zebra mussel biology, emphasis is placed in zebra mussel ecology (Claudi and Mackie, 1994; Nichols, 1996; McMahon, 1996), population dynamics (Nalepa *et al.*, 1995; Cope *et al.*, 2006), the impacts that mussel can have as a filter feeder (Fahnenstiel *et al.*, 1995; Vanderploeg *et al.*, 2002), or biofouling organism (Ram and McMahon, 1996), and the potential controls (Claudi and Mackie, 1994).

2.3. Zebra mussel ecology

The biology and ecology of zebra mussels in North America and in Europe shows some similarities and some differences (Mackie and Schloesser, 1996). North American populations are similar to European populations in their basic biological characteristic, population growth, mortality rates, dispersal mechanisms and dispersal rates. Relative to European populations differences have been demonstrated for individual growth rates, life spans, calcium requirements, pH tolerances, potential distribution limits, and population densities of veligers and adults. These commonalities and contrasts reflect the fact that this invasive species has considerable phenotypic plasticity to occupy a wide range of habitats due to its flexible reproductive cycle (Nichols, 1996; Mackie and Schloesser, 1996).

2.3.1. Identification

2.3.1.1. Classification

The zebra mussel owes its name to the zebra stripes on the shell, which presents different patterns or morphs, hence its scientific name, *Dreissena polymorpha* (Pallas, 1771). Different systems of classification have been described. Nuttall's classification (1990), described below, is the most used because it conforms to that commonly used for North America Bivalvia, where the mussel have been well studied.

Phylum: Mollusca Linnaeus, 1758
Class: Bivalvia Linnaeus, 1758
Subclass: Heterodonta Neumayr, 1884
Suporder: Eulamelliobranchia
Order: Veneroida H7A Adams, 1856
Superfamily: Dreissenacea Gray, 1840
Family: Dreissenidae Gray, 1840
Subfamily: Dreisseninae Gray, 1840
Genus: *Dreissena* van Beneden, 1835
Subgenus: *Dreissena* van Beneden, 1835

Species: ***D. polymorpha* (Pallas, 1771)**

Subgenus: *Pontodreissena*

Species: *D. (P.) bugensis* (Andrusov, 1897)

Genus: *Congeria*, Partsh, 1835

Genus: *Mytilopsis* Conrad, 1858

Genus: *Prodeissena*, Rovereto, 1898

Subfamily: Dreissenomyinae Babak, 1983

Genus: *Dreissenomya* Fuchs, 1870

2.3.1.2. Anatomy and morphology

2.3.1.2.1. Shell form

Belonging to the Bivalvia, the mussels have two-part shells with both valves being symmetrical along the hinge line. Zebra mussels are small compared to the Blue mussels, *Mytilus edulis*. The average length is about 2.3- 2.5 cm, but occasionally it can reach 4 cm (Claudi and Mackie, 1994). Externally, the anterior side has an acutely pointed umbone in anterior position. The ventral side is flattened with an acute lateral-ventral angle and concave ventral-margin; this shape allows the mussel to be well attached on hard substrates. Internally, the zebra mussel has no hinge teeth (Pathy and Mackie, 1991; Claudi and Mackie, 1994). The shell exhibits an ultrastructure: an outer crossed-lamellar structure and an inner complex crossed-lamellar structure with a thin prismatic pallial myostracum between (Pathy and Mackie, 1991). With regard to the colour and the pattern, they can vary a lot. Along the Volga River, six main phenotypes have been revealed; which in differences frequencies and relative proportions have five main population groups (Smirnova *et al.*, 1993). This pattern reveals the wide range of different shell polymorphs. Sergeeva (2008) found that the phenotypic structure of a population was influenced by both biotopic characteristics and geographic position. Only one polymorph seems to be present in Cardiff Bay.

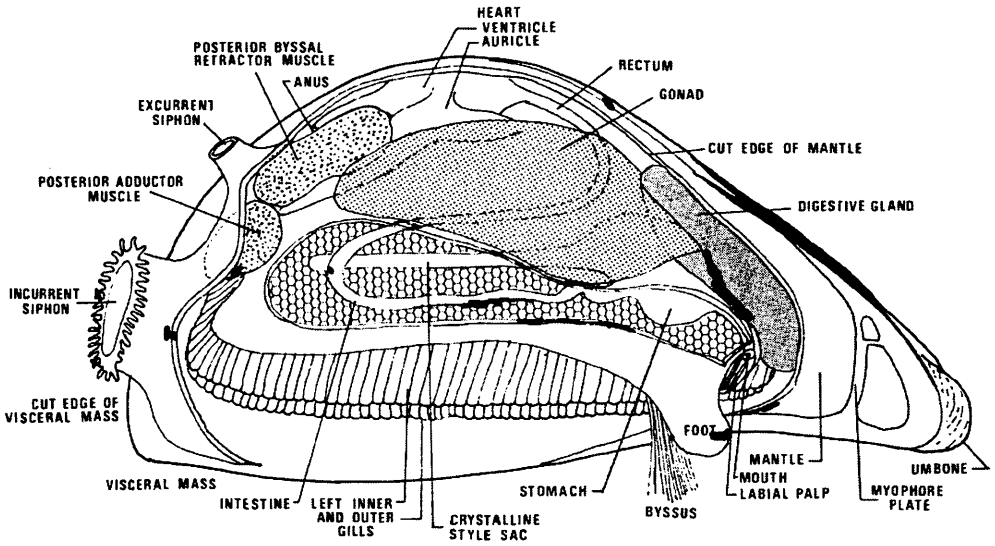


Figure 2.1: Diagram of location of major internal organs of adult zebra mussels. Mantle, right inner and outer gills, and part of the visceral mass are cut away to expose parts of the digestive system. From Claudi and Mackie (1994)

2.3.1.2.2. General aspect

The mussel body is enveloped by the mantle, tissue which secretes the shell (Figure 2.1). The space inside the mantle is called the mantle cavity. On the posterior side, there are two siphons, one inhalant and one exhalant. The incurrent siphon, the ventral and the largest one, allows the water full of food particles and nutrients to get into the mollusc. The exhalant siphon, the dorsal one, excretes the waste material. The gills are made up of filaments which form lamellae, and allow the intake of oxygen and food from the circulating water. Cilia on the gills generate water currents through the inhalant siphon, and direct digestible food particles to the mouth. The mouth is composed of labial palps which assist in guiding and selecting food to be ingested. The digestion takes place in the stomach and digestive glands. Undigested food is driven to the gut and to the anus, and is excreted as faeces by the exhalant siphon. The waste particles not directed to the mouth are wrapped in mucous secreted by cells in the gills and then rejected as pseudofaeces through the exhalant siphon (Claudi and Mackie, 1994). Another opening is

present on the anterior side, where the foot and the byssus respectively provide mussel locomotion or attachment onto substrates.

2.3.1.2.3. Byssal thread

On the ventral side, the byssal thread is produced from the byssal gland situated on the foot. The byssal apparatus is composed of a central stem and the byssal threads with an adhesive plaque at the end. The threads are composed of DOPA (3,4-dihydroxyphenyl-alanine), a prominent amino acid present in the byssus of marine molluscs. Two different types of threads exist: permanent and temporary. They are different by length, thickness, number, arrangement and plaque morphology. Permanent attachment threads are formed in clumps or arranged in rows and comprised the majority of the main byssal mass. Temporary attachment threads are few in number (1-6); arranged in a tripod pattern; originated individually; and separated spatially from the main byssal mass of permanent threads (Eckroat *et al.*, 1993). To detach from the substrate, enzymes are produced at the base of the byssal mass and all the byssal threads are released (Mackie and Schloesser, 1996). The adhesive strength of the zebra mussel byssal thread varies with the composition of the substrate on which it is settled (Ackerman *et al.*, 1994).

2.3.2. Habitat

Whilst the majority of freshwater bivalves are infaunal, living buried in the sediments, zebra mussels are epifaunal: they occupy solid substrates like rocks, floating and sunken logs, breakwaters, various debris, pipelines, intake structures, wet wells and on invertebrates (e.g. unionid, crayfish; Claudi and Mackie, 1994). However, zebra mussels can also colonise soft substrate such as macrophytes (Folino-Rorem *et al.,* 2006) and, under some circumstances, soft sediment such as sand and mud (Haltuch *et al.*, 2000). Juvenile can attach with byssal threads to individual sand grains smaller than 1mm (Berkman *et al.*, 1998).

Present in large freshwater lakes and large rivers, zebra mussels are also found in ponds, irrigation systems, canals and are capable of living in brackish waters or estuaries (Mackie and Schloesser, 1996; Strayer and Smith, 1993).

2.3.3. Reproduction

The reproductive cycle of zebra mussels is highly variable according to the geography; and differs between North America, Europe and Russia (Nichols, 1996). The high propagule pressure and the rapid growth rate are the features which result in zebra mussel invasions with high population density and rapid spread (Ram *et al.*, 1996). Zebra mussels have separate sexes, with a sex ratio around 1:1 (Claudi and Mackie, 1994) However, in case of low population densities, some individuals are able to become hermaphroditic. Fertilization is external and occurs in water: spawned oocytes contain substances that are species specific sperm chemoattractants (Ram *et al.*, 1996).

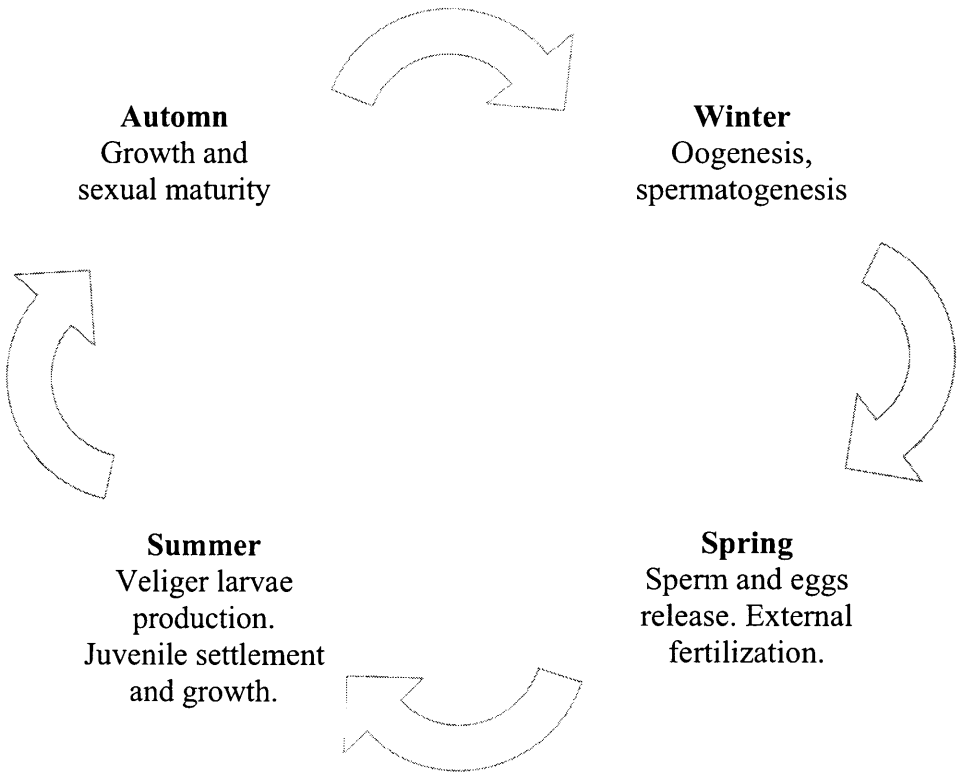


Figure 2.2: General zebra mussel reproduction cycle

Oogenesis and spermatogenesis occur during winter (Figure 2.2). Mussels can carry ripe gametes for a long time waiting for good environmental conditions. Reproduction costs a lot of energy and gonadal tissue can comprise half of the dry weight

of a female. After spawning, body weights decrease by 30-45% for male and female (Nichols, 1996). The number of eggs carried by an individual female is between 30 000-40 000 eggs/female/year and can reach 1.5 million eggs/female/year (Stanczykowska, 1977; Borcherding, 1991). Coordinated gamete maturation and synchronous spawning are keys to successful reproduction for zebra mussels which exhibit external fertilisation (Nichols, 1993; Ram *et al.*, 1996). Internal and environmental factors such as chemical regulators, water temperature and photoperiod act directly on both female and male gonads and led to coordinated gamete maturation (Ram *et al.*, 1996; Fong, 1998).

Water temperature is one of the most important factors influencing reproduction (Nichols, 1996). Spawning temperatures appear to be similar in Europe, Russia and North America, being initiated at 12°C and maximised above 17-18°C (Claudi and Mackie, 1994). The 17-18°C peak spawning threshold corresponds to the optimum temperature (18°C) for larval development and peak post veliger settlement (McMahon, 1996; Sprung, 1987). In Lake Erie, spawning seems to occur at 22- 23°C (McMahon, 1996). However, different populations will begin larval production at different temperatures (>12°C; Nichols, 1996). In many European lakes, the first veligers are often observed at 15°C (Karatayev *et al.*, 1998; Lucy, 2005). In Lake Erie, spawning typically occurs at water temperatures above 18°C, temperature considerably higher reports in several European lakes. This difference is likely a result of dissimilarities in seasonal temperature patterns and rates of warming during spring (Ram *et al.*, 1996). Moreover, veliger larvae can be found in cold waters (<5°C) and are able to overwinter. Their development is delayed or very slow: overwintering larvae can take 8 months to reach a developmental stage whilst it takes 14 days for veligers produced in summer (Kirpichenko cited in Nichols, 1996).

Gamatogenesis ceases around October. In North America the majority of adults of one year will attain sexual maturity in spring of the next year, at that time they are 8-10 mm long (Mackie and Schloesser, 1996). Some individuals are able to reproduce during the same year, if they are born early enough in the year with good environmental conditions (Claudi and Mackie, 1994). In Europe, adults become sexually mature in their second year of life (Mackie and Schloesser, 1996).

2.3.4. Life history

Zebra mussel larvae need about four weeks on average to complete the cycle. Three stages can be distinguished: veliger stage, post-veliger, settling stage (Figure 2.3). The two first stages constitute the planktonic stage, and the last one the benthic stage (Claudi and Mackie, 1994). Ackerman reviewed the early life history of zebra mussels (Ackerman *et al.*, 1994; Ackerman, 1995).

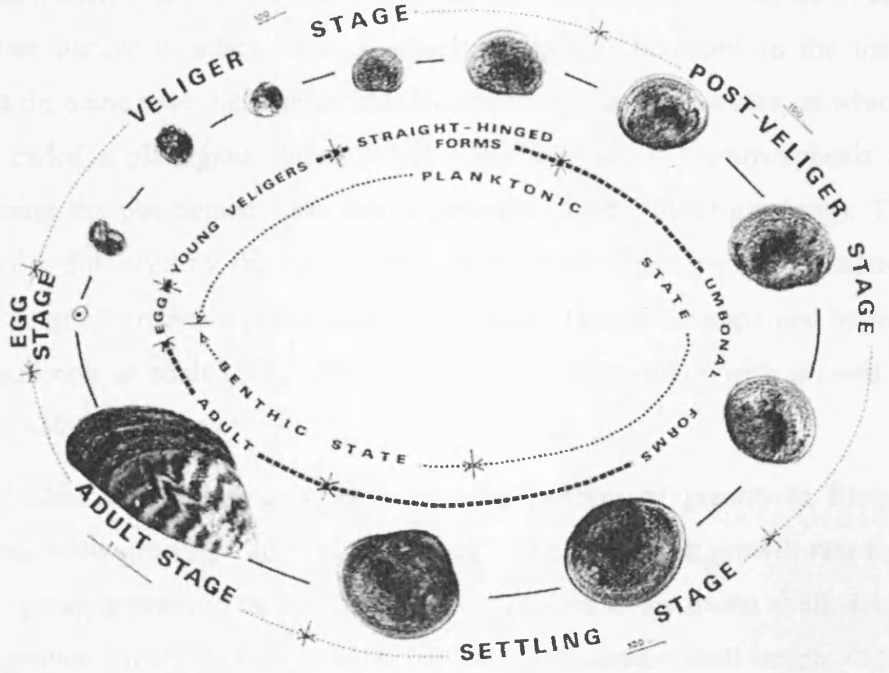


Figure 2.3: Lifecycle of *D. Polymorpha*. Taken from Claudi and Mackie, 1994.

Development begins after the external fertilization by the protostomic embryology including cleavage, blastulation and gastrulation to result in a trochophore larva after 6h-96h. Zebra mussels are characterised by the formation of veliger larvae which correspond to swimming trochophore larvae with the development of the velum, ciliated organelle of feeding and locomotion. After 2-9 days post fertilization (dpf), the veliger larva develops the first shell secreted by the shell glands, and is called prodissoconch I. This larva, 70-160 µm long, is recognisable by its shape as "D-shaped" or "straight-hinged veliger". Veligers sustain generally >99% mortality due to a lack of

suitable settling substrates, unfavourable environmental conditions, larval predation (MacIsaac *et al.*, 1991). Then, within 7-9 days, a second more ornamented larva shell is secreted this time by the mantle tissue, the prodissoconch II. It is characterised by the pronounced umbonal region, hence its name umboned veliger or veliconcha, 120-280 µm long. This is the post veliger stage, the last swimming stage typically found in the plankton. Several new organs appear, notably the acquisition of a foot which leads to a change in behaviour and to a new name: pediveliger (>167-<300 µm long). This foot allows the mussel to swim near the bottom as well as to crawl on surface to encounter an appropriate surface to attach. The development of gill filaments in the mantle cavity occurs at the same time. Settlement usually occurs on hard substrates, at which point the larva is called a plantigrade larva (>158-<500 µm long). Metamorphosis consists of transforming the plantigrade larva into a juvenile (>500-<5000 µm long). The primary settlement is followed by the loss of the velum, and its feeding filter function is taken over by the gill filaments and the mouth developed. This is accompanied by the secretion of a dissoconch or adult shell. The juvenile becomes an adult with growth and sexual maturity (>5000 µm long).

Mackie and Schloesser (1996) describe patterns of growth in European zebra mussels as "slow-growing" and "fast-growing". The maximum growth rate for the slow-growing group appears to be less than 1 cm/year with a maximum shell size of 3.5 cm; the fast-growing group exceeds 1.5 cm/year with a maximum shell length >4.0 cm. Zebra mussel growth rate is dependant on quality and quantity of food, temperature, and body size. Zebra mussel life span is highly variable through its range of colonisation and appears to be shorter for North American populations than for European populations. The average life span of zebra mussels is 3.5 years in British waters, 3-5 years in most Polish lakes, and 6-9 years in some Russians reservoirs (Morton, 1969; Claudi and Mackie, 1994; Mackie and Scloesser, 1996).

2.3.5. Feeding

Food selection is performed by a variety of cilia present in the mouth, the gills, the stomach and the midgut. Cilia in the mantle cavity and stomach select particles of 15-40 µm for food but can filter out particles as small as 0.7-1.0 µm (Claudi and Mackie,

1994). Zebra mussel feed on seston, phytoplankton and zooplankton. Mussels can ingest small zooplanktoners such as rotifers, small cladocerans, small copepods and even zebra mussel veligers (Bastviken *et al.*, 1998; Jack and Thorp, 2000; MacIsaac *et al.*, 1991). Reeders *et al.* (1993) found that zebra mussels show a high filtration rate, up to 110 ml/mussel/hour. Nevertheless, the range in values of filtration rate is considerable due to the differences in the experimental conditions such as temperature, food type and food concentration. The clearance rate depends on the phytoplankton composition and environmental conditions such as water temperature and turbidity (Alexander *et al.*, 1994; Fanslow *et al.*, 1995; Lucy, 2005). With high filtration rates and great population densities, zebra mussel is probably the dominant suspension feeder in freshwater (Jack and Thorp, 2000). Zebra mussel oxygen consumption is directly linked to the filtration rate as respiration being the dominant consumptive pathway of ingested organic material (Fanslow *et al.*, 2001).

2.3.6. Physiological ecology

2.3.6.1. Water temperature as a limiting variable

Water temperature is one of the most important limiting variables for zebra mussels. Spawning, larval development and growth rate are temperature dependant (McMahon, 1996). Through zebra mussel range of colonisation, high variations of water temperature threshold for reproduction, larval development, growth exist, with differences between North America and Europe (Mackie and Schloesser, 1996). In Europe, the temperature for a successful development of the mussel veliger is 12-24°C (Sprung, 1993). The temperature threshold for growth usually varies between 10°C and 12°C (Mackie and Schloesser, 1996). In Great Britain the temperature threshold for the initiation of growth appears to be 11°C (Morton, 1969). Zebra mussel thermal tolerance differs from European to North American lakes. Northern European data indicate an incipient upper lethal limit of 27-28°C based primarily on field studies. In contrast, North American Great Lakes mussels could be held indefinitely in the laboratory at 30-31°C (McMahon, 1996). North American zebra mussel populations come probably from the

warmest part of the Black Sea (Marsden *et al.*, 1996) and therefore, may be genetically more thermally tolerant than European mussel populations (McMahon, 1996).

2.3.6.2. Calcium and pH

As shown previously for water temperature, field and laboratory studies showed highly variable tolerance to calcium and pH (Mackie and Schloesser, 1996). Zebra mussels are less tolerant of low calcium concentrations and low pH than other freshwater bivalves. Minimum pH limits are 6.5 for adult zebra mussels and 7.4 for veligers. Required pH for moderate and maximal adult growth are 7.4 and >8.0, respectively. In European lakes, no mussels occur at pH <7.3 (McMahon, 1996). According to Mackie (Claudi and Mackie, 1994), the threshold calcium levels are: for reproduction, 12mg.l^{-1}, for survival, 3mg.l^{-1} and for a massive infestation 25mg.l^{-1}. There is a significant relationship between calcium and pH and adult zebra mussel mortality. Mortality increases as calcium concentrations increased above 25mg.l^{-1} between pH 8.5 and 10 (Hincks and Mackie, 1997).

2.4. Dispersal capacity and colonisation

2.4.1. Dispersal mechanisms

Different dispersal mechanisms of adults and larvae range from natural mechanisms (e.g., water currents, birds, insects and others animals) to human-related mechanisms (Claudi and Mackie, 1994; Carlton, 1993).

Zebra mussels employ passive methods for dispersal during both the larval pelagic state and the adult benthic state. Primary dispersal occurs during the pelagic state by transport of the veligers and post-veligers by currents. The planktonic veliger stage is probably the most effective natural dispersal phase (Horvath *et al.*, 1996; Stoeckel *et al.*, 1997). However, adult attachments with byssal apparatus to floating logs, macrophytes and debris also help to augment the dispersal. Secondary dispersal occurs by the drifting of juvenile and young adults using byssal and/or mucous threads. In such transport, the byssal or mucous threads are monofilaments, distinct in form and function from the attachment byssus threads. The threads are many times the length of the animal and increase the hydrodynamic drag to enable the young mussels to be transported in the water column by turbulence or currents. In a lake, juvenile drift and translocating adults are dictated by the direction and force of the winds (Claudi and Mackie, 1994). Although veliger transport by waterfowl is a possible dispersion vector (Carlton, 1993), it was revealed that it was relatively low and thus of a minor concern (Johnson and Carlton, 1996).

Human mechanisms are numerous and transport both veligers and adult mussels: canals, ballast water, vessel hulls, navigation buoys, fishing equipment (nets, bait-bucket water), aquarium releases, amphibious planes, scientific research (Carlton, 1993). Recreational boats are important dispersal vector with veligers found in many boat structures especially live wells, while adults are found on hulls and macrophytes entangled on boat trailers (Johnson *et al.*, 2001, Pollux *et al.*, 2003).

2.4.2. Historic expansion

The zebra mussel is a native species of south of Russia, from the Caspian, Aral and Black Seas (Skorikov, 1903 cited by Karatayev *et al.*, 2003). It was originally found in the fresh and brackish waters of the Caspian and Black Sea drainage basins including large tributary rivers. Because shipping was restricted to the largest rivers, canal systems were built in the late 1700s-early 1800s, through what is now Belarus, to connect these large rivers and the Black, Caspian and Baltic Seas for commerce (Andrusov, 1897; Starobogatov and Andreeva, 1994 cited in Karatayev *et al.*, 2003). It took around 200 years for this invasive species to spread across all of Europe and into the rest of western Russia (Walz, 1992 cited in Ludyanskiy, 1993). The invasion continued in the middle of the nineteenth century, during the industrial revolution, via the water bodies opening and their interconnections with canals (Zhadin, 1946 cited in Ludyanskiy, 1993). Zebra mussels were first recorded in Europe in: 1824 in England; 1826 in The Netherlands; in 1830 in Germany; in 1840's in Denmark and France. The spreading continued in 1940's in Scandinavia, in 1960's in Switzerland and in 1970's in Yugoslavia, Ireland, Spain and Italy (Figure 2.4; Haas *et al.*, 1929 cited in Morton, 1993; Minchin *et al.,* 2005).

Figure 2.4: Zebra mussel distribution in Europe in 1800 and its subsequent spread during the industrial Revolution. From Haas *et al.*, 1929 cited in Morton, 1993

The genus *Dreissena* had already spread widely over the Volga River and its tributaries during the Quaternary period. Its distribution then expanded into Northern, Central and Western Europe (Nuttal, 1990; Ludyanskiy *et al.*, 1993; Ricciardi, 2003). Consequently, Europe has previously been exposed to the genus *Dreissena*. During the last glacial epoch, the break-up of the Thetys isolated *Dreissena polymorpha* and other species of *Dreissena* (some extinct) in a small area the Ponton-Caspian region (Babak, 1983 cited in Morton, 1993). Unlike Europe, North America has not been exposed to *Dreissena*. The invasion in North America was more recent starting in 1980's, and was dramatically quick leading to severe economic and ecological impacts.

2.4.3. North America

The first individual was collected from Lake St Clair on the 1st of June 1988. Length-frequency analyses of populations from the Great Lakes and review of historical

benthic studies suggest that the mussel was introduced in the lake in late 1986 (Hebert *et al.*, 1989; Griffiths *et al.*, 1991). Carlton (1993) described three lines of evidence that lead to the conclusion that ballast water in transoceanic vessels transported zebra mussels from Europe to the Laurentian Great Lakes: i) the common presence of bivalve larvae in ballast water combined with patterns of vessel traffic into the lakes; ii) the likelihood that ballast water was the mechanism for the arrival in the same decade of other European aquatic organisms (fish and crustaceans); and iii) the inability to identify any other probable mechanism. It is also possible that adult mussels may have attached to anchors or vessel chains in European harbours and survived the transoceanic journey (Hebert *et al.*, 1989; Ussery and McMahon, 1994). In 6 years, zebra mussel invaded all of the Great Lakes and spread in eight river systems: Saint Lawrence, Hudson, Mississippi, Ohio, Illinois, Tennessee, Susquehanna and Arkansas rivers (Ludyanskiy *et al.*, 1993; Figure 2.5). Veliger and adult densities in the Great Lakes are among the highest reported. During the first colonisation in the Lake Erie, the peak densities of veligers reached 6-137 ind.l^{-1} (Riessen *et al.*, 1993). In western basins, when zebra mussel populations are well established, 126-268 veligers per litre in July and August are recorded. For the adult densities, 54 000 to 779 000 ind.m^{-2} were observed in the Lake Erie after few years of colonisation, and 43 000 ind.m^{-2} in the Lake Saint Clair (Mackie and Schloesser, 1996). The ecological and economic impacts are considerable (Figure 2.6).

1988: First discovery of zebra mussels in Lake Erie

1998: Zebra mussel invasion in North America

2005: Zebra mussels had spread to 22 American states and 2 Canadian provinces.

☆ Mussels on boat hulls

● Presence of zebra mussels

Figure 2.5: Rapid spread of zebra mussels in North America between 1988 and 2005. Source from USGS, 2005

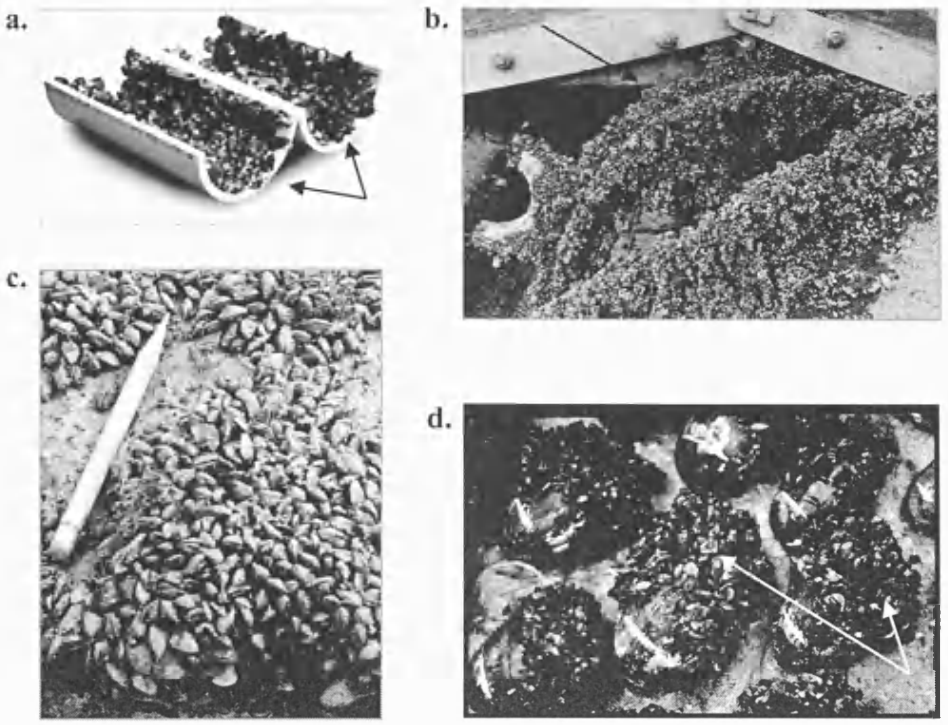


Figure 2.6: Zebra mussel colonising different structure: a. clogging a pipe, b. on gear mechanism, c. on a rock, d. on native mussels

2.4.4. Europe, Great Britain

Zebra mussel densities in Europe are lower than in North America (Mackie and Schloesser, 1996). Veliger densities comprised between 10 and 100 ind.l^{-1}. Some exceptions are recorded as in Konin Lakes (Poland) with 22-320 ind.l^{-1}. Average adult densities range from about 2 000 to about 115 000 ind.m^{-2} (Cleven and Frenzel, 1993; Stanczykowska and Lewandowski, 1993; Mackie and Schloesser, 1996). A density in one grab of 11 000 ind.m^{-2} is the highest density observed in any British waterbody (Aldridge *et al.*, 2003). Zebra mussels are present in Great Britain since 1824 but have recently increased in density and distribution. Number of sites in England and Wales where zebra mussels have colonised has shown a notable increase between 2000 and 2003 (Aldridge *et al.*, 2003). Aldridge *et al.* (2003) proposed hypotheses which can explain this phenomenon. The first hypothesis relates to water quality and climate change. With recent improvements in water quality, a much greater range of sites have become habitable, thus releasing zebra mussels from the confines of relatively localised clean

water refuges. Moreover, the warmer summers and milder winters of recent years may have enhanced the reproductive success of zebra mussel populations. Another hypothesis concerns a new gene pool. The current British invasion may be from a new introduction of zebra mussels which are better suited to the British climate than the original population. Even though zebra mussel population densities are lower than in North America, the ecological and economic impacts caused by zebra mussels are similar.

2.4.5. Origins, founder effects and genetic diversity

To understand the exact origins of invasions and to gain insight into possible reasons for population expansion, many diverse genetic methods have been used: i) the reconstruction of intraspecific phylogeographic relationships among populations ii) comparison of genetic similarities with source populations; iii) assessment of the potential number, and origins, of colonisation events; and iv) determination of the specific identity of *Dreissena* involved in each colonisation (Boileau and Hebert, 1993; Astanei *et al.,* 2005; Gelembiuk *et al.*, 2006).

Native to the Ponto-Caspian basin, zebra mussels, *Dreissena polymorpha*, are considered to be extremely variable both in morphology and habitat requirement. Three living subspecies of *Dreissena polymorpha* were discovered and currently exist: *D. p. polymorpha*, *D. p. gallandi* and *D. p. Anatolica. D. p. polymorpha* is considered as the most widespread subspecies. Gelembiuk *et al.* (2006) used DNA sequences from the mitochondrial cytochrome oxidase I (COI) gene to examine intraspecific population structure and population divergence times. Three clades corresponding to the three subspecies were found. The age of the basal split between *D. p. anatolica* and the other two subspecies (*D. p. polymorpha* and *D. p. gallandi*) was estimated as c. 730 000 years, while the age of the split between *D. p. gallandi* and *D. p. polymorpha* was estimated as c. 510 000 years.

All populations (and subspecies) within *D. polymorpha* exhibited low levels of haplotype diversity for COI and this is inconsistent with a population history of constant large effective size (Gelembiuk *et al.*, 2006). The significant differentiation between the populations studied, suggesting a series of historical bottlenecks subsequently followed by population expansion. ZM populations are effectively characterized by mutually

exclusive subsets of haplotypes, suggesting that shared haplotypes were lost in each region due to the founder effect. This scenario is consistent with fluctuating habitats in the Ponto-Caspian basin which is characterised by major variations in salinity, water level, temperature and dissolved oxygen on both seasonal and geological timescales. The range of environmental fluctuations may have promoted the evolution of broad tolerance or the maintenance of genetic variance for tolerance upon which selection could act. Therefore, the combination of habitat instability, frequent extirpation/recolonisation events, and mussel high fecundity would have predisposed *Dreissena polymorpha* to invasiveness.

Phylogeographic analyses were performed on invasive zebra mussel populations to determine the source populations (Pollux *et al.*, 2003; May *et al.*, 2006; Rajagopal *et al.*, 2009). Patterns of COI haplotype diversity indicate that all invasive populations of zebra mussels from North America and Europe corresponded to *Dreissena polymorpha polymorpha* and originated from the Ponto-Caspian Sea region. Only two haplotypes were found in the invaded regions and are originated from the Black Sea drainage, which suggests the Northern estuaries of the Black and Caspian Sea served as the source of invasive populations rather than from the more stable Ancient Lakes to the South of the seas (Gelembiuk *et al.*, 2006; May *et al.*, 2006).

High levels of genetic variability have been reported for both European and North American populations (Marsden *et al.*, 1995; Astanei *et al.*, 2005). The origin of North American mussel populations remains a matter of conjecture. Small genetic differentiation was found between North American populationswhich supports the hypothesis of a single and large founding population and/or of frequent genetic mixing (Marsden *et al.*, 1995; Astanei *et al.*, 2005; May *et al.*, 2006). However, Elderkin *et al.* (2004) revealed significant genetic differentiations between North American populations exhibiting genetic structure at large geographical. Zebra mussel colonisation dynamics may be the cause of founder events, which would increase genetic structure. The occurrence of multiple, genetically distinct, European source populations, may also explain the high genetic structure (Stepien *et al.*, 2002). Marsden *et al.* (1995) advocated that it might certainly not be possible to identify definitively the founder population from Europe. However, some studies suggest that source populations of North American zebra

mussel populations, would have come from Northwest of Europe rather than Central or East of Europe (Astanei *et al.*, 2005; Stepien *et al.*, 2002).

Nevertheless, the origin of source populations for secondary invasions within Europe is clearer. For example, recent zebra mussel invasions in Ireland and Spain were most likely to have originated from Great Britain and France respectively (Pollux *et al.*, 2003; Rajagopal *et al.*, 2009).

The discordant results for the origin of zebra mussel source populations indicate the need for further analysis using different genetic markers and a more complete sampling of source populations to understand the genetic variations of zebra mussels more comprehensively (Gosling *et al.*, 2008).

2.5. Ecological and economic impacts

2.5.1. Ecological impacts

2.5.1.1. Water quality

Zebra mussels have a high particle filtration rate (110 ml/mussel/hour), around 10 times that of unionid mussels (Vanderploeg *et al.*, 2002). Consequently, standing stocks of seston, nutrients, phytoplankton and zooplankton abundance can decrease dramatically with a concurrent increase in water clarity where zebra mussel populations become large (Leach, 1993; Fahnenstiel *et al.*, 1995). In Western Lake Erie, mean Secchi disc transparency during the May to November period between 1988 and 1989 increased by 1.24m (85%; Leach, 1993); and in 1990-1992, transparency was found to be 100% higher (Holland, 1993). Enhanced water clarity increases light transmittance and growth of benthic plants. The transport of carbon and nutrient pools to the sediment could have a major effect on the nutrients cycling within the food web. For example, in Lake Huron, Saginaw Bay, zebra mussels have a significant impact on nutrients and are a significant sink for phosphorus. Zebra mussels may alter the N:P ratio of dissolved nutrients available to the microbial community and hence potentially alter species abundance and composition (Johengen *et al.*, 1995). Furthermore, decline in dissolved oxygen concentrations attributed to zebra mussel invasion has been reported in large rivers in the United States, where zebra mussel densities have reached high densities (Effler and Siegfried, 1994; Effler *et al.*, 1996; Effler and Siegfried, 1998).

2.5.1.2. Phytoplankton and zooplankton

In shallow lakes, zebra mussels can be dominant consumers of phytoplankton and compete with zooplankton (Vanderploeg *et al.*, 2002). In Great Lakes, phytoplankton abundance decreased following zebra mussel arrival, which resulted in chlorophyll *a* decline. In Lake Erie, mean chlorophyll *a* concentrations between 1988 and 1989 declined 43% (Leach, 1993). In the Hudson River, between 1993 and 1994, grazing pressure on phytoplankton was over 10 times greater than it has been prior to the zebra mussel invasion, and phytoplankton biomass declined 85% (Caraco *et al.*, 1997). Changes in phytoplankton community composition have been observed but directions of

the species shifts have differed. In Western Lake Erie, Lake Huron and Saginaw Bay, cyanobacterial blooms have occurred after zebra mussel establishment, despite severe declines in chlorophyll corresponding to diatoms numbers decrease. Conversely, in the Hudson River, the proportion of diatoms increased, while the proportion of cyanobacteria decreased dramatically (Bastviken *et al.*, 1998; Heath *et al.,* 1995; Holland, 1993). Changes in phytoplankton species composition in a freshwater system can be due to direct clearance or indirect effects as nutrients or light regime alteration, or dominance of buoyant groups caused by the filtration at the bottom of the water column (Bastviken *et al.*, 1998).

Zebra mussels have strong impacts on zooplankton density and community structure directly by predation and indirectly by competition (Jack and Thorp, 2000). MacIsaac *et al.* (1995) showed that only rotifers and other small-bodied zooplankton are removed from the water column. Jack and Thorp (2000) concluded that zebra mussels can directly suppress rotifers but also small cladocerans and perhaps small copepods. However, Winkler *et al.* (2005) showed that in the estuarine transition zone of the St Laurence River, the zebra mussel invasion has had little or no impact on the zooplankton community in spite of the numerical dominance of veligers.

2.5.1.3. Invertebrates communities

The mussels are changing ecosystem function through mechanisms of ecosystem engineering, increasing water quality and reef building with macrophytes and mussel colonies (Vanderploeg *et al.*, 2002). This new habitat structure is of benefit for many benthic invertebrates. Zebra mussels have significant effects on benthic invertebrate communities with an increase in abundance and diversity (Mayer *et al.*, 2002; Ward and Ricciardi, 2007). Despite the oxygen depletion at the interface of zebra mussel colonies, the altered habitat structure provides refuge from predation and deposit organic material (Beekey *et al.*, 2004), and therefore, enhances the abundance of amphipods, flatworms, small gastropods, for the displacement of large gastropods and certain filterers (Ricciardi *et al.*, 1997). This single exotic bivalve species can potentially alter the entire pelagic-benthic energy balance of an ecosystem: nutrients and carbon transported to the sediments as faeces and pseudofaeces, and the increase of benthic primary production drive the energy from the pelagic zone to the benthic zone (Claudi and Mackie, 1994).

2.5.1.4. Native unionids

Freshwater mussels are the most imperilled faunal group in North America. North America contained 297 unionid species (Schloesser *et al.*, 1996): 60% of described species are considered endangered or threatened, and 12% are presumed extinct. Widespread habitat degradation has been the primary cause of extinction during this century, but a new stress was added by the introduction of zebra mussels (Ricciardi *et al.*, 1998). To date, the epizoic colonisation of unionid bivalve molluscs by zebra mussels has caused the most direct and severe ecological impact (Schloesser *et al.*, 1996). The mussels are not selective and colonise all species of unionids. The biomass of zebra mussels is so great that locomotion, burrowing and valve opening of the individuals are impaired which cause the unionid death by smothering (Claudi and Mackie, 1994). Such colonisations on unionids were also observed in Europe (Burlakova *et al.*, 2000; Lucy, 2005).

2.5.1.5. Waterfowl

Diving waterfowl consist in the rare zebra mussels predators. Zebra mussels being great filter feeders have the capacity to accumulate contaminants such as cadmium and mercury. De Kock and Bowmer (1993) examined the transfer of cadmium and organochlorine contaminants, from zebra mussels to the tufted duck (*A. filigula*), and showed a subsequent transfer to the duck eggs with resulting teratogenic effect with smaller clutch and egg size and higher embryo mortality.

2.5.2. Economic impacts

The strongly byssal feature makes the zebra mussel one of the most tenacious and potent biofoulers. Zebra mussels can infest so different structures and render them less efficient: i) navigation aids, such as fishing buoys and markers, have such heavy infestations that they sink below the surface ii) commercial fishing gear, such as trap nets and gill nets, become useless and difficult to retrieve iii) hulls of boats are so laden with mussels that their sailing efficiency is impaired (MacIsaac, 1996). Moreover, zebra mussels are known to be fouling organisms, causing severe nuisance and damage to industrial and power plant infrastructure, by aggregating in pipes and on structures (Jenner and Janssen-Mommenn, 1993; Laurier LePage, 1993; Ram and McMahon, 1996).

In the United States, industrial facility damages caused by zebra mussels are estimated to cost US$5billion each year (Khalanski *et al.*, 1997).

2.5.3. Controls

The majority of the effective controls are applied for facilities such as water intake structures, cooling systems, dams, locks. An effective zebra mussel control applicable in open body water ecosystems does not exist.

Temporary reductions of mussel density through predation are likely in areas where ducks feed during migratory periods (Hamilton *et al.*, 1994; Werner *et al.*, 2005). Part of the reason for their high biomass under most circumstances may be due to low predation pressure on settled mussels relative to zooplankton, which are prayed upon by a variety of pelagic fishes and invertebrates. Furthermore, veligers are vulnerable to predation by a variety of omnivorous or predatory zooplankton only before they form their larval bivalve shell a few days after hatching (Vanderploeg *et al.*, 2002). However, diving waterfowl are important predators of adult zebra mussels.

2.5.3.1. Chemical treatments

Mitigation by chemicals has been the common practice in Europe and North America to protect almost the entire facility, from within the intake to discharge. Chlorination is currently considered the most effective and popular method. This method does not cost much, it does work efficiently, and it provides toxic in low concentrations (Barton, 1993; Van Benschoten *et al.*, 1993, Claudi and Mackie, 1994). However, these treatments are strictly for raw water systems, and cannot be used in open water bodies because of their toxicity towards the environment. A recent method based on chemical treatment was found, called "BioBullet", which consists in the encapsulation of chlorine, in microscopic particles of edible material (Aldridge *et al.*, 2006). Mussels filter the particles and concentrate the toxin. The particles not consumed by mussels, break up and dissolve completely within a few hours, thus eliminating the risk of polluting the wider ecosystem. This method, still on trial, reveals nevertheless a high cost of money to be developed and commercialised.

2.5.3.2. Non-chemical control

Proactive and reactive techniques are distinct. The different methods used in raw water system are not always appropriate for large water volumes, have disadvantages and limits. Thermal shock, infiltration galleries sand filters, ultraviolet light, electroshock, acoustics are some of the proactive techniques (Claudi and Mackie, 1994). Antifouling agents present one of the best methods for minimizing zebra mussel attachment on structure exposed in lakes. However, toxic antifouling agents, such as organometallic and aggressive oxidisers, have negative impacts on the environment and yet are still used. Some environmental benign attractive antifouling agents were discovered but cannot be produce on a sufficient scale for commercial purposes and show expensive cost of the production (Diers *et al.*, 2006; Angarano *et al.*, 2009; Qian *et al.*, 2010).

Biological controls may be effective in reducing zebra mussel populations in ponds and lakes but not eradicating them. Many natural enemies of zebra mussels exist (220 species), both in Europe and in North America, as predators, parasites and ecological competitors (Molloy *et al.*, 1997). Although some fishes and birds consume zebra mussels at high rates (Naddafi *et al.*, 2010) they are not necessarily effective candidates for use in control programs. Round gobies for example, *Neogobius melanostomus,* also native from the Black-Caspian Sea area, are predators of zebra mussels. The gobies were probably introduced to North America in ship ballast water. Although gobies have a negative impact on zebra mussels in North American lakes, they do not eradicate mussel populations (Ray and Corkum, 1997). Furthermore, the fish also have negative impacts on native macroinvertebrates (Lederer *et al.*, 2006) As predators are typically not sufficiently specific in their prey choices; benthic competitors are not either (Molloy, 1997). Bacteria or antibiotic agents may be used as biological controls, such as *Bacillus* species, a molluscicidal agent (Singer *et al.*, 1997) but again, a biological control must be specific. The bacterium *Pseudomonas fluorescens* would be a leading candidate for the biological control of zebra mussels; research and funding for commercialisation are still ongoing (Molloy and Mayer, 2009). Parasites would likely be the most environmentally-safe biocontrols agents due to their high host specificity, and investigations are still ongoing (Molloy, 1997).

2.6. Conclusions

The flexibility in reproductive strategies, the high propagule pressure, and the tolerance of a wide range of environmental conditions make the zebra mussel a highly invasive species. Zebra mussels have very significant impacts across the globe, which have been particularly well studied in North America and Europe. The environmental and industrial damage appears to be driven by the high population densities that zebra mussels can reach and by two major traits which characterise zebra mussels: being filter feeders and fouling organisms.

The analysis of the literature reveals a large body of knowledge surrounding the zebra mussel but it is also clear that, there is limited research on zebra mussel populations in Great Britain. As an island, with many ports and rich international shipping activity, research on the zebra mussel as an invasive species is of value and interest. Furthermore, there are limited case studies on the development of zebra mussel populations in newly formed artificial water bodies, such as Cardiff Bay, where particular environmental conditions may influence, and even facilitate the establishment, development and further spread of the zebra mussel population.

3. The dynamics, seasonality and spatial distribution of zebra mussel veligers in Cardiff Bay

3.1. Summary

1. Some invasive species produce many offspring and have flexible reproductive strategies which facilitate establishment in appropriate conditions. However, few studies examine how rapidly they establish in newly-formed environments. The shallow, freshwater lake at Cardiff Bay was invaded by zebra mussels, *Dreissena polymorpha* (Pallas), soon after its creation by impoundment in 2001. This chapter appraises spatio-temporal patterns in the abundance and dynamics of the mussel's veliger larvae over a four year period (2006-2009) within five years of the lake's formation. Veliger larvae and environmental data were recorded at replicate locations across the Bay to appraise influences on reproduction and establishment in this new lake.

2. Mussels reproduced from late May to late September at >14°C. Veligers were then produced with apparent synchrony in 1-3 larval peaks in each year that were typically largest in May-June and smaller in August. Atypical conditions in 2008 revealed that seasonal reproductive patterns could be adjusted facultatively.

3. Peak veliger densities varied among years from 8 ±1.9 to 14 ±4 (±SE) ind.l^{-1}, with overall patterns affected strongly by river discharge and temperature. Densities varied with local hydraulic conditions being lowest in the mouths of the Bay's two large inflowing rivers.

4. These data are consistent with the rapid, extensive establishment in Cardiff Bay of a substantial zebra mussel population now maintained by larval production sufficient to produce high veliger densities throughout the lake. A flexible reproductive strategy, coupled with a relatively low temperature threshold for initiation, illustrate traits that have allowed this non-native species to colonise large temperate areas. High-risk periods for contamination (e.g. of boats) and dispersal beyond occupied sites extend throughout the summer. Conversely, high summer discharge apparently reduced propagule pressure in this lake, and subsequent chapters examine consequences for colonisation.

3.2. Introduction

The effects of invasive species vary according to the ecosystem and geographic regions in which they have been introduced (Ricciardi, 2003). Species establishment is a central feature in invasion, and likely traits that facilitate establishment include large numbers of offspring and flexible reproductive strategies (Nichols, 1996; Ram *et al.*, 1996). However, few studies have examined how rapidly and intensively they can colonise newly formed environments.

Now a well-documented invasive species, the zebra mussel was first recorded in Great Britain in 1824 (Morton, 1969, Sowerby, 1825). The development of the British canal system and commercial boat trade probably facilitated its dispersal, and by 1970, zebra mussels were found in Scotland, Wales and England (Morton, 1970). After some local decline during the last century, zebra mussel densities generally increased during 1980-2000 and mussel populations spread rapidly throughout Great Britain (Aldridge *et al.*, 2004). Extensive colonisation of other European countries and North America also occurred over the same time period (Stanczykowska and Lewandowski, 1993; Nalepa *et al.*, 1996; Nichols, 1996; Müller *et al.*, 2002).

One explanation for the highly invasive nature of zebra mussels is the flexibility of the reproductive cycle and a prolific reproductive rate (Nichols, 1996). Zebra mussels are apparently adapted to adjust their reproductive cycle to a wide range of environmental conditions. For example, larvae can be produced from between 6 and 52 weeks of the year, depending on local conditions (Nichols, 1996). Numbers of spawning events can also vary to create 1-5 larval peaks in any year (Waltz, 1978; Wilhelm and Adrian, 2007). Because zebra mussel fertilisation is external, through gametes released into the water column, coordinated maturation and synchronous release must occur, controlled in turn by internal and external factors (Ram *et al.*, 1996). Minimum temperature required to initiate spawning varies from 12-19°C, sometimes even 23°C (Haag and Garton, 1991; Claudi and Mackie, 1994). However, in many European lakes, veliger production starts at 12-15°C (Eisnle, 1973 cited in Lucy, 20006; Sprung, 1993; Karatayev *et al.*, 1998; Lucy, 2006). Once spawned, veligers can survive at 0-30°C (Sprung, 1993). This free-

swimming planktonic stage is, in its own right, important in dispersal (Riessen *et al.*, 1993), including movement to new sites (Morton, 1970).

As with temporal dynamics, veliger spatial distribution is related to environmental conditions. For example, water flow is a major factor which influences veliger distribution through their passive transport (Griffiths *et al.*, 1991) while retention can occur through hydrodynamic trapping in zones with high residence time (Barnard *et al.*, 2003). In brackish waters, salinity can limit veliger populations through mortality (Strayer and Smith, 1993; Barnard *et al.*, 2003). In some studies, veliger densities were positively correlated with chlorophyll *a* concentrations (Barnard *et al.*, 2003), whilst in other cases veliger densities were lower at chlorophyll-rich sites where adult zebra mussels had reduced phytoplankton through filter-feeding (MacIsaac *et al.*, 1992; Vidal *et al.*, 2004). Veliger vertical distribution in rivers tends to be homogeneous, influenced by current and water mixing (Kern *et al.*, 1994; Barnard *et al.*, 2003). Conversely, veliger vertical distribution in large lakes reflects wind-driven currents, where high veliger densities are found in the first metres from the surface (Fraleigh *et al.*, 1993).

Although these aspects of zebra mussel ecology have now been investigated extensively during the species' recent population expansion, there have been few case studies where their dynamics have been investigated in newly formed water bodies. Any such locations would provide an insight into how rapidly colonisation can occur, how populations are maintained, and what factors affect the risks of subsequent dispersal to other sites. Cardiff Bay, newly-formed by closure of the formerly tidal Taff-Ely river systems by a barrage in 2001, provided one such opportunity, because the resulting freshwater lake was occupied by zebra mussels shortly after its closure. In addition to assessing fundamental aspects of reproductive dynamics or larval density in this temperate location under contrasting conditions, data on larval dynamics and densities might help identify high-risk periods for contamination (e.g. of boats), risks of dispersal beyond this site, and factors affecting larval pressure in colonising new habitats (Chapter 3).

Work described in this chapter sought to investigate veliger population dynamics in Cardiff Bay over four consecutive years with contrasting environmental conditions

(2006-2009). Based on previous studies the following hypotheses about spatio-temporal pattern were tested through frequent data collection in the field:

i. Veliger production varies seasonally in Cardiff Bay, initiated by a water temperature threshold in the range 12-15°C, with a flexible pattern of larval peaks.

ii. Seasonal pattern and veliger density differ between years and this is related to the environmental conditions, such as river discharge and water temperature.

iii. Larval density is patterned spatially across Cardiff Bay, particularly between river and lake habitats.

3.3. Materials and methods

3.3.1. Site

Formed in 2001, Cardiff Bay is an artificial lake of 200 hectares situated on the south coast of Wales, UK, (51°27'18.9706"N 03°10'05.5186"W). The Bay was formed by the construction of a barrage impounding two rivers to create a lake with a mean depth c4.5 metres and maximum depth c13 metres. Locks in the barrage permit the passage of boats between the sea and the lake, but any seawater entering the lake is collected in an associated pump so that the lake normally has a salinity of 0.19 ‰. To reduce the risk of problem algae and stagnation of the lake, a benthic aeration system has been installed with the intention of maintaining dissolved oxygen concentrations >5mg.l^{-1} (Cardiff Harbour Authority, 2003). Further details are presented in Chapter 1 (§1.2).

3.3.2. Sampling

3.3.2.1. Temporal distribution

Veliger samples were collected at ten sites through the lake and in downstream part of the Taff and Ely rivers over the period 2006-2009. Sites were chosen to represent the fullest possible range of environmental conditions present in Cardiff Bay while allowing for the logistics of regular sampling (Figure 3.1.a): four sites (3, 4, 9, and 10) were positioned in the mouths of the Taff and Ely, two in the harbour (sites 1 and 2), two in open water (sites 5 and 7), one by the west bank (site 6) and one adjacent to the barrage (site 8). Samples were taken approximately every two weeks during the whole of 2006 and 2007, and during May-October in 2008 and 2009. Veliger samples were collected from a boat, using a conical plankton net with fine mesh (60mm) and mouth diameter 20cm (3.1.b). The net was hauled from the lake bed to the surface thereby sampling the entire water column. A square mesh (60mm) was fixed at the base of the plankton net where veligers and other zooplankton accumulated during sampling. One sample was taken at each site, and the mesh and zooplankton preserved immediately in 70% alcohol.

In the laboratory, samples were sorted and veligers counted according to Claudi and Mackie (1994), a method used in different studies (Nalepa *et al.*, 1995). Briefly the methodology was as follows:

Each sample was rinsed and diluted in 20ml of tap water. Five 1 ml sub-samples were then taken from the 20ml well-mixed sample. Using a microscope at 40x magnification, the larvae in each sub-sample were counted. The number of veligers present in the whole sample (20ml) was estimated by multiplying the mean of the five sub-sample counts by 20. When the total number of veligers in the 20ml sample was fewer than 200 individuals, the entire sample was counted. To calculate the veliger density at each site, the total of individuals present in the sample was multiplied by the volume of water sampled. The volume of water sampled was calculated by multiplying the area of the haul net aperture by the depth through which the haul net was dragged.

Volume sampled = aperture area x water column length

Veliger larvae density was expressed as number of individuals per litre ($ind.l^{-1}$). Zooplankton associated with zebra mussel veligers were collected only in 2006 and 2007 and data were obtained by Merrix (2009).

At the same time as veliger sampling, one water sample was collected at each of the ten sites with a plastic tube to assess chlorophyll *a* concentration. Samples were analysed with a fluorimeter (model bbe Moldaenke, Germany) to determine the total concentration ($\mu g.l^{-1}$) and activity of chlorophyll *a* associated with green algae, blue-green algae, diatoms and cryptophytes. Water surface temperature, dissolved oxygen, turbidity, pH, conductance and salinity were measured with a probe (model 6920, YSI Inc., USA) at each site and for each sampling occasion. Discharge in the Ely and Taff were recorded every 15 minutes at Environment Agency gauging stations at respectively St Fagans and Pontypridd. The river discharge into Cardiff Bay was then estimated by combining the two river flows; this method will accurately represent variations in discharge through time, but will slightly underestimate absolute discharge volumes from the Taff because of other small inputs downstream from Pontypridd.

Water chemistry was recorded by Cardiff Harbour Authority throughout all sampling years as part of the regular monitoring. A depth profile was weekly carried out

through Cardiff Bay at 14 sites: water temperature, dissolved oxygen, turbidity, pH, conductance and salinity were measured every 0.5 metre with a probe (model 6920, YSI Inc., USA).

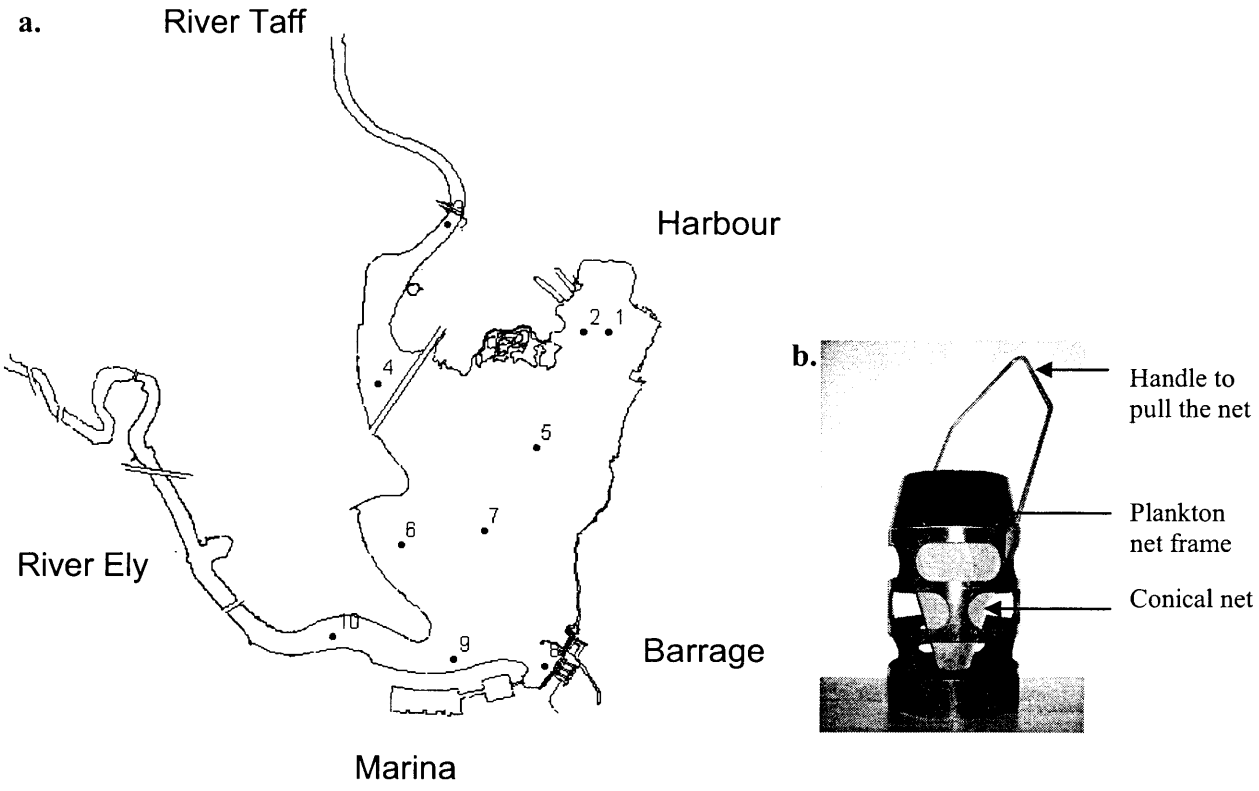


Figure 3.1: a. Positions of ten sampling sites in Cardiff Bay used to determine temporal variations in the density of zebra mussel veligers over the period 2006-20009. b. Conical plankton net used to collect veliger larvae

3.3.2.2. Spatial distribution

Detailed surveys of the spatial distribution of zooplankton were conducted in Cardiff Bay on 19th of September 2006 and 6th of September 2007 by Merrix (2009) and these same samples were used here to assess veliger spatial distribution at a finer scale than that allowed by regular sampling. Although not the period likely to have the largest

veliger densities, it was anticipated that relative patterns of distribution might be observed opportunistically from these data. Thirty sites were chosen encompassing the main body of the Bay and the lower areas of the rivers Taff and Ely Rivers (Figure 3.2). One sample was collected at each site as described in section 3.2.1 and sorted and counted using identical methods (§3.2.1). Water chemistry was also appraised using the methods described above (§3.2.1). Current velocity was assessed in the main body of Cardiff Bay only once by CHA, in February 2008 under similar discharge to the two detailed veliger surveys. Flow was measured every five seconds across a series of transects in the main body water using a 1500kHz frequency Acoustic Doppler Current Profiler (Sontek, USA) linked into a laptop running Coastal Surveyor software (Sontek, USA). Current velocity data were then interpolated using ArcGIS (ESRI, 2004) through the entire Bay.

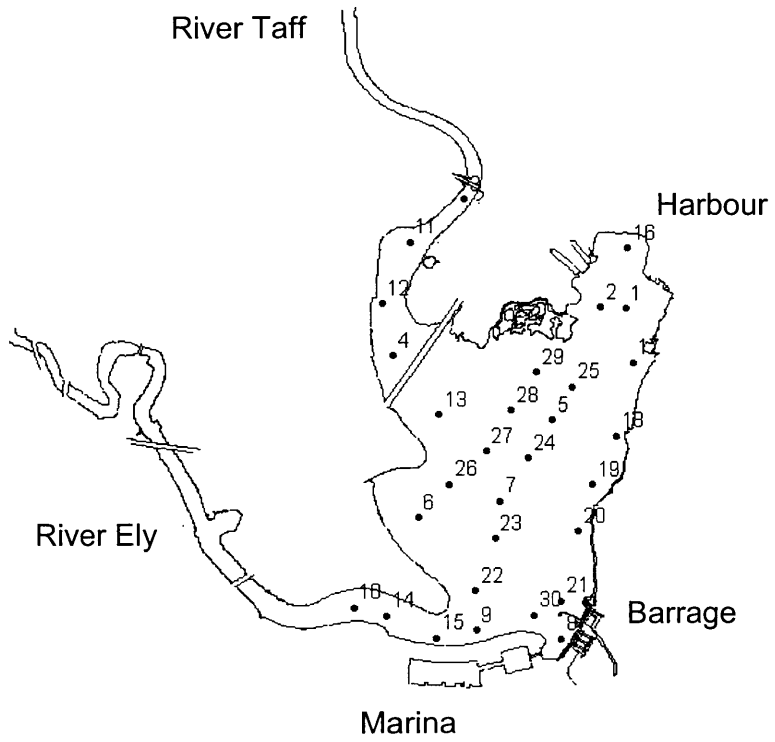


Figure 3.2: Positions of 30 sampling sites in Cardiff Bay used to determine detailed spatial variations in the density of zebra mussel veligers in Cardiff Bay in September 2006 and 2007

3.3.3. Statistical analyses

Prior to any further analysis, river discharge, chlorophyll *a* concentration, salinity, turbidity and dissolved oxygen, DO, were transformed using log, square root or exponential functions to minimise kurtosis and skewness and to ensure homogeneity of variances and normality of residuals. Also, to allow some comparisons among years, veliger density data were standardised within years by substraction of the mean and division by the standard deviation. This procedure allowed an expression of standard veliger densities across sites and months relative to the annual mean.

3.3.3.1.Temporal variations

To characterise general conditions under which zebra mussels reproduced, water temperature, river discharge and chlorophyll *a* concentration were compared between winter (November-March) and summer (April–October) using one way ANOVA. Kruskal-Wallis and Mann-Whitney tests were performed on Minitab to compare veliger densities between sites. Therefore, to assess seasonal and temporal variations in veliger density, veliger data from sites 1, 2, 5, 6, 7, 8, 9 and 10 were pooled, and the mean was calculated for each sampling date. Sites 3 and 4 were excluded from this analysis due to extremely low veliger densities, often of zero and these were, therefore, not representative of the study of temporal veliger production (Results §2.4.1.2.).

Variations in environmental data and veliger density between years (2006, 2007, 2008 and 2009) and months (May, June, July, August and September) were assessed using Generalised Linear Modelling (GLM) procedures with either a Gaussian error distribution and Identity link function (veligers) or other appropriate error terms for water temperature, discharge, chlorophyll *a* concentration, salinity, turbidity, pH and DO (Table 3.1). Data analyses were performed on R2.9.2.

Variations in veliger density through the four year study were explored in relation to environmental variations after the latter were reduced to major variants using principal components analysis (PCA) on water temperature, discharge, chlorophyll *a* concentration, salinity, turbidity, pH and DO. Veliger densities were plotted against Principal Component axes, and Pearson's correlation coefficient calculated.

3.3.3.2. Spatial variations

To assess variations in veliger density across the 30 sites in the detailed spatial survey, density data from each site were related to environmental variation derived from PCA on water temperature, chlorophyll *a* concentration, salinity, turbidity, pH and DO across all locations. Spatial veliger data were plotted against Principal Component axes, and Pearson's correlation coefficient calculated. Spatial patterns among veligers were also mapped in ArcGIS (ESRI, 2004), with environmental variables at the 30 sites displayed using spline interpolation. This procedure interpolates values for cells in a raster from a more limited number of sample data points.

3.4. Results

3.4.1. Seasonal variations

3.4.1.1. Environmental variations

Marked variations in conditions in Cardiff Bay over the year formed the backdrop for marked intra-annual variations in larval density. The summer period in Cardiff Bay was marked by increased water temperature (ANOVA, $F_{1,70}$= 160.9, P<0.001) and chlorophyll *a* ($F_{1,65}$= 61.3, P<0.001), while discharge decreased (ANOVA, $F_{1,70}$= 22.8, P<0.001; Figure 3.3). Conditions also varied between months within these periods (Figure 3.6; Table 3.1). Phytoplankton were most apparent from April to the beginning of October, and resulted in two chlorophyll *a* peaks in April and June, with a strong decline in May. Zooplankton were most apparent in the water column from the end of April to October in both 2006 and 2007, and over this period veliger larvae formed 7-10 % of zooplankton densities.

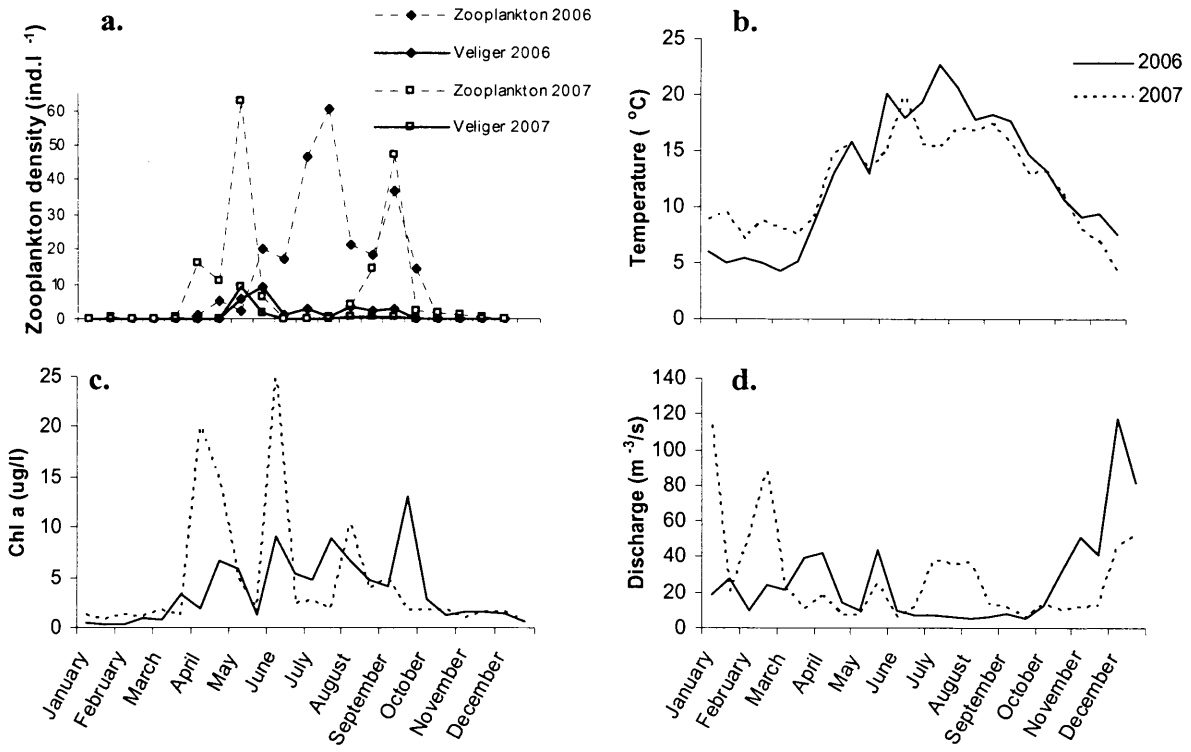


Figure 3.3: Seasonal variations in Cardiff Bay over the study period (2006-2007): a. Zooplankton and veliger density in 2006 and 2007; b, c and d: water temperature, chlorophyll *a* concentration and river discharge during 2006-2007

3.4.1.2. Variations in veliger density among regularly sampled sites

Veliger density varied between the ten regularly sampled sites (Figures 3.4 & 3.5), with mean density larger at sites 1, 2, 7 and 8 (2.4 ±0.9 ind.l^{-1} (±SE) to 3.9 ±1.3 ind.l^{-1}) than at sites 5, 6, 9 and 10 (0.21 ±0.1 ind.l^{-1} to 1.26 ±0.5 ind.l^{-1}). Veliger density at sites 3 and 4 was almost zero (0.02 ±0.01 ind.l^{-1} and 0.08 ±0.03 ind.l^{-1}), and these sites were not considered further in analyses of temporal variation (Figure 3.4). Veliger density variations elsewhere were similar, particularly among sites with greatest densities (Figure 3.5).

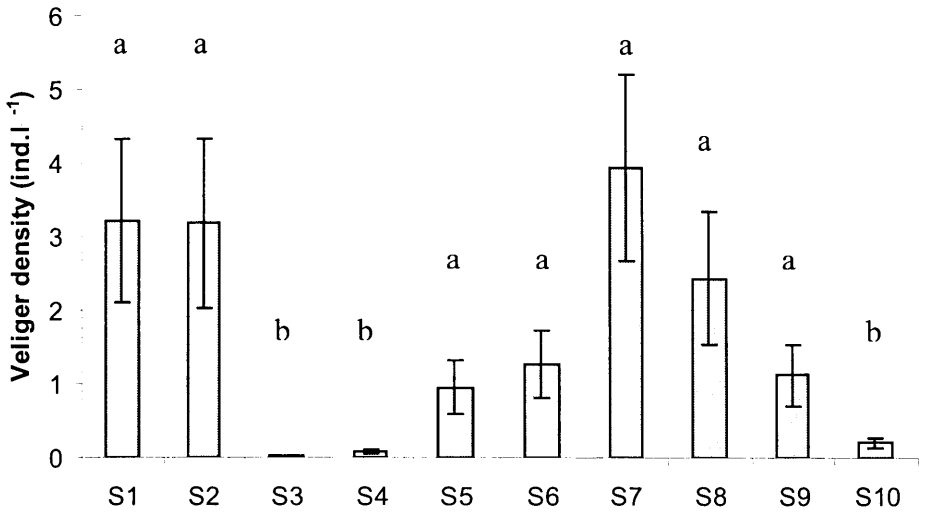


Figure 3.4: Means of zebra mussel veliger density recorded at each regularly sampled site in Cardiff Bay over four years. The 10 sites are annotated S1-S10. Error bars correspond to the standard errors. a and b are significantly different (K-W, H_9=59.3, P<0.001)

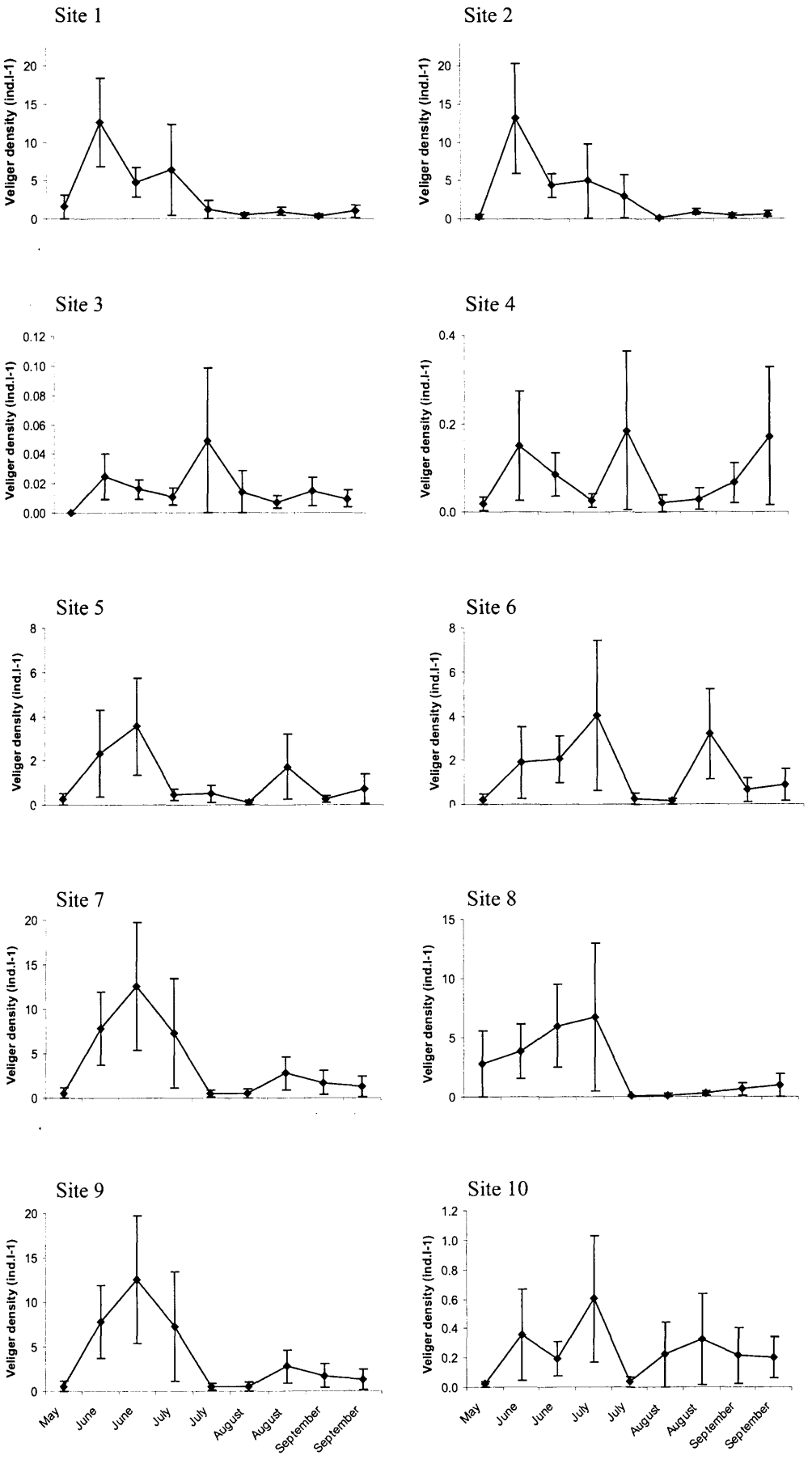


Figure 3.5: Variation of veliger densities for each of the 10 sites during the reproduction season over the four year period sampling. For each data point n=8. Error bars correspond to the standard errors

3.4.1.3.Veliger seasonal patterns

Veliger production occurred from the end of May until the end of September, except in 2006 where veliger production continued until the beginning of October (Figure 3.6). A very few veligers were found during winter sampling (2006-2007). Averaged over all years, spawning activity was strongly seasonal, with strong spawning activity in June declining through July before a second small peak in August (Figure 3.7). During these periods in June, densities reached 8.4 ±1.9 ind.l^{-1} to 14.1 ±4 ind.l^{-1} (Figure 3.7) and were significantly greater than in May (t_{36}=3.23, P=0.003), July (t_{36}=2.32, P=0.03), August (t_{36}=2.49, P=0.02) and September (t_{36}=2.75, P=0.01; Figure 3.8). At the same time, the lowest mean river discharge was observed in June corresponded with 10.3 ±0.9$m^3.s^{-1}$ (Figure 3.8). Furthermore, May showed the lowest mean temperature with 14.4 ±0.3°C while July the highest mean temperature with 18.2 ±0.5°C. June corresponded to the month with the lowest mean river discharge with 10.3 ±0.9$m^3.s^{-1}$ (Figure 3.8). However, there was also some inter-annual variability in veliger production in both peak counts and the number and timing of apparent larval peakss varied (Figure 3.6). For example, in 2006 there were three peaks in larval numbers in June, July and August, but only one occurred in 2008 during June. In all four years, the first veligers appeared when the water temperature exceeded 14°C and every peak in larval density coincided with a temperature peak (17-21°C; Figure 3.7).

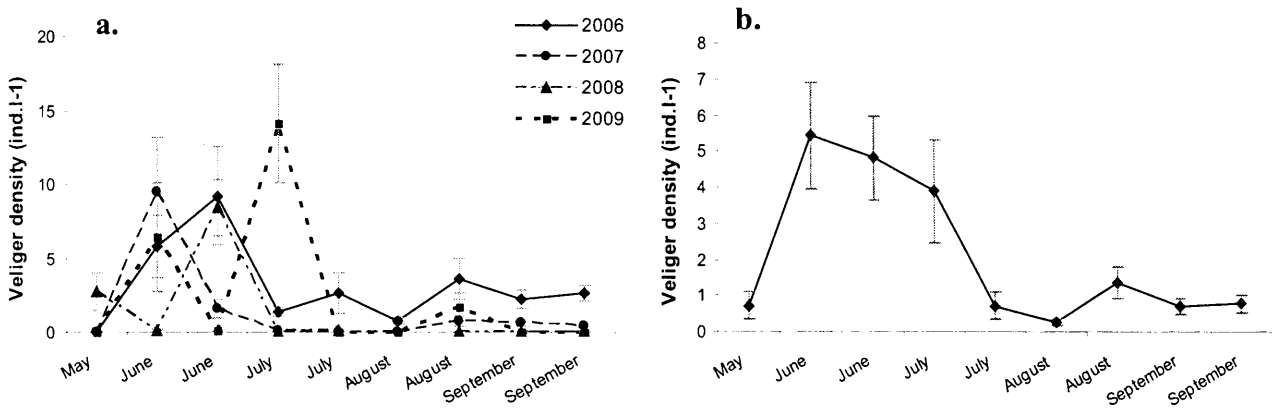


Figure 3.6: Veliger density mean in Cardiff Bay over the four year period sampling. a. Variations of veliger density during the four reproductive seasons. b. Seasonal pattern of veliger production obtained by combining the four reproductive season data. Error bars correspond to the standard error

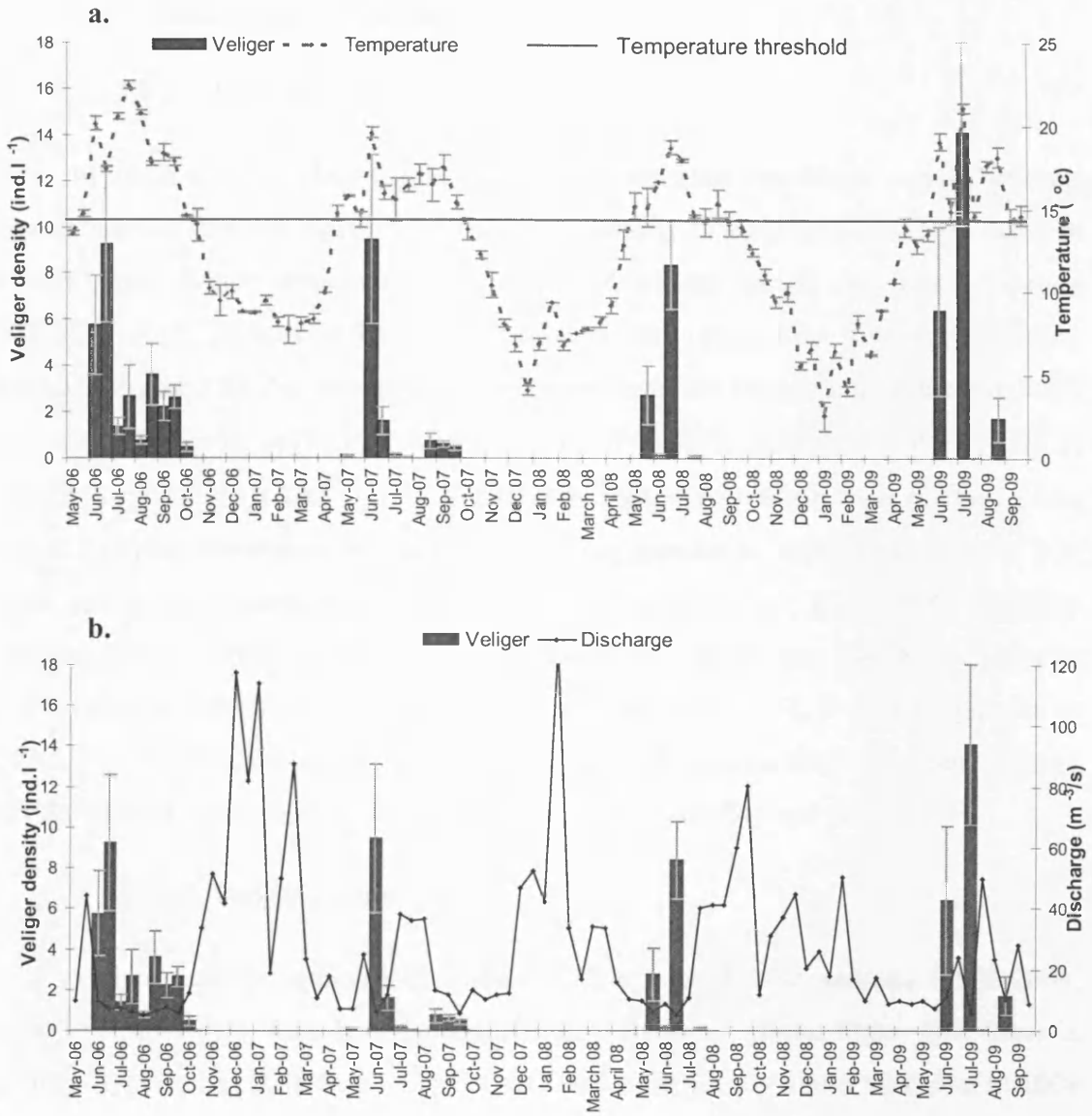


Figure 3.7: Veliger density variations in Cardiff Bay over the four-year study-period with: a. water temperature variations and water temperature threshold for the reproduction, b. river discharge variations in Cardiff Bay. Error bars correspond to the standard error

3.4.2. Inter-annual variations

3.4.2.1. Environmental variations

In addition to varying within years, environmental conditions varied between years in Cardiff Bay and again were linked apparently to variations in larval numbers between years. Water temperature, discharge and salinity varied significantly among 2006, 2007, 2008, 2009, as well as between months (May, June, July, August, September; Table 3.1, Figure 3.8). For example, water temperature was significantly greater in 2006 (18.6 ±0.4°C) than in 2007 (16.2 ±0.3°C, t =6.1, P<0.001), 2008 (16.0 ±0.3°C, t =7.1, P<0.001) or 2009 (16.3 ±0.3°C, t =6.4, P<0.001; Figure 3.8). Mean river discharge into Cardiff Bay also varied over the study years, being greatest in 2008 (30.9 ±3.7$m^3.s^{-1}$) at values almost three times greater than in 2006 (11.6 ±1.4$m^3.s^{-1}$; Figure 3.8). Probably reflecting dilution effects under these contrasting flows, salinity was significantly greater in 2006 than in 2008 (t_{36}= -3.59, P=0.001) and 2009 (t_{36}= -3.61, P=0.001), but not in 2007(t_{36}= -1.71, P=0.1; Figure 3.6). No significant differences, either between years or between months, were observed for chlorophyll *a*, DO, turbidity and pH.

3.4.2.2. Veliger density

Veliger numbers differed between the four reproductive seasons (2006-2009, Table 3.1, Figure 3.8), with densities significantly higher by almost three- four times in the drier 2006 than in 2008 (t_{36}= -2.7, P=0.01), and significantly higher also than in 2009 (t_{36}= -2.4, P=0.03). In 2006, veligers were constantly present in water from May to September when water temperature was constantly high and river discharge low (Figure 3.7). Conversely, for the three following years (2007-2009), veligers were almost absent in water between the density peaks with high river discharges and low water temperatures (Figure 3.7).

Table 3.1: Generalised Linear Model analysis on environmental variable data and veliger data. Bold typeface indicates a significant difference of the variables between 2006, 2007, 2008 and 2009, and a significant difference between May, June, July, August, September. The diverse error distributions and link functions used for each variable analysis are indicated

	Difference between years	***Difference between months***	**Error distribution and link function**
Temperature (°C)	**$F_{3,130}$=25.7** **P<0.001**	**$F_{4,130}$=19.8** **P<0.001**	Gaussian - Inverse
Discharge ($m^{-3}.s^{-1}$)	$F_{3,603}$=25.3 P<0.001	$F_{4,603}$=12.6 P<0.001	Gaussian - Log
Chlorophyll a ($\mu g.l^{-1}$)	$F_{3,36}$=0.55 P=0.65	$F_{4,36}$=1.44 P=0.25	Gaussian - Log
Salinity (‰)	**$F_{3,36}$=18.18** **P< 0.001**	$F_{4,36}$=1.68 P=0.79	Gaussian - Identity
DO ($mg.l^{-1}$)	$F_{3,36}$=0.62 P=0.61	$F_{4,36}$=0.87 P=0.49	Gaussian - Log
Turbidity (NTU)	$F_{3,36}$=1.32 P=0.29	$F_{4,36}$=0.85 P=0.51	Inverse Gaussian - Identity
pH	$F_{3,36}$=2.3 P=0.10	$F_{4,36}$=1.7 P=0.17	Gaussian - Identity
Veliger ($ind.l^{-1}$)	**$F_{3,36}$=2.97** **P=0.048**	**$F_{4,36}$=3.50** **P=0.02**	Gaussian - Identity

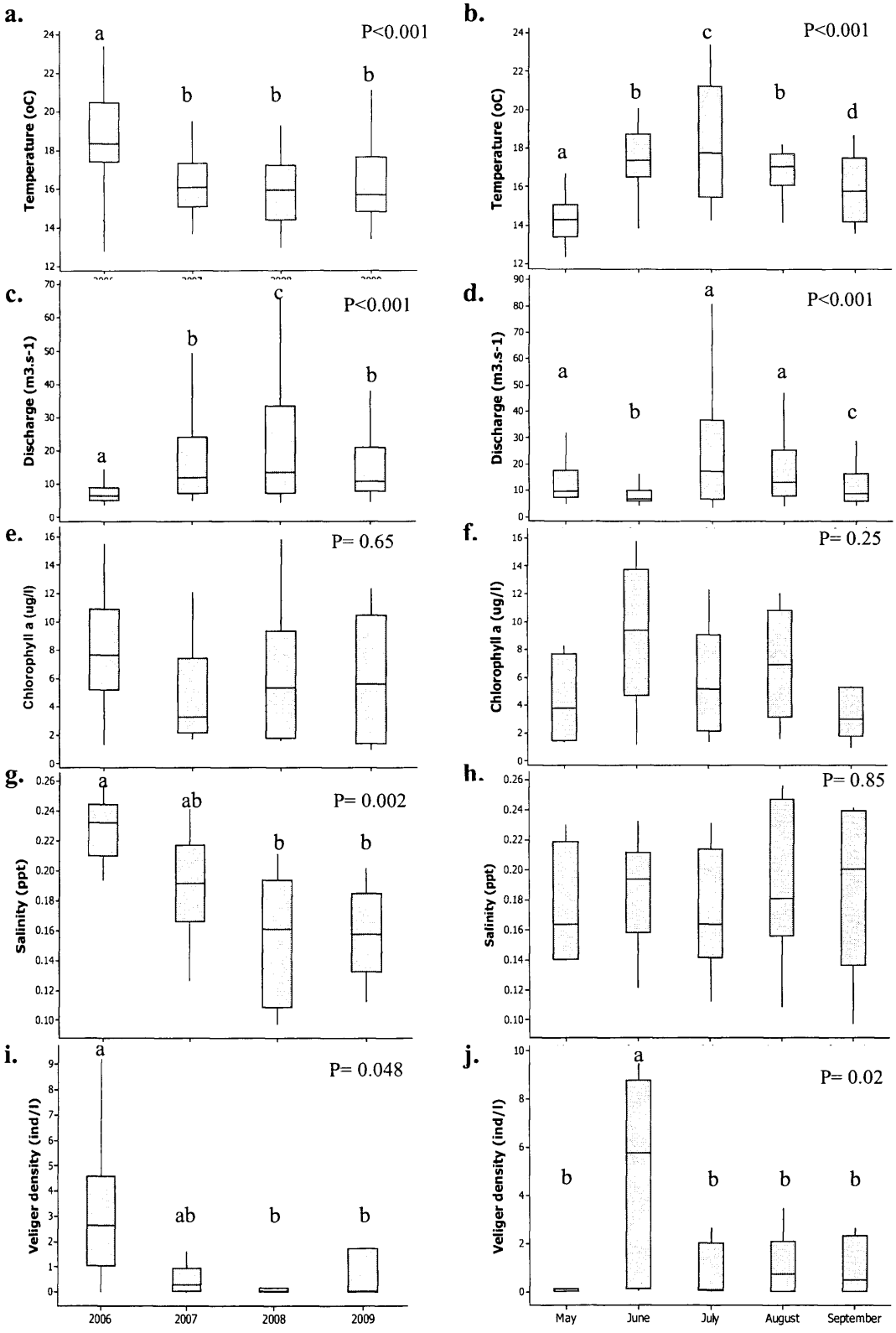


Figure 3.8: Environmental variable and veliger density variations during the reproduction season. Year data mean (2006-2009) and month data mean (May-September). Error bars correspond to standard error. P-values are obtained from Generalised Linear Model

In PCA on environmental variations within and between years, the first principle component explained 49% of the variance and reflected increasing water temperature, chlorophyll *a* and salinity but declining water discharge (Table 3.2). The second principle component explained 18% of the variance and was related to DO, water discharge and salinity (Figure 3.9). In turn, veliger density increased with PC1 ($r= 0.59$, $P < 0.001$; Figure 3.10), but did not vary along PC2. In other words, high veliger densities through time characterised increased water temperatures, high chlorophyll *a* concentration and low river discharge.

Table 3.2: Principal Components Analysis on environmental variables recorded during summer over a four year period in Cardiff Bay (2006-2009). The values are loadings by each variable on each principal component

Variables	**Axis 1**	**Axis 2**
Temperature (°C)	0.45	0.11
Water discharge ($m^3.s^{-1}$)	-0.44	-0.37
Chlorophyll *a* ($\mu g.l^{-1}$)	0.47	-0.15
Salinity (‰)	0.42	0.43
DO ($mg.l^{-1}$)	0.22	-0.65
Turbidity (NTU)	-0.22	0.24
pH	0.32	-0.40

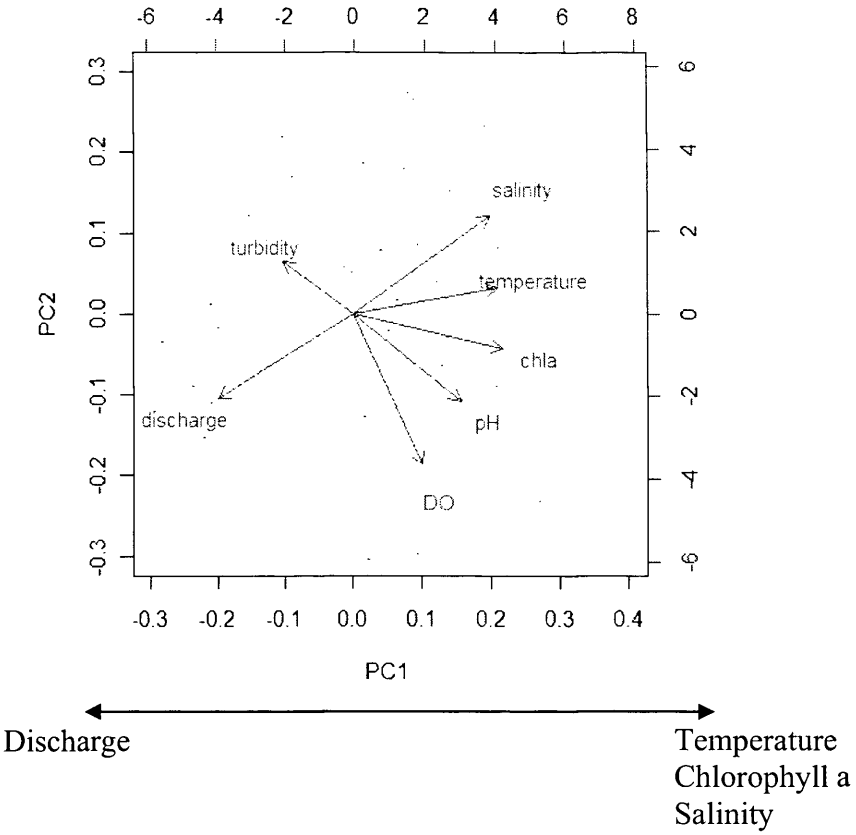


Figure 3.9: Two-dimensional PCA- ordination diagram of the environmental variables recorded in Cardiff Bay during veliger production (May-September) over the four year study period (2006-2009)

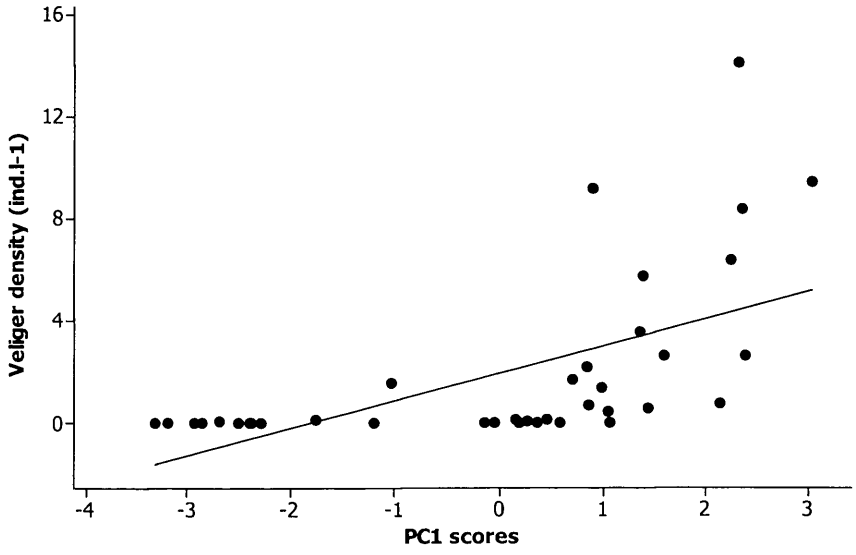


Figure 3.10: Variations in the density of zebra mussel veligers in Cardiff Bay in relation to a Principal Component of environmental variation between the summer months in over 4 years

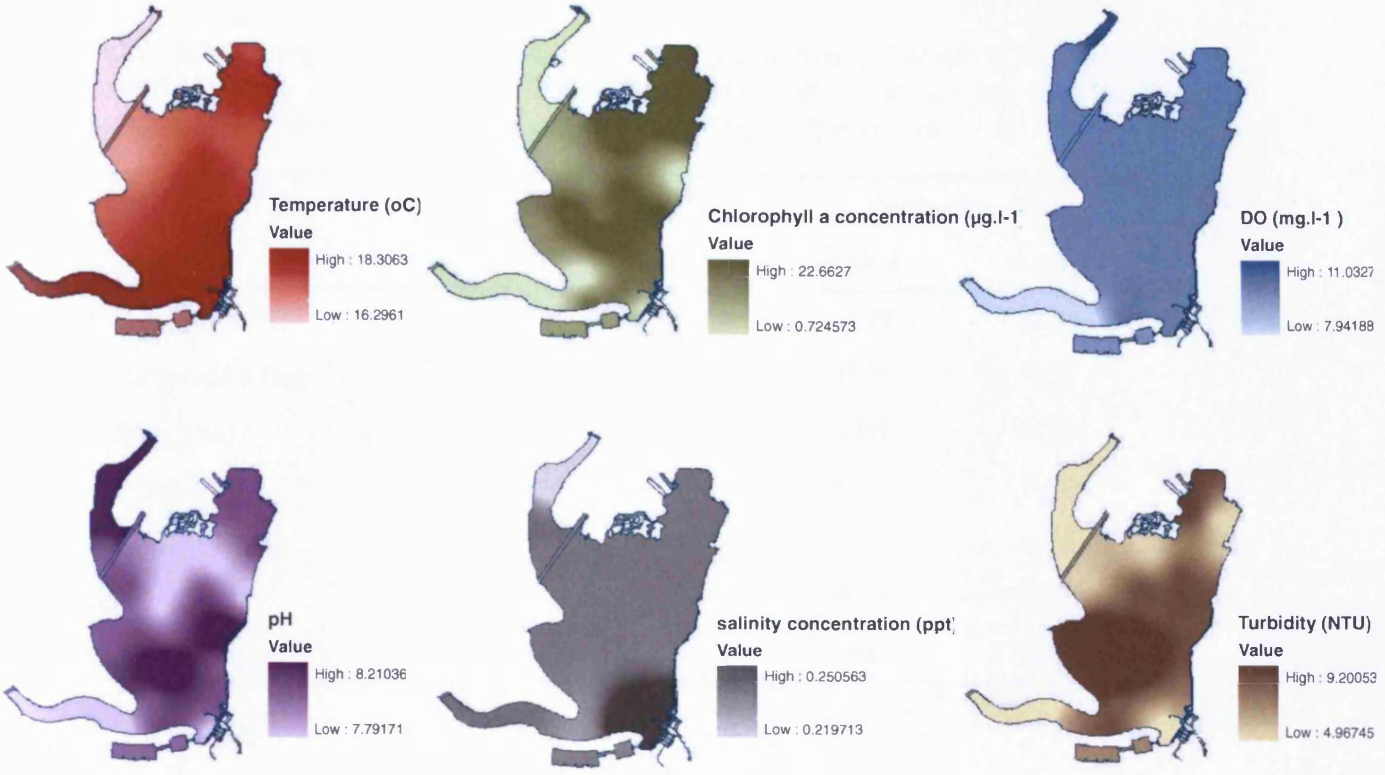
3.4.3. Spatial pattern

3.4.3.1. Environmental variations

Water chemistry varied more between riverine and lacustrine regions of Cardiff Bay than across the main Bay (Figure 3.11). Values for most variables were lower in the rivers compared to the lake, except for DO. There were also some differences between the two main rivers, with temperatures lower in the Taff River (16.3°C) than in the Ely (17-18.3°C). Conversely, DO values were greater in the Taff River (11 $mg.l^{-1}$) than in the Ely River and main Bay (8-8.4 $mg.l^{-1}$). Salinity varied by 0.4 $‰$, with values greatest near the barrage and in the Ely river mouth. Discharge in the river Taff was four times greater than in the river Ely for both years (M-W test, $n=15$, $P<0.001$). Current velocity appeared to be the highest at the Taff River mouth and in the regions of Cardiff Bay that corresponded to the former Taff river bed, but data here were limited (Figure 3.11).

Principal Component Analyses of water temperature, salinity, turbidity, DO, pH and chlorophyll *a* concentration at the 30 sites sampled in September 2006 and September 2007 mostly revealed differences between or among sites in the inflowing rivers and main Cardiff Bay (Table 3.3, Figure 3.12). In both years, PC1 represented a gradient from warmer waters rich in chlorophyll *a* to sites of higher DO and pH.

September 2006



September 2007

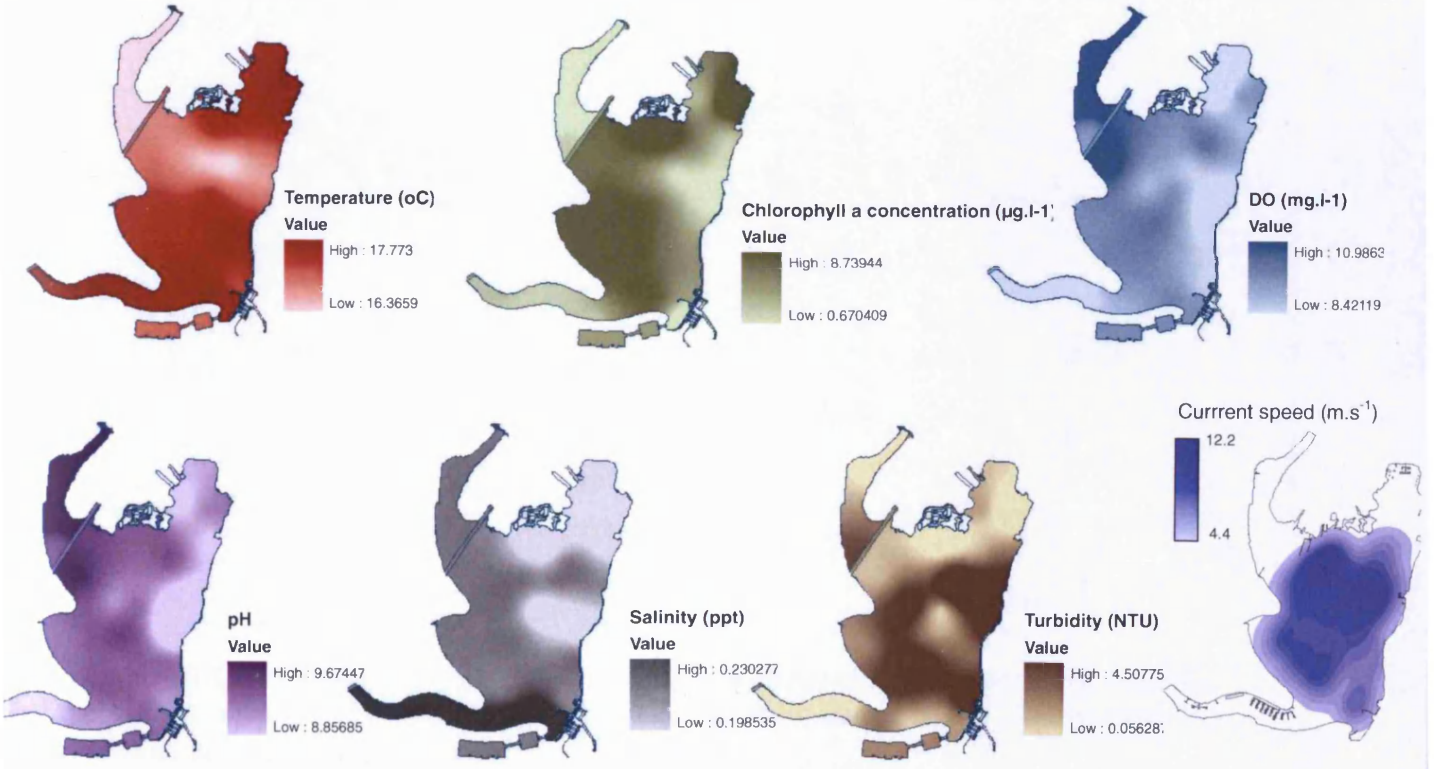


Figure 3.11: Environmental variations in Cardiff Bay interpolated from data recorded at each of 30 sites in September 2006 and September 2007. Current velocity values are recorded only in February 2008 and interpolated through Cardiff Bay

Table 3.3: Principal Component Analyses on spatial data collected in Cardiff Bay in September 2006 and September 2007. The values are loadings on each principal component, and the percentage variance explained by each is shown

	September 06		September 07	
	Axis 1	**Axis 2**	**Axis 1**	**Axis 2**
Temperature (°C)	-0.54	0.008	-0.49	-0.23
Chlorophyll a ($\mu g.l^{-1}$)	-0.36	0.49	-0.41	0.08
Salinity (‰)	-0.45	0.004	0.09	-0.95
DO ($mg.l^{-1}$)	0.27	0.59	0.51	0.08
Turbidity (NTU)	-0.42	-0.45	-0.27	0.16
pH	0.42	0.38	0.50	0.04
Variance (%)	52	33	55	17

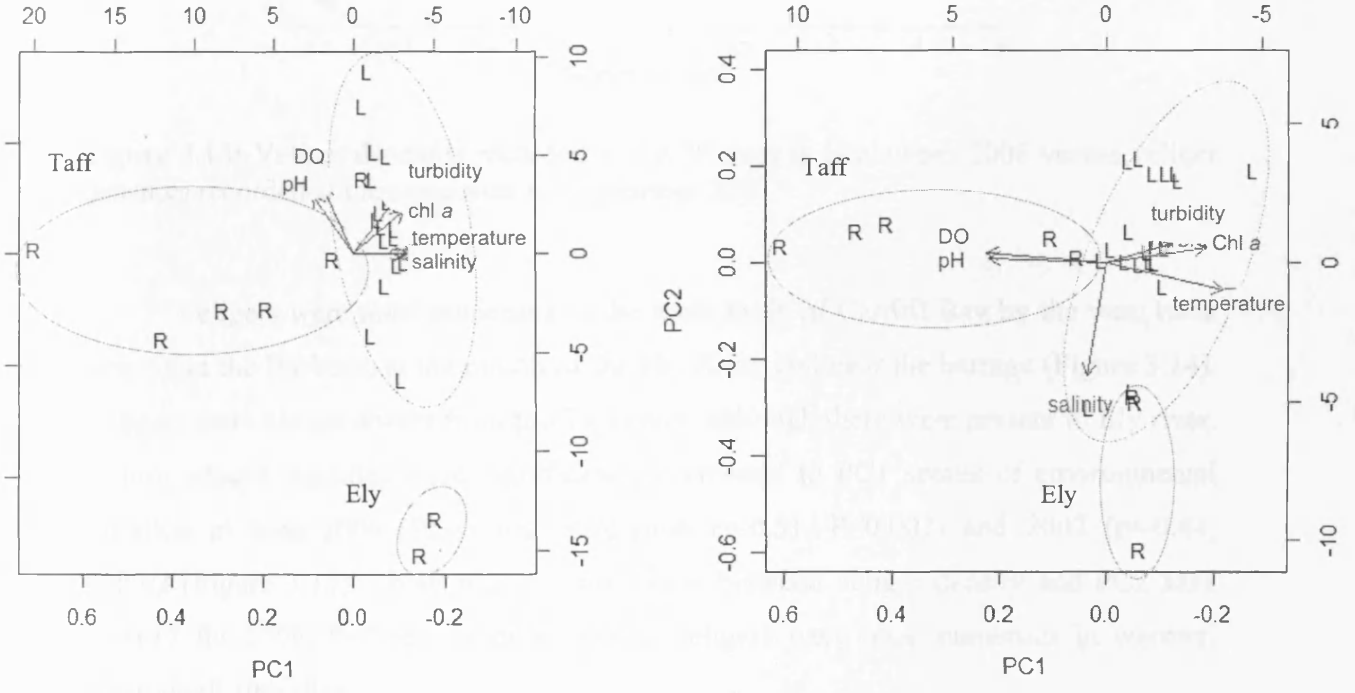


Figure 3.12: Two-dimensional PCA- ordination diagram of the environmental variables recorded at 30 sites in Cardiff Bay. a. data for 2006, b. data for 2007. R represents the riverine sites, L the lacustrine sites

3.4.3.2. Veliger spatial distribution

Veliger densities assessed at the 30 sites in September 2006 were significantly correlated to the densties at the same 30 sites in September 2007 implying some consistency through time, although the match was not perfect (r=0.49, P= 0.01) (Figures 3.13).

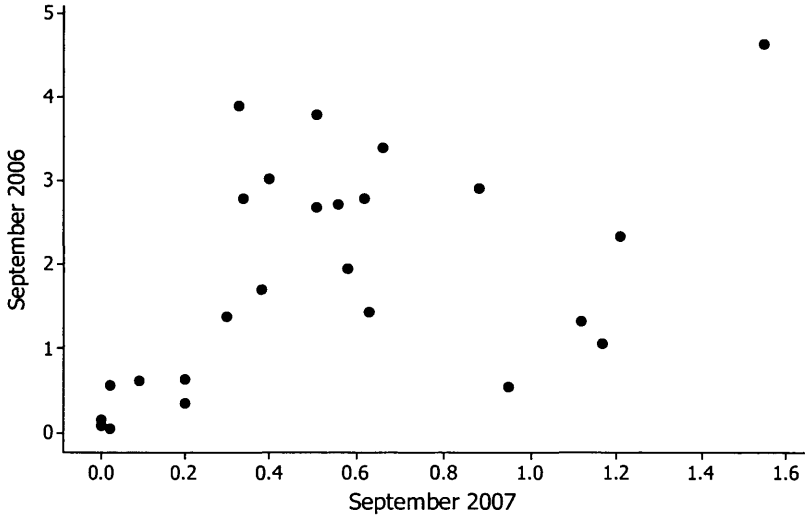


Figure 3.13: Veliger densities recorded at the 30 sites in September 2006 versus veliger densities recorded at the same sites in September 2007

Veligers were most numerous in the main body of Cardiff Bay by the west bank (site 6), in the Harbour, at the mouth of the Ely River and near the barrage (Figure 3.14). Veligers were almost absent from the Taff river, although there were present in Ely river. In turn veliger densities were significantly correlated to PC1 scores of environmental variation in both 2006 (Pearson's correlation r=-0.51, P=0.007) and 2007 (r=-0.44, P=0.02 (Figure 3.15). There was no correlation between veliger density and PC2 axes (P=0.17 for 2006, P=0.68). In other words, veligers were most numerous in warmer, chlorophyll-rich sites.

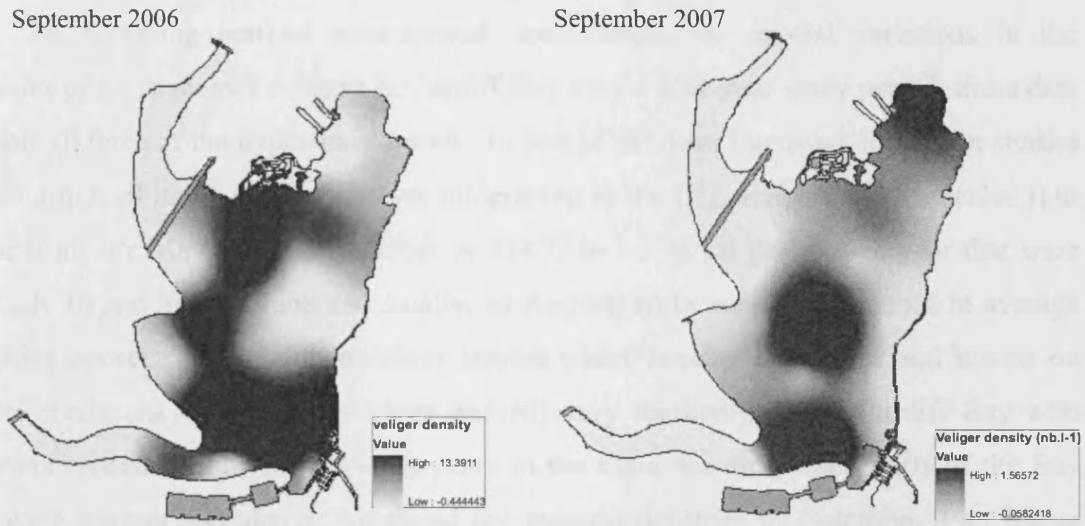


Figure 3.14: Veliger densities interpolated from data recorded at 30 sites in September 2006 and 2007

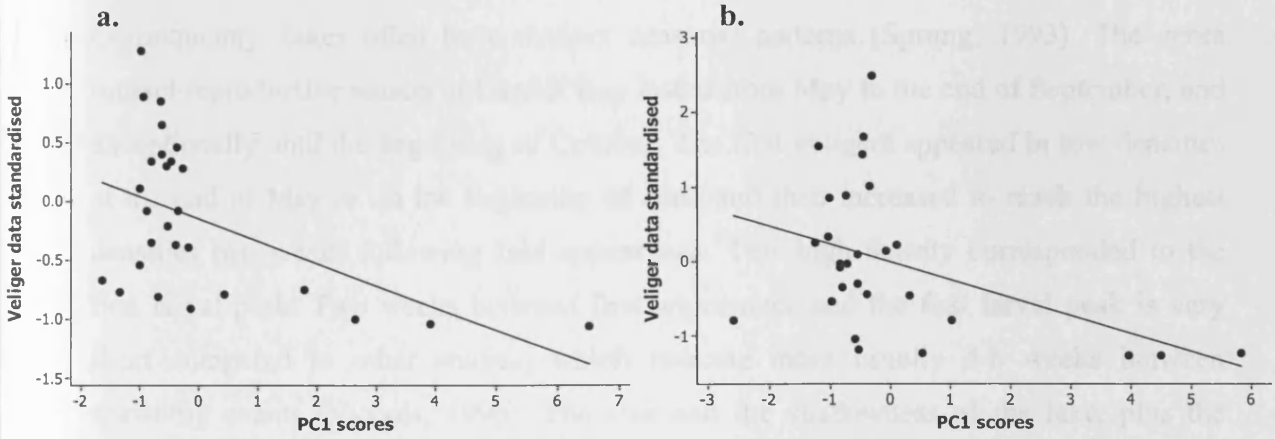


Figure 3.15: Spatial veliger data versus PC1 scores of the PCA done on spatial environmental variables. a. Data recorded in September 2006 b. Data recorded in September 2007

3.5. Discussion

In revealing marked intra-annual, inter-annual and spatial variations in the densities of zebra mussel veligers in Cardiff Bay over a four-year study period, these data support all three of the hypotheses tested. In one of the most intensive, long-term studies of zebra-mussel larval production ever undertaken in the UK, veligers were revealed i) to occur from late May to late September at >14°C in 1-3 larval peaks each year that were typically largest in May-June and smaller in August; ii) to vary by three fold in average densities between years, with numbers largest under hot-dry conditions and lowest on cooler conditions with high discharge and iii) vary markedly across Cardiff Bay with numbers consistently higher between years in the main standing water parts of the Bay that were warmer and also accumulated the greatest densities of Chlorophyll *a*. These patterns are both intrinsically interesting in revealing some of the traits that have led to this species in rapidly establishing a substantial population in Cardiff Bay, but also in revealing possible controls on recruitment or dispersal to new sites.

3.5.1. Seasonal pattern

The zebra mussel reproductive cycle varies considerably throughout its invasive range, and this is strongly influenced by the environmental conditions (Nichols, 1996). Consequently, lakes often have distinct seasonal patterns (Sprung, 1993). The zebra mussel reproductive season in Cardiff Bay lasted from May to the end of September, and exceptionally until the beginning of October. The first veligers appeared in low densities at the end of May or on the beginning of June and then increased to reach the highest densities two weeks following first appearance. This high density corresponded to the first larval peak. Two weeks between first appearance and the first larval peak is very short compared to other studies, which indicate more usually 4-8 weeks between spawning events (Nichols, 1996). The size and the shallowness of the lake, plus the homogeneity of the water column may facilitate the transmission of physical and chemical factors which activate synchronous spawning (Nichols, 1993). Intrinsic to the zebra mussel, serotonin is a chemical produced and released by adults. The chemical acts directly on both female and male gonads and led to coordinated gamete maturation (Ram *et al.*, 1996; Fong, 1998). Coordinated gamete maturation and synchronous spawning are

keys to successful reproduction for freshwater mussels (Galbraith and Vaughn, 2009); especially zebra mussels which exhibit external fertilisation (Ram *et al.*, 1996).

The combination of temperature and food availability also appeared to affect spawning in Cardiff Bay and this has been seen previously (Galbraith and Vaughn, 2009). As in other studies, the overall duration of veliger production occurred in response to the water temperature regime (Nichols, 1996). In Cardiff Bay, 14°C appeared to be the temperature threshold to initiate the larval production and 17- 21°C, the temperature threshold for peak spawning. Also, the maximum water temperatures recorded in the Bay, 22- 23°C, did not exceed the maximal limit of water temperature (24- 26°C) above which one larval development becomes bad (McMahon, 1996). These results correspond with the range of water temperature thresholds required for the onset of egg development and spawning described in the literature. In most populations, 12°C is the threshold for zebra mussel reproduction (Claudi and Mackie, 1994) while 17-18°C the threshold where spawning activity is maximised. In different European lakes, larval production starts when water temperatures are between 14 and 19°C (Tourari *et al.*, 1988; Sprung, 1993; Nichols, 1996; Karatayev *et al.*, 1998, Lucy, 2005). In North America, zebra mussel succeeded in adapting their reproductive cycle to higher water temperatures (Nichols, 1996): in Lake Erie, veligers appeared at 18°C and the first spawning occurred when the water reached 22-23°C (Haag and Garton, 1991; McMahon, 1996).

Food supply for adult zebra mussels is considered to be a second key environmental factor that controls reproduction by affecting the volume and fecundity of the mussel gonads. Food quantity and food quality affect reproductive investment (Wacker and von Elert, 2003; Galbraith and Vaughn, 2009). In Cardiff Bay, phytoplankton were abundant in the lake from April until mid October, with a decline in May. Phytoplankton plays an energetic role in gamete production, but also releases chemicals that stimulate zebra mussel spawning (Ram and Nichols, 1993). Larval peaks in Cardiff Bay were thus synchronous with phytoplankton blooms. This synchrony might also increase the survival of planktotrophic larvae: available food increases veliger growth rate, which reduces the planktonic phase during which they are at risk of planktonic predators (Ram *et al.*, 1996).

The highest veliger densities found in this study (from 8 ±1.9 ind.l^{-1} to 14 ±4 ind.l^{-1}) was comparable with the range of the maximum densities recorded in American, European and Russian lakes – of between 7-700 ind.l^{-1} (Sprung, 1993). However, veliger densities in Cardiff Bay were relatively small compared to some results reported from other European lakes. For example, in Lough Key, Eire, veliger densities comprised between 3 and 20 ind.l^{-1} at the beginning of the colonisation, and reached densities of 39-45 ind.l^{-1} three years after the colonisation (Lucy, 2005). A study of a German shallow lake recorded densities between 43 and 160ind.l^{-1} (Wilhelm and Adrian, 2007). The toxicity of the water due to the presence of heavy metals in the sediments (due to historic activity in Cardiff Bay) may explain the lower veliger densities in Cardiff Bay than in other European lakes.

The very few veligers found during winter with water temperatures below 10°C, were probably overwintering. Indeed, veligers produced at the end of the reproductive season are able to overwinter when the conditions become inappropriate for larval development (Haag and Garton, 1992; Reed *et al.*, 1998). Veligers which overwintered and were retained in Cardiff Bay probably restarted their development the following spring with rising water temperatures.

3.5.2. Inter-annual variations

Zebra mussels in Cardiff Bay appeared to have one to three spawning events per year. For three reproductive seasons, the major larval peak occurred between the end of May and the end of June, depending on the environmental conditions, and a smaller one occurred in August. Exceptionally, a third larval peak occurred in Cardiff Bay in 2006, while only a single occurred in 2008. Zebra mussels across their acquired range vary in their number of spawning events, probably reflecting a balance between intrinsic biological capacity and environmental plasticity (Sprung, 1993). Waltz (1978) studied zebra mussel spawning in the laboratory and observed that gamete release does not occur at once, but tends to happen in 2-6 spawning events. About half of the eggs produced are released during the first spawning event, with subsequent spawnings corresponding to the release of residual gametes (Haag and Garton, 1991). In Lake Erie in the United States, one spawning event occurs (Haag and Garton, 1991) while more than two were observed

in Constance Lake and Muggelsee lake, Germany (Waltz, 1978; Wilhelm and Adrian, 2007). Borcherding (1991) observed two spawning events in two German lakes, as did Bacchetta *et al.* (2001) in Italy.

In Cardiff Bay, veliger production and the number of larval peaks clearly showed a degree of plasticity that reflected environmental variations such as river discharge and water temperature. The environmental conditions in 2006 included exceptionally high water temperature (18-23°C), with veliger larvae present from June until the beginning of October. By contrast, from July to October 2008, the river discharge in Cardiff Bay was unusually high, with correspondingly low water temperatures of 14°C; and almost no veliger larvae were present in the water column. As the water temperature in Cardiff Bay is linked to discharge in the Bay's two main inflowing rivers, probably because factors affecting rainfall and temperature are related. For example, in a two month period in 2009, the water temperature fell from 20°C to 14°C, subsequently increased to 21°C and decreased again to 15°C. At low temperatures and high discharge rates, veligers were absent from the lake and, therefore, river discharge had a direct impact on the veliger density in the Bay. Free-swimming larvae are bond to be displaced by water currents and downstream flow (Griffiths *et al.*, 1991; Carlton, 1993), and in the bay, high river flows almost certainly flushed veligers out to sea as residence time in the Bay declined. Furthermore, water turbulence damages veligers and increases mortality rate (Horvath and Lamberti, 1999). A river discharge of 15-20$m^3.s^{-1}$ in Cardiff Bay appeared to be the threshold to reduce veliger density.

Even in the coolest summer conditions, the water temperature in Cardiff Bay never fell below the threshold needed for mussel reproduction over any of the 4 summer's studies in Cardiff Bay. Whether veligers were still produced and flushed to the sea, damaged by the flow current, or veliger production ceased because of a sudden environmental change cannot be categorically determined from the results. However, in some areas of the bay which were sheltered from the water current such as the Inner Harbour, a very small number of veligers were found under high discharge conditions. That veliger density fell even in areas sheltered from the effects of the current suggest that veliger production stopped or slowed due to the sudden drop in water temperature. In

addition to draining veligers, high discharge also flushed phytoplankton from the bay. Discharge, therefore had an indirect impact on veliger production through decreasing water temperature and by reducing food availability. A laboratory experiment on adult mussels could help to assess the effect of sudden changes in environmental conditions on veliger production.

Constant environmental conditions in Cardiff Bay, combined with low river discharge, high water temperature (17-22°C) and abundant food availability facilitate continuous and prolific veliger production during the entire potential reproductive season. Conversely, a wet summer, with high river discharge and cold water temperature led to the occurrence of a single larval peak in 2008.

3.5.3. Spatial distribution

Water temperature, chlorophyll *a* concentration and turbidity were all lower than in the two main inflowing rivers to Cardiff Bay whilst the dissolved oxygen concentration was higher than in the main Bay. River flow was probably a key reason for the observed differences. Water current carries veligers, explaining the low veliger densities observed in both rivers (Griffiths *et al.*, 1991). Boat traffic and circular currents observed in the rivers (pers. comm., CHA) could be at the origin of the presence of veligers in the rivers (Johnson and Carlton, 1996; Barnard *et al.*, 2003).

However, more veligers were found in the Ely River than in the Taff and there are two possible reasons for this. First, lower current speed in the Ely River: river current velocity was only recorded in the mouths of both rivers and was higher in the Taff mouth than the Ely, suggesting a lower current velocity in the Ely than in the Taff. These conditions will have allowed zebra mussel veligers to disperse into the Ely thereby establishing spawning populations. Secondly, there is a yacht club with boats at pontoons in the Ely River at sites 10, 14, and 15. Boats are a major vector for zebra mussel dispersion (Johnson and Carlton, 1996; Pollux *et al.*, 2003). The first mussels which colonised Cardiff Bay were probably brought by boats, attached on the hulls. The combination of a likely lower current velocity and the presence of available substrates in the port may explain the higher veliger density in the Ely River than in the Taff River.

Water chemistry in the main body of Cardiff Bay was relatively homogeneous during the investigation linked to the aeration system and its effects in mixing the water column. Water temperature, chlorophyll *a* concentration, and DO presented the greatest variations through Cardiff Bay. The pH did not vary spatially, and was in the range required (7.4-9.4) for successful veliger development (McMahon, 1996). There was apparently no influence of salinity on veliger distribution since the maximum rate found in the bay (0.23-0.25‰ near the barrage) was far below the lethal salinity of post-veligers (2‰) and zebra mussel salinity tolerance (0.5- 2‰; Kilgour *et al.*, 1994; McMahon, 1996).

Throughout the whole of Cardiff Bay, the greatest veliger densities were observed in the Inner Harbour, in shallow areas (sites 6), in the middle of the Bay and surprisingly near the barrage. Veliger densities were observed in areas sheltered from the main river flow, with high water temperatures and high chlorophyll *a* concentrations. These results suggest that river current was the main environmental variable which influenced veliger distribution through Cardiff Bay. The two river currents meet in the lake and let to the formation of circular currents particularly in the Inner Harbour, at site 6 and near the barrage which act as retention zones (pers. comm., CHA) and could explain high veliger densities in these areas (Barnard *et al.*, 2003). Nevertheless, another spatial veliger sampling session in June when veliger densities are the highest, coupled with the measurement of current velocity throughout the entire main body and rivers, would bring more information to understand the mechanisms which control spatial veliger distribution in Cardiff Bay.

3.5.4. Dispersal risk

Larval dispersion and colonisation from Cardiff Bay to other water bodies is very unlikely by natural mechanism such as flow because the Bay empties into a saline environment. Horvath *et al.* (1996) described how zebra mussel populations dispersed between lakes and streams, with source populations is always upstream, and the colonisation of downstream habitat occurs by larval drift (Stoeckel *et al.*, 1997). Although birds could be a potential dispersal vector by carrying eggs or veligers suspended in water droplets on their body (Carlton, 1993), it was revealed that it was

relatively low and thus of a minor concern (Johnson and Carlton, 1996). Conversely, equipments used by fishermen such as net, cages, bait-bucket water, nets are other vectors for zebra mussel dispersion. Fishermen should use their equipment with a lot of care through the entire lake and in the downstream part of the rivers during the whole veliger production to reduce the dispersal risk of zebra mussels. Seasonal and inter-annual data collected here suggest that these effects are mostly likely in June and in particular in dry years.

In the Taff River, almost no veligers were observed at the most upstream sites. Nevertheless, veliger density in the Ely was quite substantial; it would be valuable, therefore, to take more samples at additional upstream sites to determine the point at which veligers are no longer found.

3.6. Conclusions

Four years of data collection during the zebra mussel reproductive season in Cardiff Bay, have allowed a thorough description of the seasonal pattern in veliger production and an examination of the factors controlling it. River discharge is the main factor which influences both temporal and spatial veliger distribution in Cardiff Bay.

The results mirror those of many previous studies: veliger production season stretched from late May to late September, water temperature threshold for veliger production was 14°C, and the highest veliger densities were comprised between 8-14 ind.l^{-1}. These data support the idea that a substantial and self sustaining zebra mussel population has established in Cardiff Bay.

The variation of the number of spawning events in response to the stressful environmental conditions in the artificial lake Cardiff Bay highlights the flexible productive strategies and the strong propagule pressure of zebra mussels.

The eutrophication and limited depth of the lake facilitate the successful zebra mussel population establishment in Cardiff Bay. The aeration system maintaining a suitable oxygen level for zebra mussels and creating water chemistry homogeneity through Cardiff Bay contributes to the successful veliger development and spatial veliger distribution in the lake.

The study of juvenile settlement variations over time and across Cardiff Bay, coupled with veliger distribution data will be developed in chapter 3 and will bring more information on zebra mussel population dynamic and on the recruitment of the mussel population in Cardiff Bay. The study of the spatial adult distribution in Cardiff Bay in the chapter 5 will help to understand the spatial veliger distribution.

4. Patterns of colonisation and substrate selection by zebra mussels in Cardiff Bay

4.1. Summary

1. Invasion by non-native species requires not only dispersal, but also colonisation and establishment in new habitats. Sessile organisms depend particularly on local establishment, but factors affecting it are only partly understood. The invasive zebra mussel, *Dreissena polymorpha*, has sessile adults, and this chapter reports on a three-year study (2007-2009) into factors affecting their local establishment in Cardiff Bay. Experiments involving artificial substrates were used to test hypotheses about the effects of substrate type, environmental conditions and veliger density on local colonisation and recruitment.

2. Artificial substrates exposed during veliger release by zebra mussels in Cardiff Bay were colonised within months. However, settled juvenile densities varied significantly from 32 800 ±3 000ind.m^{-2} (±SE) in 2007 to 270 ±70ind.m^{-2} in 2008, when discharge was high and veliger densities low. Juvenile settlement was apparently reduced by increased river discharge into Cardiff Bay, and hence reduced water residence time.

3. Spatial patterns in juvenile settlement also suggested flow effects, with recruitment apparently greatest in areas with reduced current and increased veliger densities. Spatial patterns in veliger distribution and juvenile settlement were correlated between years.

4. Juvenile settlement was greater on pebbles and clay tiles, than on steel substrates. However, post-veliger settlement was lowest in 2008 than 2009 irrespective of substrate type.

5. Overall, these data illustrate how juvenile settlement and recruitment in zebra mussels is determined at fine scales by substrate availability, but at larger scales by variations between sites and years in flow patterns and veliger density. Low flows and increased residence time apparently enhance zebra mussel recruitment, suggesting that these factors might have affected the evolution of zebra mussel breeding strategies as well as the probability of invasion of new sites.

4.2. Introduction

For sessile invasive invertebrates with a larval planktonic stage, population recruitment reflects a combination of 'propagule pressure', caused by variations in larval density, and variations in their survival once settled.

In the invasive zebra mussel, propagule pressure reflects the dispersal, distribution, abundance and arrival of veliger larvae (Stanczykowska and Lewandowski, 1993; Wacker and von Elert, 2003). Variations in larval numbers can arise from the distribution and abundance of adult sources. However, veliger mortality rates can exceed 70-99% (MacIsaac *et al.*, 1991; Sprung, 1993; Mackie and Schloesser, 1996, Nichols, 1996), with survival then affecting both veliger abundance as well as juvenile settlement intensity (Garton and Haag, 1993; Martel *et al.*, 1994; Nalepa *et al.*, 1995; Wacker and von Elert, 2003; Lucy, 2005). Systematic effects on veliger survival arise from environmental factors such as water current, turbulence, patchy food resources, predation, and passage into adverse locations (Nalepa *et al.*, 1995; Marsden and Lansky, 2000; Kobak, 2005). For all these reasons, the spatial and temporal variations in veliger distribution, as well as the environmental factors involved, provide fundamental and potentially important predictive information on mussel distribution and population dynamics between years (Sprung, 1993; Nichols, 1996).

Once in locations where populations are being established, internal population dynamics can affect recruitment. New populations are often characterised by initial high density followed by population reduction, while established populations can fluctuate irregularly over years to decades (Stanczykowska and Lewandowski, 1993; Nalepa *et al.*, 1995). Intrinsic factors might explain these effects, but possible inter-annual variations in juvenile settlement rates and even larval predation by adult mussels might also be involved (MacIsaac *et al.*, 1991; Garton and Haag, 1993).

Substrate effects are particularly important in supporting the settlement and recruitment of zebra mussels, and soft, depositing sediments are unsuitable. Otherwise, many hard substrates can support juvenile settlement (Smit *et al.*, 1993; Nichols, 1996). For this reason, zebra mussels are often damaging as fouling organisms and responsible

for severe nuisance in industrial or power plant infrastructure where they can occlude pipes, and settle on hard structures (Jenner and Janssen-Mommenn, 1993; Laurier LePage, 1993; Ram and McMahon, 1996). Previous studies have shown how substrate preferences, shape, orientation affect colonisation, but also work on antifouling agents has appraised how substrate treatment affects mussel colonisation on important structures (Kilgour and Mackie, 1993; Kobak, 2000; Czarnoleski *et al.*, 2004; Folino-Rorem *et al.*, 2006). So far, most of the latter studies have appraised antifouling agents with potentially large non-target effects, for example organometallic and aggressive oxidisers with only a small number assessing more benign agents such as aaptamine derivatives or cannabinoids (Diers *et al.*, 2006; Angarano *et al.*, 2009; Qian *et al.*, 2010). In this study, antifouling agents made from natural components of myrrh, were tested on zebra mussels.

Despite all of this existing information, case-studies in individual lakes into spatial and inter-annual variations in zebra mussel colonization and recruitment are scarce. This is particularly true under novel circumstances, for example where new lakes have been created in urban locations. As noted elsewhere in this thesis (Chapters 1, 3), the newly formed lake in Cardiff Bay has provided unique circumstances for one such case study. Valuable features for understanding larval settlement here arise because i) the newly formed Bay was invaded by zebra mussels between 2001-2003, so that the population is still in early phases of development; ii) existing data illustrate how zebra mussels vary spatio-temporally across Cardiff Bay in relation to varying environmental conditions (Chapter 3) and iii) these variations, coupled with data collected simultaneously on veliger abundances, provide a context for experimental work aimed at understanding larval settlement.

This chapter describes a range of experiments carried out on veliger settlement over three years (2007-2009 inclusive) using artificial substrates. The following hypotheses were tested respectively about broad- and fine-scaled effects on recruitment:

i. Varying environmental conditions and varying veliger density between years (Chapter 3) are matched by variations in post-veliger settlement.

ii. Spatial variations in veliger numbers across Cardiff Bay are also matched by variations in post-veliger settlement between areas of the Bay characterised by high and low veliger numbers (Chapter 3).

iii. At finer scales, post-veliger settlement is expected to vary between substrate types: settlement on hard, natural porous surfaces (e.g. pebbles) should be higher than on artificial smooth surfaces such as tiles and steel.

iv. For the same substrate type, natural antifouling agents reduce post-veliger settlement. Understanding the possible effects of anti-fouling agents has management importance in Cardiff Bay since anti-fouling agents are used on some structures.

4.3. Materials and methods

4.3.1. Site

Formed in 2001, Cardiff Bay is an artificial lake on the coast of South Wales, UK formed by the construction of a barrage across the mouths of two rivers, the Taff and the Ely. The lake has an area of c 200 hectares lake with a mean depth around 4.5 metres. The lake bed is composed mainly of soft sediments, mud and silt while the lake surrounds are composed of hard substrata, principally of pebbles, walls and locks (Figures 4.1, 4.3). An aeration system is installed on the lake bed and presents another hard substrate, composed of rubber pipes and joint in stainless steel. The fish pass is coated with an antifouling agent to prevent colonisation by invertebrates (Hempel anit-foulant, globic 81900, and Hempadur 45143 curing agent).

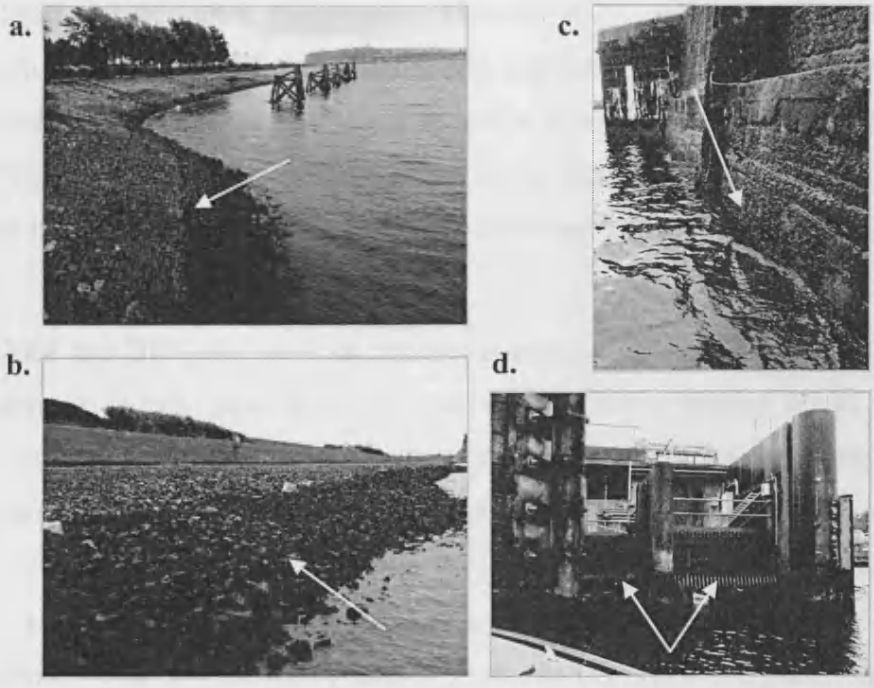


Figure 4.1: Hard substrates found on the shore of Cardiff Bay: a. cemented pebbles near the Inner Harbour, b. pebbles between CHA and the barrage, c. wall of a former lock in the Inner Harbour, d. fish pass

4.3.2. Artificial substrates, installation and experimental design

Experiments investigating juvenile settlement on artificial substrates were carried out in Cardiff Bay in 2007, 2008 and 2009. In each case, replicate artificial substrates were installed in replicate crates that were then exposed in various locations across Cardiff Bay during the summer period of veliger production and settlement. However, the first experiment, in 2007, was affected by heavy silt deposition onto the artificial substrates which required a change in the equipment design for subsequent years. Some data from the 2007 experiment were salvaged because veliger settlement occurred on the crates used to hold the substrate units.

Experimental crates contained three types of hard substrate: clay tiles, pebbles and steel. The tiles (15x 15x 1.3cm) were intended to resemble smooth surfaces such as the walls present around in Cardiff Bay. Pebbles were dredged from the River Taff to recreate pebble substrates, and were ovoid with approximate size of 6cm length and 4cm diameter, fixed into non-toxic epoxy resin (Horvath *et al.*, 1999). Stainless-steel tubes (12cm length x 5cm diameter) were intended to represent the joint-sections of the Bay aeration system, as well as the steel parts found on the fish pass and lock gates of the barrage. Wood was used as an artificial substrate in 2007, however it is not commonly found in the lake. Therefore, the experiments in 2008 and 2009 were focused on the other substrates.

In 2008 and 2009, the artificial substrates were positioned vertically in crates by wooden supports, which were screwed onto plastic crates (Figure 4.2.a). Substrate orientation can affect zebra mussel settlement (Kilgour and Mackie, 1993; Marsden and Lansky, 2000) but not always (Czarnoleski, 2004; Kobak, 2005). However, experience gained in 2007 showed that horizontally-oriented substrates were inundated by fine sediments. Artificial substrates were located on each crate using a random allocation among positions, with two replicates of each substrate per crate. The plastic crates were ultimately deployed upside down to elevate the substrates above the lakes bed to reduce the risk of smothering by soft sediments.

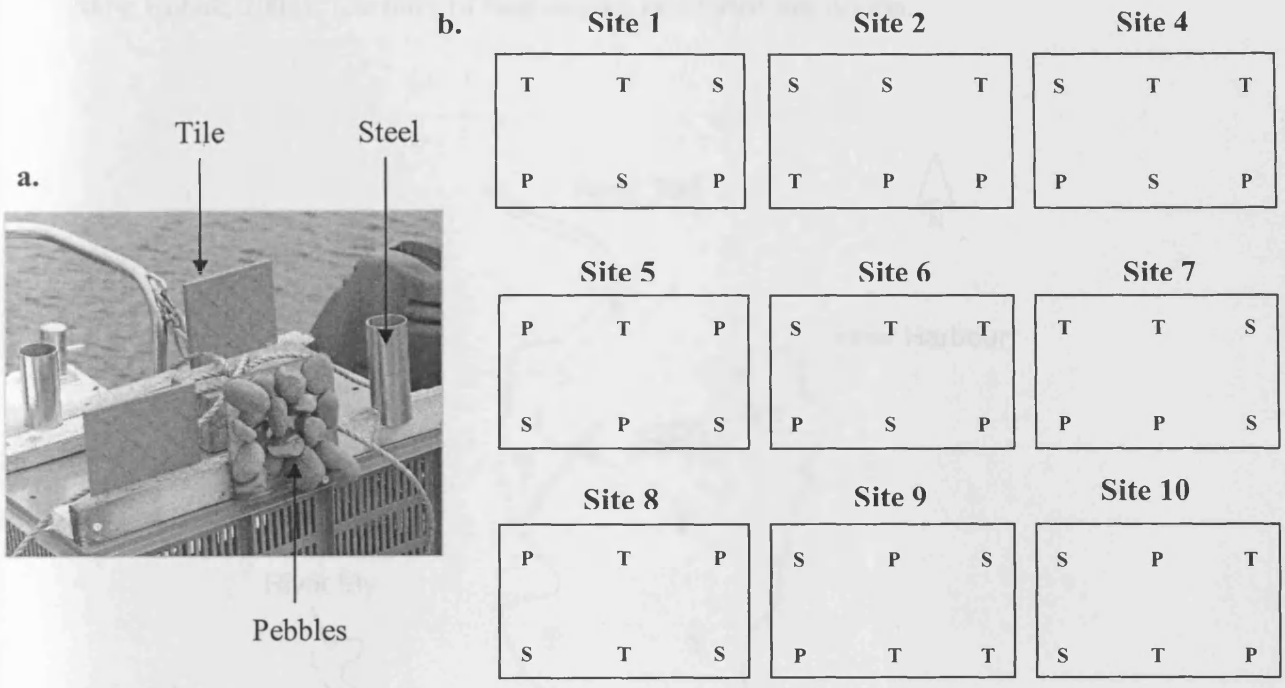


Figure 4.2: Design of the experimental crates. a. Artificial substrates fixed vertically on the crate. b. Random allocation of artificial substrates on crates for the experiment in 2009. T: tile, P: pebbles, S: steel

Crates were deployed on the lake bed from a boat in June (the beginning of the veliger period), and attached by rope to a buoy or a wooden dolphin as close as possible to the veliger sampling sites (Figure 4.3; Chapter 3) at sites in the Taff and Ely rivers (sites 4, 9, 10), the inner harbour (1 and 2), the open Bay (sites 5 and 7), by the bank (site 6) and near the barrage (site 8). No crate was deployed at site 3 in 2009 (the most upstream site in the River Taff) because the water current was too strong. The average depth where plastic crates were deployed was recorded for each site (Table 4.1). Flows in the Ely and Taff rivers were recorded on a daily basis at Environment Agency gauging stations. The river discharge in Cardiff Bay was calculated by summing the two river flows.

It should be noted that human activity, boat traffic and use of the lake constrained experimental design. For example, artificial substrates would ideally have been set in the water column at different depths to limit soft-sediment deposition and represent zebra

mussel distribution in relation to plankton (Garton and Haag, 1993; Czarnoleski *et al.*, 2004; Kobak, 2004). The risks of boat impact precluded this option.

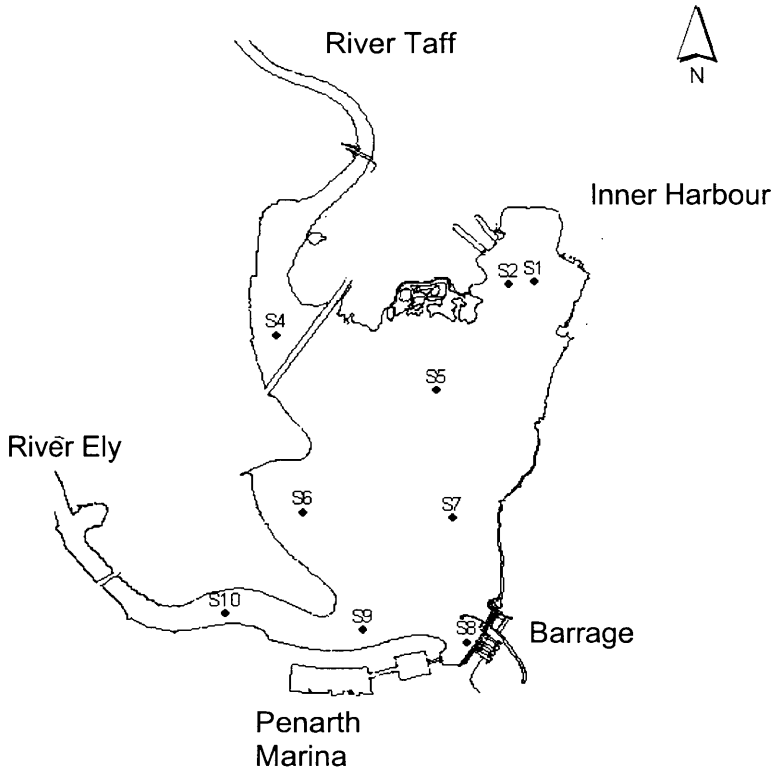


Figure 4.3: Location of the nine sites in Cardiff Bay where crates with artificial substrates were deployed

Table 4.1: Approximate depths in metres where the crates with the artificial substrates were deployed at the nine sites

Sites	Average depth (metres)
S1	5.1
S2	3.9
S4	2.7
S5	7.9
S6	2.7
S7	7.3
S8	3.0
S9	6.6
S10	4.3

Crates and substrates were removed at the end of September, at which point artificial substrates were detached, sealed in plastic bags, and transported in a cool box to the laboratory. Visible juvenile mussels on each artificial substrate were then removed, counted and preserved in 70% alcohol (Figure 4.4). Dimensions of the tiles and steel tubes were measured, and their surface area calculated. Pebble substrates were wrapped in aluminium foil to determine their surface area (Mackie, 1993), with foil pieces being removed, flattered and scanned using the software Image J. Juvenile settlement was expressed as number of individuals per metre square (ind.m^{-2}).

Although experiments in 2007 were unsuccessful because of mud deposition, there was substantial juvenile settlement on the plastic crates. Rather than discard these data, the plastic crates were considered opportunistically as artificial substrates and juvenile counts were made in quadrats (10 x 10cm) placed on each side of the crate and scraping mussels. Quadrat locations were chosen randomly by dividing the crate sides into six sections and generating random numbers. Four samples were taken on single crates while six samples were taken on the double crates, which were composed of one crate containing the artificial substrates and a second crate containing the artificial

substrates coated with antifouling agents (sites 1, 2, 6 and 9). Individuals were counted as for artificial substrates, and the juvenile density estimated as number of individuals metre square (ind.m^{-2}). Similar data were collected in 2008 in addition to the artificial substrate samples.

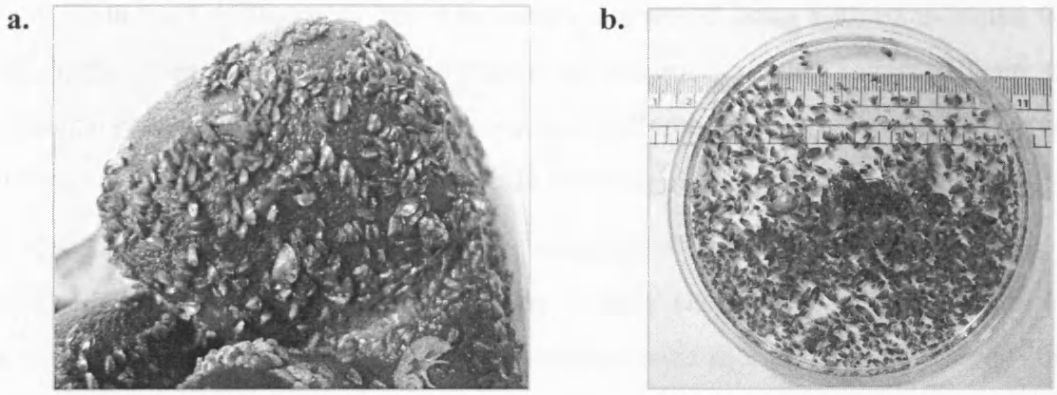


Figure 4.4: a. Juveniles settled on a pebble substrate. b. Juveniles to be counted after being detached from an artificial substrate

4.3.3. Antifouling agents

In 2008, three antifouling agents (Opoponax essential oil (Sigma-Aldrich, Poole, Dorset, UK), Ocimene (RcTreatt, Suffolk, UK) and Frankincense oil - *Boswellia carteri* (Hargeysa, Somalia) were examined for their repellent capacity to zebra mussel settlement on natural substrates. All of the chemicals tested are natural components of myrrh and have been used in previous works on barnacles *Balanus amphitrite* (Ifor Bowen, pers. comm.). Each chemical deterrent was mixed with a based solution composed of Brazilian rosin 50% and xylene 20%, to obtain a 30% concentration (weight: volume), and effects were compared against a control of Brazilian rosin 50% mixed with xylene 50%. In each case, two layers of deterrent or control agent were applied with a brush to the tiles and to the steel samples, the only substrates tested. A minimum of two days was required to allow each coat to dry before substrates were immersed in the lake. Each antifouling agent and the control mixture were applied to four tiles and four steel samples, creating a total of 16 tiles and 16 steel samples in the lake. The four tiles and four steel samples were fixed on a second crate using identical

procedures to those described above. Artificial substrates with deterrents were placed at only four sites: 1, 2, 6 and 9, where the juvenile settlement was expected to be the greatest and where human disturbance was the lowest. The crate with treated substrates was attached to the one with untreated artificial substrates.

4.3.4. Statistical analysis

Hypothesis 1 (differences between years) was tested using a Kruskal-Wallis test (K-W) on the density of juvenile settlement on quadrat samples collected from the experimental crates in 2007 and 2008. Inter-annual differences were also tested as part of the analysis of the effects of substrate type (see below).

Hypothesis 2 (spatial variations) was tested by first mapping spatial patterns in juvenile settlement throughout Cardiff Bay on ArcGIS (ESRI, 2004), focussing on the years with the highest densities when spatial patterns were most clearly expressed (2007 and 2009). Linear correlation between juvenile settlements across sites was also calculated using Pearson's correlation to assess whether spatial patterns were preserved between years. Relationship between juvenile settlement in crate quadrats (2007, 2008) or artificial substrates (2008, 2009) and veliger density were analysed using regression (Veliger density from Chapter 3).

Hypothesis 3 (effects of substrate) was tested using ANOVA with Generalised Linear Mixed Modelling (GLMM) on juvenile density on artificial substrates in 2008 and 2009, simultaneously assessing variations between years and the effect of depth where the substrate were set. A residual maximum likelihood (REML) linear mixed model on juvenile densities, in association with year, artificial substrate type and depth was performed using R 2.9.2. It was necessary to define site as a random term because of repeated sampling within the lake. The significance of terms was tested using the Wald statistic, which is distributed as chi-square. Juvenile density data were log transformed to minimise kurtosis and skewness and to improve residual normality and homogeneity of variance.

Hypothesis 4 (effects of the antifouling agents) was tested using one way ANOVA was carried out on juvenile density following square-root transformation to harmonise variances.

Except where stated, analyses were performed using Minitab 14 with alpha set at 0.05 as the significant level.

4.4. Results

In addition to the adverse effects of sedimentation on substrates in 2007, some crates or artificial substrates were lost or disturbed during the experiment, probably by boats. As a result there were no data on juvenile settlement for sites 5 and 6 in 2009.

4.4.1. Variation in juvenile settlement between years

Juvenile settlement on plastic crates varied between years. On the crates, juvenile density was significantly higher in 2007 than in 2008, with mean densities in the former (32 800 ±3000 ind.m^{-2} (±SE)) over 120 times greater than in the latter (270 ±70 ind.m^{-2}; M-W test, W=3296, P<0.001; Figure 4.5, a). The maximum density recorded in 2008 at site 9 was 54 700 ±700 ind.m^{-2}.

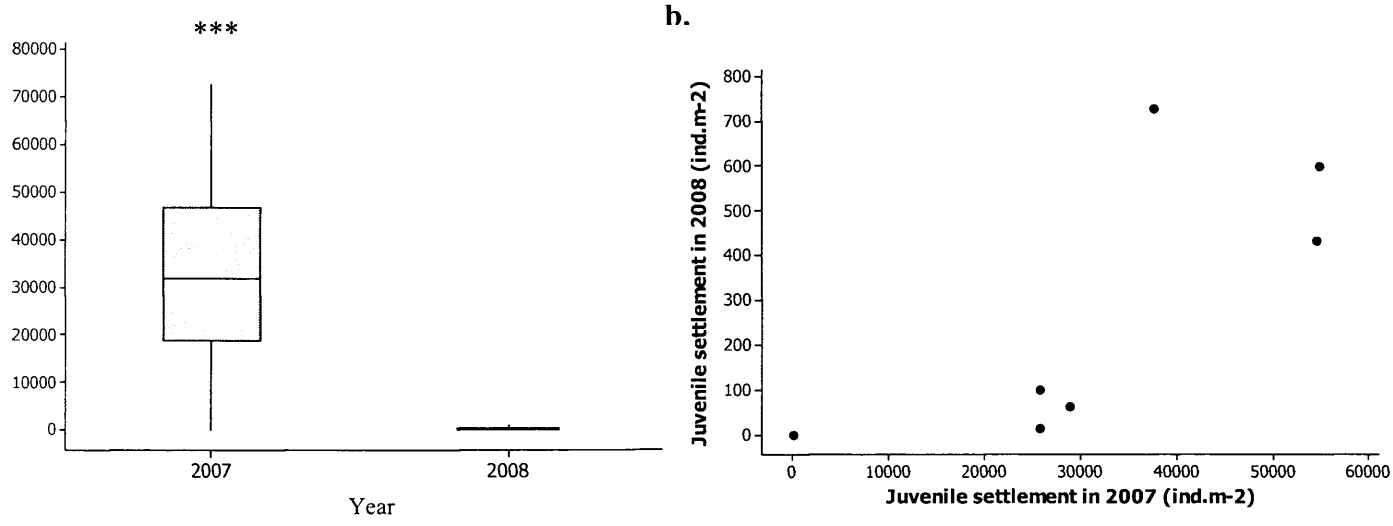


Figure 4.5: Juvenile settlement densities on plastic crates deployed over the summer periods of 2007 and 2008 in Cardiff Bay. a. Box-plots of juvenile settlement (ind.m^{-2}) on plastic crates at sites 1, 2, 4, 5, 6, 8, and 9 in 2007 (n=52) and 2008 (n=41; K-W test *** P<0.001) b. Correlation between juvenile settlement in 2007 and 2008

Juvenile settlement on artificial substrates also varied between years, with higher densities in 2009 than in 2008 on all substrate types. For example, on pebbles, average

values in 2009 (7 200 ± 2 400 ind.m^{-2}) exceeded those in 2008 (300 ± 100 ind.m^{-2}) by 24 times (F1,80=8.130, P<0.001; Figure 4.8 and Table 4.2). REML showed that juvenile settlement was higher in 2009 than 2008 on all artificial substrates (Figure 4.8).

Working from all substrate types, including crate surfaces, juvenile settled densities were 29 000- 55 000 ind.m^{-2} in 2007, 270-300 ind.m^{-2} in 2008 and 6 000- 10 000 ind.m^{-2} in 2009.

4.4.2. Spatial variations in juvenile settlement across Cardiff Bay

Despite differences in absolute density, juvenile settlement densities for zebra mussel were inter-correlated between 2007 and 2008 across Cardiff Bay, although values were not quite formally significant (r = 0.74, P = 0.057; Figure 4.5.b). This result implied spatial patterns in colonisation that were consistent across years, with the highest densities being found in the Inner Harbour, at the Ely River mouth and near the barrage (Figure 4.6).

Substrate depth had no effect on juvenile settlement (Table 4.2). However, settlement was significantly higher in areas with a greater concentration of veligers in 2009 ($F_{1,6}$= 14.45, P=0.009), with the same tendency apparent in 2007 ($F_{1.7}$=4.31, P = 0.08; Figure 4.7).

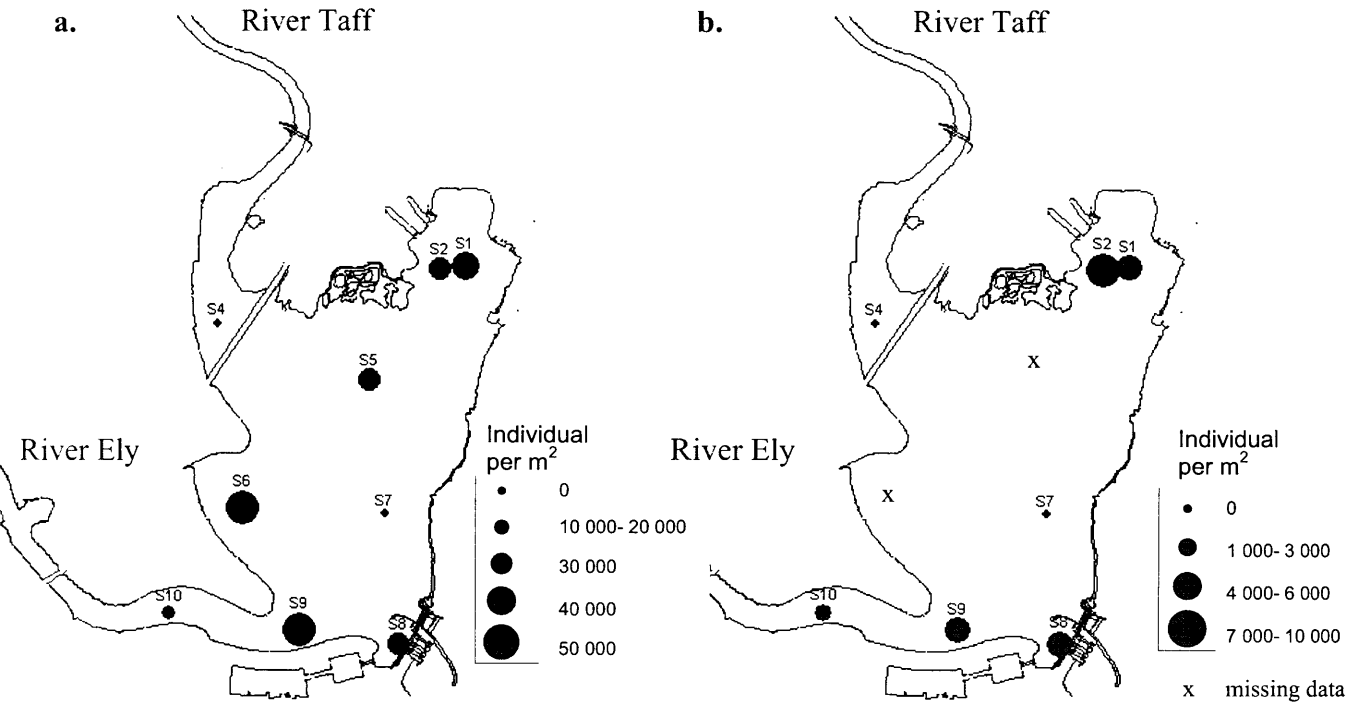


Figure 4.6: Settlement of zebra mussels at different experimental sites in Cardiff Bay: a) densities estimated from quadrat samples collected on plastic crates in 2007; b) densities estimated from artificial substrate in 2009

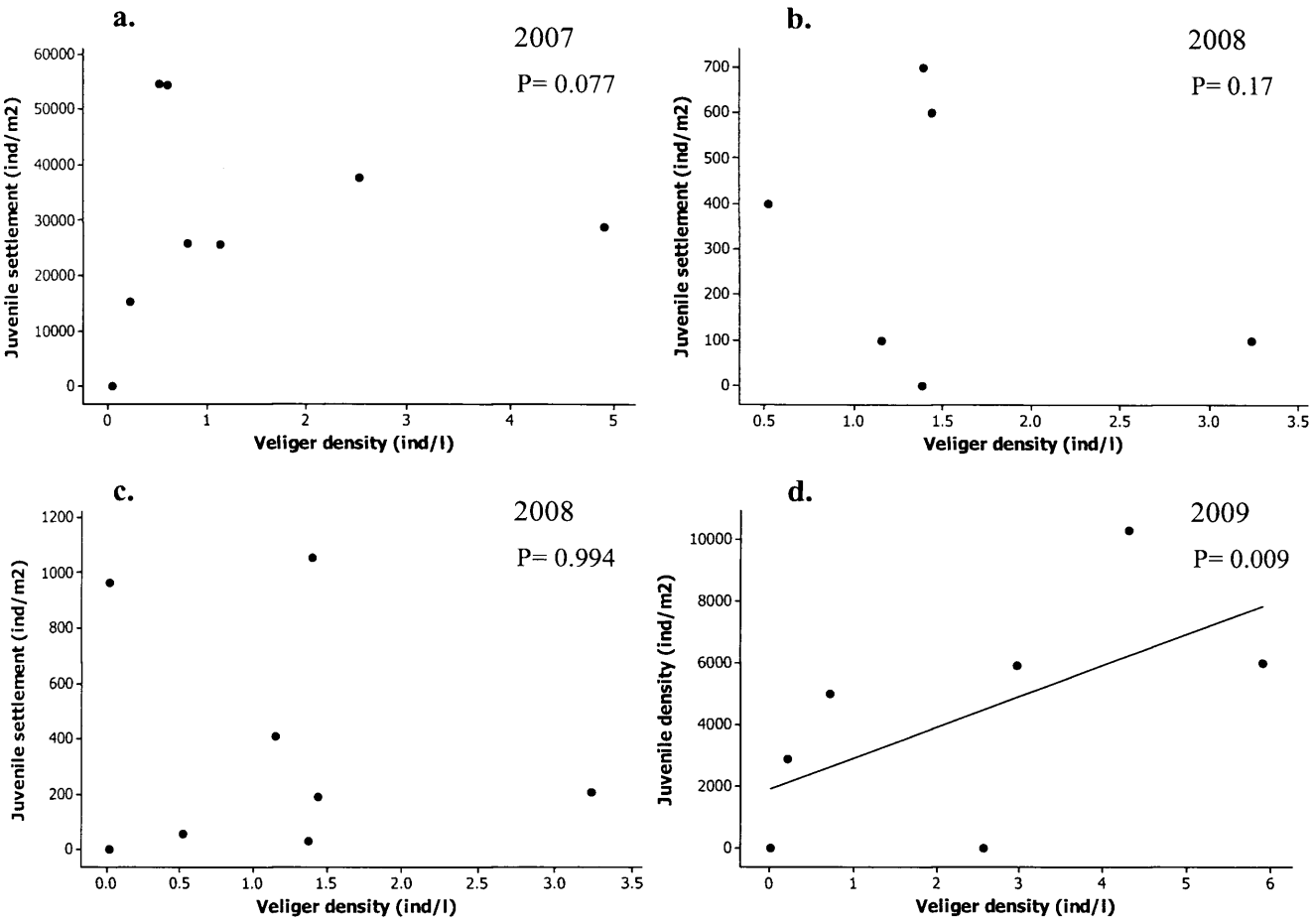


Figure 4.7: The relationship between the settlement of zebra mussel juveniles and the abundance of veligers at sites in Cardiff Bay. a. Juvenile settlement estimated from crate samples taken at sites 1, 2, 4, 5, 6, 8, 9 and 10, in 2007 ($F_{1,7}$=4.31) b. Juvenile settlement estimated from crate samples taken at sites 1, 2, 5, 6, 8 and 9, in 2008 ($F_{1,5}$=2.51) c. Juvenile settlement estimated from artificial substrates at sites 1, 2, 4, 5, 6, 8, 9 and 10, in 2008 ($F_{1,7}$=0.00) d. Juvenile settlement estimated from artificial substrates at sites 1, 2, 4, 8, 9, 10 and 11, in 2009 ($F_{1,6}$= 14.45)

4.4.3. Juvenile settlement on artificial substrates (2008-2009)

Having accounted for variations among years, juvenile settlement varied significantly between artificial substrate types ($F_{2,80}$=8.130, P<0.001; Figure 4.8, Table 4.2). REML showed that juvenile settlement was greater by 2 times on pebbles than on steel substrates, and similar to tiles (Figure 4.9). Antifouling agents had no effect on

juvenile settlement compared to controls using the same substrate (ANOVA, $F_{3,27} = 0.26$, P=0.851).

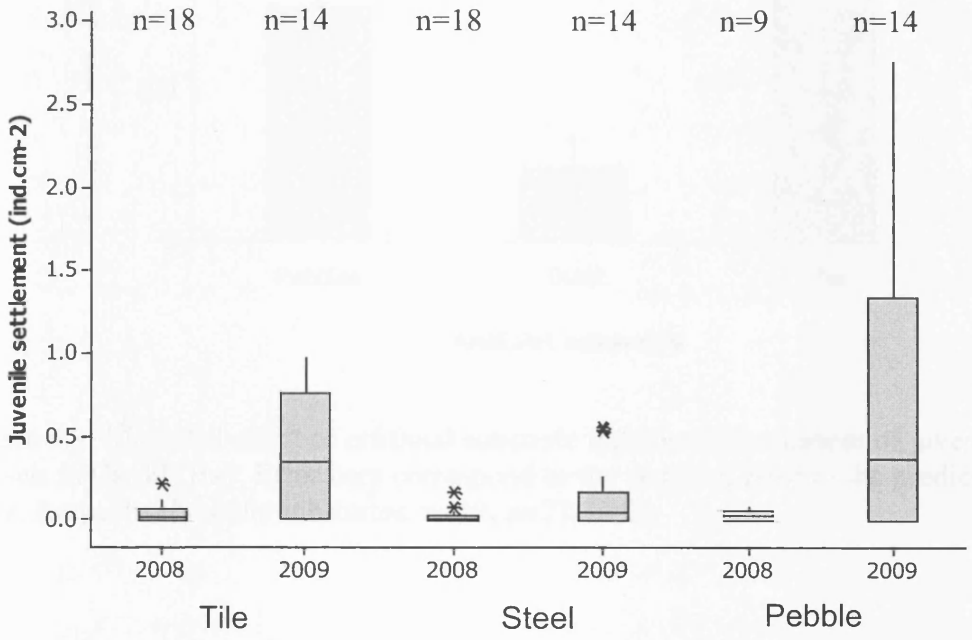


Figure 4.8: Juvenile settlement (ind.cm^{-2}) on the three artificial substrates of tile, steel and pebble in 2008 and 2009 in Cardiff Bay

Table 4.2: Minimal restricted maximum likelihood (REML) modelling showing how the settlement of juvenile zebra mussels varied among years, substrates and depth (random terms= sites)

Model term	Wald statistic (χ^2)	d.f	*P*
Artificial substrate	7.12	2, 62.4	0.002
Year	27.56	1, 63.6	<0.001
Depth	0.89	1, 46.1	0.35

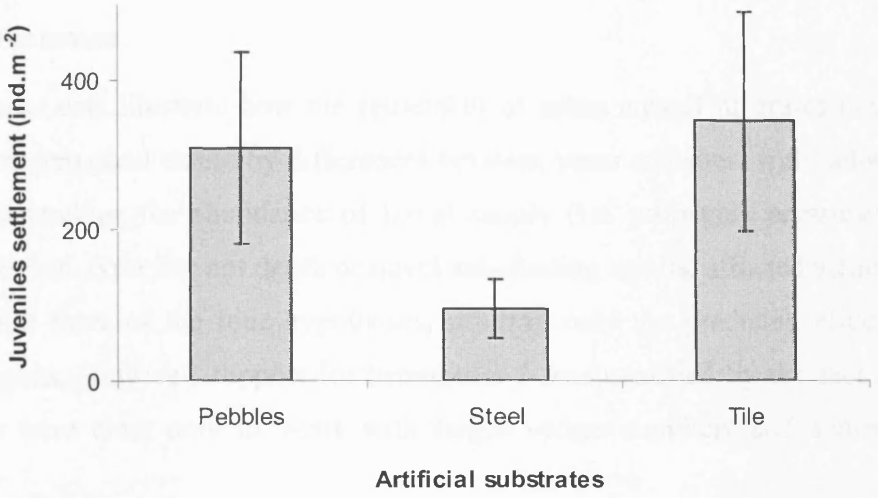


Figure 4.9: Modelled effect of artificial substrate type on the settlement of juvenile zebra mussels in Cardiff Bay. Error bars correspond to the standard error of the predicted mean value. Respectively to the substrates: n=19, n=27, n=28

4.5. Discussion

These data illustrate how the settlement of zebra mussel juveniles is affected at broad spatio-temporal extent by differences between years and sites, with colonisation in both cases tracking the abundance of larval supply (i.e. propagule pressure). At finer scales, substrate type, but not depth or novel anti-fouling agents, affected settlement. The data support three of the four hypotheses, refuting only the predicted effects of anti-fouling agents. However, support for hypothesis 2 was qualified by the fact the spatial variations were clear only in years with larger veliger numbers and settled juvenile densities.

4.5.1. Temporal variations in juvenile settlement

Zebra mussel recruitment in Cardiff Bay varied between years, with settled mussel density over 100 times greater in 2007 than in 2008. Variations continued also into 2009, as indicated by the data from artificial substrates. Inter-annual variations of this magnitude have been observed previously (Garton and Haag, 1993), for example in the Lake Huron where mean juvenile density fell between two years from 33 200 to 4 200 ind.m^{-2} in 1993 (Nalepa *et al.*, 1995). While these latter values are of similar magnitude to Cardiff Bay, newly settled mussel densities in the North American Great Lakes can reach 700 000 ind.m^{-2} (Griffiths *et al.*, 1991). In Lough Key, Ireland, juvenile densities ranged between 4 000-17 0000 ind.m^{-2}, again encompassing the range found in Cardiff Bay (Lucy, 2005).

Variations between years in juvenile settlement among zebra mussels can sometimes be explained by intrinsic factors (Nalepa *et al.*, 1995): new populations are typified by an initial high density followed by population reduction, while established cycle irregularly over decades (Stanczykowska and Lewandowski, 1993; Nalepa *et al.*, 1995). Low recruitment may then be explained by density dependant regulation: at high adult densities, with veliger predated by adults thereby reducing population recruitment (MacIsaac and Sprules, 1991; Garton and Haag, 1993; Chase and Baley, 1999). Zebra

mussel population in Cardiff Bay is relatively new and such intrinsic factors could therefore explain in part the recruitment variations.

Physical factors might also explain inter-annual change: total river discharge in 2008 was significantly higher than in 2007 and 2009, respectively with mean values of 31 ± 4 $m^3.s^{-1}$, 19 ± 2 $m^3.s^{-1}$, and 18 ± 2 $m^3.s^{-1}$ (§3.4 Chapter 3). Post-veliger settlement in zebra mussels is highly influenced by water currents and turbulence (Sprung, 1993; Garton and Haag, 1993; Orlova and Panov, 2004), with current velocity over 1-1.5 $m.s^{-1}$ inhibiting mussel settlement (Kobak, 2005) and velocities of 0.27$m.s^{-1}$ suppressing byssal production (Clarke and McMahon, 1995). Data on current velocities in Cardiff Bay are very patchy, and none are available to illustrate the effects of varying river discharge from the Taff and Ely. However, with a volume of approximately 9 million cubic metres (200 ha x average depth 4.5 m), average residence times of water in Cardiff Bay will have declined by almost half from 5.7 to 3.3 days in 2008 as average discharge increased. Peak discharges during July 2008 will have further reduced residence, leading to increased veliger loss and washout from the Cardiff Bay system. Chlorophyll *a* concentrations also decreased in Cardiff Bay with increased discharge in 2008 with possible consequences for food availability in the early settlement phase (Chapter 3).

Differences between years in veliger density would also explained recruitment variations (Chapter 3). One interesting permutation of this experiment would have been to make juvenile settlement assessments monthly during the reproduction season by collecting substrate samples at this time step, coupled with veliger sampling. Such a methodology would reveal the initial settlement, settlement peaks and settlement intensity variations as described in some studies (Nalepa *et al.*, 1995; Cope *et al.*, 2006).

4.5.2. Spatial variations in juvenile settlement

Despite inter-annual variations in settled juvenile density, spatial patterns in settlement were preserved between years: the highest juvenile settled densities were in the Inner Harbour, in shallow parts of the barrage (site 6), in the Ely River mouth and in the nearby the barrage where veliger densities were the greatest. These areas were also characterised by increased veliger density, and juvenile settlement were correlated with

veliger numbers in both 2007 and 2009. In other words, juvenile settlement closely tracked the number of zebra mussel propagules arriving in each location. The influence of veliger density on juvenile settlement was also reported in the Erie Lake with a strong relationship between daily settlement rates and local concentrations of veligers (Martel *et al.*, 1994). In addition, however, juvenile settlement is influenced by water current and turbulence (Clarke and McMahon, 1995; Kobak, 2005), and areas with the largest veliger residence and juvenile settlement in Cardiff Bay are likely to be those area sheltered from the main river current.

In contrast, there was no relationship between juvenile settlement and depth. Elsewhere, Wacker and von Elert (2003), showed that juvenile settlement was a function of depth in Lake Constance (mean depth= 90m; Fraleigh *et al.*, 1993), but perhaps the shallow depths of Cardiff Bay (mean depth = c 4.5m), coupled with mixing effects caused by the aeration system, creates more homogeneous physico-chemical condition through the water column.

4.5.3. Mussel settlement on the artificial substrates

As with all experimental approaches to represent real ecological processes, some caution is required in interpreting the data on artificial substrates. For example, the data from 2007 illustrated that fine sediment deposition could interfere with colonisation pattern. Movement and disturbance of some creates by boat traffic or possibly water-flow also reveals a possible lack of stability in the experimental units. More subtle artefacts might have been possible in 2008 and 2009, for example because substrate surfaces might have lacked the microbial biofilms typical of natural substrates, at least initially. The development of a natural biofilms can positively influence post-veliger attachment, although a period as short as two weeks should be sufficient a biofilm to develop (Kavouras and Maki, 2003). Substrates deployed in June would have rapidly acquired conditions suitable to support attachment during peak veliger numbers. Moreover, periods in excess of two-three weeks are typically required between veliger production and post-veliger settlement, making it more likely that mature biofilms would be present on substrates by the time of larval settlement (Fraleigh *et al.,* 1993; Nichols, 1996; Lucy,

2005). In total, artificial substrates were left *in situ* for four months during the veliger production period.

Notwithstanding the above aspects of design, other aspects allowed some inferences about the effects of artificial substrates that were replicated across locations and years. Previous data show how hard surfaces constitute suitable substrate for zebra mussel settlement (Smit *et al.,* 1993), and juvenile settlement on artificial substrates in 2008 and 2009 was greatest on pebbles and tile, where mussel density was significantly higher than on steel. Settlement density was not, however, significantly greater on pebbles than on tile as expected. The shape of the pebble substrates, with interstices and localised areas with reduced flow, was supposed to protect mussels from predation and turbulence, and leading therefore to higher juvenile densities on pebbles (Kobak, 2005). Juvenile densities on tile were higher than on steel as its porous structure provided a suitable substrate for mussel attachment. Surface textural effects on either mussel attachment or biofilm development might also have been involved, with the smooth steel surfaces less likely to provide suitable substrates for mussels than tiles or pebbles (Marsden and Lansky, 2000). Although steel presented the lowest juvenile densities, it was colonised. Previous studies have shown how zebra mussels can colonise metal (Kilgour and Mackie, 1993), albeit at low density, illustrating how zebra mussels can cause a nuisance on metal structures, and even parts of the aeration system in Cardiff Bay. Regular cleaning and control of the aeration system and barrage structure is necessary.

While these data illustrate that hard substrates are most affected by mussel colonisation, one interesting question arises about whether zebra mussels could ever colonise the extensive soft sediments in Cardiff Bay. In 2007, mud was tested as a soft artificial substrate, but no mussels settled. Moreover, mud deposition on other artificial substrates appeared to impede zebra mussel settlement, with sedimentation rates apparently large. Nevertheless, zebra mussels can colonise soft sediment through initial settlement on sand or other localised patches of hard debris such as shell fragments (Berckman *et al.*, 1998; Bially and MacIsaac, 2000). These patches then provide a nucleus from which the mussel population can expand, providing population increase as faster than rates of fine-sediment deposition or erosion; environmental stability is an

important factor for a successful juvenile settlement (Stanczykowska and Lewandowski, 1993; Nichols, 1996). The potential zebra mussel colonisation of soft sediment in Cardiff Bay is investigated further in Chapter 5.

4.5.4. Antifouling agents

Given the potential nuisance effects of zebra mussels, work on antifouling agents has been an important research line. In this case, however, anti-fouling agents derived from myrrh had no significant repelling effect to zebra mussel settlement. One possible explanation is that this experiment was carried out in 2007, when the artificial substrates were affected by soft sediment, and in 2008, when juvenile settlement was very low. As a result it is difficult to draw definitive conclusions about the effectiveness of the antifouling agents used in this study. Further work on these agents is required.

4.6. Conclusions

The results from these experiments on the temporal and spatial distribution of juvenile settlement have revealed some key information about the colonisation dynamics of zebra mussels in Cardiff Bay. At broad extents, recruitment varies in time and space, with varying river discharge and local flow paths probably acting in concert with local veliger density to affect settlement pattern. Variations between years were large, and it is interesting to speculate that very low flows and high temperatures in 2003 – very shortly after the arrival of zebra mussels in Cardiff Bay – must have provided ideal conditions for population establishment. High risk areas for fouling by zebra mussels appear to be areas with low velocity and high veliger density in areas sheltered from river currents and water turbulence such as the Inner Harbour, near to the barrage and the Bay's west bank.

At more local scales, and notwithstanding the caution required in extrapolating from experimental data, hard, irregular and porous substrates appear to favour colonisation, although even steel structures are not free from colonisation risk. As a result, the greatest zebra mussel colonisation is likely to occur around the bay shoreline where embedded pebbles, barrage structure and dock walls are the dominant substrate. Steel structures found on the barrage structure, the fish pass, the aeration system and in locks support also mussel settlement in probably smaller densities.

Overall, the research presented here can form an important foundation for understanding the spread and recruitment of the mussel population in Cardiff Bay, the associated risks posed by mussel colonisation, and as a result information on which to base management decisions.

5. The density and age-structure of adult zebra mussels in Cardiff Bay

5.1. Summary

1. Zebra mussels affect many freshwaters outside their native range due to their rapid population development and high density. However, there have been few lake-wide estimates of total population size, particularly in newly formed lakes such as Cardiff Bay. This chapter illustrates inter-biotope variations in zebra mussels across the Bay within 7-8 years of its formation in 2001 and presents a lake-wide population estimate.

2. The range of biotopes present in Cardiff Bay required a range of survey methods including i) Side Scan Sonar (SSS) surveys, coupled with confirmatory grab samples, on the lake's extensive soft sediments (2007/2008); ii) quadrat samples in representative pebble areas (2008/2009) iii) under-water video imagery on representative vertical surfaces in three regions of the Bay (2009) and iv) samples on the Bay's aeration system (2009).

3. Zebra mussels were mostly absent from Cardiff Bays's soft sediments: SSS revealed few reefs, and only seven of 34 sites grab-sampled contained mussels. They held densities of 3 300 ±1 200 m^{-2} and 2 700 ±1 900 m^{-2} respectively in the Inner Harbour and by the CHA pontoon.

4. By contrast, vertical hard surfaces were occupied extensively over a depth range of 0.5-7 m, with densities increasing from 450 ±180 m^{-2} at 0.5m to 1 600 ±220 m^{-2} in deeper water. Densities also varied moderately but significantly among sites from c 600 m^{-2} in the Marina to c 2 000 m^{-2} near the Inner Harbour.

5. Quadrat samples revealed variation in mussel densities between pebble areas ranging from 950 ±250 m^{-2} by CHA and the barrage, to 3 700 ±370 m^{-2} in the Inner Harbour.

6. Colonisation of the aeration system was variable, with some parts unoccupied and mean densities elsewhere ranging from 700 ±200 m^{-2} to 6 600 ±900 m^{-2}. Patchiness and limited sampling of this biotope precluded its inclusion in any lake-wide population estimate.

7. Length frequencies from all samples suggested that Cardiff Bay's zebra mussels were characterised by four cohorts probably dominated by a 2-3 years lifespan and individuals of 1 year old (shell length 11-19mm).

8. These data suggest that Cardiff Bay now holds a large, extensive, and young zebra mussel population in all except areas of soft-sediment. However, occupied areas mostly have overall densities within an order of magnitude (c 250- 6 600 m^{-2}). Accounting as far as possible for variations in occupancy, density and biotope availability, the adult population surrounding the lake is estimated to be around 9.5 and 30.5 million individuals (excluding the aeration system), consistent with the large density of veligers recorded in the lake. The ecological effects of such a large population are likely to be substantial.

5.2. Introduction

The zebra mussel (*Dreissena polymorpha*, Pallas) is one of the most invasive of all freshwater species and capable of large ecological effects (Ludyanskiy *et al.*, 1993; MacIsaac, 1996; Vanderploeg *et al.*, 2002). Native to the Caspian and Black Seas, zebra mussels began to occur outside their native range in European water bodies in the 19th century as a result of increasing commercial boat trade, whilst the invasion in North America occurred in the 1980s (Morton, 1970; Müller *et al.*, 2002). On both these continents, pronounced population growth over the period 1980- 2000 has led to serious ecological and economic impacts (Stanczykowska and Lewandowski, 1993; Nalepa *et al.*, 1995, Nichols, 1996). Key effects include i) changes in lake food webs through the selective removal of phytoplankton (Fahnenstiel *et al.*, 1995); ii) modification of lake biogeochemistry, notably affecting oxygen and nutrient dynamics (Effler *et al.*, 1996; Effler and Siegfried, 1998); iii) fouling of natural or artificial structures, out-competing native bivalves for space (Schloesser *et al.*, 1996) and iv) clogging of water treatment facilities or industrial infrastructure with consequences for costs and efficiency (Kovalak *et al.*, 1993; Laurier LePage, 1993; Ram and McMahon, 1996). A major pre-requirement for understanding such effects in any given occupied lake is some assessment of population distribution, density and structure (Naddafi *et al.*, 2010).

While many studies have examined the population and distribution of zebra mussels (Stanczykowska and Lewandowski, 1993; Nalepa *et al.*, 1995; Sprung 1995; Mackie and Schloesser, 1996), there are few lake-wide inventories, and almost none have involved newly formed water bodies. These could provide an interesting insight into zebra mussel invasion because they might reveal how rapidly new populations are formed. Such an undertaking is clearly complicated: zebra mussels colonise almost any hard substrates (e.g. rock/pebbles, wood, various metals, cement and plastic) but also soft substrates such as macrophytes and algae (Kilgour and Mackie, 1993; Stanczykowska and Lewandowski, 1993; Lucy, 2005; Folino-Rorem *et al.*, 2006; Berkman *et al.*, 1998). Individual case studies, such as in Lake Erie, have sometimes illustrated also the capacity of mussels to colonise fine sediments (Berkman *et al.*, 1998; Bially and MacIsaac, 2000; Haltuch *et al.*, 2000). In addition, zebra mussels are influenced by factors such

turbulence, water temperature, oxygen concentration, light intensity, food availability and predation (Mellina and Rasmussen, 1994; Nalepa *et al.*, 1995; Sprung, 1995; Naddafi *et al.*, 2010). These factors vary not only spatially across individual lakes, but also with depth (Garton and Johnson, 2000; Wacker and Von Elert, 2003; Naddafi *et al.*, 2010) so that only large-scale assessments across all available biotopes, coupled with depth-distributional surveys, can permit a population estimate.

In addition to variations in density, the ecological effects of zebra mussels depend on population age and size-structure, for example mussels of different size have contrasting filtration rates and oxygen uptake (Kryger and Riisgard, 1988; Reeders and de Vaate, 1990; Effler *et al.*, 1996; Young *et al.*, 1996). Age-structure also gives some indication of population history and dynamics, for example through information on the growth and survival of individual cohorts. Zebra mussel lifespan is highly variable across its range, varying apparently from 1.5-2 years in North America, through 3-5 years in European lakes to 6-9 years in some Russian reservoirs (Nalepa *et al.*, 1995; Mackie and Schloesser, 1996; Garton and Johnson, 2000). However, it is unclear whether these variations are representative of different regions, or whether they reflect the data available in individual case studies or from populations in different stages of development. The age structure of zebra mussels in newly occupied lakes is particularly interesting in providing information on the possible invasion history.

Assessing the distribution, population size and age structure of zebra mussels in Cardiff Bay has now become urgent. This 200 ha urban lake was formed in 2001 by the construction of a barrage which impounded the rivers Taff and Ely. The lake is managed for amenity, but there is also a legal requirement on the body responsible, Cardiff Harbour Authority, to maintain oxygen concentrations at >5 $mg.l^{-1}$ to support aquatic life, especially for the passage of migratory salmonid fishes into the Taff drainage system (Cardiff Bay Barrage Act, 1993). This is facilitated by an aeration system deployed across the lakebed to maintain water mixing and oxygen diffusion. However, the lake receives large quantities of nutrients and organic matter from the Taff and Ely which lead to oxygen depletion through sediment uptake and net algal respiration overnight. Potentially more important, Cardiff Bay was invaded by zebra mussels sometime after its formation and counts of veliger larvae suggest a substantial population (Chapter 3). The

potential for effects on the lake's ecology and oxygen dynamics is large, for example through i) direct oxygen uptake; ii) modification of algal numbers and patterns of photosynthesis and iii) modification of nutrient dynamics. So far, however, there has been no lake-wide inventory of zebra mussel numbers. This chapter presents such an inventory. Through a range of methods designed to assess zebra mussel numbers as widely as logistically possible on the different substrates (= biotopes) in the lake, the following predictions were tested:

i. Because zebra mussels are vulnerable to sediment deposition (Chapter 4), numbers were expected to be low in the lake's soft sediments.

ii. In contrast, zebra mussel colonisation was expected to occur on all hard substrates except those most exposed. This includes the rubber pipe work of the aeration system.

iii. Vertical variation in physical factors down the water column was expected to cause depth-related variation in zebra mussel density, with numbers greatest in euphotic, algal-rich zone within c 1-3 m from the surface.

While testing these predictions, mussel size and age-structure was determined opportunistically on individuals collected during density estimation.

5.3. Study area and methods

5.3.1. Site

The history of Cardiff Bay explains the heterogeneity of biotopes, especially man made habitats present in the lake. Before the barrage's construction, Cardiff Bay was a harbour for coal trade, and was characterised by mudflats and inter-tidal marshlands. The barrage construction impounded two rivers, the Taff and the Ely, to create an artificial lake of 200ha with a mean depth c 4m, and maximum depth c 13m. The lake bed is mainly composed by soft sediments, silt and mud. However, the aeration system, composed of long rubber pipes lain on the lakebed constitutes a real pipe network which corresponds to hard substrate (Figure 5.1). The lake is surrounded by different hard substrates which can be grouped into two main substrates: vertical surfaces which correspond to the walls and the locks of the previous harbour, the actual barrage and structure in Cardiff Bay; and pebble banks, which correspond to cemented pebbles at an angle of $<45^{\circ}$ and area of loose pebbles (Figures 5.1; 5.4). Soft sediment levels vary over time (month scale) which make difficult to determine the depth where pebble banks penetrate into the sediment.

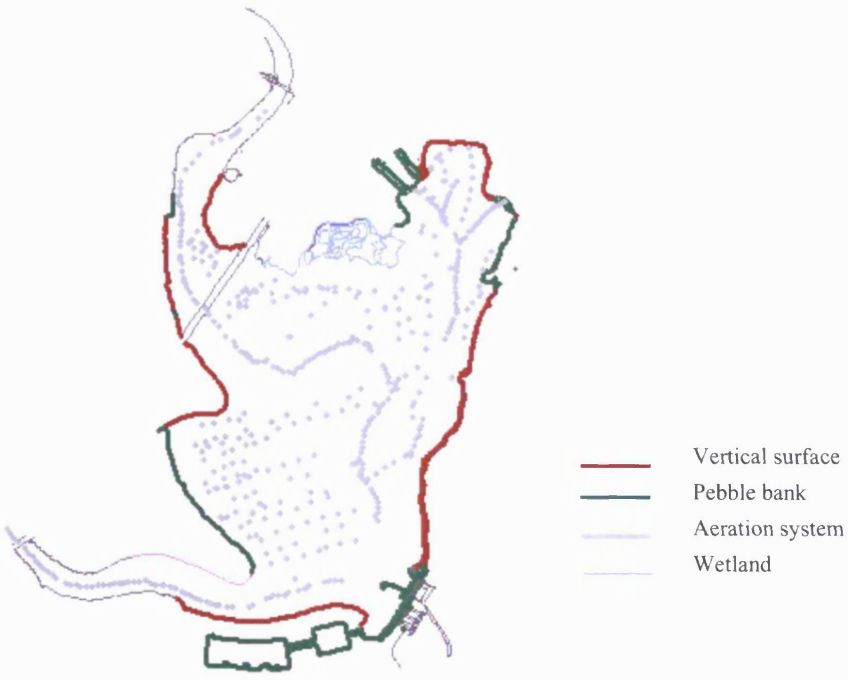


Figure 5.1: Location of the air diffusers of the aeration system and of the two main hard substrates surrounded Cardiff Bay (vertical surfaces and pebble bank)

5.3.2. Direct observation survey

An important step in understanding the extent of occupancy by zebra mussels across the entire area of Cardiff Bay was a lake-wide survey involving all types of structure. Initial walk-over observations and information from CHA staff prior to all other surveys suggested that colonisation was widespread, but the opportunity to illustrate this was validated when water levels were intentionally dropped by 0.5m during the construction of a bridge in the Ely River on the August 3 2009. At each of 15 sites around the lake shore the presence/absence of mussels was noted and photographs taken (Figure 5.8).

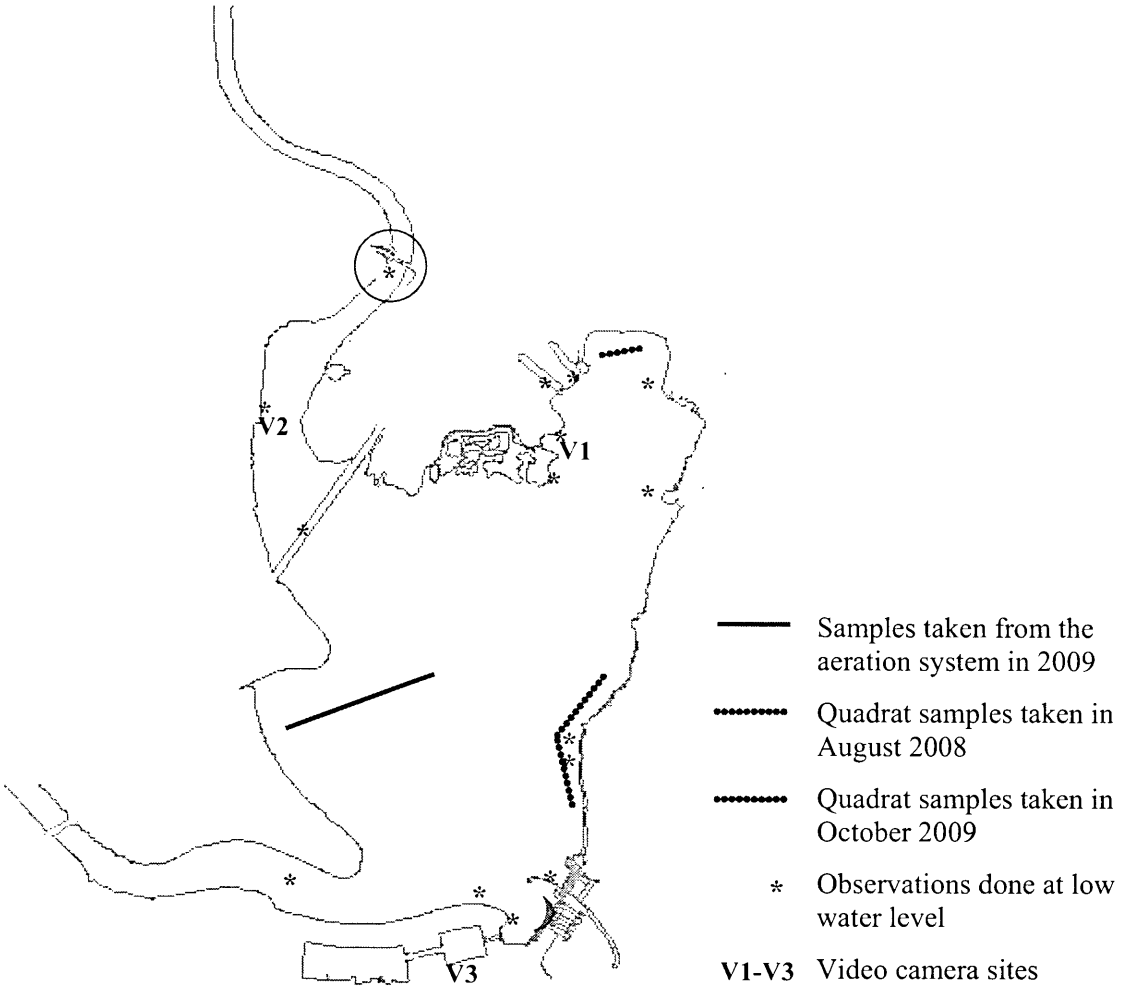


Figure 5.2: Sites where zebra mussel density was assessed with different techniques and observation survey through Cardiff Bay. The encircled site corresponds to Clarence Road Bridge

Population estimates next required methods appropriate for each substrate type as follows.

5.3.3. Lake bed and fine sediments

The Bay's extensive fine sediments were surveyed using Side Scan Sonar (SSS) imaging coupled with grab samples to ground-truth any areas of apparent reef formation

(Coackley *et al.*, 1997; Sauriau *et al.*, 1997; Haltuch *et al.*, 2000; Allen *et al.*, 2005). Side Scan Sonar (SSS) surveys were carried out in November 2007, allowing image mapping of the lakebed based on sound-reflection from bed material of contrasting type and texture (see below). Side Scan Sonar has been used recently to assess mollusc formations in both marine and freshwater environments (Sauriau *et al.*, 1997; Haltuch *et al.*, 2000; Allen *et al.*, 2005).

Surveys were arranged to provide North East to South West parallel transects through the entire lake as well as transects in both the Taff and Ely River mouths. The SSS system, model CM2 (C-MAX.Ltd, Dorset, UK), comprised a towfish, tow cable, processing and display device, and a global positioning system (GPS; Figure 5.3.a.b.). The towfish (Figure 5.3.a) transmitted acoustic pulses (350 kHz) at right angles to the moving boat (2.5 knots) that were reflected weakly by soft sediments and strongly by hard substrates (Haltuch *et al.*, 2000). The aeration system in the Bay was turned off during the survey since air bubble diffusion would inhibit the effective transmission of acoustic pulses. The lakebed was visualised on a screen (Figure 5.3.b) in such a way that soft sediments appeared as grey- white zones whilst hard substrates were represented as black zones. The sonar images were recorded for subsequent analysis using Multiviewer, part of Side Scan Sonar processing toolkit (Coda Octopus) and zones highlighted on a map was created using ArcGis 9.2 (ESRI, 2004).

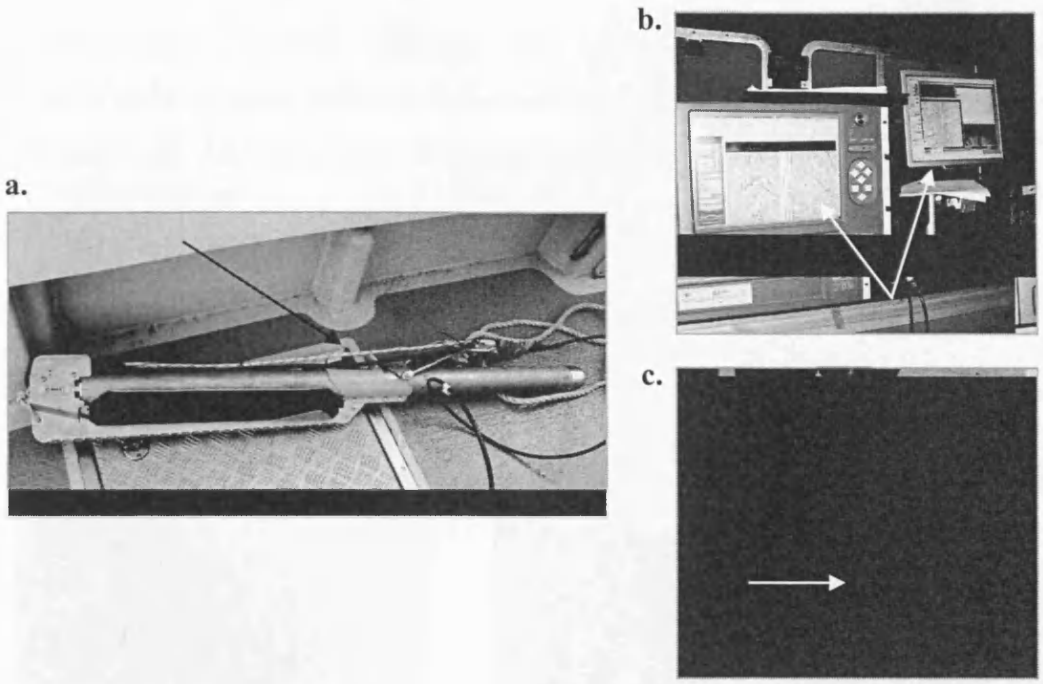


Figure 5.3: a. Side Scan Sonar system model CM2: towfish, b. Processing and display device. c. Grab sampler closed after sampling

Any possible mussel-bed formations were investigated using a sediment grab sample technique. The grab sample had a capacity of 2L which corresponded to a sample area of 27 cm^2. It was manually let down to the lakebed with the jaws locked open, and once it touched the bottom, it was strongly pulled up to close the jaws into the substrate. Thirty-four such samples were taken on 9th July 2008, and the samples sorted into a basin (Figure 5.3.c). Any mussels found in each sample were stored in plastic bags and preserved in ice prior to counting and measurement in the laboratory.

5.3.4. Lake margins

Only two areas were composed of loose pebbles, and this made sampling relatively straightforward, at least in wadeable depths. Sampling of the entire depth range was not prevented by depth. Otherwise, areas of loose pebbles were accessible from land, and zebra mussels could be freely sampled. In each of four quadrats (24 x 24cm; Lucy, 2005) in the Inner Harbour (August 2008; Figure 5.4.a), and six in the area between CHA and the barrage (October 2009; Figures 5.2, 5.4.b), pebbles were removed randomly

along with unattached mussels. Although reflecting coverage of just over half a square metre, these samples were considered representative of what were extensive, relatively uniform substrates. All were placed in bags and preserved on ice for further analysis.

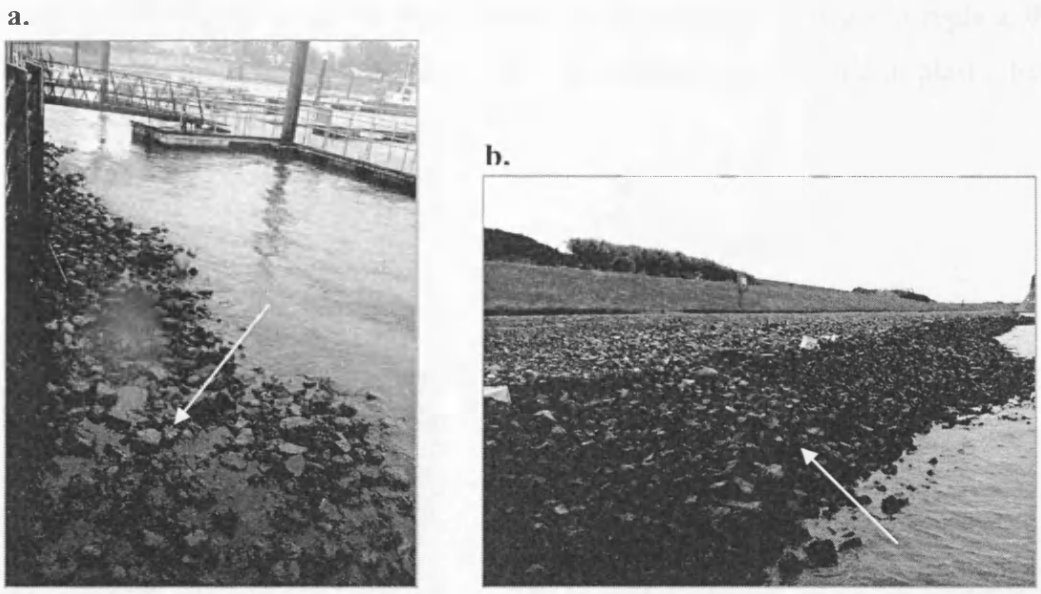


Figure 5.4: Area of loose pebbles in: a. Inner Harbour. b. Area between CHA and the barrage. Both pictures were taken during a building operation done in the River Ely which required a low water level of Cardiff Bay, dropped of half a metre

5.3.5. Aeration system

The aeration system is monitored by CHA and the air diffusers are regularly replaced to maintain a sufficient mixing in the water column. The aeration pipes can be pulled from the water using a pulley on board a boat (Figure 5.5.a) from which diffuser replacement takes place once the pipe system is brought on board (Figure 5.5.b). Because of logistical restrictions in accessing the aeration system, only one day of field work was carried out.

To determine zebra mussel densities on the aeration pipes, zebra mussel were collected in the west part of Cardiff Bay on May 21st 2009, along a transect known to

have a range of veliger densities (Figure 5.2). At four site along a 500 m length of aeration pipe, three or four replicate samples were taken randomly by scraping mussels from the exposed part of the pipe, as opposed to the part lain in the mud, on a length of 20cm, which corresponded to a surface of approximately 300 cm^2. Each site corresponded to a portion of the pipe chosen by the monitoring team to replace the air diffuser. The average depth of the sites was 3 m. Mussels were placed in plastic bag and preserved in ice.

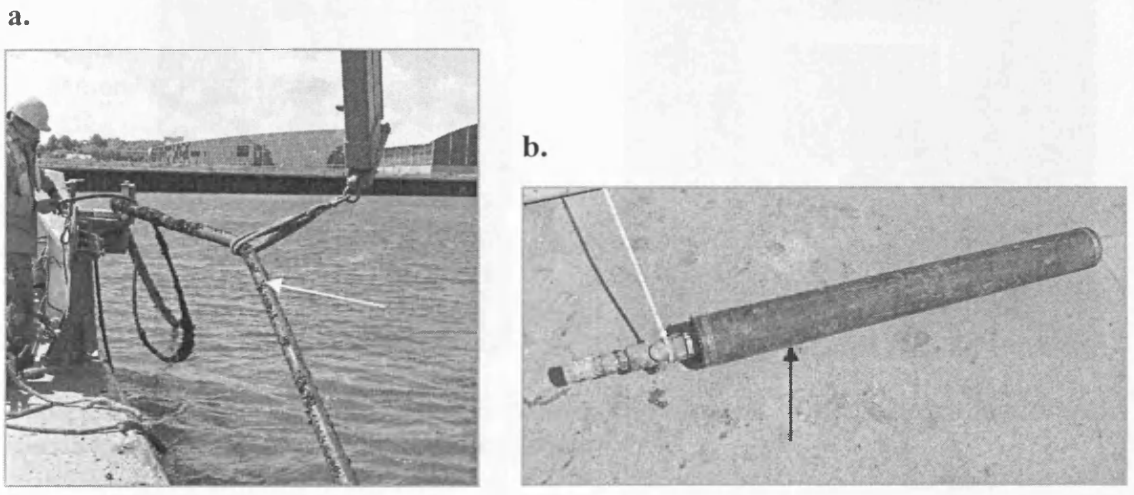


Figure 5.5: Aeration system maintenance realised by Cardiff Harbour Authority. a. Pipe of the aeration system pulling out of the water with a pulley. b. New air diffuser to set up

5.3.6. Vertical hard substrates

On the Bay's underwater walls, zebra mussel density, distribution and occupancy were recorded along vertical transects using an underwater video camera (ROVTECH Systems Colour U/W). The camera, connected to a remote-control pan and tilt unit, was mounted on a steel frame set at 22cm from the vertical wall surface (Figure 5.6). Area coverage was inter-calibrated by filming graph paper (1mm) from the same distance. The area being imaged was illuminated by two lights fixed respectively on the top of the camera and on the frame. Operations were controlled via a surface monitor which enabled control of light intensity and camera movements in four directions: up, down, left

and right, while a calibrated rope attached to the frame recorded camera depth (Figure 5.6). To prevent the apparatus from being carried along by the river Taff current, a plastic tube was fixed at the base of the frame, parallel to the wall, to stabilise the frame against the wall.

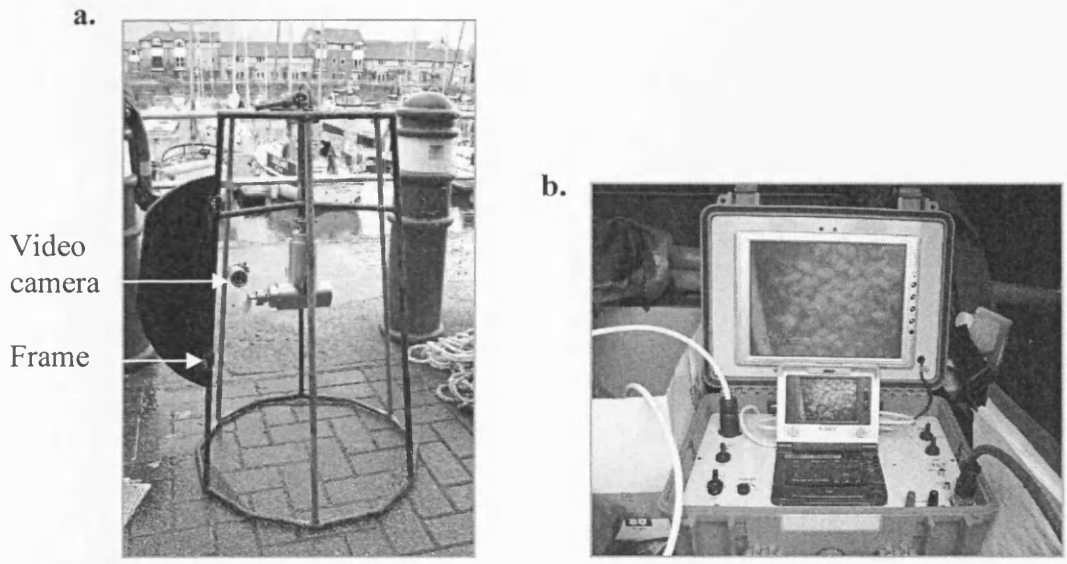


Figure 5.6: Underwater video camera system equipment a. Video camera mounted on a steel frame, b. Remote-control pan and tilt unit

Filming surveys were possible only in areas accessible by land from where the operations were carried out. Images were recorded from three locations representing different environmental conditions across the lake: in the main water body nearby the Inner Harbour (site V1, Saint David's Hotel), in the Taff River (site V2) and in Penarth Marina (site V3; Figure 5.2). At each site, three depth-transects were chosen randomly and filmed from the water surface to the lakebed. Each film consisted of two steps: i) a video was recorded from the water surface to the lake bed with stops every 50cm, for approximately 15 seconds to change the light intensity to obtain the optimal image; ii) a second video was recorded along the same transect from the bottom up to the surface, as a precaution against the first videos being of poor quality, with again stops every 50cm. At the same time, a larger area around the transect was filmed by moving the camera on the right and on the left to see if the colonisation cover was constant along the wall.

All video data were transferred to computer and saved using Windows Movie Maker. Screen images were taken at each 50cm depth step, and mussels counted over a 16 x 16 cm surface unaffected by edge distortion (Figure 5.7). It should be noted that, beneath 2m, poor visibility prevented the detection and counting of individual mussels.

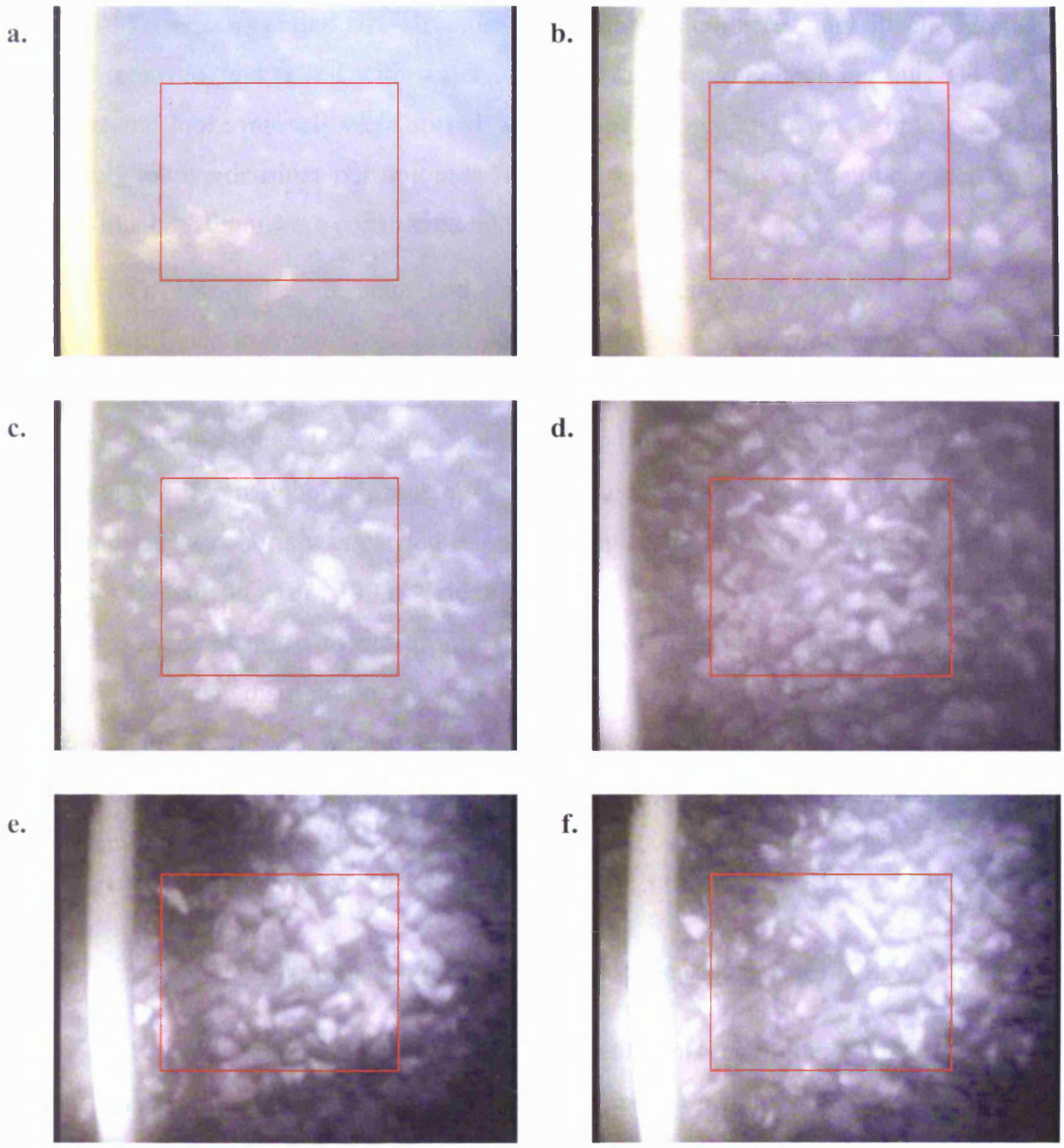


Figure 5.7: Pictures caught from video taken at Saint David's Hotel showing zebra mussel colonisation at different depths: a. 0.5m, b. 1m, c. 1.5m, d. 2m, e. 2.5m, f. 2.8m, the deepest part of the lake in this location. The red frame represents the area in which zebra mussels were counted

5.3.7. Size and age structure

To assess age and size-structure of all mussels collected, any attached to pebbles were removed, and rinsed with water in a 500μm sieve to collect any mussels <0.5mm. These and loose mussels were sorted with the naked eye and each sample was counted entirely to give densities per unit area (ind.m^{-2}). Empty shells were not counted and only individuals >10mm were considered adults.

To assess the life span and age structure, length frequency distribution were analysed. Since it is not possible to determine the age of zebra mussels by examining growth rings on the shell as is the practice with freshwater bivalves (Chase and Baley, 1999), length-frequency structure was used to infer age distribution and to estimate growth in different cohorts (Mackie, 1993; Chase and Baley, 1999). The determination of the individual cohorts was carried out according to the cohort description done in other studies (Neumann *et al.*, 1993; Nalepa *et al.*, 1995) and was not evident for every year. Cohorts were assumed to correspond to group of individuals from any one year. For length-frequency distribution, mussels with a shell length <5mm were placed in a single size category. Mussels with a shell length >5mm were measured with digital callipers (shell length from the umbo to the posterior growing edge) and placed into size categories of 1mm intervals.

5.3.8. Data analysis and total zebra mussel density in Cardiff Bay

To estimate mussel density variations between sites on the studied section of the aeration system, a one-way ANOVA was performed on mussel densities using Minitab 15.

For densities on vertical surfaces, site and depth effects on zebra mussel densities were examined by analysis of variance performed with the PROC GLM procedure of SAS (SAS Institute Inc., 1999) only over this top 2 m. Transects were treated as random factors nested within sites. Where sites or depths differed ($P < 0.05$), least squares means were sorted with the PDMIX800 macro (Saxton, 1998).

Zebra mussel population in Cardiff Bay was estimated by considering only zebra mussels on the lake's shoreline. The estimation of mussel density on the aeration system was not possible since data were neither sufficient nor known to be representative. Otherwise, the area surrounding the lake was composed of two major biotopes described in the methods section: vertical surfaces (barrage structure included) and pebble banks. The area of each habitat was estimated by measuring the total length of habitat sections with ArcGIS (ESRI, 2004; Figure 5.1), and by multiplying the obtained perimeter length value by the mean depth of each habitat. The estimation of the mean depth of the vertical surfaces was obtained by calculating the average of all the transect depths at the three sites where video estimates were made, which corresponded to 3.5 m. The depth range of the pebble banks was estimated at 3 m based on knowledge of the Bay's structure prior to inundation (pers. comm., CHA). The calculations are shown in the results section.

An estimation of zebra mussel density on the barrage and the fish pass was used to compare the current estimates of the zebra mussel biomass removed annually during the cleaning of the barrage. The length of the barrage structure was measured with ArcGIS (ESRI, 2004; Figure 5.1) and the mean depth of vertical surface was estimated to be 6m (pers. comm., CHA). The conversion of the mussel density into biomass was obtained by weighing and counting the mussel samples taken during the oxygen experiment (Chapter 6): 100 individuals weighed, on average 96 g. This conversion was also used to estimate the biomass of the mussel population at the shoreline in Cardiff Bay.

5.4. Results

The lake-wide survey of different structures across Cardiff Bay confirmed that zebra mussel colonisation had occurred on all types of hard substrate (e.g wood, pebbles, cement, metal; Figure 5.8) across the entire lake, including the barrage infrastructure, the fish pass, and in both rivers (Figure 5.2). Only the most upstream site on the Taff, Clarence Road Bridge (Easting 318549.9, Northing 174584.3), was free of mussels (Figures 5.2, 5.8.h). At occupied sites, zebra mussels generally occurred from a depth of 0.5 m below the surface at normal water levels, and visual observations suggested they were present at depths of >2m in all sites, and even to 7m at the deepest site in the Taff.

5.4.1. Lake bed and fine sediment

Side Scan Sonar confirmed that the majority of the lakebed was composed of soft sediments, mostly silt and mud, although there were localised hard substrates (Figure 5.9.a). Subsequent grab sampling showed that zebra mussel distribution on these harder areas was very patchy, occurring in only 7 samples over the 34 taken and only in two regions of the Bay: in the Inner Harbour, mussels on hard patches of the lake benthos reached a mean density of 3 300 ±1 200 m^{-2} (±SE) and nearby CHA pontoon, they reached 2 700 ±1 900 m^{-2} (Figure 5.9.b). The majority of mussels found were present in mud, while some were attached to woody debris or dead mussel shells (Figure 5.10). Distributions in these occupied areas were again patchy, as indicated by the high coefficients of variation (76% and 118%).

Figure 5.8: Water level dropped of half a metre in August 2009 in Cardiff Bay and zebra mussel colonisation on a. b. c. pebbles in the Inner Harbour; d the pontoon at CHA; d. e. f. lock walls; g. wood; i. j. the infrastructure of the fish pass and the barrage; h, no zebra mussel colonisation was observed on Clarence Road bridge

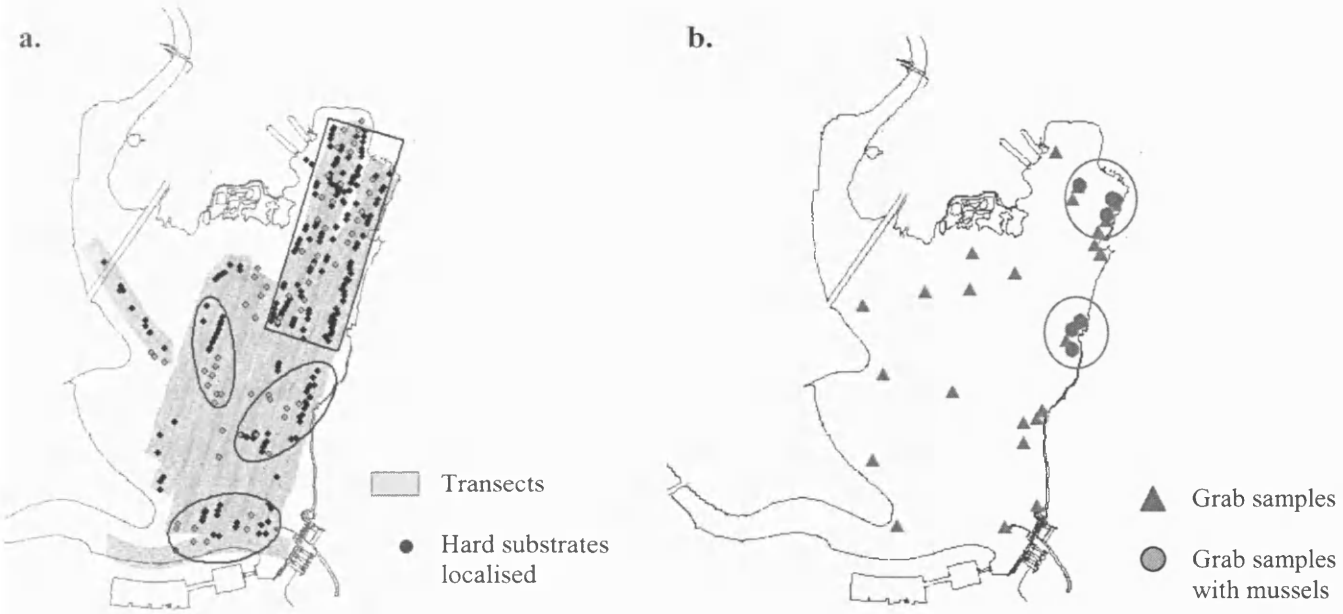


Figure 5.9: a. Side Scan Sonar survey transects in November 2007 in Cardiff Bay. Each point represents hard substrate. b. Sites where grab samples were taken to determine the presence and density of zebra mussel colonisation. Circles represent the two sites were mussels were found

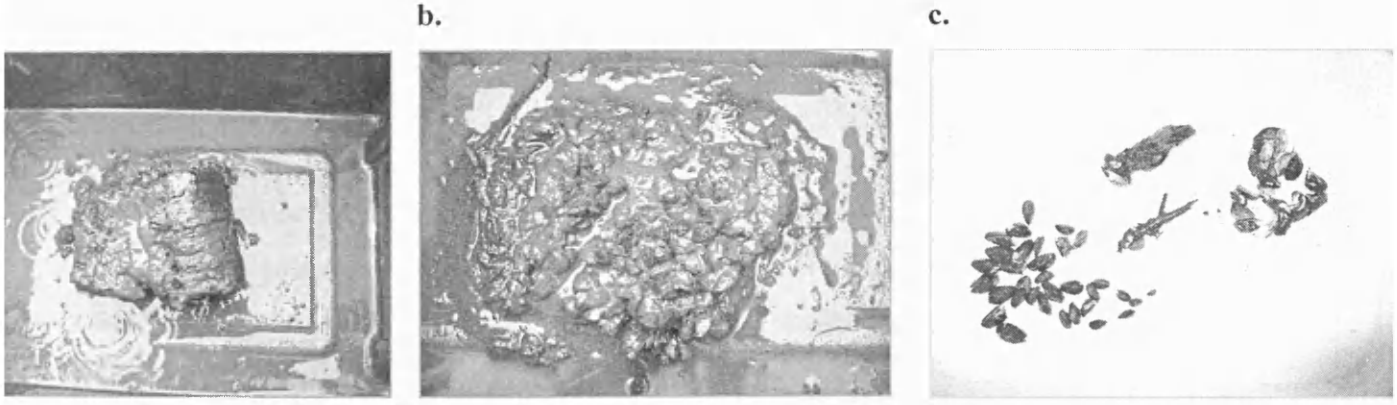


Figure 5.10: Grab samples: a. showing mud and silt substrate, b. containing zebra mussels in the mud, c. following sorting showing mussels on piece of wood and dead mussel shells

5.4.2. Aeration system

Zebra mussels occupied the aeration system in clusters along its length (Figure 5.11.a). Mussel densities varied significantly between sites (one-way ANOVA, F_9= 13.24, P=0.004) with densities 680 ±230, 6 200 ±1 200 and 7 700 ±1 100 m^{-2} at three sites. Coefficients of variation were lower here (29%-59%) than on soft sediment, indicating a more regular distribution on occupied parts of the pipe work. However, there was no colonisation at site 4, the closest site to the Taff River channel (Figure 5.11.b). There were no zebra mussels on any air diffusers.

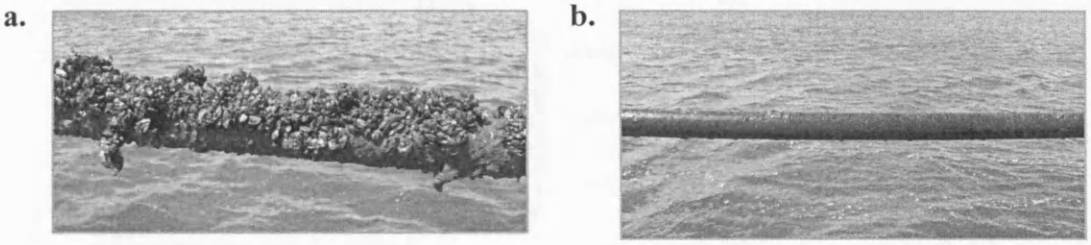


Figure 5.11: a. Zebra mussel colonisation on one of the pipe of the aeration system, in the west part of Cardiff Bay. b. Pipe of the aeration system with no zebra mussel colonisation at site 4

5.4.3. Lake margins

Densities of zebra mussels on pebble substrates as estimated using quadrat sampling, ranged from 950 ±250 m^{-2} around the CHA and barrage to 3 700 ±370 m^{-2} in the Inner Harbour, with mussels fairly regularly distributed in each case (CV= 10%-26%).

5.4.4. Vertical surfaces

Analyses of variance (REML linear mixed model) showed that zebra mussel densities on vertical hard surfaces in Cardiff Bay were significantly lower at the surface (0.5m) with 450 ±180 m^{-2}, than at 1, 1.5 and 2m where densities were more

homogeneous with 1 400 ±200, 1 600 ±200, 1 600 ±400 m^{-2} respectively (Figure 5.12.a, $F_{3,21}$=27.13, P<0.001). Densities also varied between sites, being lower in the Marina with 700 ±130 m^{-2}, than in either at Saint David's hotel or in River Taff with 1 550 ±220 and 1 600 ±220 m^{-2} respectively (Figure 5.12.b, $F_{2,8}$=16.04, P=0.0017). There were also interactions between site and depth ($F_{5,21}$=4.84, P=0.0044), for example there were low mussels densities in shallow water at the Saint David's hotel (Figure 5.12).

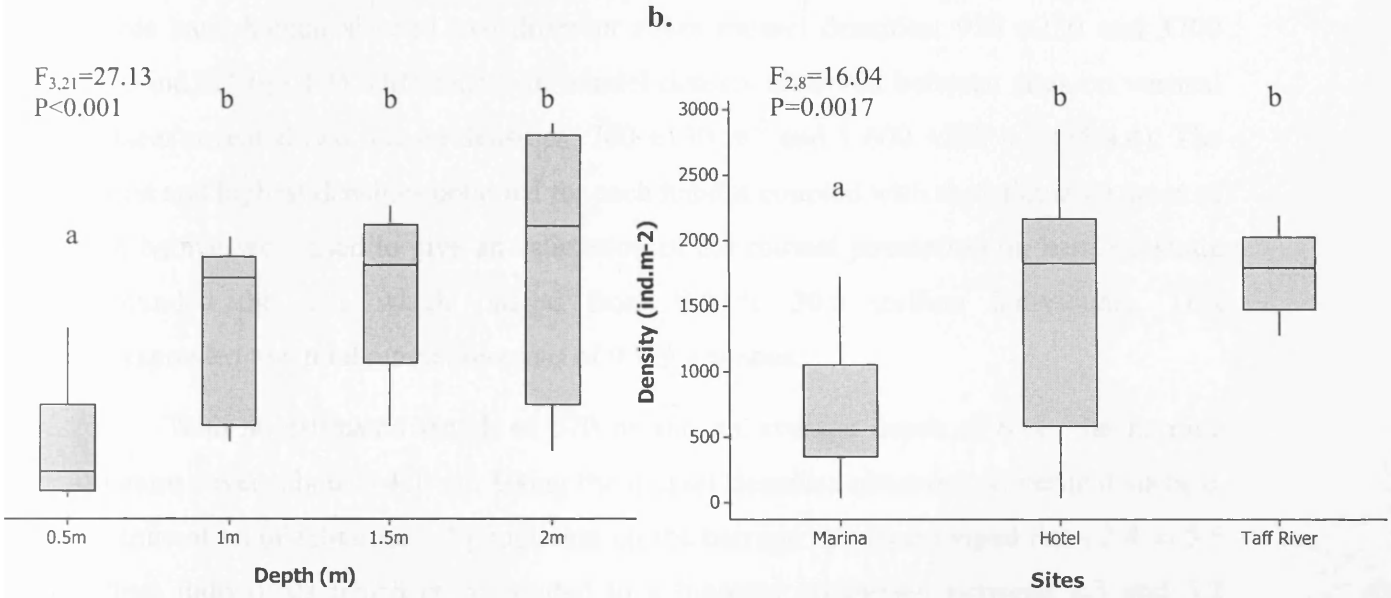


Figure 5.12: Zebra mussel density variations estimated using a video camera in Cardiff Bay: a. along a depth profile 0.5-2m, b. at different sites in Cardiff Bay. Error bars correspond to standard error. P-values are obtained by REML linear mixed model, with alpha set at 0.05 as the significant level. Respectively to the depths: n=11, n=11, n=11, n=7; respectively to the sites: n=15, n=18, n=9

5.4.5. Zebra mussel population estimate

With such extensive zebra mussel occupancy on hard substrates across the whole of Cardiff Bay, and in increasing numbers at depths from 0.5m below the water surface, mussel populations across the whole of Cardiff Bay are bound to be substantial (Figure 5.13). Mussel densities increased with the depth from c 500- 1 600 ind.m^{-2} at 0.5-2.5 m, to c 2 200- 5 100 ind.m^{-2}, at 3- 4.5 m.

Observations on the shoreline and the video camera surveys confirmed that mussel cover was fairly constant on vertical surfaces and on pebble areas, therefore density estimates were assumed to representative of these habitats. Vertical surfaces and pebble banks, habitats on which zebra mussel population density was assessed, covered lengths of 4 500 and 3 900 m respectively in Cardiff Bay. Therefore, areas of 13 500 and 7 800 m^2 for the vertical surfaces and pebble banks respectively, were obtained based on the mean depth of the habitat (3.75m and 3m respectively), minus 0.5 m since zebra mussel settlement started at 0.5 m below the water surface. The two sites sampled in the pebble bank habitat showed two different zebra mussel densities: 950 ±250 and 3700 ±370 ind.m^{-2} (§5.4.3). Differences in mussel density observed between sites on vertical surfaces revealed two mussel densities: 700 ±130 m^{-2} and 1 600 ±220 m^{-2} (§5.4.4). The lowest and highest densities obtained for each habitat coupled with the calculated areas of each habitat were used to give an estimation of the mussel population on hard substrate surrounded the lake which ranged from 9.5 to 30.5 million individuals. This corresponded to a total mussel biomass of 9 -29.4 tonnes.

With an estimated length of 570 m and an average depth of 6 m, the barrage structure covers about 3 420 m^2. Using the mussel densities observed on vertical surface, the estimation of zebra mussel population on the barrage structure ranged from 2.4 to 5.5 million individuals which corresponded to a biomass comprised between 2.3 and 5.2 tonnes.

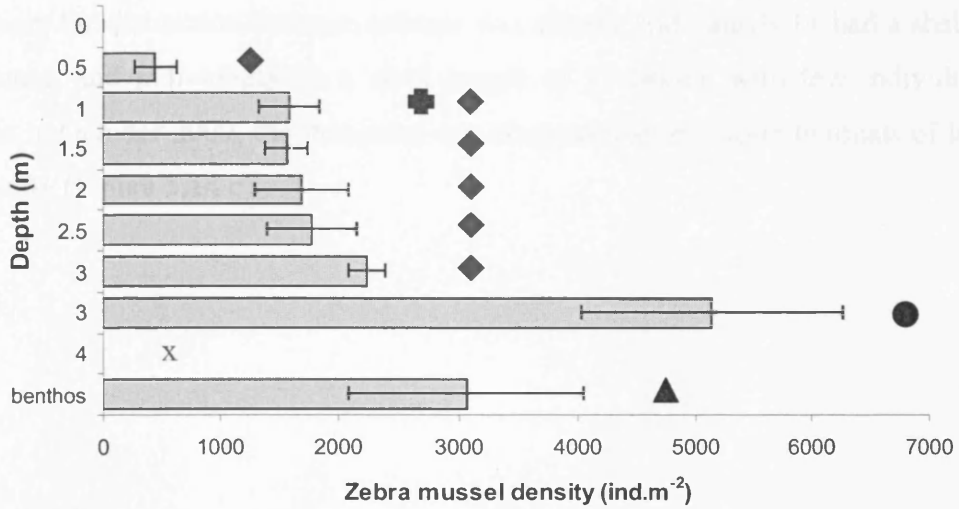


Figure 5.13: Synthesis of zebra mussel colonisation with mussel density variations along a depth gradient in Cardiff Bay. Error bars correspond to standard error. Symbols indicate the technique with which zebra mussels were collected: ▲ grab samples, ● samples from the aeration system, ✚ quadrat samples in pebbles banks, ◆ video camera survey, x density was not estimated at this depth

5.4.6. Size and age structure

Mussel size structure in Cardiff Bay revealed that the smallest individuals had shell length <10mm, corresponding to juveniles that had settled during the year of sampling. Beyond this group, the size structure suggested four age groups, with the dominant age class generally being 1 year old (referred to as 1+; Figure 5.14).

In 2008, the distinction between the age groups of individuals 1+ and 2+ was unclear because the cohorts of 2006 and 2007 overlapped and this made it difficult to distinguish shell length for each age group (Figure 5.14.a.). The presence of large mussels >25mm (to a maximum of 33mm) may represent an older cohort of mussels 3+ or older individuals (Figure 5.14.a.b; Lucy, 2005) that must have first occurred in the lake as veligers as 2004.

In 2009, cohort determination was carried out on mussels collected both before and after the veliger production (Figure 5,14.b.c). In May 2009, before veliger production, the distinction between cohorts was clearer: individuals 1+ had a shell length of 9-16mm, and individuals 2+ a shell length of 17-24mm, with few individuals 0+. Whereas in October 2009, the dominant age class corresponds to individuals of less than one year 0+ (Figure 5.14.c).

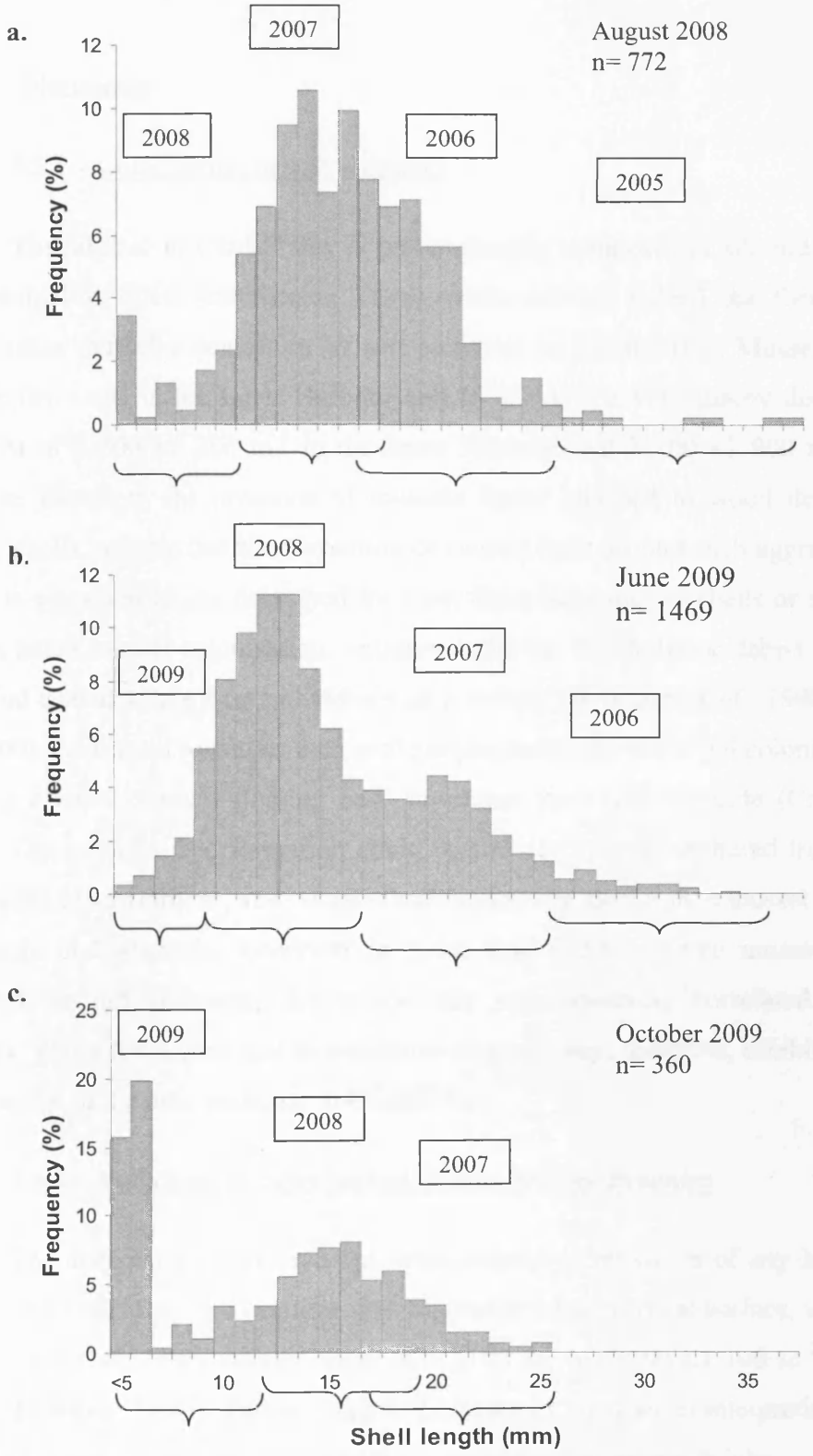


Figure 5.14: Length frequency distribution of zebra mussels in Cardiff Bay. Individuals collected: a. at the Inner Harbour with quadrats and grab sampler in 2008, b. on the aeration system in 2009, c. between CHA and the barrage with quadrats in 2009

5.5. Discussion

5.5.1. Colonisation of soft sediment

The lakebed in Cardiff Bay is predominantly composed of silt and mud, limiting the potential for direct colonisation. These results showed, indeed, that there has been no major zebra mussel colonisation of soft sediment in Cardiff Bay. Mussels were found only at two sites, in the Inner Harbour and by CHA in a very patchy distribution with densities of 3 300 ±1 200 m^{-2} in the Inner Harbour and 2 700 ±1 900 m^{-2} near CHA pontoon. However, the presence of mussels found attached to wood debris and dead mussel shells indicate that the formation of mussel beds around such aggregations in the future is a possibility. As described for Lake Erie, dead mussel shells or sand grain can initiate zebra mussel colonisation: veligers settle on the shells or debris present in the mud and constitute the first individuals of a colony (Berkman *et al.,* 1998; Berkman *et al.,* 2000). Additional juveniles then settle subsequently on the initial colonisers gradually forming a zebra mussel 'floating bed' formation over soft substrate (Coackley *et al.*, 1997). This potential bed formation could occur only in areas sheltered from the current such as the Inner Harbour, and where siltation rates are slower than mussel accumulation. As Bially and MacIsaac observed in Lake Erie (2000), where mussel colonisation occurred on soft sediment, destruction rate was positively correlated with current velocity. Water turbulence and strong water currents may, therefore, inhibit zebra mussel colonisation of the mud susbtrate in Cardiff Bay.

5.5.2. Variations in zebra mussel density and uncertainties

The different surveys revealed zebra mussel colonisation of any hard substrates present in Cardiff Bay: the aeration system, pebble areas, vertical surface, with variations in density between sites ranging from 450 ±180 m^{-2} to 7 700 ±1 100 m^{-2} and between depths. However, several factors need to be borne in mind when interpreting the density data, and in particularly the total population estimates for the whole lake.

First, not only were there variations in mean mussel density within areas, but representative and extensive sampling throughout the barrage would have been a very large undertaking. Some extrapolation is therefore inevitable.

Second, the density estimates and total population inventory required the combination of different methods of data collection which, coupled with varying performance in the Bay's, heterogeneous set of biotopes, provide another source of uncertainty. For example, the majority of pebble areas, composed of pebbles cemented in the banks, could not be sampled. Only two accessible sites with lose pebbles could be sampled and were surveyed but limited since only to the uppermost first metre. The video recording technique used to estimate zebra mussel density also had some limitations: mussels forming clusters could not be all identified since the image was in a two dimension plane.

Finally, some biotopes could not be sampled to a satisfactory extent – notably the aeration system. Some of the highest densities, 5 500 ±1 100 m^{-2}, were observed on parts of the aeration system. At other sites, however, zebra mussels were absent, and sufficient sampling to make representative measurements were not possible. For this reason, the aeration system has been left out of the total population estimate.

Despite these difficulties, the data can be amalgamated to produce a crude estimate of total population with a range that reflects the difficulties and uncertainty involved.

5.5.2.1. Between sites

The absence of zebra mussel on the aeration system closest site to the Taff River channel, and on Clarence Road bridge, the most upstream site in The Taff River, indicates that zebra mussel distribution and density in Cardiff Bay are probably, influenced in part, by the water currents. This pattern was also shown for veliger densities and juvenile settlement (Chapters 3 and 4; Mellina and Rasmussen, 1994). Previous work also showed that high mussel densities occur in areas with low water disturbance while low densities are found in areas with high water turbulence (Mellina and Rasmussen, 1994; Kobak, 2005). The Taff River seems to constitute an upstream barrier to zebra mussel dispersion. Therefore, it was unexpected to observe similar mussel densities on vertical surfaces both in the main water body and in the Taff River, where especially veliger densities and juvenile settlement were very low (Chapters 3 and 4). Nevertheless,

veliger collection and juvenile settlement experiments were carried out in the middle of the riverbed, where the water current is the strongest, whereas adult densities were observed on the shoreline. Physical factors, such as current velocity, vary between the inshore zone and the main river channel (Schiemer *et al.*, 2001). With low water turbulence, inshore zones in rivers constitute retention zones which facilitate nutrient accumulation, phytoplankton and zooplankton development (Reckendorfer *et al.*, 1999). Therefore, these are also suitable habitat for veliger development and juvenile settlement. In Cardiff Bay, boats and circular currents observed in the rivers (pers. comm., CHA) enhanced probably zebra mussel invasion and dispersion. Unfortunately, no veliger or water chemistry data are available to explain the lower colonisation of zebra mussels in the Marina. The absence of zebra mussels on the air diffusing structures of the aeration system suggests that their design prevents mussel colonisation.

5.5.2.2. Between depths

Zebra mussel densities varied also between depths. Mussel distribution followed a depth gradient, where mussels were absent in the first 0.5 metre from the water surface, and in low densities between 0.5 and 1m. The maximum population densities were then reached between 1- 2m, although data on the estimation of the colonisation after 2m were not considered in the data analysis because of missing data for some sites and the difficulty to assess the density in deeper areas. Zebra mussels were found up to 3 m at Saint David's Hotel site, and up to 7 m at the Taff River site. The absence of zebra mussels in the first 0.5 metre was most likely due to physical factors such as light intensity and wave action. Kobak (2000) showed in laboratory studies that zebra mussels preferred shaded sites over illuminated ones which were avoided. Since lake turbidity is relatively high in Cardiff Bay, light transmission is largely reduced after the first metre (pers. comm. CHA) where zebra mussels are found in substantial densities. Furthermore, waves on the surface of the lake create water turbulence on the shoreline and most likely inhibit mussel settlement (Clarke and McMahon, 1995; Chase and Baley, 1999; Kobak, 2005). The absence of zebra mussels in the first 0.5m may also be explained by waterfowl predation. White swans (*Cygnus olor*) and mallard ducks (*Anas platyrhynchos*) feed on zebra mussels in Cardiff Bay (pers. obs.) and are likely to prefer foraging for

mussels in shallow waters. However, waterfowl predation is more likely to occur on horizontal surfaces such as pebble areas rather than on vertical surfaces which appear to be more difficult to reach for birds. Studies of most European lakes have shown maximum zebra mussel densities at depths between 1 and 5m with water temperature, oxygen concentration and food availability as limiting factors (Mellina and Rasmussen, 1994; Karatayev *et al.*, 1998). In Cardiff Bay, however, the same factors are not limiting: the water column is well mixed due to the aeration system. Furthermore, experiments on juvenile settlement showed that there was no relationship between depth and juvenile settlement (Chapter 4). Zebra mussels in Cardiff Bay may, therefore, show similar densities from 1 to 7 m depth.

5.5.3. Zebra mussel lifespan

Cohort determination and analysis showed that the mussel population was generally composed of 1 year old individuals with a shell length between 10-16mm; while the adults of 2 years old have a shell length of 17-24mm. Elsewhere, shell length averages for individual 1+ and 2+ zebra mussels vary between cohorts, since mussel growth is influenced by water temperature, food availability and water chemistry (Nalepa *et al.*, 1995; McMahon, 1996; Jantz and Neumann, 1998). Growth also varies with season, being almost zero during winter (Sprung, 1995; Cope *et al.*, 2006). Cardiff Bay's eutrophic character would be expected to generate a high growth rate of zebra mussels (Karatayev *et al.*, 1998), although high turbidity might reduce growth potential (Schneider *et al.*, 1998). However, shell length of mussels from Cardiff Bay compare to others in Europeans lakes, with shell lengths between 9-15mm reported for individuals 1+ (Morton, 1969; de Vaate, 1991; Lucy 2005). Filtration rate and oxygen consumption rate increase with the shell length of the mussel (Kryger and Riisgard, 1988; Reeders and de Vaate, 1990; Effler *et al*, 1996). The general dominance of individuals 1+ should be therefore taken in consideration during the determination of the filtration rate and oxygen consumption rate of the mussel population in potential future studies.

The dominance of individuals 0+ in October 2009 and the overlapping of the cohorts 2007 and 2008 are probably explained by variations in population recruitment between years and by the year-class strength. In fact, variations in environmental factors

between years, such as food availability and water temperature, influence population recruitment and the growth rates in zebra mussels elsewhere (Jantz and Neumann, 1998; Chase and Baley, 1999). Low water temperature and low food availability due to high water discharge in 2007 and 2008 in Cardiff Bay (Chapter 3) probably led to poor growth and poor recruitment for the individuals of the cohort 2007 and 2008. Environmental conditions were better in 2009 with low water discharge and high food availability which generated a higher recruitment in 2009 than the previous years (Chapter 3).

Measurement and cohort determination revealed that the lifespan of zebra mussels in Cardiff Bay is probably c. 2- 3 years, with a small number of mussels surviving for 3 or more years. It is, however, difficult to determine the age of individuals with a shell length >30mm since mussel growth rate is considerably slower in the old mussels than in the younger ones (Neumann *et al.*, 1993). Zebra mussel growth rates and mussel lifespan observed in Cardiff Bay concur with the results found in Lough Key, UR (Lucy, 2005). However, zebra mussel lifespan in Cardiff Bay appeared to be shorter than the one described by Morton (1969) which estimated a lifespan of 3.5 years for a mussel population present in the East of England. With some individuals attaining a maximum shell length around 30-37mm and a lifespan around 2 years, zebra mussel population in Cardiff Bay is considered as a fast growing population as Mackie observed (1993). This contrasts with zebra mussel populations in the East of Europe which have slow growth (maximum shell length >40mm) and a lifespan between 3-7 years.

The presence of older individuals corresponding to the cohort 2005 suggests that zebra mussels must have been spawning already in Cardiff Bay already by 2004, implying that adults were present and that colonisation must have occurred already in 2003.

5.5.4. Zebra mussel density

The mean density of zebra mussels in Cardiff Bay ranged from 450 to 5100 ind.m^{-2}. This range of zebra mussel densities concurs with studies executed in some European lakes: an average density of 3 900 m^{-2} was found in Lough Key_UR (Lucy, 2005), 1 500 m^{-2} and 4 700 m^{-2} in Polish Lakes (Burlakova *et al.*, 2000; Wolnomiejski

and Wozniczka, 2008); 2 200 ±800 m^{-2} in Finland (Orlova and Panov, 2004). However, these mussel densities are low compared to other European lakes where mussel densities can reach 60 000 m^{-2} in Lake Constance for example (Cleven and Frenzel, 1993), up to 115 000 m^{-2} in some Polish lakes (Mackie and Schloesser, 1996).

The estimation of the total mussel population in the lake, ranging from 9.5 and 30.5 millions individuals, needs to be considered cautiously as described above. The estimation of the mussel population colonising the barrage comprise between 2.4 to 5.5 millions individuals, e.g. 2.3-5.2 tones, corresponded to the value of the mussel biomass removed every year from the barrage structure which is around 4 tonnes. This comparison shows that the estimation of mussel density based on our data accord with the mussel densities present in the lake.

However, to obtain a more accurate estimation of the total mussel population in Cardiff Bay, an estimation of the population colonising the aeration system as well as the pontoon, bridges and boats would also be required.

5.5.5. Potential impacts of the mussel population in the lake

Zebra mussel invasion in Cardiff Bay probably occurred by at least around 2003-2004, leading to colonisation over time of all hard substrates in the lake. Zebra mussels are now extensive and widely established. The estimations of the mussel population density and distribution in the lake allow the determination of the potential ecological impacts in the lake, and the setting up of measures for a control of the population. These themes are explored with respect to oxygen concentrations in Chapter 6, but potential effects on algal stocks, zooplankton and nutrient dynamics should also be considered.

5.6. Conclusions

Surveys carried out in Cardiff Bay, with the collection of zebra mussel samples, allowed some conclusions about its adult zebra mussel population.

The use of SSS revealed the absence of any significant mussel bed formations on the lakebed. Conversely, zebra mussels colonised some parts of the aeration system with densities between 700 ±200 m^{-2} and 7 700 ± 1 100 m^{-2}, but without any impacts for the air diffusers. Zebra mussels have colonised every hard substrate present across the entire lake and river mouths. River flows nevertheless, seem to constitute a barrier to zebra mussel dispersion upstream. Mussel densities followed a depth gradient where the highest densities were found between 1 and 2m, although the density of mussels in deeper areas (up to 7m) could not always be estimated. Based on the mussel density data determined on the shoreline areas surrounding the lake, a first estimation of the mussel population was assessed around 9.5 and 30.5 millions individuals.

The densities and distribution of zebra mussel population indicate the presence of a well and widely established population in Cardiff Bay. Even if zebra mussel densities compared to others in European lakes are relatively low, the population is large enough to suggest further studies to assess the severity of any associated ecological impacts. The determination of impacts on dissolved oxygen concentration (See chapter 6) and on the phytoplankton concentration will bring information on the need for control measures based on better understanding of impacts.

6. Potential seasonal changes in the respiration of zebra mussels from Cardiff Bay

6.1. Summary

1. As one of the world's most invasive freshwater organisms, zebra mussels can have a range of negative effects on occupied ecosystems. Among them are potentially large effects on dissolved oxygen dynamics through effects on primary producers and directly through oxygen uptake to support respiration. Also called zebra mussel oxygen demand (ZOD), the latter effects reflect the total area of colonisation, population density and individual uptake. In the newly-formed Cardiff Bay, the potential for effects on oxygen concentrations is large and important as a risk factor for other aquatic organisms. Legislation also requires oxygen to be maintained in the Bay at > 5 $mg.l^{-1}$.

2. Laboratory mesocosms were used to simulate and assess seasonal changes in zebra mussel respiration in Cardiff Bay. Winter-like conditions corresponded to a water temperature of 10°C with low food availability, whereas summer-like conditions corresponded to a water temperature of 17°C with high food availability created by adding the algae Chlorella sp. Mussel biomass was also varied (0, 125, 250, 500g per 2400cm2). Summer temperature and food-rich conditions were hypothesised to increase oxygen uptake.

3. Oxygen consumption by zebra mussels increased with rising water temperature and increased food availability: uptake was 3 times greater at 17°C with high food availability than at 10°C with low food. The highest oxygen consumption, due to the environmental conditions and to mussel metabolic activity, would therefore be expected to occur in Cardiff Bay in summer. Temperatures here typically reach 17-20°C, and sometimes exceed 20°C.

4. Although no accurate determination of mussel respiration rate was possible, oxygen consumption increased linearly with mussel biomass, and further investigations using respiratory chambers at varying mussel densities are advocated. The combined effects of mussel respiration, temperature, phytoplankton removal by mussels, and atmospheric re-diffusion also require careful investigation given the potential for zebra mussels at the densities observed to deplete oxygen in Cardiff Bay under hot summer conditions and low flow.

6.2. Introduction

Non-indigenous species are considered to be invasive when a given population is established, dominant, has a high propagule pressure and occurs at densities sufficient to have negative environmental impacts (Colautti and MacIsaac, 2004). Due to its severe economic and ecological effetcs, the zebra mussel (*Dreissena polymorpha*, Pallas) has been the object of numerous studies (e.g. Laurier LePage, 1993; MacIsaac, 1996; Schloesser *et al.*, 1996; Khalanski *et al.*, 1997; Vanderploeg *et al.*, 2002; Descy *et al*, 2003; Wu *et al.*, 2010). As a fouling organism, zebra mussels cause a severe nuisance and damage to industrial and power plant infrastructure, by aggregating in pipes and on structures, substantially increasing the cost of maintenance (Jenner and Janssen-Mommenn, 1993; Laurier LePage, 1993; Ram and McMahon, 1996). In ecosystems, zebra mussels smother benthic organisms, alter nutrient dynamics, increase water clarity by removing suspended particles, and alter the community composition of small zooplankton (MacIsaac *et al.*, 1992; Holland, 1993; Caraco *et al.*, 1997; Vanderploeg *et al.*, 2002).

Potentially one of the most important of all effects of zebra mussels is their effect on oxygen dynamics. At high densities, they have the potential to reduce concentrations by direct uptake, by reducing phytoplankton populations (a major oxygen source) and by contributing to sediment oxygen demand by the deposition of decomposing faeces and pseudofaeces (Effler and Siegfried, 1994; Caraco *et al.*, 2000; Gelda *et al.*, 2001; Denkenberger *et al.,* 2007). Reduced oxygen concentrations attributed to zebra mussels have been reported in large rivers in the United States, where zebra mussel densities have reached 61 000 m^{-2}. The average areal zebra mussel oxygen demand (ZOD) was comprised between 34 and 39 $g.m^{-2}.d^{-1}$ while sediment oxygen demand (SOD) of highly enriched organics deposits was around 5 $g.m^{-2}.d^{-1}$ (Effler and Siegfried, 1994; Effler *et al.*, 1996; Effler and Siegfried, 1998). In the Seneca River, reduced concentration occurred even at moderate zebra mussel densities of 2 000 m^{-2} (Caraco *et al.*, 2000).

Laboratory and field studies have shown that zebra mussel oxygen demand (ZOD) is related with the area of colonisation and the population density (Effler and Siegfried, 1994; Caraco *et al.*, 2000; Gelda *et al.*, 2001). Zebra mussel oxygen

consumption not only varies with population density, but also with environmental conditions such as water temperature, food quantity and quality, turbidity and flow rate (Alexander *et al.*, 1994; Aldridge *et al.*, 1995; Caraco *et al.,* 2000). Zebra mussel respiration may also vary with zebra mussel metabolic activity, peaking for example during spawning (McMahon, 1996).

The potential effects of zebra mussels on Cardiff Bay provide a particularly interesting case study. This artificial lake was created in 2001 by the construction of a barrage impounding the formerly tidal Taff and Ely Rivers. Modelling prior to barrage construction suggested that a range of possible consequences of this ecosystem transformation and that might arise once the lake was completed (Cardiff Bay Barrage Bill, 1991). One important prediction was that high water temperatures and low river flows might reduce dissolved oxygen (DO) concentrations in the lake below those required (5 $mg.l^{-1}$) to support aquatic organisms, and in particularly migratory salmonids (Cardiff Bay Barrage Bill, 1991). Moreover, the two urban rivers discharging into the Bay carry a large volume of nutrients and treated organic matter which, by decomposition and benthic deposition, contributes to the sediment oxygen demands. Although an aeration/mixing system has been installed over the 200 ha to maintain oxygen level at >5 $mg.l^{-1}$, values have occasionally fallen below this key legal and environmental threshold. A mobile, water based oxygenation unit then operates. However, since formation Cardiff Bay has been invaded by a large zebra mussel population (Chapter 5), there is a real potential for additional adverse impacts (Effler *et al.*, 1996). With such concerns, studies on ZOD in Cardiff Bay are crucial for understanding the potential oxygen impact of zebra mussel invasion.

The following brief investigation was carried out to begin to estimate the possible effects of zebra mussels at different density, temperature and food abundance on oxygen uptake. Using factorial experiments in mesocosms, I tested the initial hypotheses that:

i. zebra mussel oxygen consumption increases with rising temperature and increased food availability;

ii. oxygen intake increases with zebra mussel density.

6.3. Materials and methods

6.3.1. Experimental design

Zebra mussels were collected on Day 1 from crates used in previous colonisation studies (Chapter 4). Mussels were measured, weighed (wet weight) and counted for each replicate sample. Only adults between 15 and 25 mm shell length (1.5 and 2 years old) were selected for the experiment. Quigley *et al.* (1993) showed that oxygen consumption in zebra mussels remained roughly constant across shell lengths of 15-16mm to 24-25mm and was higher than for smaller mussels. Mussels were initially acclimated for 24h in a large tank (800x 600x 425mm) filled with oxygenated water from the bay (Figure 6.1).

On Day 2, each experiment began when the mussels were transferred to the closable (but not airtight) experimental mesocosms of 600x 400x 235mm, filled with 40 litres of lake water. Each experimental set was composed of three replicates with the same biomass of mussels (125g, 250g, 500g per 2400 cm^2) and one control with no mussels (Figure 6.1). Target temperatures were achieved by controllable heaters fixed to the tank, and both temperature and DO concentration were recorded every 15 minutes during exposure using a probe (model 6920, YSI Inc., USA) calibrated prior each experiment. The mussels were added under fully oxygenated starting conditions and target temperature. The experiment ended on day 3, following between 20 and 24 hours since commencement. Unfortunately, the experiment could not be conducted for a longer duration. It would have been necessary to delay the set of experiments with some of them being conducted during the spring. Therefore, the change in Bay's water chemistry (used in the experiments) would have been a confounding variable in the experiment.

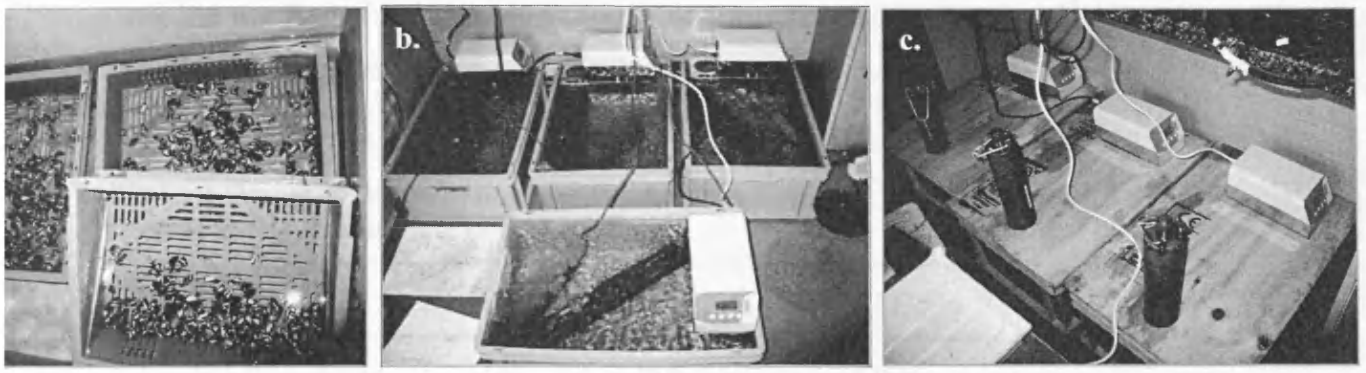


Figure 6.1: Experimental design: a. zebra mussels after sorting and acclimation to laboratory conditions, b. tank preparation before the experiment: four tanks full of lake water over oxygenated and heated, c. tanks during the experiment with the probes recording data and the lid on to reduce oxygen diffusion

In addition to mussel biomass, water temperature and food availability were varied factorially to produce four different conditions (Table 6.1): low temperature x low food availability, low temperature x high food availability, high temperature x low food availability, high temperature x high food availability. High temperature corresponded to the average summer temperatures in Cardiff Bay (17°C; Chapter 3), while low water temperature was approached typical winter conditions as far as possible (10°C). Low food availability corresponded to conditions during winter when plankton density in Cardiff Bay is low (Merrix, 2009). High food availability was obtained by adding Chlorella spp. after Nichols (1993): 0.38g for 125g of mussels, 0.76g for 250g of mussels, 1.52g for 500g of mussels. For each condition, three series of experiment were carried out with different zebra mussel biomasses (Table 6.1).

6.3.2. Data analyses

Data analyses were performed using the PROC GLM procedure of SAS (SAS Institute Inc., 1999). Repeated-measures ANOVA was used to test whether DO concentration differed through time with water temperature, food availability, mussel biomass or pair wise interactions between these factors. The effects of the different

treatments (Table 6.1) on DO concentration were tested on data recorded every 50 min and up to 500 min exposure, since all depletion curves had reach plateaus by this point. As data did not meet the sphericity assumption (Mauchly's sphericity test; $\chi^2_{54}=760.72$, $P<0.0001$), a multivariate approach to within-subjects tests was used.

Table 6.1 : Design of the oxygen experiment with a total of 12 experiment sets. Each set consists of three replicates and one control

	10°C	**17°C**
	125g	**125g**
Low food level	250g	**250g**
	500g	**500g**
	125g	**125g**
High food level	**250g**	**250g**
	500g	**500g**

6.3.3. Zebra mussel oxygen consumption

Zebra mussel oxygen consumption for each treatment was obtained by subtracting the final DO value (value at the beginning of the plateau; Table 6.2) from the initial DO value calculated by the statistical model (Table 6.2). Oxygen depletion in controls, which accounted for bacterial and planktonic respiration, was used to adjust mussel respiration, by subtracting the mean value of oxygen depletion in controls to the total value of oxygen depletion in mesocosms with mussels. Oxygen consumed by zebra mussels was expressed in milligrams of oxygen per litre ($mg.l^{-1}$).

6.4. Results

Zebra mussel oxygen consumption was significantly greater with increased food availability, higher water temperature and larger mussel biomass. A linear regression was observed between zebra mussel biomass and DO depletion.

6.4.1. Dissolved oxygen depletion during the experiment

Overall, DO concentration showed a clear depletion through time (Wilks' Lambda=0.0162; $F_{10,27}$=164.31, P<0.0001), which significantly depended on rising temperature (F_{10}=10.65, P<0.0001), increased food (F_{10}=235.32, P<0.0001), and increased mussel biomass (F_{30}=137.27, P<0.0001; Table 6.2). The effects of the biomass by food and biomass by temperature interactions were also significant (F_3=52.25, P<0.0001 and F_3=25.46, P=0.0056, respectively), indicating that these factors do combine to influence DO depletion. For example, combining increased food availability and increased mussel biomass resulted in a significantly stronger DO depletion than any other combination of these two factors (Table 5.3; F_3=10.2, P<0.0001).

Considering all experiments irrespective of mussel biomass, mean DO concentration depletion at 17°C with high food availability was more than three times greater than at 10°C with low food availability. Mean DO depletion was 1.91 $mg.l^{-1}$ and 0.64 $mg.l^{-1}$ respectively at these two temperatures compared with ambient and fully oxygenated conditions.

Table 6.2: Repeated-measures ANOVA to test the effects of temperature, food availability and zebra mussel biomass on the oxygen depletion after 500 minutes. F-ratios, p-values and the error mean square term were used to test all effects are shown. Separate errors were used for the between subjects (i.e. time-averaged) values and the depletion over time. P values are significant with $\alpha = 0.05$

Source	*df*	*ss*	*F*	*P*
Between subjects				
Biomass	3	205.6	47.92	<0.0001
Food	1	62.49	43.69	<0.0001
Temperature	1	511.6	357.7	<0.0001
Biomass x Food	3	52.25	12.18	<0.0001
Biomass x Temperature	3	25.46	5.93	0.0022
Food x Temperature	1	11.74	8.21	0.007
Within subjects				
Time	10	84.03	1543.47	<0.0001
Time x Biomass	30	29.72	181.96	<0.0001
Time x Food	10	16.98	311.94	<0.0001
Time x Temperature	10	0.77	14.12	<0.0001
Time x Biomass x Food	30	4.98	30.52	<0.0001
Time x Biomass x Temperature	30	1.43	8.77	<0.0001
Time x Food x Temperature	10	0.69	12.72	<0.0001

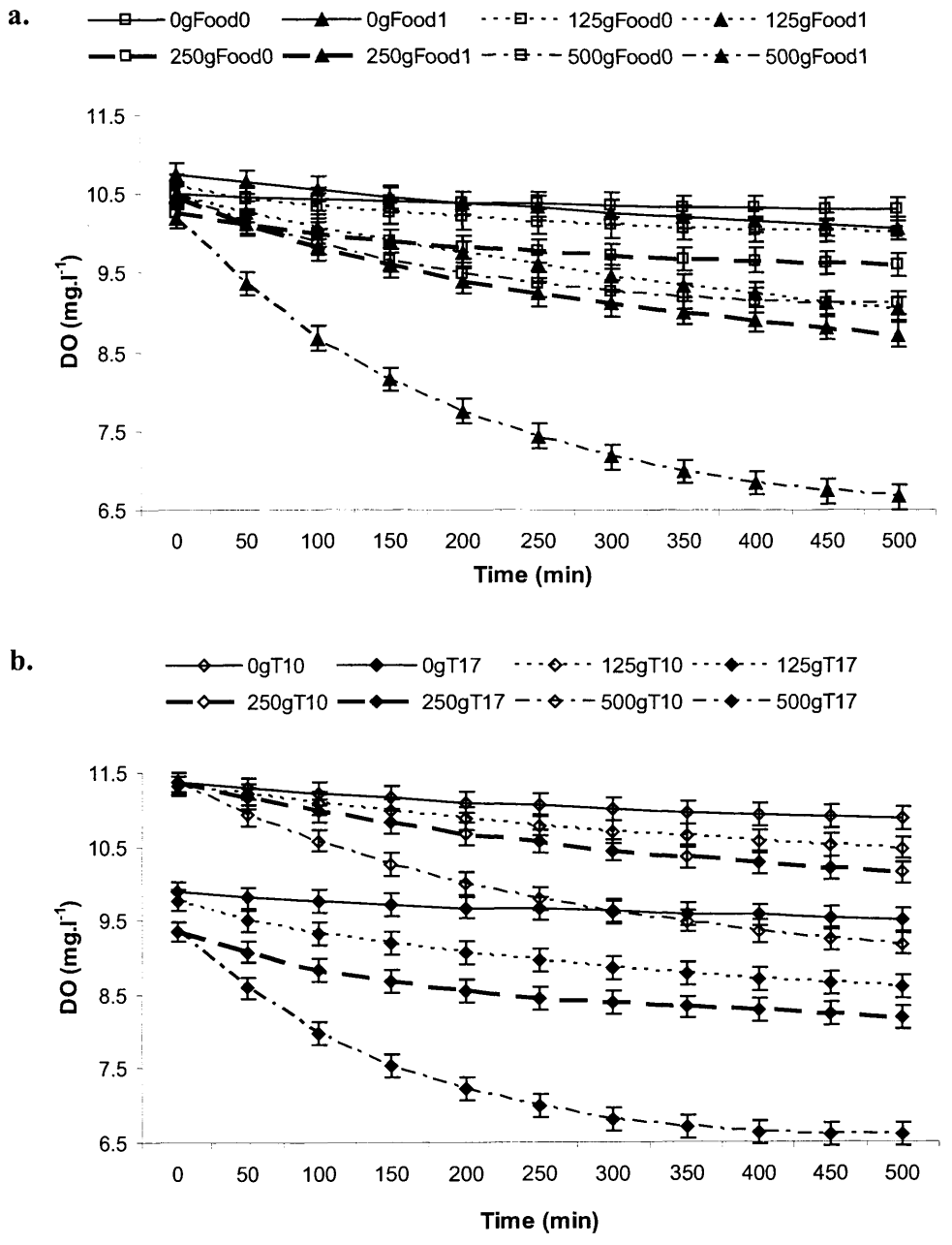


Figure 6.2: Mean DO concentrations calculated by the statistical model over time a. for each mussel biomass with food concentration variations and b. for each mussel biomass with water temperature variations. Error bars correspond to standard error

6.4.2. Zebra mussel oxygen consumption

Mean oxygen depletion plotted against the different mussel biomass (0-500g) under two different treatments revealed a significant correlation between zebra mussel biomass

and oxygen depletion for both treatments: at 10°C with low food availability (P=0.006, r= 98) and at 17°C with high food availability (P=0.018, r=95; Figure 6.3).

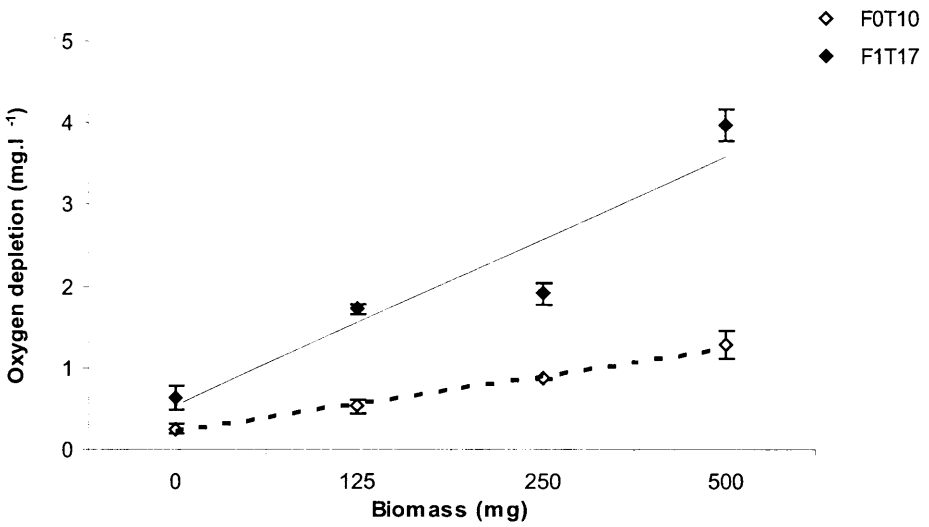


Figure 6.3: Oxygen depletion in presence of different biomasses of zebra mussels (0-500g) under two treatments: F0T0, with low food level at 0°C; F1T17, with high food level at 17°C. Error bars represent standard errors

6.5. Discussion

Although based on preliminary methods, key conclusions from this study are that DO depletion increased with increasing mussel biomass, increased temperature and increased food availability. The effects of mussel biomass indicated that DO concentration corresponded directly to zebra mussel oxygen consumption. As a result, reductions in DO concentration greater than in controls units could be attributed to the effect of mussel respiration.

6.5.1. Effects of water temperature and food concentration rising in summertime

Zebra mussel oxygen consumption increased with increasing water temperature and increasing food concentration: mean oxygen consumption rate at 17°C with high food availability was three times larger than at 10°C with low food availability. These effects have mostly been supported in other studies, but not always and the reasons are unclear (Garton and Stoeckmann, 1991; Quigley *et al.*, 1993). Effects of food can also be complex: Madon *et al.* (1998) found high food concentrations increased zebra mussel respiration, but Schneider *et al.*, (1998) found that respiration rate did not change with either food quantity or quality. Food quality sometimes affects mussel respiration more than food quantity (Madon *et al.*, 1998; Fanslow *et al.*, 2001), with respiration declining particularly under poor food conditions, with high turbidity (Alexander *et al.*, 1994).

Overall, however, temperature is often reported to be a major environmental control increasing zebra mussel oxygen consumption, consistent with my results (Alexander *et al.*, 1994; Aldridge *et al.*, 1995; Sprung, 1995; Fanslow *et al.*, 2001). Higher mussel respiration would be expected in summer than in winter in Cardiff Bay, and similar results have arisen in studies of zebra mussel respiration in Germany and North America during field experiments over 2 years (Sprung, 1995; Fanslow *et al.*, 2001). Hamburger and Dall (1990) showed that crustaceans and insects have a higher respiration rate than worms and molluscs, including zebra mussels. The decrease in DO concentrations in body waters is then due to the high densities that zebra mussels can reach in some areas.

Reductions in DO concentrations in Cardiff Bay during hot summers would already be expected with lower oxygen solubility with increasing temperature. More rapid decomposition of organic matter present in the lake and reduced water flow would further deplete oxygen, and summertime deoxygenation of the deepest parts of Cardiff Bay has already been observed (CHA, pers. comm.). The effects presented here suggest that further additional depletion could occur in Cardiff Bay as increased water temperature and phytoplankton concentration increased the metabolic demands of zebra mussels. Total effects on the Bay's oxygen dynamics will then reflect complex interactions between aggregate mussel metabolism, local replenishment by diffusion and oxygen supply from photosynthesis. Assessments of gradients in oxygen concentrations near zebra mussel beds would now prove useful.

6.5.2. Experiment design: advantages and limitations

There are a range of advantages and disadvantages in the approach used in this work, and some caveats that arise from the design used. For example, in studies observing increased mussel respiration at high summer temperatures also accompany increased reproductive activity (Sprung, 1995), when oxygen consumption peaks (McMahon, 1996; Fanslow *et al.*, 2001). Zebra mussels manipulated during this experiment, carried out during winter, would be expected to be less metabolically active than in summer. While this feature provides clear evidence that zebra mussels increasing respiration with increasing temperature, and reveals the value of mesocosms, the data might underestimate the true effects of metabolically active, spawning mussels during summer.

In every experiment carried out here, DO concentration curves reached a plateau after approximately 500 minutes, irrespective of the percentage of oxygen saturation in the tank (48-90%). Mussel respiration rates are highly influenced by ambient DO concentration, and oxygen consumption declines with the decreasing ambient oxygen concentration (Quigley *et al.*, 1993; Alexander and McMahon, 2004). This feature might explain the shape of the curves. However, the reasons for the reduced rate of oxygen depletion occurring at percent oxygen saturation reached 80-90% are still not clear. The curves could also be explained by the fact that the tanks were not airtight and oxygen

diffusion occurred sufficiently to balance uptake. Zebra mussels possibly depleted DO to the point at which uptake equalled oxygen diffusion, at which point depletion curves stabilised. Another hypothesis to explain the plateau occurrence could be the existence of a daily respiration cycle in zebra mussels, with interval periods of high and low respiration. Experiments under similar conditions over several days would be interesting to determine any regular variations in mussel respiration over long time period.

Only two water temperatures were tested in my experiments, corresponding to the average of the water temperature in winter and in summer. Fuller assessment of the effects of temperature on zebra mussel oxygen consumption would ideally use more temperatures over the range 10°C-24°C, corresponding to the water temperature peak in Cardiff Bay. Similarly, experiments carried out with a greater range of mussel biomass (e.g. 750g-1kg of mussels) would have provided more certainty about effects. The linear relationship between oxygen consumption rate and mussel density would then allow the estimation of mussel respiration for a given density. The results nevertheless accord with the relationship between zebra mussel density and zebra oxygen demand (ZOD) found by Effler *et al.* (1996).

ZOD has been estimated in some North American rivers, based on the respiration rate of a given mussel population in an area and by the population density within that area (Effler and Siegfried, 1994; Caraco *et al.*, 2000). However, my experiment did not permit such an accurate measurement of mussel respiration rate (due to oxygen diffusion) that could be extrapolated to estimate ZOD in Cardiff Bay. According to Effler *et al.* (1996), ZOD depends on the size structure of the population and increases with individual shell length. In my experiment, mussel respiration was estimated for mussels of 15-25mm shell length, for which ones respiration rate is higher than for smaller individuals (Effler *et al.*, 1996). Therefore, the use of a respiratory chamber as described in some studies would allow more measurement of respiration rate accurately on a few individuals of different size (Hamburger and Dall, 1990; Aldridge *et al.*, 1995; Sprung, 1995; Alexander and McMahon, 2004). My results showed that, to measure the greatest zebra mussel oxygen consumption rates of the year, such experiments should be done during summer using mussels with high metabolic activity, a water temperature of about

17°C and high food availability. Such an experiment would permit the accurate estimation of the highest mussel respiration rates which, when combined with mussel densities data (Chapter 5), would allow the calculation of ZOD in Cardiff Bay in summer and its potential impact on DO concentration in the lake.

6.6. Conclusions

These preliminary results show that mussel respiration was influenced by key environmental factors: water temperature and food availability. Since oxygen consumption increased with rising water temperature and increasing food availability, the highest mussel respiration rate is expected to occur in summer. An estimation of oxygen consumption rate in summer, associated with mussel densities in the lake, would allow the calculation of ZOD which would indicate the potential impact that zebra mussels may have on DO concentrations in Cardiff Bay.

Studies carried out before barrage completion recommended monitoring water quality in the lake and the maintenance of the oxygen level at $5mg.l^{-1}$, but no account of ZOD as made in these initial estimates. Since the impact of ZOD has not been fully assessed yet in Cardiff Bay, regular water oxygen monitoring should continue to appraise any possible changes in conditions as zebra mussel population grows. The combined effects of mussel respiration, temperature, phytoplankton removal by mussels, and atmospheric re-diffusion also require careful investigation given the potential for zebra mussels at the densities observed to deplete oxygen in Cardiff Bay under hot summer conditions and low flow.

7. General discussion

7.1. Zebra mussel population in Cardiff Bay

Zebra mussels are now among the most widespread aquatic invasive species in the world, capable of establishing large populations and causing major environmental change in occupied systems. However, case studies of population establishment and development are still scarce, particularly in newly formed water bodies in urban settings such as Cardiff Bay. Here, four years of field surveys have permitted this baseline description of the reproductive cycle, the colonisation dynamics and distribution of zebra mussels in this 200 ha lake. The overwhelming evidence is of a large, widespread, reproductively active zebra mussel population. Once colonisation occurred probably in 2003 or 2004, the maintenance of this well and widely established zebra mussel population in Cardiff Bay probably reflects a combination of: i) reproductive strategies which are flexible enough to accommodate the intra- and inter-annual environmental variation in the Bay's conditions; ii) a high propagule pressure (i.e. large larval numbers) throughout the entire lake; iii) the presence of suitable hard substrates surrounding the lake and iv) relatively homogeneous water chemistry, rich in nutrients, calcium, plankton and dissolved oxygen that in combination are able to support population development.

The investigations carried out were designed to examine the extent and size of the population, the key life cycle stages in zebra mussel population establishment, the colonisation dynamics, and to make some initial assessments of one of the potential effects (i.e. on oxygen). The investigations began with the veliger stage, and progressed through juvenile settlement to adult distribution and mussel oxygen demand. Ideally and with more time, other ecosystem effects would have been investigated. Veliger production (generally May to September) and the number of larval peaks (usually two) varied between years and were influenced apparently by environmental conditions such as water temperature and discharge. Linked to veliger distribution and river flow pattern, juvenile recruitment fluctuated spatially through the lake and temporally between years. Although zebra mussels colonised any hard substrates present in the lake and in the two river mouths, both natural and man made, direct dispersal from Cardiff Bay to other

water bodies by natural means is unlikely because conditions downstream are unsuitable for zebra mussels while upstream movement is prevented by river flows. There remains, however, the potential for onwards population dispersal by human mechanisms, and this must be a large risk in view of the apparent population size, prolonged period of veliger production, veliger density in the water column and substantial human activity that links Cardiff Bay with other locations.

Even though veliger and adult densities recorded revealed the presence of a well established zebra mussel population, densities were comparable only to the lowest densities reported in other European lakes (Chapters 3 and 5; Mackie and Schloesser, 1996; Sprung, 1993; Wilhelm and Adrian, 2007; Cleven and Frenzel, 1993). Limits on development include varying water discharge from the inflowing rivers and lakebed composition of mainly soft sediments (Chapters 4 and 5). However, it is also possible that the population is still in an early phase of development. Even at current numbers, ecological impacts are possible due to the high filtration rate of zebra mussels (Vanderploeg *et al.*, 2002). There is a risk of decreasing dissolved oxygen concentrations due to zebra mussel respiration may happen especially during summer (Chapter 6). The investigation of these and other possible ecological effects of zebra mussels in Cardiff Bay are now priorities for further work, as well as the continued assessment of population development (see below).

7.2. Limitations and strengths of the study

All field investigations are characterised by strengths and weaknesses, and this study has been no exception. Typical problems arise from survey approaches, which permit no appraisal of cause-and-effect in patterns detected, and from the experiments, which are invariably are characterised by artefacts in the methods used. In this investigation, a blend of these two approaches was used in an attempt to maximise the opportunities from both.

Veliger production assessment in any water bodies requires a study period long enough to provide valuable information on variability between years and with varying environmental conditions. The four year study period used here highlighted this

variability, and revealed the particularity of the year 2006, characterised by a high veliger densities over a long period in comparison with the three following years, due certainly to favourable environmental conditions. The lack of current velocity data through the entire lake did not permit, however, a full assessment of the relationship between flow pattern and spatial veliger distribution. Nevertheless, this work highlighted the value of a long-term study of veliger production in newly invaded water bodies as a method to determine the establishment level of zebra mussel population and its evolution over time (Chapter 3; Lockwood *et al.*, 2005). Factors both favourable and unfavourable to settlement were clear.

The experimental design to assess juvenile settlement was problematic because of diverse factors: the sporadic occurrence of large river discharge, the mud and silt deposition on substrates and the monitoring disturbance which limited the experimental design and the choice in sites. However, the experiments carried out over three years with change in experimental design and the use of the crates as substrates permitted the collection of data which revealed variations in recruitment between years. It also showed spatial patterns in juvenile settlement that were in accord with veliger pattern suggesting some robustness in the data collected.

The investigation of the zebra mussel adult population in Cardiff Bay produced some of the most important data with respect to the potential impacts of zebra mussels in Cardiff Bay, but these data are also characterised by the largest uncertainties. The collection of mussel samples on man-made structure surrounding the lake was difficult to carry out both logistically and because of the sheer spatial extent involved. The use of divers was unfeasible because of prohibitive cost and safety implications, while limited accessibility to sites was a constraint. The distribution and density of zebra mussel population in Cardiff Bay were, however, assessed with the use and development of other techniques. Side Scan Sonar coupled with under-water video imagery provided usable methods to investigate the presence/absence of adults and to estimate population density and total population size. Whilst video camera coupled with SSS (to investigate mussel density on lakebeds) has been carried out in other studies (Coakley *et al.*, 1997), the use of a video camera to investigate vertical surfaces is innovative. This method is particularly valuable for studying sites inaccessible to divers. However, only mussel

populations on hard substrates in the lake were estimated because coverage of the aeration system was insufficient to estimate the mussel population through the entire lake. An exhaustive estimation of the total zebra mussel population in Cardiff Bay would also require an assessment of mussel colonisation on boats and pontoons.

The experiment on zebra mussel oxygen consumption did not permit a precise measurement of zebra mussel respiration rate in a closed system. As a result, the determination of ZOD in Cardiff Bay was not possible. The mesocosm experiment carried out in winter nevertheless revealed seasonal variations in zebra mussel oxygen consumption with an increase in the oxygen uptake during summer time. These indications confirm the summer period as that most likely to be affected by potential oxygen loss to the Bay's large zebra mussel population.

7.3. Zebra mussel invasion in a newly formed lake

The opportunity to study the zebra mussel population in a newly formed lake is rare and on the results from Cardiff Bay may be valuable to consider where any similar artificial lake project is planned or taken forward.

Initial veliger sampling was carried out in 2006, five years after the completion of Cardiff Bay, and this year revealed to have the highest veliger densities recorded and the longest veliger production period over the four year study period. These results reveal how rapidly zebra mussels established a large population in Cardiff Bay. The results on size structures suggest that the zebra mussel population was probably present already in 2003 (Chapter 5) - a year with a very hot summer which probably created optimal conditions for a significant expansion of the zebra mussel population in this newly formed lake.

The fact that invasion occurred so rapidly implies some vulnerability of Cardiff Bay to invasion – probably reflecting its ideal environment, large size and use by boats in large numbers. This invasion risk was not anticipated, but should be foreseen in similar new environments: the numerous case studies of zebra mussel invasion in both North America and Europe, the knowledge of severe impacts caused by zebra mussels, the difficulties in controlling populations once established, and the lack of technologies for

eradication (Chapter 2), underline the importance of preventing dispersal into newly formed lakes as far as is possible. Preliminary studies carried out prior to a comparable development project should consider the risk of zebra mussel colonisation and examine colonisation prevention measures that could be taken. As boats are considered to be a main dispersal vector, stricter regulations on boat cleaning (hull, ancillaries and any form of on board water) may help to minimise the risk of zebra mussel invasion. However, other dispersal pathways (e.g. contaminated equipment, nets, etc) should not be overlooked. Boat owners and fishermen should take precautions during the veliger production periods, in spring and summer, to minimise the risk of veliger dispersal. Boat hulls and fishing equipment should be well cleaned whilst water carried on board should not be released anywhere but should be treated.

7.4. Management of zebra mussel population in Cardiff Bay

As it is large, well established and widespread, the Cardiff Bay zebra mussel population already poses a risk of causing impacts on the Cardiff Bay environment. For example, mussel colonisation of the barrage structure and the aeration system has been substantial. The estimation of zebra mussels in the surrounding areas of the lake - between 10 and 31 million individuals – indicates the presence of a mussel population in Cardiff Bay with the potential to cause substantive ecological impacts in this artificial lake. Veliger production, recruitment and growth rate of zebra mussels are enhanced by warm environmental conditions. Aldridge (2004) suggested that warmer seasons in Great Britain could be one reason for the enhanced recent expansion of British zebra mussel populations. With warmer summers and winters expected to occur more often in Cardiff Bay in future because of climate change, zebra mussel population could increase further yet to reach densities similar to those recorded on the European mainland. With increased densities, mussel bed formation on soft sediments is possible in areas sheltered from the current, where debris and dead mussel shells could accumulate (Chapter 5; Berckman *et al.*, 1998).

Although the barrage structure was treated with an antifouling agent, a large amount of zebra mussels colonise the infrastructure (Chapters 4 and 5). Consequently, a regular cleaning of the barrage structure, at least once a year, is necessary to preserve it

from damage, and the appliance of the antifouling agent should be renewed regularly to maintain its efficiency. Furthermore, the aeration system, highly colonised, requires regular cleaning (Chapter 5): the mussels must be removed from the structure, collected and incinerated. Any mussels returned to the water substantially limits the value of the cleaning process as live mussels replaced into water can resettle on available hard substrates and dead mussels constitute a suitable hard substrate for new mussel colonisation as it was observed in chapters 4 and 5. However, regular cleaning can only control zebra mussels in Cardiff Bay very locally, and population persistence is extremely likely through the prolific veliger production even if adult populations are substantially reduced.

A potential control on zebra mussel reproduction success would consist of leaving the barrage locks open for 1-2 days during veliger peaks to allow salt water ingress to the lake. As a salinity rate of 2‰ is fatal for veligers (Kilgour *et al.*, 1994), allowing seawater into the lake should eliminate veligers. There would however also be impacts on the zooplankton and other freshwater organisms in Cardiff Bay, notably fish. Whist zooplankton can regenerate; this process may have a substantial impact on both the population composition and density (Merrix, pers. comm.). If salt water ingress caused a significant effect on zebra mussel reproduction success, such a control may warrant serious consideration. However, negative impacts on other organisms would be large, and it is debateable whether water of sufficiently high salinity would reach all locations occupied by zebra mussels in the upper Bay.

Zebra mussels have no specific predators which could operate as a biocontrol agent (Molloy, 1997). However, the presence of white swans (*Cygnus olor*), mallard ducks (*Anas platyrhynchos*) and roaches (*Rutilus rutilus*) in Cardiff Bay may have a limited control effect on zebra mussel densities. Wintering waterfowl such as tufted ducks (*Aythya fuligula*), pochards (*Aythya farina*) and coots (*Fulica atra*) caused significant impacts on zebra mussel populations in Lake Constance (Werner *et al.*, 2005). Furthermore, predation pressure by roach, locally decreased zebra mussel density (Naddafi *et al.*, 2010). An increase of water bird and roach populations in Cardiff Bay may, therefore limit zebra mussel expansion, but effects are likely to be marginal given

the large settlement rate. The search for other natural enemies remains a possibility that could be explored more fully.

7.5. Future work

This work has highlighted the presence of a large and well established zebra mussel population in Cardiff Bay which has the potential to case large ecological impacts on its aquatic ecosystem.

Zebra mussels are filter feeders, filtering large volumes of water and particles ranging in size from 0.7 μm up to 750 μm (Johengen *et al*, 1995). Decreases in concentrations of seston (Fanslow *et al.*, 1995), dissolved nutrients (Johengen *et al*, 1995), planktonic diatoms (Holland, 1993), micro zooplankton (MacIsaac *et al.*, 1995) and chlorophyll *a* (Mellina *et al.*, 1995) in many lakes were attributed mainly to the filtering activity of zebra mussels. One concern in the long term is the shift of energy from the pelagic (phytoplanktonic) to the benthic components and resultant alterations in the Bay's food web. The filtration rate is influenced by seston concentration (Reeders *et al.*, 1993; Fanslow *et al.*, 1995), water temperature (Fanslow *et al.*, 1995) and population size structure (Young *et al.*, 1996). The determination of zebra mussel filtration rate in Cardiff Bay, coupled with zebra mussel population density and its size frequency distribution, would allow an assessment of possible effects on phytoplankton and zooplankton concentration in the lake, but also the potential indirect impact of zebra mussel on fish populations by altering the food web. Impacts can be assessed by determining mussel filtration rate in laboratory as per Fanslow *et al.* (1995), or by using models based on data from the literature as described by Descy *et al.* (2003). However, field experimentation could prove extremely valuable.

Chapter 5 showed that high zebra mussel densities can lead to the decrease of dissolved oxygen concentrations through zebra mussel respiration (Effler, 1994; Caraco *et al.*, 2000; Gelda *et al.*, 2001; Denkenberger *et al.*, 2007). The determination of zebra mussel respiration rate in laboratory coupled with zebra mussel density would allow the assessment of ZOD and the impact on dissolved oxygen concentrations in the field. Given the legal importance of oxygen concentrations in Cardiff Bay, and the potential for

both direct and indirect effects on oxygen by zebra mussels under the most sensitive periods (summer), these and other aspects of work in relation to oxygen dynamics should take the highest priority for further studies.

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