The Ecology and Palaeoecology of Diatom – Duckweed Relationships

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by

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I, **David Emson** confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

This thesis focuses on the ecology and palaeoecology of diatom-duckweed relationships and utilises a combined experimental, ecological and palaeoecological approach.

In particular, the study sought to determine the potential of the epiphytic diatom *Lemnicola hungarica* to be utilised as a proxy indicator of past dominance of duckweed (*Lemna*) in small ponds. To this end, contemporary sampling of epiphytic diatom assemblages from a variety of macrophytes (including multiple samples of free-floating plants) were collected from around the world and analysed for diatom epiphytes. In this study, even despite significant environmental gradients, *L. hungarica* showed a significant association with free-floating plants (including *Lemna* spp.) as did *Sellaphora seminulum*. To determine whether this relationship might be used to infer *Lemna*-dominance in sediment cores, diatom assemblages were analysed in surface sediments from English *Lemna* and non-*Lemna* covered ponds and in a core from a pond (Bodham Rail Pit, eastern England) known to have exhibited periods of *Lemna*-dominance in the past. In both cases, the data suggested that both *L. hungarica* and *S. seminulum* were excellent predictors of past *Lemna*-dominance.

Finally, to infer the consequences of *Lemna*-dominance for the long-term biological structure and ecosystem function of the Bodham Rail Pit, the sedimentary remains of diatoms, plant pigments, and plant and animal macrofossils were enumerated from two sediment cores. These stratigraphic data were compared with the diatom *Lemna*-indicator metric which indicated three distinct *Lemna* cycles. Sediment core analyses suggested major compositional, structural and ecological changes brought about by the *Lemna* cycles, especially in the submerged macrophyte community and in fish-invertebrate relationships. These data reveal that duckweed proliferation, often brought about by eutrophication and terrestrialisation in ponds, can result in dramatic ecological changes due to a strong physical ecosystem engineering effect.

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Chapter 1. Introduction

1.1 Research background

All types of freshwater aquatic systems have been subjected to anthropogenic modifications over the last two centuries. The most recent impact by human activities upon aquatic systems have been eutrophication through increased loading of nutrients, particularly phosphorus and nitrogen (James *et al*., 2005, Elser *et al*., 2007, Barker *et al*., 2008).

Eutrophication stimulates primary production in aquatic systems resulting in increased macrophyte and phytoplankton biomass often leading to significant and profound changes to freshwater ecosystems (Mason & Bryant 1975). Although this process affects all types of freshwater systems, it is small water bodies such as shallow lakes and ponds where it often has the greatest impact upon ecological structure and function (Oertli *et al*., 2002, Nicolet *et al*., 2004, Williams *et al*., 2004, Søndergaard *et al*., 2005, Scheffer *et al*., 2006, Declerck *et al*., 2006). Moreover, other driving changes such as climate change and global warming impact upon freshwater ecosystems (Mooij *et al*., 2005, Liboriussen *et al*., 2005, Feuchtmayr *et al*., 2007). Changes in water levels, seasonality and precipitation are predicted with rising global temperatures (IPCC 2007), and it has been suggested that the direct effects of increasing temperature may also be acting as a driver of ecological changes (McKee *et al.,* 2003, Mooij *et al*., 2005, Kosten *et al.*, 2009). These drivers are likely to interact with and exacerbate existing symptoms of eutrophication such as algal dominance and cyanobacterial blooms (Moss *et al*., 1996). In some cases increasing floating plant dominance, particularly lemnid cover may result (Feuchtmayr *et al*., 2007) likely presenting significant additional stresses to freshwater systems such as impacting upon the availability of light for submerged macrophytes, particularly in small lowland ponds, over the coming century.

1.2 Small lowland ponds

1.2.1 Definition

Although there is no universal agreement on what defines a water-body as a pond rather than a lake, it is possible to recognise four broad categories, reflecting the fundamental concepts most frequently used and repeated: (i) it is difficult (if not impossible) to define a pond, (ii) ponds are small in area and shallow in depth, (iii) ponds are shallow enough to support rooted macrophyte growth throughout the year, and (iv) a miscellany of other physical characteristics (Biggs *et al.,* 2005). According to Williams *et al.,* (1999) a pond is defined as:

Water bodies between 1 m^2 and 2 ha in area which may be permanent or seasonal, including both man-made and natural water bodies.

The study sites in this research project (see Chapters 5 and 6) are shallow ponds. They are not shallow lakes which cover larger areas, or deep lakes, or even deep ponds which are naturally very rare such as quarry pits. Furthermore, there is evidence that small shallow ponds (perhaps <50-80m diameter) can exhibit oxygen stratification (Sayer *et al*., 2013), but this stratification was not recorded from a set of 39 shallow lakes within the same geographical location (C. D Sayer, pers. com.). There are relatively few pond studies dedicated to ecosystem structure, functioning and ecological processes; previous studies are limited to species surveys, and to date there are virtually no documented palaeoecological studies on small ponds.

1.3 Pond or lake: does size make a difference?

Much of the previous research, both ecological and palaeoecological, into the role of environmental factors on freshwater systems has traditionally focussed upon relatively large lakes (Wetzel 2001, Søndergaard *et al.,* 2005), providing a broad ecological understanding of human impact, but the overall ecological functioning of ponds has been less well elucidated (Palik *et al.,* 2001, Tessier & Woodruff, 2002). Furthermore, smaller

lakes, and ponds in particular, have received less attention despite their relatively high prevalence in the landscape, together with their rich biodiversity and consequent conservation value (Biggs *et al.,* 1999, Oertli *et al.,* 2002, Williams *et al.,* 2004). Studies that have been undertaken on ponds have thus far tended to focus upon surveys of specific taxa, such as macrophytes (Palmer *et al.,* 1992), amphibians (Swan & Oldham, 1989, Oertli *et al.,* 2002) and invertebrates (Foster & Eyre, 1992) resulting in species lists but giving little information on the ecological structure and functioning of the water bodies. Data from small shallow lakes may provide a useful insight into the function of ponds (Søndergaard *et al.,* 2005), but there have been few comparative studies along a gradient of lake size (Tonn & Magnusson 1982, Wellborn *et al.,* 1996, Tessier & Woodruff 2002) which makes it difficult to determine the extent to which existing knowledge of large lakes may be applied to small lakes or ponds and vice versa (Søndergaard *et al.,* 2005). Moreover, another problem is that it is difficult to discriminate between large lakes and small lakes or ponds because the lake size gradient comprises an environmental continuum without any clear delimitation (Wellborn *et al.,* 1996, Søndergaard *et al.,* 2005).

Some studies have suggested that there are fundamental differences between large lakes and small lakes and ponds. Firstly, small lakes or ponds have a relatively greater littoral zone and closer contact with the adjacent terrestrial environment than large lakes (Palik *et al.,* 2001), resulting in a higher terrestrial-aquatic interchange of organisms and allochthonous organic matter. Secondly, smaller water bodies are relatively more isolated and insular when compared with the large catchments and riverine inflows of large lakes. Thirdly, small sites may potentially lack fish because of winter fish kills and summer dry out events, thus greatly affecting both the structure and functioning of the ecosystem. The former arises because of the strong cascading effects of fish on multiple trophic levels in lake ecosystems (Wellborn *et al.,* 1996, Jeppesen *et al.,* 1997, Jones & Sayer 2003), often resulting in the increased importance of invertebrate predators in the absence of fish (Yan *et al.,* 1991, Hobaek *et al.,* 2002). Fourthly, small lakes often contain relatively stagnant water. Fifthly, small water bodies have a relatively low water volume with enhanced benthic-pelagic coupling and a greater impact of the sediment on the water's nutrients (Tessier & Woodruff 2002). Finally, many small sites typically have a shallow and wind-

protected morphometry allowing submerged and floating-leaved macrophytes to potentially cover large areas (Van Geest *et al.,* 2003, Søndergaard *et al.,* 2005).

1.4 Pond size and conservation value

In a study of nearly 800 Danish lakes (0.01 to 4200 ha) Søndergaard et *al.,* (2005) found that the number of macrophyte species was highest in the largest lakes and lakes with relatively higher alkalinity. Nonetheless Williams *et al.,* (2003) and Biggs *et al.,* (2005) found that ponds which were not degraded by human activities supported similar numbers of wetland plants to lakes. In these same studies of comparative biodiversity (Williams *et al.,* 2003) and pond assessment (Biggs *et al.,* 2005) it was reported that, at the UK level (even though individual ponds varied considerably in biodiversity), ponds supported slightly more macro-invertebrate species than rivers, and more uncommon species. Indeed, in terms of regional biodiversity, these studies showed that ponds make a significantly greater contribution than any other aquatic habitat, supporting considerably more species, more unique species and more scarce species. This contrasts markedly with their relative status in national monitoring and protection strategies, where small water bodies are relatively ignored (Williams *et al.,* 2003).

Wetzel (2001) suggested that biodiversity relative to lake size can be expected to be higher in small lakes and ponds where littoral habitat heterogeneity interfaces with the pelagic regions. Similarly, in a study of odonates from 80 Swiss ponds Oertli (2002) concluded that a set of small ponds may host more species than a single large pond of the same total area. In a recent study investigating the importance of ponds for biodiversity at the European level, Davies *et al.,* (2008) found that ponds (and ditches) displayed a broader range of physical and chemical characteristics than lakes and rivers. In addition ponds were more strongly influenced by local geology, altitude and catchment land-use, and in smaller catchment areas, resulting in different characteristics for ponds even though they could be relatively close to each other. The study by Davies *et al.,* (2008) showed that: (i) at the local, individual site level the greatest water plant and macro-invertebrate diversity was found in rivers, then ponds and lakes, streams and finally

ditches, (ii) at the regional level, however, the greatest diversity of both aquatic plants and macroinvertebrates was to be found in ponds, with much lower diversity in the other aquatic habitats.

The relatively small catchment size of ponds is both a benefit and a disadvantage with respect to their protection and conservation. Ponds are highly vulnerable to environmental impacts and degradation caused by surface water pollution because their small water volumes provide little possibility of dilution or buffering of pollutant inputs. However, because of their small catchments, especially where pollution is largely absent, ponds can often be of exceptionally high quality which may explain the relative richness in biodiversity of these small water bodies (Biggs *et al.,* 2005). In a study of 126 small farmland ponds across Belgium, Declerck *et al.,* (2006) concluded that catchment type and land use impacted upon pond ecological characteristics, with trampling by cattle and percentage cover of nearby crop land both positively associated with turbid conditions. Conversely, they showed that ponds with high forest coverage in their catchments and immediate surroundings tended to be more associated with the clear water state.

Despite their small surface area, ponds can contribute significantly to both local and regional biodiversity because they support heterogeneous communities of aquatic organisms, often including rare or unique endemic species (Oertli *et al.,* 2002, Nicolet *et al.,* 2004). Many of these include UK Biodiversity Action Plan (BAP) species, such as species of vascular plants (*Chara connivens*, *Nitella tenuissima* and *Tolypella prolifera*), several invertebrates (e.g. *Donacia aquatica*, *Anisus vorticulus*) and vertebrates such as Great Crested Newt, *Triturus cristatus* (Williams *et al.,* 2004, Biggs *et al.,* 2005, Davies *et al.,* 2008) and the rare and culturally important Crucian Carp *Carassius carassius* (Sayer *et al.,* 2011).

Ponds are threatened due to eutrophication, chemical pollution, terrestrialisation and even physical destruction (Heath & Whitehead 1992, Boothby, 2003, Biggs *et al.,* 2005) and extensive droughts can bring about rapid and extreme changes in plant communities with a loss of most of the aquatic vegetation (Painter & May 1997). In terms of the number of ponds in the UK it is clear that ponds are at an historic low. It has been estimated that

there were 1.2 million ponds in Great Britain in 1880 but less than 400,000 in the late 1990s (Haines-Young *et al.,* 2000, Biggs *et al.,* 2005). Approximately 1% of ponds per annum are filled in by natural and artificial processes. Equally in recent decades, conservation initiatives have led to the creation of many new ponds (Williams *et al.,* 1998a). The net effect on the conservation value of the pond resource of this rapid turnover is not known, and even less is known about trends in the quality of existing ponds (Biggs *et al.,* 2005).

The conservation value of inland water bodies, including ponds, must be based upon integrated catchment management whereby land and water are considered together at the catchment level to ensure long-term ecological and socio-economic sustainability (Williams *et al.,* 2003). This fundamental premise has been incorporated into legislation and policy via the implementation of the EC Water Framework Directive (2000/60/EC) which emphasises catchment management for the protection of water bodies, and the maintenance of ecological quality of freshwater systems through monitoring and restoration. Nonetheless, the Water Framework Directive (WFD) only considers lakes >50 ha and ponds are not included.

1.5 Pond and shallow lake ecology, eutrophication and alternative stable states

Although there is a paucity of work focussing on the ecological characteristics of small freshwater ponds there have been a plethora of such studies undertaken for shallow lakes (<3m), particularly with respect to eutrophication impacts. Small ponds are expected to differ from larger ponds and lakes in several aspects (as discussed in section 1.3 above). Nevertheless, it is reasonable to assume that much knowledge of shallow lake functioning can be applied to ponds. Ponds and shallow lakes both have extensive littoral zones, which cover much of the lake area with the potential for the entire water body to be within the photic zone.

Primary production within shallow lakes and ponds can be described as pelagic (that is production by phytoplankton) or benthic which is production from attached algae

associated with surfaces of the sediment and attached epiphytic algae on the surfaces of macrophytes. Changes in the relative balance of benthic and pelagic production are known to occur over time in shallow lakes as a response to eutrophication (Vadeboncoeur *et al*., 2003). Generally, over a wide range of nutrient concentrations shallow lakes exist as either a clear water system dominated by submerged aquatic plants, or as turbid water systems characterised by phytoplankton dominance with a marked reduction in, or absence of, submerged plants (Canfield *et al.,* 1984, Irvine *et al.,* 1989, Moss 1989, Jeppesen *et al.,* 1990a). These two contrasting ecosystem states have been described as alternative equilibria and they are thought to exist due to positive biological feedback mechanisms, particularly interactions between submerged vegetation and turbidity (Scheffer *et al.,* 1993). Some disturbance or perturbation must occur to precipitate the switch between community equilibria (Bender *et al.,* 1984). Shifts between the alternative stable states, due to changes in nutrient loading are thought to be characterised by a hysteresis in ecosystem response resulting in catastrophic shifts or switches between states. Such shifts usually occur unannounced and 'early-warning signals' of the approaching catastrophic change remain elusive (Moss 1977, Phillips *et al.,* 1978, Stansfield *et al.,* 1989, Jones & Sayer 2003) although whole-lake experiments that temporarily and massively reduced fish biomass resulted in a return to a permanent clear water state when nutrient levels were excessive (Meijer *et al.,* 1994). A reduction of nutrient concentrations is often insufficient to restore the vegetated clear water state, and the restoration of clear water occurs at substantially lower nutrient levels compared to the concentrations at which the collapse of the vegetation occurred (Scheffer *et al.,* 1993, Meijer 2000). This pattern of hysteresis, which is a forward and backward switching process, occurs at different critical conditions, and shallow lakes can have a pronounced hysteresis in response to nutrient loading (Scheffer *et al.,* 2001).

Aquatic vegetation has been shown to increase water clarity, thereby enhancing plant growing conditions (Scheffer *et al.,* 1993) which causes this clear state to be a self-stabilising alternative to the turbid regime (Scheffer *et al.,* 2001). This reduction in phytoplankton biomass and turbidity involves a suite of mechanisms, such as reducing nutrient concentrations in the water column, affording physical protection to grazing

Cladocera against fish predation, whilst plant roots prevent the re-suspension of sediment. Conversely, fish are thought to be central in maintaining the turbid state through grazing-induced reductions in large open water cladocerans and/or through sediment resuspension and enhanced nutrient recycling, thereby exacerbating the turbidity (Scheffer *et al*., 2001, Zambrano *et al*., 2006).

The evidence for the existence of Alternative Stable States comes from species surveys, and rarely from long-term studies. Therefore, the background to these changes, the suddenness and the permanence are rarely investigated. As an alternative to the Alternative Stable State model, Sayer *et al.,* (2010a) suggest a gradual change in shallow lakes induced by nutrient enrichment, where submerged plant loss is caused by progressive nutrient-enrichment. A palaeoecological approach would be useful as it would be possible to see any changes in aquatic plant composition over real timescales (i.e. decadal-centennial) and the effects of eutrophication on shallow lakes.

The question arises: do such states also exist in ponds? This is largely unknown, but it is thought that dense coverage by floating plants may form an Alternative Stable State in small ponds (Scheffer *et al.,* 2003).

1.6 Floating plant dominance as an ecological stable state

Scheffer *et al.,* (2003) demonstrated that free-floating plant dominance can also be a self-stabilising ecological state in freshwater ecosystems. In temperate climate zones, it is known that dense mats of duckweeds in small water bodies are symptomatic of high-nutrient loading (Portielje & Roijackers 1995). Free-floating plants are dependent on high nutrient concentrations in the water column as they have no access to nutrients within the sediment. A large proportion of their leaf surfaces are exposed to the atmosphere rather than to the water, which further reduces the possibility of utilising nutrients from the water column through their leaves (Scheffer *et al.,* 2003), although they can utilise atmospheric carbon via leaf assimilation (Scheffer *et al.,* 2003). Rooted submerged plants can utilise sediment-based nutrients (Hutchinson 1975) and water column nutrients through their shoots (Sculthorpe 1967). Although free-floating plants are superior

competitors for light, the presence of submerged plants which can utilise nutrients in the water column can adversely affect the growth of free-floating plants. Indeed in experiments with low nutrient concentrations *Elodea nuttallii* strongly reduced the growth of *Lemna gibba* supporting the hypothesis that submerged plants can prevent colonisation by floating plants (Szabó *et al.*, 2010). Through experiments, field data and models, Scheffer *et al.*, (2003) found evidence for alternative domains of attraction in environments prone to duckweed domination (i.e. small ponds and ditches) in which the final state of the system depends upon the initial biomass of the free-floating plants, and nutrient enrichment reduces the resilience of rooted submerged plants. However, in shallow waters with submerged plants and a moderate nutrient level, a single drastic harvest of free-floating plants led to a permanent switch to rooted, submerged plant dominance.

1.7 Ecosystem engineers

1.7.1 Definition

Ecosystem engineers are organisms that directly or indirectly modulate and control the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials as they modify, maintain and create habitats (Jones *et al.*, 1994, 1997b). Ecosystem engineering is essentially the creation, destruction or modification of habitats, the physical alteration of ecosystems typically having cascading effects on other biota (Crooks 2002).

1.7.2 Effects of ecosystem engineers

Ecological engineers directly create non-food resources such as living space, directly control abiotic resources, and indirectly modulate abiotic resources that affect resource use by other organisms. Ecosystem engineering does not involve direct trophic interactions and resource competition between species (Jones *et al.*, 1994, 1997b). Organisms act as engineers when they modulate the supply of a resource or resources other than themselves; the direct provision of resources to other species in the form of living or dead tissues is not engineering. Engineers differ from keystone species in their

impacts on ecosystem structure, even though many engineers are keystone species, as they play relatively minor direct roles in structuring community food webs (Jones *et al*., 1994, 1997b). An example of ecological engineers from freshwater systems are submerged macrophytes that grow to create extensive weed beds that impact upon attenuated light, steepen vertical temperature gradients, retard flow, enhance sedimentation and oxygenate rhizospheres (Carpenter & Lodge 1986). Jones *et al*., (1997b) argue that engineering can have both negative and positive effects on species richness and abundances at small scales, and models of the population dynamics of engineers suggest that the engineer/habitat equilibrium is often locally stable and may show long-term cycles, with potential ramifications for community and ecosystem stability.

The concept of ecosystem engineering can provide vital information on the impacts of exotic species and associated modification of habitats (Crooks 2002). However, do exotic ecosystem engineers have predictable effects upon integration into novel or foreign ecosystems? One consequence of physically altered habitats could be changes in the abundance and diversity of structural components which could affect habitat complexity or heterogeneity (McCoy & Bell 1991). This relationship between exotic species and their ecosystem engineering effects can be used to examine the community-level effects of exotic or alien species on habitat complexity (Crooks 2002).

It is interesting to speculate if the dense mats of free-floating plants, particularly Lemnids, can also 'engineer' the structure of small water bodies, and indeed be classified as ecosystem engineers of these under-studied systems. This thesis will attempt to investigate the ecological impacts of dense mats of *Lemna* spp. on a small pond, to determine if it indeed can be classified as an ecosystem engineer.

1.8 Invasive aquatic plants

1.8.1 Biological invasions and ecosystem engineering

Biological invasions are regarded as natural processes but the current rates of species invasions around the globe are wholly unprecedented (Williamson 1996, Vitousek *et al.*,

1997). Alteration of ecosystems by the activities of exotic invaders can be dramatic (Crooks 2002), affecting resource availability for other species, altering the flow of energy or biomass, changing food webs, and even changing the physical structure of the ecosystem itself (Simberloff 1991, Crooks 2002). In Britain the water plants, New Zealand Pigmy Weed *Crassula helmsii* and Parrot's Feather *Myriophyllum aquaticum* are well-known habitat modifiers. These plants create thick beds that limit water movement and light penetration, but they also offer habitat for invertebrates and predation refugia for fish (Schmitz *et al.*, 1993, 1997, Crooks 2002).

Biological invasions are a major cause of biodiversity loss (Willby 2007). Their impacts on native biodiversity include displacement of indigenous species through competition or predation, structural damage to aquatic habitats, and a loss of genetic integrity. Invasive species are of critical concern to conservation bodies worldwide (Willis & Birks 2006) as they can often threaten native species with extinction (Gurevitch & Padilla 2004). Invasive alien species are assigned with an 'Impact Status' according to risk assessments by the UK Environment Agency (Environment Agency, Water Framework Directive Programme, 2004). Water bodies most vulnerable to biological invasions are often subject to multiple pressures for which the key drivers are agricultural intensification and urbanisation (Kercher & Zedler 2004, Ervin *et al.*, 2006). The presence of invasive species can, therefore, impair the ecological status of such sites. In an attempt towards urgent and effective control of invasive aquatic plants, together with post-control recovery of native communities, the European Water Framework Directive requires the restoration of degraded water bodies to 'good ecological status' by 2015 (EU 2000/60/EC).

1.8.2 Invasive free-floating plants

The invasion of dense mats of free-floating plants is acknowledged as among the most important threats to the functioning and biodiversity of freshwater systems (Scheffer *et al.*, 2003). Duckweeds (Lemnaceae) are well known to cause physico-chemical changes in the water beneath them (Pokornŷ & Rejmankova 1983, Goldsborough 1993, Portielje & Roijackers 1995) by interfering with light penetration, reducing photosynthetic active

radiation (PAR) by up to 99% with associated temperature fluctuations which can lead to diurnal temperature stratification (Dale & Gillespie 1976, Goldsborough 1993). Moreover, they reduce gaseous exchange causing the predominance of respiratory activity beneath the mats by reducing dissolved oxygen and increasing carbon dioxide levels (Janes 1998), causing a reduction in pH (McLay 1976, Janes 1998) and an increase in conductivity (Sayer *et al*., unpublished data). Furthermore, dense duckweed mats are detrimental to ecological structure, functioning and biodiversity due to the loss of submerged macrophytes that often arises when they are present (Janse & Van Puijenbroek 1998). The anoxic and cold conditions, together with an increase in carbon dioxide can also cause fish kills (Lewis & Bender 1961) and loss of macroinvertebrates (Janse & Van Puijenbroek 1998). These prolonged anoxic conditions could result in just a few invertebrate species surviving these harsh conditions in small ponds, examples include *Cloeon dipterum* (Ephemeroptera) larvae that have adapted to anoxic conditions in small ponds in regions with long and cold winters (Nagell 1977).

Clearly, further work is required to assess the impacts of introduced invasive species on specific water bodies, both to enable an accurate assessment of ecological status and to design appropriate response measures (Environment Agency, Water Framework Directive Programme 2004). This thesis intends to contribute to future assessments of pond ecological status by attempting to shed light on the ecological impacts and effects of *Lemna minuta* dominated duckweed mats in small ponds.

1.8.3 *Lemna minuta* Kunth.

Lemna minuta (American duckweed) is a native of temperate regions of North and South America (Preston & Croft 1997, Lucey 2003) and has now become naturalised in Europe. It was first recorded in the British Isles in Coe Fen, Cambridge in 1977 (Landolt 1979). It was first recorded in Eire in 1993 at Blarney Castle, Cork (Lucey 2003). It has spread rapidly across the British Isles since the 1980s (Bramley *et al.*, 1995) and is listed as one of the species showing the most dramatic increase in range and abundance in Britain during the twentieth century (Walker 2007). It has a significantly broader tolerance to nitrate concentration than the native species *L. minor* (Lüönd 1980), and according to

Bramley *et al.,* (1993) the abundance of *L. minuta* is not significantly controlled by water chemistry. Despite its relatively small size it occurs in considerable quantities, often excluding other free-floating aquatic plants, and there is evidence that it is more aggressive than other lemnids (Leslie & Walters 1983). According to Landolt (1980) this is a species that favours a more Mediterranean climate and there is a significant risk that climate change will allow a rapid northerly expansion of some invasive aquatic plants that are already established in the south of England (Willby 2007). This will likely favour the rapid establishment of *L. minuta*. It has been seen to out-compete indigenous Lemnaceae (Leslie & Walters 1983, Oliver 1991) becoming the most dominant aquatic plant species, covering the surface area in dense and thick mats, creating anoxic conditions leading to high fish mortalities, and declining aquatic invertebrate diversity. *L. minuta* appears to be prone to devastating and alternating boom-bust cycles leading to the marginalisation of indigenous Lemnaceae (Bramley *et al.,* 1995, Dussart *et al.,* 1993). Despite the above knowledge, *L. minuta* is classified as 'Unknown Impact' and for which a full risk assessment is required (Environment Agency, Water Framework Directive Programme, Technical Assessment Method, 2004).

L. minuta appears to be capable of withstanding British winter temperatures (Leslie & Walters 1983) and even sub-zero temperatures where protection is afforded by growth in thick, dense mats. Consequently it tends to over-winter more successfully than monolayer growths of native *L. minor* (Janes 1998). The introduction, spread and the biological interactions of invasive plant species have provided fascinating ecological and evolutionary insights (Walker 2007) and according to Max Walters (1970): "Most of them are unplanned experiments [sic], but if we watch we can learn a great deal from them". This thesis intends to take this insight on board by studying the recent ecological impacts of *L .minuta* in a small pond.

1.9 Tracking ecological change in shallow lakes and ponds

The ecological status of nutrient-enriched shallow lakes has been variously assessed by contemporary monitoring, water quality assays and biological manipulations. Palaeolimnological techniques that provide historical data for defining baseline

restoration targets for lake management and ecological trajectories have also been widely applied to shallow systems (Osborne & Moss 1977, Madgwick & Phillips 1996). The various multi-proxy techniques employed in shallow lake palaeolimnological studies have included diatoms, plant macrofossils, pollen, cladocerans, chironomids, pigments, chrysophytes, molluscs and fish remains (Bennion & Battarbee 2007, Davidson *et al.*, 2010b, Sayer *et al.*, 2010a). However, there has been comparatively little attention afforded to the ecological and biological status of ponds. There is a general lack of knowledge on the structure, diversity and functioning of these systems and how they are affected by anthropogenic influences (Wood *et al.*, 2003, Williams *et al.*, 2004, Declerck *et al.*, 2006). Furthermore there is a paucity in knowledge, understanding and information regarding their aquatic histories.

Palaeoecological analyses employ techniques that utilise the chronologically accumulated sediment record by investigating micro and macrofossil assemblage changes in time. Diatoms, in particular, have been widely used in palaeolimnological studies of environmental change because of their importance in aquatic ecosystem ecology, their sensitivity to changes in water quality and their good preservation in aquatic sediments (Stoermer & Smol 1999). The sediment record, coupled with reliable dating techniques, can not only be used to track environmental change such as eutrophication and acidification but can also provide an archive of ecosystem dynamics over long time frames. Moreover, according to Smol (1992) this historical information can provide data on early baseline 'reference' conditions and natural variability of the community and therefore can isolate and identify anthropogenic influences affecting the water body. This historical data and information over such time scales is not available to contemporary ecological investigations (Anderson & Battarbee 1994).

Palaeoecological techniques can infer and track whole ecosystem changes as sub-fossil species are preserved from multiple biological groups across all trophic levels. An example of utilising sub-fossil remains to reconstruct chironomid community changes in relation to the succession and disappearance of aquatic macrophytes (Brodersen *et al.*, 2001) is presented in Figure 1.1 below. By combining palaeoecological and contemporary ecological studies (e.g. of modern analogues) it is possible to observe

changes in habitat structure and plant architecture, biodiversity, species succession an trophic structure in response to environmental drivers, especially eutrophication and climate change. This combination of seasonal/inter-annual to decadal/centennial timescales affords a powerful means of understanding ecological changes in patterns and processes on multiple timescales in shallow freshwater systems (Sayer *et al*., 2010a).

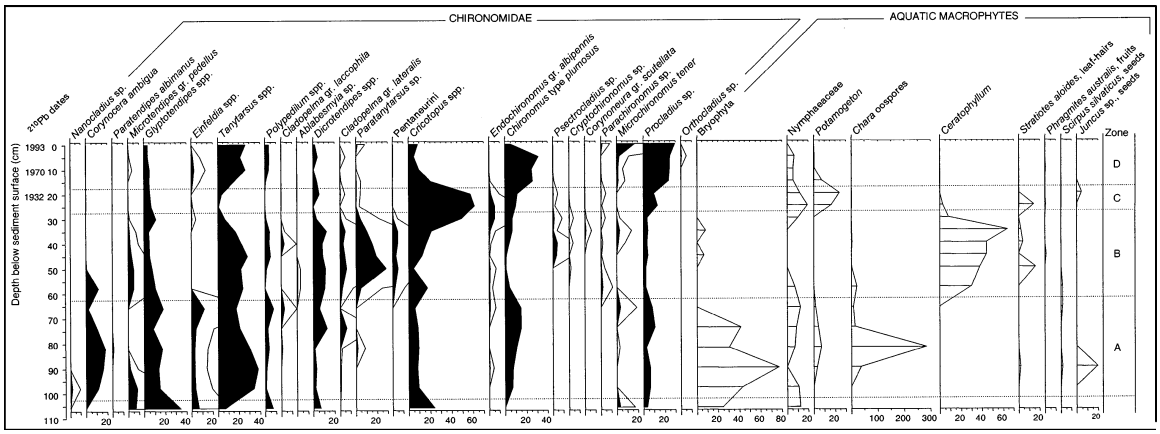


Figure 1.1. Chironomid and aquatic macrophyte stratigraphies for Lake Søbygaard, Jutland, Denmark (Taken from Brodersen *et al.*, 2001).

This thesis will explore the palaeoecological potential of small ponds by employing similar palaeoecological techniques and analyses on a sediment core taken from a small pond. The pond was also subjected to a parallel seasonal monitoring study.

Whilst the focus of palaeoecological techniques and analyses has been on shallow lakes, there has been very little work on the palaeoecology of ponds. Furthermore, there have been no documented studies investigating the ecological impacts and effects of past *Lemna* histories on small ponds, even though *Lemna* dominance is likely to be a major driver of ecological changes. This could be explained by the lack of a tool to determine past *Lemna* dominance, given the known poor preservation of *Lemna* fronds in sediments (Hilary Birks, pers. com.). In addition, *Lemna* rarely produces flowers, resulting in a lack of pollen production (Hillman 1961, Landolt 1986). Therefore, a palaeoecological 'test tool' of a proxy *Lemna* indicator needs to be devised to provide a robust technique to infer past *Lemna* dominance. A possible indirect solution to this problem could be to use the potential association between the diatom, *Lemnicola hungarica* and the Lemnaceae.

1.10 *Lemna*-epiphytic diatom history

Diatoms (Bacillariophyta) are unicellular, eukaryotic, photosynthetic microscopic algae with siliceous walls. The first documented record of a diatom was in 1703 where an English gentleman using a simple microscope looked at the roots of the duckweed *Lemna* and "saw adhering to them (and sometimes separate in the water) many pretty branches, compos'd of rectangular oblongs and exact squares" (Round *et al.,* 1990). This anonymous gentleman's observation was communicated to the Royal Society of London and there is little doubt that the diatom that he reported was *Tabellaria*, probably *Tabellaria flocculosa.* It is remarkable that he came to the conclusion that his rectangles and squares "made up of two parallelograms joyn'd longwise" were indeed plants. On Christmas day in 1702 Van Leeuwenhoek also looked at the roots of *Lemna* from a ditch near Delft in Holland, and he also probably saw diatom species, although he described his findings as 'animalcula'. His recordings and descriptions of diatom-like organisms were published in the Philosophical Transactions of the Royal Society (Van Leeuwenhoek 1703) but it was not possible to identify the diatom species from his personal drawings (Round *et al.,* 1990).

1.10.1 Diatoms and their importance in the aquatic environment

Epiphytic algal communities are an integral part of freshwater ecosystems (Godward 1937, Round 1965, Straskraba & Pieczynska 1970, Wetzel 1975). They are important mediators between freshwater nutrient status and primary productivity (Wetzel 1964, Wetzel & Allen 1970, Brock 1970, Allen 1971, Hickman 1971b, Wetzel *et al.,* 1972, Cattaneo & Kalff 1979); are significant components in the diets of aquatic herbivores (Brook 1975, Mason & Bryant 1975, Denny *et al.,* 1978) and have been used extensively as indicators of water quality (e.g. ter Braak & Van Dam 1989, Anderson *et al.,* 1993, Bennion 1994, O'Connell *et al.,* 1997, Bennion *et al.,* 2001) and for tracking environmental change via palaeolimnology (e.g. Battarbee 1984, Birks *et al.,* 1990a, Charles & Smol 1994). However, even though attached algal assemblages are often dominant primary producers in shallow lentic systems, little is known about their

geographical distributions, population dynamics and microhabitat utilisation (Wetzel 1975, Millie & Lowe 1983).

1.11 Epiphytic diatom assemblage structure

In any given freshwater aquatic system there are a variety of substrates on which benthic diatoms can readily colonise (Round 1981). The diatom species that colonise and form communities on macrophytes are termed the 'epiphyton' or 'periphyton'. The epiphytic diatoms firmly attach to the macrophyte surfaces, where the upright algae extend above the substratum on the end of basal mucilaginous structures, such as stipes, tubes, stalks and apical pads (Round 1981). The adnate or adpressed species position themselves against the substratum (Müller 1999), effectively forming a dense carpet over the substratum where they are motile. Meulemans and Roos (1985) subdivided the periphyton into three layers with the basal layer consisting of adpressed diatoms or diatom species with very short stalks; an intermediate layer consisting of species with long stalks, and a top 'canopy' layer consisting of species that form very long chains of cells (Fig. 1.2).

Hoagland *et al.,* (1982) examined the three dimensional structure of periphyton communities through time and found that their micro-succession is analogous to higher plant succession. The colonisation sequence commenced with an organic coating and bacteria, followed by low profile diatoms, and finally an upper-storey of long-stalked and large-rosette diatoms and filamentous green algae with a consistent change in vertical community structure from low to high physical stature. Diatom mucilage also contributed to the community structure by binding particulates and entrapping other algae and serving as a mechanism for substrate attachment.

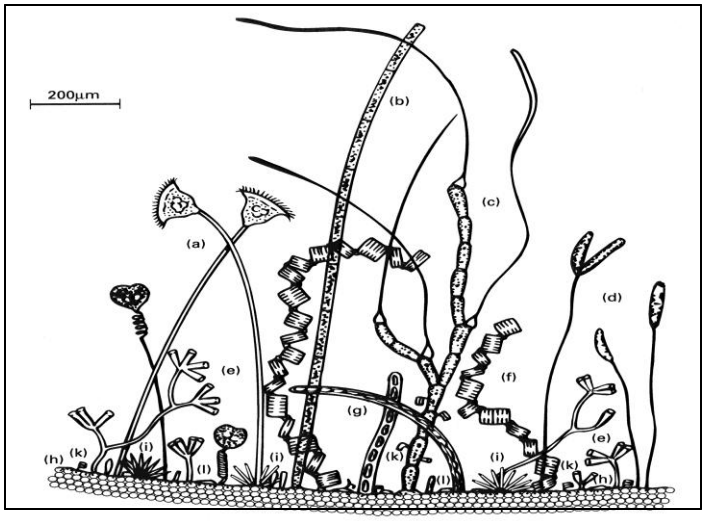


Figure 1.2. Schematic diagram of epiphytic diatom species distinct micro-niches and growth forms on the surface of a macrophyte. [long upright filamentous stalks seen in (a), (b) and (c); the shorter stalks and chains (d), (e), (f) and (g); the rosette forms (i), (j) and (k), and the closely attached and motile adnate forms (h). The epiphytic community structure is analogous to the layers or strata of vegetation seen in higher plant succession. (Taken from Waterford & Driscoll 1992).

These individual layers and their structural complexities are primarily determined by light and nutrient availability and grazing pressure from herbivorous invertebrates, with the thickness of the periphyton layers and their species diversity increasing with duration of the colonisation (Korte & Blinn 1983, Ács & Kiss 1993a). However, species diversity decreases together with a reduction in biomass when the periphyton is exposed to rapid currents and strong wind action (Luttenton *et al.*, 1986, Peterson & Stevenson 1990, Peterson & Hoagland 1990, Ács & Kiss 1993b). This reduction is mainly due to the loss of tall upright species; the adpressed species holding firmly to the substratum giving less resistance to water currents and wave action (Luttenton & Rada 1986, Cattaneo 1990). It is noteworthy that species diversity in the epiphyton is typically less than seen in both epilithic and epipelic habitats (Round *et al.*, 1990).

1.11.1 Relationship between epiphytic diatoms and aquatic macrophytes

The epiphytic diatom communities associated with aquatic macrophytes are living on the surfaces of biologically active and growing plants. Aquatic macrophytes absorb and

secrete substances to the water column (Hasler & Jones 1949, Khailov & Burlakova 1969, Wetzel 1969, Wetzel & Manny 1972) and because of the reduced mixing of water within the macrophyte communities this may alter adjacent water chemistry (Carter 1955, Dvorak 1970, Howard-Williams & Lenton 1975). For example, O'Neill Morin and Kimball (1983) found that dense growths of *Myriophyllum heterophyllum* influenced temperature, dissolved oxygen, pH and light levels in the waters of the littoral zone of Lake Winnipesaukee, New England, U.S.A. Some of these excretions or exudates are considered to be allelopathic with antibiotic effects that discourage the growth of epiphyton (Wium-Andersen *et al.*, 1982, Weaks 1988, Elakovitch & Wooten 1989). Dodds (1991) observed that the filamentous algae, *Cladophora glomerata* inhibited the photosynthetic rate of a pure culture of *Nitzschia fonticola* established from the alga's own epiphyton in a controlled laboratory experiment. Fitzgerald (1969) conducted nutritional studies on cultures of *Myriophyllum* sp., *Ceratophyllum* sp., and *L. minor* and found that they remained relatively free of epiphytes or competing phytoplankton if the cultures were nitrogen limited, and that this antagonistic activity may be due to a 'nitrogen sink' effect in which the aquatic plants prevent the growth of contaminating algae by competition for nitrogen compounds. However, other workers have reported that, where there is enrichment of the nutrient supply and changes in the P: N ratio, there is an increase in periphytic biomass but a reduction in species diversity and changes in species composition (Fairchild *et al.*, 1985, 1989, Fairchild & Everett 1988, Carrick & Lowe 1989, Stevenson *et al.*, 1991).

Grazing may have an important effect on the structure and production of periphyton (Hickman & Round 1970, Hargrave 1970, Elwood & Nelson 1972, Mason & Bryant 1975). It is thought that some aquatic macrophytes have a high rate of new leaf production and discard older epiphytic-laden leaves to combat this heavy inhibiting growth (Sand-Jensen 1983), but many plants tolerate quite dense epiphytic growth (Eminson & Moss 1980). It has been suggested that grazing snails and Ephemeroptera (Mayfly) nymphs favour the easily available and dense epiphytic covered leaves and are, therefore, diverted away from the sensitive growth tips of host plants, thereby conveying some advantage to the host plants (Hutchinson 1975). In a study of fish-invertebrate-periphyton relationships in seventeen shallow lakes, Jones and Sayer (2003) found that

plant biomass was negatively correlated to the density of periphyton, but the density of periphyton on the plants was correlated with the density of grazing invertebrates and not nutrient concentrations, and in turn the biomass of fish determined the density of invertebrates. They concluded that the periphyton appeared to have a stronger influence on plant growth than phytoplankton, and that fish were a prime determinant of community structure in shallow lakes, through a cascading effect of predation on grazing invertebrates which influenced the biomass of periphyton and, therefore, the biomass of the plants. Rogers and Breen (1981, 1983) also noted that snails grazing on the epiphytic community and associated necrotrophic bacteria, which 'condition' the macrophyte host tissues, on *Potamogeton pectinatus* reduced the rate of development of the bacteria and probably extended the life of the plants. Thus it was demonstrated that epiphyte/grazer interactions can play an important role in determining the fate of submerged macrophyte production. This grazing pressure by invertebrate grazers can also alter the floristic composition of the diatom communities by selective grazing on the longer filamentous epiphyton (Allan 1995). Indeed, Hutchinson (1975) considered that the physiochemical factors associated with macrophyte-host surfaces are less important than the external variables in influencing periphyton community composition and structure. Further, the importance of host-plant specificity has been disputed by many other workers (Cholnoky 1927, Fritsch 1931, Simonsen 1962, Main and McIntire 1974, McIntire & Moore 1977).

As the surfaces of macrophytes are not inert but are biologically active, it would be reasonable to expect some degree of macrophyte-epiphyte interaction. An example is the proposed macrophyte-periphyton metabolic interaction model (Wetzel & Allen 1970, Allen 1971) where the epiphytic algal uptake of extra-cellular organic products of macrophytic origin by simple diffusion was demonstrated and confirmed by Allanson (1973). Nonetheless, Carignan and Kalff (1982) estimated that between 3.4-9% of the phosphorus present in the loosely attached fraction of epiphytes was derived from their host macrophytes and, therefore, they obtained most of their phosphorus from the surrounding water. They concluded that macrophytes are principally important as physical supportive structures for the active microbial community rather than as a phosphorous source to their epiphyton and surrounding waters. There is some evidence that carbon is 'leaked' by intact duckweed plants. Wetzel and Manny (1972) reported

0.02-0.07% of recent photosynthetically fixed CO_2 was externally secreted by *Lemna perpusilla* whilst Baker and Farr (1982) reported approximately 2% of fixed carbon from Lemnaceae to be secreted as low molecular weight dissolved organic carbon (DOC). However, the significance of such secretions and their potential uptake and utilisation by epiphytes has again been disputed (Carignan & Kalff 1982).

Several studies have demonstrated host-plant specificity (e.g. Prowse 1959, Pip and Robinson 1985; see Chapter 3) but there is also conflicting evidence. Siver (1997) reported no diatom community composition differences across five macrophyte species. Similarly, Gons (1979) and Millie & Lowe (1983) found no significant differences between collected periphytic assemblages on their sampled macrophytes. Delbecque (1983) compared diatom epiphyton assemblages on the undersides of floating leaves of *Nuphar lutea* and *Nymphaea alba* and found that there was no difference in the diatom flora between the two nymphaeid species. The apparent affinities of some periphytic algae for specific substratum surfaces is thought to be because of factors such as the surface area of the host plant (Rho & Gunner 1978), surface micro-texture (Brown 1976), differential calcium carbonate encrustation between different macrophytes, age of the leaves and also the light variation between the upper and lower leaf surfaces (Allanson 1973, Cattaneo 1978, Cattaneo & Kalff 1978).

The influence of nutrient chemistry in conjunction with the influence of the macrophyte hosts would be expected ultimately to affect the epiphytic communities. Moss (1976) suggested that the type of macrophyte and the external water nutrient levels are both important factors in determining epiphyte composition. Indeed, a comparison between three aquatic macrophyte species in a relatively infertile ‘oligotrophic’, moderately fertile ‘mesotrophic’ and very fertile ‘hyper-eutrophic’ systems revealed high host specificity at low nitrogen and phosphorus levels, and a decreasing degree of specificity at the higher nutrient levels, even though some specificity always persisted (Eminson & Moss 1980).

However, Pip and Robinson (1985) reported considerable specificity in eutrophic waters highlighting further conflict. Furthermore, comparative studies on the same macrophyte types from different sites must be carefully interpreted as community structure

differences may not be solely due to the direct effects of the different external water nutrient chemistries but also to the variations in the macrophyte metabolism at different sites (Pip & Robinson 1985).

In a comparative study between natural and artificial macrophytes (*Potamogetons*) in a phosphorous limited lake, Burkholder and Wetzel (1989) found that mean cell size of loosely attached algae on the artificial leaves was smaller than on natural plants, suggesting that nutrient supplies may have been more limiting on the former. Moreover, they reported that there was a development of distinct epiphytic communities on natural and artificial plants, as both the loosely attached and adnate communities that developed on natural substrata were distinct in taxonomy, cell number and/or biomass from those found on inert artificial surfaces over much of the growing season. This supports previous studies reporting differences in algal communities growing on natural substrata when directly compared with artificial substrata in both mesotrophic and oligotrophic water bodies (Tippett 1970, Cattaneo 1978, Cattaneo & Kalff 1978, Morin 1986) and provides corroborating support for the premise of Eminson and Moss (1980) that the role of macrophytes in eutrophic systems may be secondary to the water column in supplying nutrients for their epiphytes (Burkholder & Wetzel 1989).

1.11.2 Physical and chemical hypotheses of periphyton and substrate

The controversy surrounding the nature of the relationships between periphytic algae growing on substrata within freshwaters is based upon limited evidence (Wetzel 1983), culminating in two distinct claims and hypotheses. The first 'physical hypothesis' states that there is no significant interaction between algae and the substrata upon which they are found; the second 'chemical hypothesis' states that very complex metabolic relationships do exist between the attached microflora and their substrata. However, Wetzel (1983) argues that the first, non-functional, viewpoint may not only direct research away from investigations of periphyton-substrata inter-relationships but it is also based upon insufficient information.

The two viewpoints put forward to explain these associations are based upon the physical or chemical characteristics of the host macrophytes. The 'physical hypothesis' relates to the possibility of unique physical micro-niches provided by the architecture and growth habits of the host plants, to which the attached diatoms directly respond. The alternative 'chemical hypothesis' emphasises the distinct chemical micro-environment existing around the host plant resulting from the active metabolism of the plants, such as nutrient exudates where particular epiphytic algae can utilise the exudates; or the production of allelopathic compounds which inhibit epiphytic growth and production. However, it is entirely feasible that any given association may well be the product of both physical and chemical influences (Goldsborough & Robinson 1985). This physical and/or chemical relationship between host plant and epiphyte has an important bearing on the use of artificial substrata as replicable and uniform bases for the ecological investigations of periphyton (Sládecková 1962, Hickman 1971).

1.11.3 Artificial substrata and epiphyton

Glass microscope slides, 'Perspex', plastic, 'Plexiglas', porcelain, slate, granite, wood, expanded polystyrene, polypropylene ropes and unglazed tiles have all been used extensively since the 1930s (Godward 1937, Newcombe 1949, Patrick *et al.*, 1954, Cooke 1956, Yount 1956, Grzenda & Brehmer 1960, Castenholtz 1960 & 1961, Sládecková 1962, Hohn & Hellerman 1963, Wetzel 1964, Slàdecek & Sládecková 1964, Szczepanski & Szczpanska 1966, Harper & Harper 1967, Wetzel & Westlake 1969, Allen 1971, Rosemarin & Gelin 1978, Gale *et al.*, 1979, Hudon & Bourget 1981, Cattaneo & Amireault 1992, Goldsmith 1996, Kelly *et al.*, 1998).

Many authors have found that the periphyton community closely resembles that found on natural substrata (see Castenholtz 1960, Sládecková 1962, Pieczynska & Spodniewska 1963, Dor 1970, Mason & Bryant 1975). However, other authors have reported significant differences in algal species diversity and abundance between artificial substrata and aquatic macrophytes (see Godward 1934, Tippett 1970, Brown 1976, Foerster & Schlichting 1965). Cattaneo (1978) and Cattaneo and Kalff (1978) observed differences in the composition and distribution of epiphytes between artificial substrata

and natural macrophytes. They concluded that this was largely attributable to the $CaCO_3$ encrustations on the natural plants which reduced light penetration to the leaf surface. However, Losee and Wetzel (1983) and Burkholder and Wetzel (1989) demonstrated that even thick calcium carbonate encrustations only minimally reduced light penetration through periphyton layers. Cattaneo and Kalff (1979) showed that species composition, epiphyte biomass and production were no different on natural *Potamogeton* specimens and their plastic mimics in a mesotrophic system. They concluded that living macrophytes appear to be a neutral substrate for algal growth.

Clearly, the effects of allelochemicals, macrophyte nutrient exudates and macrophyte architecture upon epiphyton community composition are currently poorly understood.

1.12 Overall aims and specific research questions to be addressed

The overall aim of this research is to explore the potential impacts of Lemnids on the ecological structure and function of small freshwater ponds within the agricultural landscape. A major aim is to develop and test a palaeoecological 'tool' for inferring periods of past *Lemna* dominance and then to apply this tool to a case study site. Previous workers have suggested that there is an association between the epiphytic diatom *Lemnicola hungarica* and duckweeds, particularly the Lemnaceae. Indeed, *L. hungarica* has been commonly recorded in high abundances on *L. minor* (Hustedt 1930, Patrick and Reimer 1966, Round 1973 & 1981, Marvan & Komárek 1978, Bowker & Denny 1980, Germain 1981, Zuberer 1984, Goldsborough & Robinson 1985, Goldsborough 1993, Goldsborough 1994, Round & Basson 1997). Furthermore, in a diatom-substrate specificity study of five Lemnaceae species from herbarium specimens, Buczkó (2007) found that *L. hungarica* dominated the diatom assemblages of *L. minor, Lemna gibba*, *Spirodela polyrhiza* and *Wolffia arrhiza*. *L. hungarica* dominated the undersides of the leaf fronds, in marked contrast to the assemblages found on *Lemna trisulca,* which was dominated by *Cocconeis placentula.* Therefore, this thesis will explore diatom-duckweed relationships further by investigating the strength of the association between *L. minor* and *L. hungarica.* The rationale for this is as follows: if there is strong statistical support for a

specific host-plant association, then it may be feasible to employ this association as a proxy indicator of past *L. minor* dominance of a small pond in a palaeoecological study.

The Bodham Rail Pit (North Norfolk, eastern England) is a small farmland pond likely to have been formed by groundwater flooding of an excavated and later abandoned marl pit that is at least two hundred years old. The pond has previously experienced periods or cycles of Lemnaceae dominance in recent times, where the whole surface area was completely covered in dense floating mats (see Chapter 5 for a detailed site description and characteristics).

(i) The specific aim is:

- To explore and assess the palaeoecological potential of ponds using a multi-indicator approach to reconstruct the aquatic history of these relatively under-studied water bodies.

(ii) The specific objectives to be addressed:

- Are there any specific epiphytic diatom species associated with free-floating plants and *L. minor* in particular?
- How strong is the documented association between the epiphytic diatom *L. hungarica* and Lemnaceae, and therefore, can *L. hungarica* be used as a biological proxy to model past Lemnid abundances?

- What is the 'nature' of the relationship between *L. hungarica* and *Lemna*? Is there a nutrient or chemical interaction whereby *L. hungarica* receives leachates from *Lemna* or is the relationship due to the physical location at the water-air interface?

- What is the 'nature' of past *Lemna* abundances in the Bodham Rail Pit? Is there any evidence of cyclicity? What is the ecological impact of dense mats of *L. minor* and also of the recent arrival of the invasive *Lemna minuta*? Is *Lemna* functioning as an

ecological engineer on the structure and function of the plant and animal communities in a small farmland pond?

- Is there any evidence that explosive blooms of *Lemna* have occurred throughout the history of the pond, or are the dense floating mats a direct signal, and a consequence of, the onset of eutrophication? Moreover, what are the potential ramifications for the management of small farmland ponds with respect to their relative importance for maintaining aquatic species richness and diversity?

1.13 Structure and outline of thesis

This study comprises six main sections (Fig. 1.3). Each section is intrinsically linked to each other and there is a logical progression from a contemporary epiphytic diatom investigation and ecological experiments to an analysis of sedimentary fossil diatom assemblages, culminating in a multi-proxy palaeolimnological investigation of a small, shallow freshwater pond. The findings of each stage of the thesis directly inform the development of later stages. The six linked sections or chapters of the thesis are outlined below.

Chapter 2

A description of the pilot study sites and the methods used in the analysis of the pilot study data are presented.

Chapter 3

A global investigation of the epiphytic diatom assemblages associated with various types of freshwater macrophytes was undertaken covering a wide gradient of physical and chemical parameters. The investigation focussed upon the diatom assemblages associated with free-floating plants and *Lemna minor* in particular, to determine whether there is any evidence of a strong and robust association between *L. minor* and the epiphytic diatom, *L. hungarica*. If there is an association, then can this diatom be used as a *Lemna*-indicator species in a palaeolimnological study? Is it feasible that *L. hungarica* can be utilised as a biological proxy indicator of the presence of *L. minor,* or other free-floating plants? To

this end, statistical techniques were employed to analyse patterns and potential associations within the macrophyte and diatom data. The validity of utilising this approach of past *Lemna* abundance was tested by comparing diatom assemblages in surface sediment samples from *Lemna* dominated ponds and from non-*Lemna* ponds.

Chapter 4

The nature of the association and the unequivocal establishment of a specific host-plant relationship between *L. hungarica* and the Lemnaceae is a concern of this study. This chapter complements the findings of Chapter 3 by investigating the mechanism that underpins the relationship between *L. hungarica* and *L. minor*. A series of laboratory-based experiments were undertaken to directly determine the habitat preference, growth rates and micro-distribution of cultured cells of *L. hungarica* on artificial '*Lemna*', axenic and photosynthetically inert *L. minor* and axenic, photosynthetically active *L. minor* under controlled conditions. The hypothesis that there is a statistically significant difference in the relative abundances and growth rates of *L. hungarica* on live biological samples compared with inert artificial surfaces was tested.

Chapter 5

In this chapter *L. hungarica* was utilised as an indicator species for inferring past *Lemna* abundance in a palaeolimnological study of the Bodham Rail Pit, Norfolk, England. This identified any past blooms of *L. minor* and the sequential timing of these potential blooms. The epiphytic/benthic and planktonic diatoms recorded from this stratigraphic investigation were analysed to identify any historical phases between submerged macrophyte dominance of the water column and open water conditions. There have been several major boom-bust blooms of *L. minor*, and more recently *L. minor* with *L. minuta*, over the last 30 year history of the Bodham Rail Pit. The surface sediments of the Bodham Rail Pit, covering the recent history of the pond, were analysed for their diatom assemblages and were directly compared with the known historical *Lemna* dominated periods.

Chapter 6

The simple diatom *Lemna*-indicator model developed in Chapter 5 was applied and compared with the sedimentary macrofossil analysis from the same site (Bodham Rail Pit) and from the same sedimentary core profile (RAIL1). A stratigraphic analysis of the macrofossils included plant and animal macrofossils including seeds, vegetative remains, cladoceran ephippia, fish scales, aquatic invertebrates and ostracod assemblages.

The findings of the stratigraphic diatom analysis, and the presence and timing of *L. hungarica* assemblages in particular, were directly compared with the findings of the stratigraphic microfossil and macrofossil analyses to determine if dense mats of duckweed can indeed be classified as physical ecosystem engineers on small freshwater bodies. This was determined by examining the sub-fossil record to identify the consequences of past *Lemna* abundance to see if there have been losses of submerged plants, invertebrates and fish species.

Furthermore, an analysis of the sedimentary fossil plant pigments from both RAIL1 and RAIL2 sediment cores was undertaken to explore past algal and bacterial community composition (Züllig 1981; Yacobi *et al.,* 1990) of the Bodham Rail Pit. This provided valuable information on past food-web interactions (Leavitt *et al.,* 1989, 1994a, 1994b), changes in the physical structure (Hodgson *et al.,* 1998), the mass flux within the pond (Carpenter *et al.,* 1988) and the past UV radiation environment (Leavitt *et al.,* 1997, 1999). The sedimentary analyses of fossil pigments therefore provided further insight into the anthropogenic impacts on the Bodham Rail Pit, such as eutrophication and changes in land-use practices.

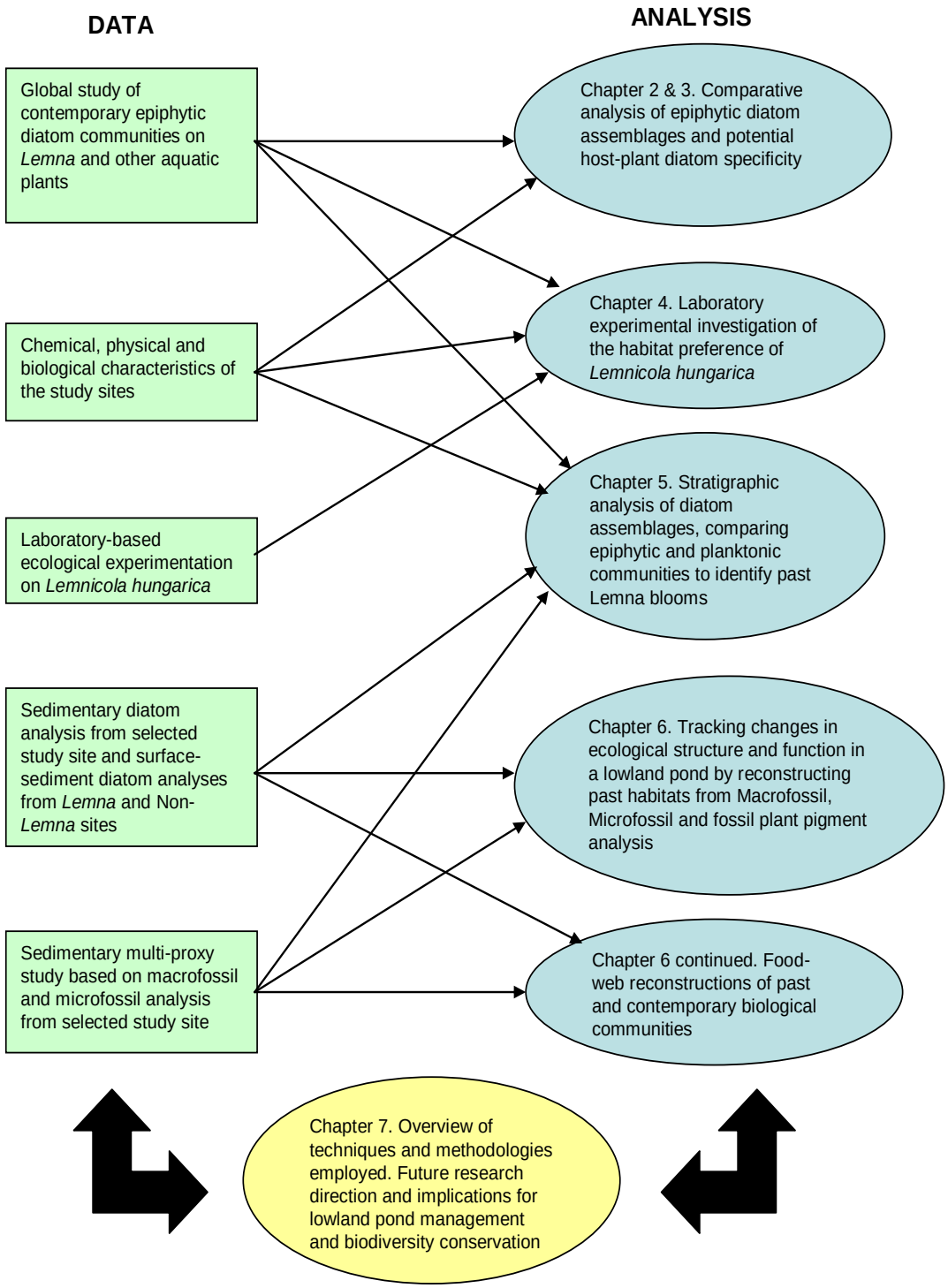
A direct comparison of the species richness of the various communities before and after the onset of agricultural eutrophication was made to inform the effects of increased nutrients on the conservation status of the pond. Moreover, the early history of duckweed at the Bodham Rail Pit was likely represented by the common duckweed, *L. minor*, but since the late 1990s the non-native duckweed, *L. minuta*, has become established and is now the dominant duckweed species at this site. Therefore, a high resolution stratigraphic

analysis of the microfossils from a short sediment core (RAIL2) was undertaken to elucidate the potential impacts upon the biodiversity of a non-native species of duckweed.

Chapter 7

This chapter presents an overview and a summary of the findings and conclusions from the preceding sections. The various problems encountered and their attempted solutions are discussed. An appraisal of the various techniques employed is presented together with a brief discussion of the direction of future research and the implications for the management and conservation of small ponds set within the agricultural landscape.

Figure 1.3. Conceptual diagram of the structure of the thesis outlining the sources of data and an overview of the chapters.



Chapter 2. Pilot study sites and diatom analysis methods

2.1 Introduction

The fundamental aim of the pilot study was to establish whether there are any specific epiphytic diatom species, such as *Lemnicola hungarica*, associated with free-floating plants and *Lemna minor* in particular. To this end, a range of aquatic plants was collected from standing waters around the world and were examined for their epiphytic diatom assemblages. Details of the study sites and methods employed in the pilot study are presented in this chapter.

2.1.2 Pilot study sites

A total of 131 macrophyte samples were collected from 63 sites from ten countries covering the continents of North and South America, Europe, Southern Africa, Australia and Asia. The sites are not ordered systematically along any geographical or chemical gradients. They do, however, cover a diverse range of macrophyte types including free-floating macrophytes and *L. minor* in particular, submerged plants including *Lemna trisulca* and attached-floating leaved-plants (such as Water-lilies and *Potamogeton* species). Of the 63 sites, 49 had recent and readily available, but limited, water chemistry data. The sites vary considerably with respect to water chemistry (e.g. pH, alkalinity, nutrients, conductivity, and colour), water-body characteristics (area, altitude, depth, shoreline extent) and catchment characteristics (geology, hydrology, vegetation, soils). The aim was that the selected water bodies should represent a wide range of environmental conditions, ranging from small shallow artificial ponds to large, deep lakes, and covering a broad gradient of water chemistry characteristics. This approach was deemed necessary to negate any potential biases in terms of biogeographical peculiarities and morphological differences in the sampled macrophytes and their

epiphytic diatom communities that could influence the structure and dynamics of the diatom assemblages (Battarbee *et al.*, 2011a, b).

The European study sites covered Northern Ireland (12 sites), Scotland (3 sites), England (28 sites) and Denmark (6 sites); southern Africa (1 site); Asia including China (1 site), SE China (1 site) and NE China (6 sites); SE Asia (1 site); Australia (2 sites); North America (1 site) and South America (1 site). Of the 28 sites in the UK, 20 were based in Norfolk, E. England (see Sayer *et al*., 2010b). The six Danish sites in the study were selected from Davidson (2006) and Davidson *et al*., (2007).

An example of the dominance of free-floating mats of lemnids (*Lemna* cf. *aequinoctialis* and *Lemna gibba*) is shown in the widespread cover of Inner Puno Bay, Lake Titicaca, Peru (Fig. 2.1). These free-floating mats are approximately 5cm thick and cover an area ranging from 179 to 393 hectares in Inner Puno Bay and the mats are regularly harvested for cattle fodder (Cruz *et al*., 2006).

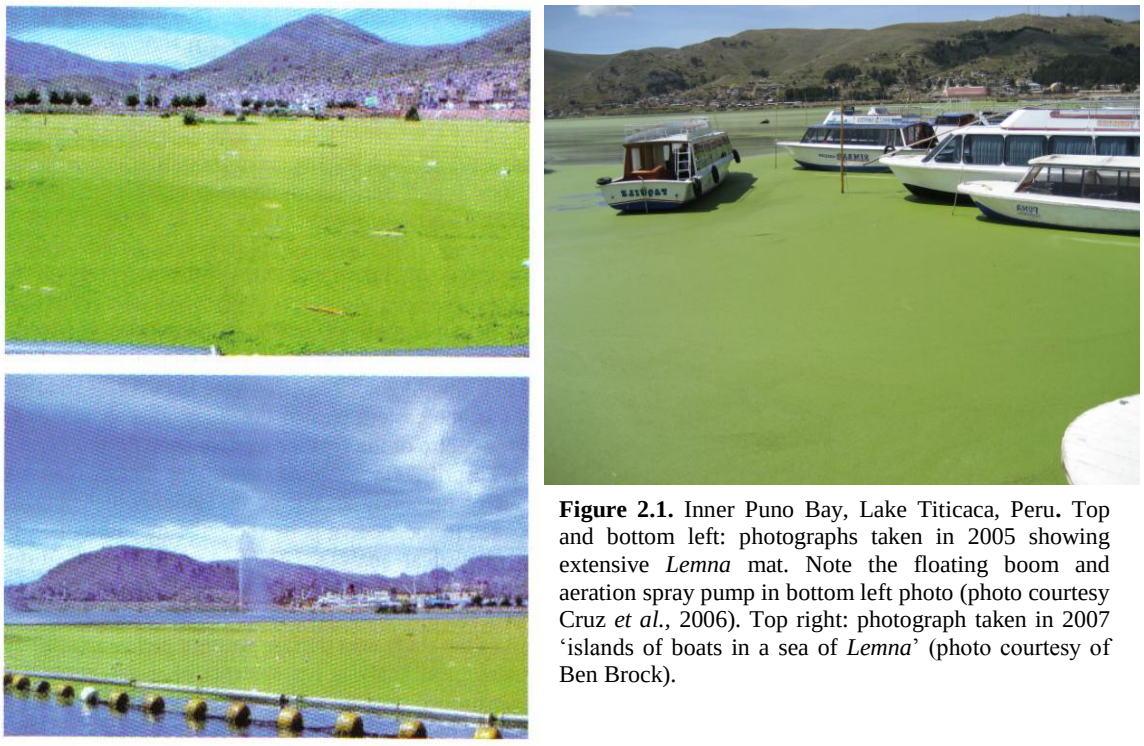


Figure 2.1. Inner Puno Bay, Lake Titicaca, Peru**.** Top and bottom left: photographs taken in 2005 showing extensive *Lemna* mat. Note the floating boom and aeration spray pump in bottom left photo (photo courtesy Cruz *et al.*, 2006). Top right: photograph taken in 2007 'islands of boats in a sea of *Lemna*' (photo courtesy of Ben Brock).

Further details of the specific site locations, the macrophyte species samples and ecological types, and the physical morphometric characteristics of the sites are given in Table 2.1. The water chemistry data available for the 49 sites are given in Table 2.2. The locations of the thirteen worldwide study sites are presented in Figure 2.2.

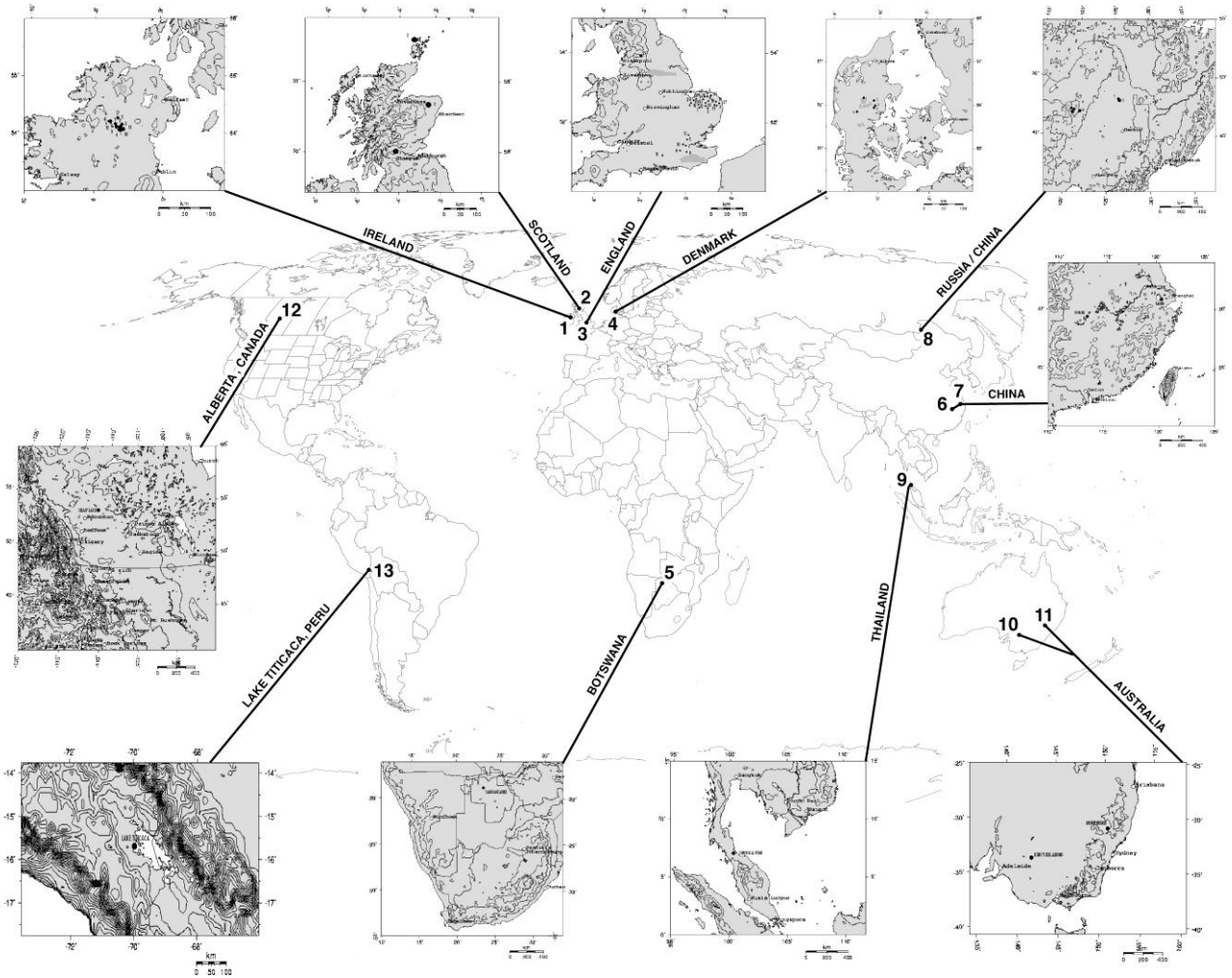


Figure 2.2. Location and distribution of the thirteen global study sites (Europe, Africa, Asia, SE Asia, Australia, N. America and S. America) where a variety of freshwater macrophyte species covering a broad range of ecological types were collected and sampled for their epiphytic diatom assemblages.1 - Northern Ireland; 2 – Scotland; 3 – England; 4 – Denmark; 5 – Botswana; 6 – S China; 7 – SE China; 8 – NE China; 9 – Thailand; 10 – Victoria, Australia; 11 – New South Wales (NSW), Australia; 12 – Alberta, Canada; 13 – Peru. (See Tables 2.1 and 2.2 for specific site descriptions of the 63 water bodies and the identification of the 131 macrophyte samples).

2.2 Methods

2.2.1 Macrophyte sampling

A total of 131 freshwater macrophyte samples, from 39 different species, were harvested from the littoral margins of the pilot study sites. The free-floating samples were collected by carefully teasing whole plants (fronds and roots) from monocultural mats where possible. These were placed into sterile plastic or glass specimen vials by gently detaching intact plants (by hand) in order to minimize any potential loss of loosely attached epiphytic diatoms. The leaves of attached-floating plants, such as *Nuphar* and *Nymphoides*, were carefully excised from their supporting stems and placed into plastic sampling bags and distilled water was added. Submerged specimens, including *Lemna trisulca* were gently placed into plastic sampling bags, labelled and sealed for safe transportation to the laboratory and stored in the refrigerator prior to analysis.

As several of the macrophyte samples were collected from isolated sites around the world and forwarded to the laboratory invariably these samples arrived degraded and, therefore, some samples were impossible to identify to species level, but could be identified to the level of genus (i.e. *Potamogeton*, *Nymphoides*, *Sparganium*, *Ceratophyllum*, *Utricularia*, *Myriophyllum*, *Chara* and *Littorella*). Furthermore, this meant that a quantitative analysis of the density of the epiphytic diatoms was not possible. However, as the primary aim of this investigative pilot study was to examine the diatom assemblages as a qualitative analysis, a count of the relative abundances of the diatoms was considered to be both adequate and appropriate. Many of the macrophyte samples were collected on more than one occasion throughout the year as a way of incorporating and negating the potential seasonal variation effects of the epiphytic diatoms and their host plants.

For the scientific integrity of studying the distribution and potential host-plant specificity of *L. hungarica* on Lemnaceae species it was essential to sample duckweed mats consisting of just one species and not a composite sample taken from a community of

several Lemnaceae species; thus as far as practically possible Lemnaceae samples were collected from monocultural mats.

As the primary aim was to investigate the host-plant diatom specificity of the Lemnaceae in general and *Lemna minor* in particular, 45 of the 131 macrophyte samples (i.e. 34%) collected comprised of *L. minor*. Other free-floating macrophytes sampled included *Lemna minuta, Lemna gibba, Lemna* cf. *aequinoctialis, Spirodela polyrhiza, Wolffia arrhiza, Azolla filiculoides, Azolla pinnata, Riccia fluitans, Salvinia natans* and *Salvinia molesta* (i.e. 19%). Over half (i.e. 53%) of the total macrophytes sampled for their epiphytic diatom assemblages comprised of free-floating macrophytes. As well as sampling the epiphytic floras of free-floating macrophytes, submerged and emergent (attached-floating) macrophytes were also included in this study to determine whether *L. hungarica* is restricted to the Lemnaceae. Table 2.1 provides details of all the macrophyte samples collected.

2.2.2 Epiphytic diatom slide preparation

The macrophyte samples were transferred into individual 250 ml glass beakers, after the beakers were cleaned by dissolving sodium hydroxide (NaOH) in distilled water, and then thoroughly rinsed out with distilled water. Samples were then digested to remove residual organic matter, a process that also facilitated removal of epiphytic diatoms from the macrophyte surfaces. Digestion involved oxidizing the macrophyte samples by boiling them in distilled water together with 20-30 ml of 30% hydrogen peroxide (H_2O_2) on a hotplate set at 90^{o}C situated within a fume cupboard for between 2-6 hours. After excessive effervescence had finished, the beakers were 'topped-up' with distilled water to prevent the samples from drying out.

The chemical digestion process was augmented by vigorously shaking and stirring the samples. Bowker *et al*., (1986) concluded that 100% removal of epiphyton is rarely achieved and Cattaneo and Kalff (1979) reported that simply shaking macrophytes in water removed a highly variable proportion (6-68%) of the epiphyton. However, Gough

and Woelkerling (1976) found that employing both a hydrolyzing agent in conjunction with shaking resulted in a higher removal efficiency of epiphyton to 96-99.7%. This efficient 'dual' technique was employed in this study and resulted in complete oxidization of the free-floating macrophytes and almost complete oxidization of the physically bulkier submerged and attached-floating macrophytes, giving greater confidence that practically all the epiphytic diatom frustules present could be sampled.

After all of the organic material was oxidized the beakers were allowed to cool to room temperature. The H_2O_2 was removed from the samples by rinsing the beakers for a minimum of four times with distilled water and leaving at least 24 hours between washes as an adequate settling interval. This allowed the diatoms to sink and to settle on the bottom of the beaker. The sides of the beakers were rinsed with distilled water to wash any diatoms into the beaker bottom and then covered with 'cling film' to prevent deposition of extraneous material. The supernatant was decanted using a water suction pump so as not to disturb the bottom 50 ml which contained the diatom frustules (modified from Battarbee 1986, Barker 1990). This particular settling method was used as the centrifuge method can cause damage and breakage to some delicately silicified diatom frustules, hampering identification.

After all traces of H_2O_2 had been rinsed from the diatom suspension it was diluted with distilled water to a volume that would yield a suitable and homogenized concentration of diatom valves for microscope slide preparation (the suspension looked neither totally clear nor milky in appearance to the naked eye). Using a 1ml micro-pipette, 0.5ml quantity of solution was evenly spread onto grade 0, 19mm circular glass cover-slips placed on a clean metal settling-out tray. The tray was covered to prevent any airborne dust from contaminating the cover-slips and left to evaporate at room temperature over a period of about 48 hours. A small drop of Naphrax^{TM} was placed on glass microscope slides, and the cover-slips were carefully inverted with the dried diatoms placed directly over the high optical mountant Naphrax$^{TM.}$ The slides were then placed on a hotplate at 130^{o}C in the fume cupboard for approximately 15 minutes to drive off the toluene content of the Naphrax$^{TM.}$ After cooling at room temperature, the cover-slips were

checked to see if they were securely fixed to the slide. The permanent mounted slides were carefully labeled.

2.2.3 Diatom slide preparations from surface sediments

In conjunction with producing epiphytic diatom preparations from macrophytes, diatom slide preparations were also produced from the surface sediments collected from *Lemna* (25-100% water surface cover; n=12) and from non-*Lemna* covered ponds (n=14) in Norfolk, England. A gravity Glew corer (Glew 1991) and a ladle attached to a long metal rod ('Pond Putter') were used to carefully collect surface sediment samples for diatom analysis. This space-for-time study was undertaken to determine if diatoms, especially epiphytic diatom taxa, were successfully transferred to surface sediments from the floating mats of *Lemna*. The successful deposition of epiphytic diatoms from the *Lemna* mats to the surficial sediments is a fundamental prerequisite to enable future palaeolimnological studies of past diatom assemblages to be undertaken with confidence. The diatom slide preparations followed standard methods (Battarbee 1986, Battarbee *et al*., 2001). All samples were mounted on microscope slides using Naphrax$^{TM.}$ and absolute numbers of diatoms present in 0.1g of sediment were counted using a light microscope at x1000 magnification (see paragraph 2.2.2).

2.2.4 Diatom counts

As the fundamental purpose of the diatom analysis was to identify any potential host-plant and diatom specificity, which could indicate any potential diatom 'indicator species' of specific macrophyte species, it was considered appropriate to count a minimum of 500 valves (Lund *et al*., 1958, Battarbee 1986). The transect method was employed to enable maximum coverage of the slide for good representation of the diatoms. Given that eutrophication reduces the number of rare species and increases the abundance of meso-eutraphentic to hyper-eutraphentic species (Van Dam & Mertens 1993), a high count was deemed necessary to reduce the dominance of common diatom species whilst also allowing rarer taxa to be captured. Furthermore large counts were

required to identify any associated interspecific competition effects of common diatom species by increasing the probability of the numbers of diatom species found (Hughes 2002). Several slides had insufficient numbers of valves to use the transect method so the whole slide was counted to obtain the required 500 valves for analysis. Diatom valves were identified to species level and counted at 1000x magnification with an oil immersion lens under phase-contrast illumination using a Leitz 'Laborlux S' light microscope. The relative abundances of each of the diatom species per sample were calculated. The main taxonomic keys and nomenclature followed were Krammer and Lange-Bertalot (1986-1991, 1997, 2000, 2001, 2002, 2004), Round and Basson (1997), Sonneman *et al.,* (1999), Patrick and Reimer (1966), Schoeman and Archibald (1976) and Round *et al.,* (1990).

2.2.5 Water chemistry

Conductivity and pH were measured in field using pre-calibrated meters. Total alkalinity was also measured in the field using a digital Hach® field titration kit. Total phosphorous (TP) was determined using the method described by Johnes and Heathwaite (1992). Nitrate-nitrogen (NO_3-N) and soluble reactive phosphorous (SRP) were determined using standardised methods (see APHA 1990, Murphy & Riley 1962).

2.2.6 Numerical methods

To explore the variation in the biological data, indirect ordination methods of detrended correspondence analysis (DCA), correspondence analysis (CA), non-metric multidimensional scaling (NMDS) with Bray-Curtis distance, homogeneity test of multivariate dispersion (HMD), analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (ADONIS) were used to identify the gradients of diatom species composition change, dissimilarities of species composition and an assessment of statistically significant differences in species assemblages between the different macrophyte species and their ecological groups/types. Canonical correspondence analysis (CCA) and linear regression were also performed to explore the relationships between the water chemistry variables and the biological data. Ordination analyses were performed

using CANOCO 4.5 program (ter Braak & Šmilauer 2002), and NMDS plots were produced using PC-ORD metaMDS of the *R* language programme, dispersion (Anderson 2006). Macrophyte samples/diatom species assemblage dissimilarities and the statistical analyses ANOSIM and ADONIS were performed using the vegan package in *R* (Oksanen *et al*., 2011). The data were further explored using post hoc analysis of variance (ANOVA), Dunnett's t-tests and exploratory diatom boxplots to compare the differences in epiphytic diatom assemblages between the macrophyte groups, with analyses performed using SPSS version 13.0 (SPSS, Inc., 2004). To identify any potential diatom-macrophyte indicator species, two different methodologies were employed. TWINSPAN (Two Way Indicator Species Analysis, Hill 1979, Hill *et al.,* 1975) was performed using WinTWINS version 2.3 (Hill & Šmilauer 2005) and INDVAL was performed using Indicator Species Analysis in PC-ORD 4.2 software (McCune & Grace 2002). TWINSPAN produces a tabular matrix arrangement approximating the results of a Braun-Blanquet table (Dufréne & Legendre, 1997). INDVAL was used to explore potential host-plant specificities of diatoms with their host-plant ecological groups.

The TWINSPAN dendrograms were based *a priori* upon the main pre-defined macrophyte groups. INDVAL analysis requires that two or more groups of samples with species abundances are provided prior to the analysis (McCune & Grace, 2002). The relative abundance of a diatom species is combined with its relative frequency of occurrence in the various groups of sites (Dufréne & Legendre, 1997), or macrophyte samples in this analysis. In contrast to TWINSPAN, INDVAL is a flexible asymmetric classification where its value is highest (maximum) when all individuals of a species occur in a group of sites (i.e. macrophyte ecological groups) and when the species is present in all the sites of that group. This indicator value is defined as the 'Maxgroup' for the diatom species and statistically identifies the macrophyte group indicated by diatom species s*pecificity*, the extent to which a species is found only in that group, and diatom species *fidelity*, the measure of the proportion of the samples of a group where the species is found in (Legendre & Birks, 2012). Furthermore, the INDVAL index of one given species is independent from other species percentage abundances and therefore arbitrary

uses of pseudo-species (as used in TWINSPAN) are not necessary with INDVAL analysis (Dufréne & Legendre, 1997).

2.3 Availability of supporting data

The collection of freshwater macrophyte samples from several countries and continents was kindly provided by other workers at the Environmental Change Research Centre (ECRC) University College London, as part of concurrent freshwater surveys and monitoring programmes. The *L. minor* sample collected from Santantadibe, Botswana was part of the Darwin Initiative Project, funded by DEFRA (Department for the Environment, Food and Rural Affairs) investigating the aquatic biodiversity of the Okavango Delta. Macrophyte samples from NE China were collected by colleagues at the Chinese Academy of Sciences, Beijing and the free-floating macrophytes from Lake Titicaca were collected by the Universidad Nacional del Altiplano, Puno, Peru. The *Salvinia molesta* samples from Thailand were kindly provided by K.P. Ruddy.

Water chemistry data for many of the UK sites were made available by Carl Sayer of the ECRC (Jones & Sayer 2003, Sayer et *al*., 2008). The water chemistry data for the Northern Ireland sites were made available by ENSIS Ltd; data for the NE China sites were made available by the Chinese Academy of Sciences, Beijing; data for the Botswana site were provided by the Darwin Initiative Project; data for Malham Tarn were kindly provided by Natural England; data for Lake Titicaca were made available by the Freshwater Biological Association and the data for the Danish sites was provided by the ECRC and courtesy of the Natural Environment Research Institute (NERI), Silkeborg, Denmark. The site description details of Alresford Lake were made available by the School of Geography, University of Southampton.

Table 2.1. Summary of the key descriptive data for the 63 pilot study sites including site location, macrophyte sample and ecological type, date collected and the specific site number used in the Correspondence Analysis Ordination diagrams.

Key: A – Australia, B – Botswana, Ca – Canada, Ch – China, D – Denmark, E – England, NEC – North East China (Inner Mongolia & Heilongjiang) NI – Northern Ireland, P – Peru, S – Scotland, T – Thailand. Lake Titicaca (a) – Inner Bahia de Puno; Lake Titicaca (b) – Bahia de los Incas; Lake Titicaca (c) – Bahia Interna Huaje. * Extreme outliers and excluded from analysis. NB. Free-floating leaves and submerged roots (i.e. modified leaves) were analysed separately (see Figures 3.3 & 3.4 below), *n/a* – data not available).

Site No.	Site Name	Country	Latitude	Longitude	Macrophyte	Ecological Type	Date	Surface Area (ha)	Maximum Depth (m)
1	Sichi (i)	NEC	48º 46′N	126º 11′E	*Lemna minor*	Free-floating	11.7. 2006	10.4	3
2	Døj Sø (i)	D	56º 01′ 45. 04″N	9º 54′24. 18″E	*Lemna minor*	Free-floating	16.6. 2003	2	2.5
3	Døj Sø (ii)	D	56º 01′ 45. 04″N	9º 54′24. 18″E	*Lemna minor*	Free-floating	15.7. 2003	2	2.5
4	Shaw lake (i)	Ca	54º 45′ 43. 67″N	111º 49′ 19. 71″W	*Lemna minor*	Free-floating	1.9. 2006	4.5	n/a
5	Gub Sø (i)	D	56º 12′ 07. 09″N	9º 31′ 45. 50″E	*Lemna minor*	Free-floating	18.6.2003	0.6	1.5
6	En Sø (i)	D	55º 56′ 6. 39″N	9º 20′ 48. 28″E	*Lemna minor*	Free-floating	6.8.2003	10.6	3.5
7	Beeston Hall Lake (i)	E	52º 9′ 13. 63″N	1º 2′ 95. 28″E	*Lemna minor*	Free-floating	20.6.1999	2.6	2.5
8	Gub Sø (ii)	D	56º 12′ 07. 09″N	9º 31′ 45. 50″E	*Lemna minor*	Free-floating	9.6.2003	0.6	1.5
9	Beeston Hall Lake (ii)	E	52º 9′ 13. 63″N	1º 2′ 95. 28″E	*Lemna minor*	Free-floating	14.7.1999	2.6	2.5
10	Strumpshaw Broad	E	52º 36′ 25. 19″N	1º 27′ 13. 73″E	*Lemna minor*	Free-floating	9.9.1999	2.8	2.5
11	Cromes Broad	E	52º 43′ 22. 75″N	1º 30′ 53. 53″E	*Lemna minor*	Free-floating	10.8.1999	2.3	3
12	Gub Sø (iii)	D	56º 12′ 07. 09″N	9º 31′ 45. 50″E	*Lemna minor*	Free-floating	9.6.2003	0.6	1.5
13	Gub Sø (iv)	D	56º 12′ 07. 09″N	9º 31′ 45. 50″E	*Lemna minor*	Free-floating	9.6.2003	0.6	1.5
14	Little Downham Pond	E	52º 25′ 14. 84″N	0º 14′ 44. 09″E	*Lemna minor*	Free-floating	28.8.2006	0.3	2.5
15	Sorte Sø (i)	D	56º 02′ 01.06″E	9º 54′ 53. 13″E	*Lemna minor*	Free-floating	15.7.2003	4.6	3.5
16	Cornabrass Lough (i)	NI	54º 10′ 01″N	7º 23′ 12″W	*Lemna minor*	Free-floating	21.7.2006	20.7	2.5
17	Cheshunt Pit 2C	E	51º 42′ 43. 62″N	0º 1′ 14. 06″W	*Lemna minor*	Free-floating	22.8.2006	21	2.2
18	Bodham Rail Pit (i)	E	52º 54′ 20. 62″N	1º 09′ 21. 23″E	*Lemna minor*	Free-floating	21.4.2006	0.12	1.65
19	Gammelmose	D	55º 25′ 01. 93″N	10º 38′ 12. 24″	*Lemna minor*	Free-floating	19.6.2003	1.6	2.5
20	Corraleash Lough	NI	54º 8′ 85″N	7º 27′ 72″W	*Lemna minor*	Free-floating	25.7.2006	7	1.6
21	Wandsworth Common (i)	E	51º 26′ 56. 39″N	0º 10′ 6. 81″W	*Lemna minor*	Free-floating	23.7.2003	1.5	1.2
22	Papercourt Small Lake	E	51º 17′ 38. 77″N	0º 30′ 40. 50″E	*Lemna minor*	Free-floating	21.6.2006	1.6	1
23	Hedgecourt Lake	E	51º 8′ 85. 96″N	0º 3′ 59. 27″W	*Lemna minor*	Free-floating	20.5.2006	5	1.1
24	Beeston Hall Lake (iii)	E	52º 9′ 13. 63″N	1º 2′ 95. 28″E	*Lemna minor*	Free-floating	20.8.1999	2.6	2.5
25	Wandsworth Common (ii)	E	51º 26′ 56. 39″N	0º 10′ 6. 81″W	*Lemna minor*	Free-floating	23.7.2003	1.5	1.2
26	Beeston Hall Lake (iv)	E	52º 9′ 13. 63″N	1º 2′ 95. 28″E	*Lemna minor*	Free-floating	11. 9.1999	2.6	2.5
27	London Wetland Centre	E	51º 28′ 42. 84″N	0º 13′ 47. 29″W	*Lemna minor*	Free-floating	15.2.2006	42	n/a
28	Dichi Pond	NEC	47º 18′ 14. 75″N	120º 26′ 35. 76″E	*Lemna minor*	Free-floating	7.7.2006	0.03	0.5
29	Derrymacrow Lough (i)	NI	54º 10′ 42″N	7º 26′ 34″W	*Lemna minor*	Free-floating	20.7.2006	21	4.4

30	Upper Lough Erne (i)	NI	54º 15′ 5. 73″N	7º 34′ 51. 32″W	*Lemna minor*	Free-floating	16.8.2006	5835	22.7
31	Sorte Sø (ii)	D	56º 02′ 01. 06″N	9º 54′ 53. 13″E	*Lemna minor*	Free-floating	1.8.2003	4.6	3.5
32	Derrykerrib Lough (i)	NI	54º 8́′ 02″N	7º 22′ 95″W	*Lemna minor*	Free-floating	22.7.2006	24.5	1.6
33	Alresford Lake (i)	E	51º 5′ 37. 78″N	1º 9′ 32. 71″W	*Lemna minor*	Free-floating	5.10.2006	52	2
34	Salhouse Little Broad	E	52º 41′ 27. 60″N	1º 25′ 11. 84″E	*Lemna minor*	Free-floating	22.8.1999	1.2	2
35	Upper Lough Erne (ii)	NI	54º 15′ 5. 73″N	7º 34′ 51. 32″W	*Lemna minor*	Free-floating	16.8.2006	5835	22.7
36	Balls Wood Ponds (i)	E	51º 46′ 58. 23″N	0º 3′ 16. 23″W	*Lemna minor*	Free-floating	7.4.2006	0.15	0.5
37	Lough Sarah (i)	NI	54º 8′ 54. 24″N	7º 22′13. 08″W	*Lemna minor*	Free-floating	23.7.2006	3	1
38	Santantadibe	B	19º 65′ 81. 50″S	23º 34′ 60. 40″E	*Lemna minor*	Free-floating	12.9.2006	2	0.5
39	Briston Pond (i)	E	52º 51′ 03. 57″N	1º 03′ 53. 83″E	*Lemna minor*	Free-floating	20.4.2006	0.05	1
40	Doagh Lough	NI	54º 25′ 03″N	7º 52′ 83″W	*Lemna minor*	Free-floating	31.7.2006	5	4
41	Lowes Pond (i)	E	52º 55′ 29. 87″N	1º 5′ 15. 50″E	*Lemna minor*	Free-floating	14.8.1999	0.5	2.5
42	Lowes Pond (ii)	E	52º 55′ 29. 87″N	1º 5′ 15. 50″E	*Lemna minor*	Free-floating	12.9.1999	0.5	2.5
43	Lough Corry	NI	54º 15′ 05. 37″N	7º 23′ 24. 77″W	*Lemna minor*	Free-floating	28.7.2006	6.5	8
44	Blickling Hall Lake (i)	E	52º 48′ 72. 90″N	1º 13′ 90. 80″E	*Lemna minor*	Free-floating	30.9.2006	10.1	4
45	Bayfield Hall Lake	E	52º 55′ 37. 30″N	1º 2′ 61. 79″E	*Lemna minor*	Free-floating	12.8.1999	2.7	3
46	Balls Wood Ponds (ii)	E	51º 46′ 58. 23″N	0º 3′ 16. 23″W	*Lemna minuta*	Free-floating	7.4.2006	0.15	0.5
47	Bodham Rail Pit (ii)	E	52º 54′ 20. 62″N	1º 09′ 21. 23″E	*Lemna minuta*	Free-floating	21.4.2006	0.12	1.65
48	Balls Wood Ponds (iii)	E	51º 46′ 58. 23″N	0º 3′ 16. 23″W	*Lemna minuta*	Free-floating	7.4.2006	0.15	0.5
49	Beeston Hall Lake (v)	E	52º 9′ 13. 63″N	1º 2′ 95. 28″E	*Lemna gibba*	Free-floating	20.8.1999	2.6	2.5
50	Lake Titicaca (a)	P	15º 50′40.22″S	70º 0′ 55. 23″W	*Lemna gibba*	Free-floating	22.2.2007	837,200	281
51	Lake Titicaca (a)	P	15º 50′40. 22″S	70º 0′ 55. 23″W	*L.*cf. *aequinoctialis*	Free-floating	22.2.2007	837,200	281
52	Lake Titicaca (b)	P	15º 49′57. 02″S	70º 0′ 56. 74″W	*L.*cf.*aequinoctialis*	Free-floating	22.2.2007	837,200	281
53	Lake Titicaca I	P	15º 49′30. 25″S	70º 0′ 0. 34″W	*L.*cf. *aequinoctialis*	Free-floating	22.2.2007	837,200	281
54	Døj Sø (iii)	D	56º 01′ 45. 04″N	9º 54′ 24. 18″E	*Lemna trisulca*	Submerged	16.6.2003	2	2.5
55	Denderup (i)	D	55º 15′ 0. 66″N	11º 57′ 19. 13″E	*Lemna trisulca*	Submerged	9.7.2003	4.5	3
56	Døj Sø (iv)	D	56º 01′ 45. 04″N	9º 54′ 24. 18″E	*Lemna trisulca*	Submerged	15.7.2003	2	2.5
57	En Sø (ii)	D	55º 56′ 6. 39″N	9º 20′ 48. 28″E	*Lemna trisulca*	Submerged	18.6.2003	10.6	3.5
58	Scottow Pond (i)	E	52º 46′ 37. 68″N	1º 8′ 26. 98″E	*Lemna trisulca*	Submerged	19.5.1999	2	2.5
59	Knockballymore Lough	NI	54º 11′ 19″N	7º 16′ 4″W	*Lemna trisulca*	Submerged	13.8.2006	15	12.5
60	Scottow Pond (ii)	E	52º 46′ 37. 68″N	1º 8′ 26. 98″E	*Lemna trisulca*	Submerged	15.8.1999	2	2.5
61	Summerhill Lough	NI	54º 11′ 51″N	7º 14′ 55″W	*Lemna trisulca*	Submerged	14.8.2006	5	11.3
62	Selbrigg Pond	E	52º 54′ 49. 37″N	1º 11′ 47. 78″E	*Lemna trisulca*	Submerged	13.5.1999	3.1	3.5
63	Burdautien Lough (i)	NI	54° 11′ 56”N	7° 14′ 33”W	*Lemna trisulca*	Submerged	14.8.2006	6.5	7.8
64	Scottow Pond (iii)	E	52° 46′ 37. 68”N	1° 8′ 26. 98”E	*Lemna trisulca*	Submerged	9.9.1999	2	2.5
65	Denderup (ii)	D	55° 15′ 0. 66”N	11° 57′ 19. 13”E	*Lemna trisulca*	Submerged	13.8.2003	4.5	3
66	Upper Lough Erne (iii)	NI	54° 15′ 5. 73”N	7° 34′ 51. 32”W	*Lemna trisulca*	Submerged	16.8.2006	5835	22.7
67	Tai Hu Lake (i)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Spirodela polyrhiza*	Free-floating	26.4.2006	233,800	4
68	Hong Hu lake (i)	Ch	29° 54′ 14. 68”N	113° 16′ 19. 64”E	*Spirodela polyrhiza*	Free-floating	10.9.2004	34,800	2.2
69	Corracoash Lough (i)	NI	54° 8′ 85”N	7° 27′ 72”W	*Spirodela polyrhiza*	Free-floating	25.7.2006	7	1.6
70	Tai Hu Lake (ii)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Spirodela polyrhiza*	Free-floating	2.11.2006	233,800	4
71	Derrykerrib Lough (ii)	NI	54° 8′ 02”N	7° 22′ 95”W	*Spirodela polyrhiza*	Free-floating	22.7.2006	24.5	1.67
72	Lough Sarah (ii)	NI	54° 8′ 54. 24”N	7° 22′ 13. 08”W	*Spirodela polyrhiza*	Free-floating	23.7.2006	3	1
73	Derrymacrow Lough (iii)	NI	54° 10′ 42”N	7° 26′ 34”W	*Spirodela polyrhiza*	Free-floating	20.7.2006	21	4.4
74	Upper Lough Erne (iv)	NI	54° 15′ 5. 73”N	7° 34′ 51. 32”W	*Spirodela polyrhiza*	Free-floating	16.8.2006	5835	22.7
75	Shaw Lake (ii)	Ca	54° 45′ 43. 67”N	111° 49′ 19. 71”W	*Wolffia arrhiza*	Free-floating	1.9.2006	4.5	n/a

76	Murrurundi	A	31° 45′ 51. 19”S	150° 50′ 8. 72”E	*Azolla filiculoides*	Free-floating	15.1.2007	2.5	n/a
77	Shaw Lake (iii)	Ca	54° 45′ 43. 67”N	111° 49′ 19. 71”W	*Azolla filiculoides*	Free-floating	1.9.2006	4.5	n/a
78	Lake Titicaca (a)	P	15° 50′ 40. 22”S	70° 0′ 55. 23”W	*Azolla filiculoides*	Free-floating	22.2.2007	837,200	281
79	Tai Hu Lake (iii)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Azolla filiculoides*	Free-floating	2.11.2006	233.800	4
80	King’s Billabong	A	142° 13′ 25. 18”S	34° 14′ 32. 38”E	*Azolla pinnata*	Free-floating	7.11.2006	200	n/a
81	Briston Pond (ii)	E	52° 51′ 03. 57”N	1° 03′ 53. 83”E	*Riccia fluitans*	Free-floating	20.4.2006	0.05	1
82	Tai Hu Lake (iv)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Salvinia natans*	Free-floating	2.11.2006	233,800	4
83	Tai Hu Lake (v)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Trapa natans*	Attached-floating	2.11.2006	233.800	4
84	Sichi (ii)	NEC	48° 46′N	126° 11′E	*Trapa natans*	Attached-floating	11.7.2006	10.4	3
85	Tai Hu Lake (vi)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Trapa natans*	Attached-floating	2.11.2006	233.800	4
86	Sanchi	NEC	48° 43′N	126° 13′E	*Trapa natans*	Attached-floating	11.7.2006	221	3.6
87	Sichi (iii)	NEC	48° 46′N	126° 11′E	*Trapa natans*	Attached-floating	11.7.2006	10.4	3
88	Sichi (iv)	NEC	48° 46′N	126° 11′E	*Potamogeton natans*	Attached-floating	11.7.2006	10.4	3
89	Upper Lough Erne (v)	NI	54° 15′ 5. 73”N	7° 34′ 51. 32”W	*P. gramineus*	Attached-floating	16.8.2006	5835	22.7
90	Sichi (v)	NEC	48° 46′N	126° 11′E	*Potamogeton crispus*	Attached-floating	11.7.2006	10.4	3
91	Tai Hu Lake (vii)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*P. malaianus*	Attached-floating	2.11.2006	233,800	4
92	Stradsett Hall Pond	E	52° 37′ 35. 70”N	0°27′ 41. 50”E	*P. pectinatus*	Attached-floating	23.8.1999	8.3	2.5
93	Upper Lough Erne (vi)	NI	54° 15′ 5. 73”N	7° 34′ 51. 32”W	*P. x nitens*	Attached-floating	16.8.2006	5835	22.7
94	Woniu Paozi	NEC	47° 34′N	121° 17′E	*Nymphoides peltata.*	Attached-floating	9.7.2006	22	2.2
95	Sichi (vi)	NEC	48° 46′N	126° 11′E	*Nymphoides peltata*	Attached-floating	11.7.2006	10.4	3
96	Tai Hu lake (viii)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Nymphoides peltata.*	Attached-floating	2.11.2006	233,800	4
97	Cornabrass Lough (ii)	NI	54° 10′ 01”N	7° 23′ 12”W	*Nuphar lutea*	Attached-floating	21.7.2006	20.7	2.5
98	Alresford Lake (ii)	E	51° 5′ 37. 78”N	1° 9′ 32. 71”W	*Nuphar lutea*	Attached-floating	5.10.2006	52	2
99	Derrymacrow Lough (iii)	NI	54° 10′ 42”N	7° 26′ 34”W	*Nuphar lutea*	Attached-floating	20.7.2006	21	4.4
100	Burntfen Broad	E	52° 42′ 68. 04”N	1° 27′ 46. 33”E	*Nuphar lutea*	Attached-floating	17.8.1999	5.6	3.5
101	Dujuan Hu	NEC	47° 25′N	120° 34′E	*Nuphar lutea*	Attached-floating	6.7.2006	24.1	1.5
102	Corracoash Lough (ii)	NI	54° 8′ 85”N	7° 27′ 72”W	*H. morsus-ranae*	Attached-floating	25.7.2006	7	1.6
103	Derrykerrib Lough (iii)	NI	54° 8′ 02′N	7° 22′ 95”W	*H. morsus-ranae*	Attached-floating	22.7.2006	24.5	1.6
104	Xianhe Hu	NEC	47°21”N	120° 27′E	*Sparganium emersum.*	Attached-floating	6.7.2006	31.3	7
105	Tai Hu Lake (ix)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Sparganium emersum.*	Attached-floating	2.11.2006	233,800	4
106	Upper Lough Erne (vii)	NI	54° 15′ 5. 73”N	7° 34′ 51. 32”W	*Persicaria amphibia*	Attached-floating	16.8.2006	5835	22.7
107	Lowes Pond (iii)	E	52° 55′ 29. 87”N	1° 5′ 15. 50”E	*Persicaria amphibia*	Attached-floating	14.8.1999	0.5	2.5
108	Tai Hu Lake (x)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”N	*C. demersum*	Submerged	2.11.2006	233,800	4
109	Hong Hu lake (ii)	Ch	29° 54′ 14. 68”N	113° 16′ 19. 64”E	*Ceratophyllum* sp.	Submerged	10.9.2004	34,800	2.2
110	Sichi (vii)	NEC	48° 46′N	126° 11′E	*Ceratophyllum* sp.	Submerged	11.7.2006	10.4	3
111	Cromes Broad (ii)	E	52° 43′ 22. 75”N	1° 30′ 53. 53”E	*C. Demersum*	Submerged	10.8.1999	2.3	3
112	Bluestone plantation Pond	E	52° 46′ 22. 97”N	1° 9′ 12. 91”E	*C. demersum*	Submerged	25.8.1999	3.6	3
113	Melton Constable	E	52° 50′ 56. 85”N	1° 00′ 36. 30”E	*C. Demersum*	Submerged	13.8.1999	7.4	3.5
114	Gunthorpe Hall Lake (i)	E	52° 52′ 35.29”N	0° 58′ 94. 63”E	*C. demersum*	Submerged	12.8.1999	1.7	3
115	Balls Wood Ponds (iv)	E	51° 46′ 58. 23”N	0° 3′ 16. 23”E	*Callitriche* sp.	Submerged	7.4.2006	0.15	0.5
116	Malham Tarn (i)	E	54° 05′ 59. 26”N	2° 10′ 07. 95”E	*Hypericum elodes*	Submerged	27.10.2006	62	2.4
117	Burdautien Lough (ii)	NI	54° 11′ 56”N	7° 14′ 33”W	*Utricularia vulgaris*	Submerged	14.8.2006	6.5	7.8
118	Lake of Menteith	S	56° 10′ 52. 75”N	4° 17′ 10. 70”W	*M. alterniflorum*	Submerged	2.6.2000	259	6
119	Tai Hu Lake (xi)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Myriophyllum verticillatum.*	Submerged	2.11.2006	233,800	4
120	Loch of Harray	S	59° 3′ 06. 13”N	3° 13′ 52. 64”W	*M. spicatum*	Submerged	2.7.1998	1010.1	2.8
121	Tai Hu Lake (xii)	Ch	31° 0′ 36. 22”N	120° 4′ 42. 69”E	*Hydrilla verticillata*	Submerged	2.11.2006	233,800	4

122	Beeston Hall Lake (vi)	E	52° 9′ 13. 63”N	1° 2′ 95. 28”E	*Chara* sp.	Submerged	20.8.1999	2.6	2.5
123	Blicking Hall Lake (ii)	E	52° 48′ 72. 90”N	1° 13′ 90. 80”E	*Chara* sp.	Submerged	25.8.1999	10.1	4
124	Wolterton Hall Lake	E	52° 50′ 10. 94”N	1° 12′ 35. 96”E	*Chara* sp.	Submerged	19.8.1999	4.1	3
125	Gunthorpe Hall Lake (ii)	E	52° 52′ 35. 29”N	0° 58′ 94. 63”E	*Hippurus vulgaris*	Submerged	12.8.1999	1.7	3
126	Upton Great Broad	E	52° 39′ 55. 09”N	1° 31′ 53. 95”E	*Najas marina*	Submerged	18.8.1999	6.9	3
127	Green Plantation Pond	E	52° 55′ 26. 24”N	1° 05′ 46. 43”E	*Elodea nuttallii*	Submerged	13.8.1999	1.6	2
128	Malham Tarn (ii)	E	54° 05′ 59. 26”N	2° 10′ 07. 95”E	*Elodea canadensis*	Submerged	27.10.2006	62	2.4
129	Loch Kinord	E	57° 4′ 52. 57”N	2° 55′ 16. 07”W	*Littorella uniflora*	Submerged	9.7.1998	82.9	1.5
*	Songkhla	T	7° 12′ 22”S	100° 35′ 48”E	*Salvinia molesta*	Free-floating	19.3.2007	0.002	0.3
*	Songkhla	T	7° 12′ 22”S	100° 35′ 48”E	*Salvinia molesta*	Submerged	19.3.2007	0.002	0.3

Table 2.2. List of sites with available water chemistry data: pH, Conductivity (EC-µS cm^{-3}), Alkalinity (Alk-mg L^{-1} $CaCO_3$), Total Phosphorus (TP-µg L^{-1}), Soluble Reactive Phosphorus (SRP-µg L^{-1}) and Nitrate-nitrogen (NO_3^--N mg L^{-1}).

Site	pH	EC	ALK	TP	SRP	Nitrate – nitrogen
Bayfield Hall Lake	8.2	651	244	262	159	1.7
Beeston Hall Lake	8	616	166	110	23	0.23
Blickling Hall Lake	8.7	487	120	83	4	0.2
Bluestone Plantation Pond	8	515	160	42	6	0.1
Burntfen Broad	7.9	638	150	157	13	0.4
Cromes Broad	8.2	603	179	389	127	0.2
Denderup	8.1	395	171	55	15.4	0.01
Døj Sø	8.3	384	107	110	33	0.01
En Sø	8.6	346	106	76	11	0.01
Gammelmose	8.7	447	153	157	223	0.03
Green Plantation Pond	7.9	536	141	31	19	0.8
Gub Sø	7.9	266	98	225	117	0.02
Gunthorpe Hall Lake	7.9	522	210	98	5	0.3
Lowes Pond	7.9	624	186	30	27	2.1
Melton Constable Hall Lake	8.4	339	140	243	74	0.01
Salhouse Little Pond	7.9	640	216	84	66	1.1
Scottow Pond	7.6	657	210	51	15	0.2
Selbrigg Pond	7.9	566	188	34	7	0.01
Sorte Sø	7.9	1022	310	4056	3065	0.1
Stradsett Hall Lake	8.5	490	148	283	86	0.1
Strumpshaw Broad	8.5	1664	212	151	70	0.0
Upton Great Broad	8.4	490	150	33	7	0.0
Wolterton Hall Lake	8.2	526	158	63	26	0.01
Burdautien Lough	7.6	353	154	27	13.2	1.94
Doagh Lough	7.9	197	73	76	6.1	1.19
Derrymacrow Lough	7.5	268	89.5	61	29.1	0.9
Knockballymore Lough	7.4	294	122	25	14	0.5
Lough Corry	5.8	61	1.7	45	9.5	0.92
Summerhill Lough	7.6	320	140	80	13.6	2.21
Corracoash Lough	7.9	285	107	113	20.9	3.89
Cornabrass Lough	8.2	381	150	101	34.9	0.97
Derrykerrib Lough	8	252	81.7	29	18.5	1.86
Lough Sarah	8.2	251	62.3	40	16.7	0.78
Upper Lough Erne	8.5	262	94	50	-	-
Wandsworth Common Lake	8.02	555	-	51	25.6	0.03
Loch of Harray	7.5	-	25	-	-	-
Loch Kinord	-	-	20	-	-	-
Lake of Menteith	-	-	19	-	-	-
Dujuan Hu	7.3	76.3	-	10.4	5.09	0.0
Xian Hu	7.39	68.5	-	1.4	5.22	0.0
Woniu Paozi	8.69	182.6	-	59.1	4.94	0.42
Sanchi	8.62	181.2	-	16.9	1.48	0.0
Sichi	7.61	169.8	-	46.9	5.11	0.69
Hong Hu	7.38	-	-	80	-	1.19
Tai Hu	7.74	-	-	60	-	1.38
Bodham Rail Pit	7.65	348	113	351	42	1.09
Santantadibe	5.58	117.8	85.4	0	-	0.23
Malham Tarn	7.9	143	56.5	20	-	0.35
Lake Titicaca	7.4	1048	285	7	-	-
Mean	**7.89**	**1664**	**136.7**	**177.7**	**111.6**	**0.64**
Minimum	**5.38**	**61**	**1.7**	**0**	**1.48**	**0**
Maximum	**8.69**	**4372**	**1372**	**4056**	**3065**	**3.89**

Key: - data not available; Nitrate-nitrogen data readings of 0.0 indicate levels below detection.

Chapter 3. Is there a reliable host macrophyte-diatom association between the Lemnaceae and *Lemnicola hungarica*: developing a novel approach for inferring past duckweed cover?

3.1 Introduction

3.1.2 Host macrophyte and epiphytic diatom specificity

Several workers have investigated host-macrophyte relationships for freshwater diatoms. Prowse (1959) reported a significant association between *Gomphonema* (possibly *Gomphonema gracile*) and *Utricularia* and to a lesser degree with *Najas graminea* in studies from Malaysian rice paddy fields. Furthermore, a significant association between *N. graminea* and *Eunotia* (possibly *Eunotia pectinalis*) was found. In an investigation of the epiphytic diatoms associated with *Chara* species, Allanson (1973) found that *Achnanthidium minutissimum*, *Eunotia arcus* and several Naviculoid species prevailed on the surfaces of *Chara* leaves, whilst *Synedra nana* and *Synedra ulna* dominated axillary regions of the macrophytes. Pip and Robinson (1985) compared algal periphyton composition on several species of submerged macrophyte and found substantial compositional differences in the periphyton assemblages associated with different macrophyte species. Other workers have found a degree of specificity in the periphytic algal community composition on different macrophyte species over many decades (Godward 1937, Prowse 1959, Foerster & Schlichting 1965, Edsbagge 1968, Rautiainen & Ravenko 1972, Ramm 1977, Allanson 1973, Gough & Woelkerling 1976, Moss 1976, Eminson & Moss 1980). However, there is conflicting evidence from some studies on host-plant diatom specificity. For example Siver (1977) Gons (1979) Millie and Lowe (1983) and Delbecque (1983) reported no evidence of specificity between host-plants and their epiphytic diatom communities. To explain

these divergent findings, Moss (1976) proposed that water chemistry was important in determining epiphyte community composition in ecosystem experiments undertaken at Cornell University Agricultural Experiment Station, USA. It was found that relatively high host-plant specificity was associated with low water nutrient status with a decreasing degree of specificity at higher nutrient levels (Eminson & Moss 1980).

3.2 *Lemnicola hungarica* and duckweed

In 1863 Grunow first described a species called *Achnanthes hungarica* from Lake Balaton in Hungary, and it was already noted that this species was found in lakes that had *Lemna* present. Indeed, the initial descriptions of *A. hungarica* suggested that its habitat preference was "in lacunis parvis inter radicula Lemnarum". Hustedt (1930) also commented upon the occurrence and prevalence of *A. hungarica* as being "anscheinend mit Vorliebe an *Lemna*": *A. hungarica* appears to have a predisposition towards *Lemna*. The observation of an association between *A. hungarica* and *Lemna* was confirmed by Round (1973). Further, Round and Basson (1997) placed *A. hungarica* into its own monospecific genus of *Lemnicola*, within which it is now known as *Lemnicola hungarica* (Grunow) Round & Basson. Light microscopy images of *L. hungarica* are presented in Figure 3.1.

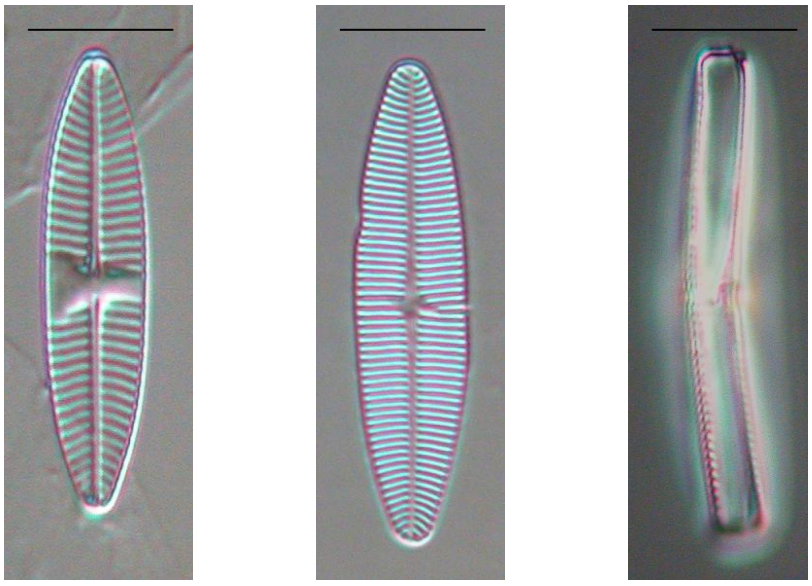


Figure 3.1. Light microscopy images of *Lemnicola hungarica*. Raphe view (left), Non-raphe view (centre) and girdle view (right). Scale bar = 10 μm. (Images by Patrick Rioual).

There have been few quantitative studies of epiphytic communities associated with duckweed mats, and little is known regarding the environmental factors regulating the abundance and composition of diatoms attached to duckweed. In a study of the diatom epiphytes on *L. minor* in western Canada, Goldsborough (1993) observed that the species richness of the diatom flora was low, suggesting that duckweed mats are environments to which few species are adapted. Others have shown a low density of diatoms on *Lemna*. For example Bowker & Denny (1980) observed reduction in diatom density on *L. minor* (in comparison with epipelic and epipsammic assemblages) and suggested this could be related to low silicon levels, rapid frond growth, high temperatures or oxygen depletion. It was also noted by Bowker & Denny (1980) that *L. hungarica* and *Amphora veneta* were abundant on duckweed surfaces. Goldsborough (1994) used an artificial substratum positioned vertically through the duckweed mat and found that *L. hungarica* was the only diatom species occurring among the partially dried *Lemna* fronds, wrapping the substratum immediately above the waterline. It was found that *L. hungarica* comprised more than 90% of the total diatom species recorded at the air/water interface. However, below the surface amongst the *L. minor* roots, *L. hungarica* was replaced by *Epithemia turgida*, *Gomphonema parvulum*, *Eunotia curvata* and *Sellaphora seminulum* (Fig. 3.2).

Goldsborough (1994) suggested that the occurrence of *L. hungarica* at the air/water interface was due to the high irradiance, depletion of inorganic nutrients by the *Lemna* plants, and the accumulation of organic mat leachates. It has been shown that *L. hungarica* is a motile diatom which can concentrate its abundance in certain locations such that it is not distributed randomly (Zuberer 1984). It has also been shown that motile species can reposition themselves for their maximum benefit in relation to areas of high nutrient status or irradiance (Pringle 1990) and that other diatom species observed on *Lemna* roots must be adapted to survival in a low irradiance environment. The vertical sequence of diatoms on the substratum that Goldsborough (1994) observed was also very similar to a study using Scanning Electron Microscopy (SEM) micrographs of intact *L. minor* plants taken from a duckweed mat in Texas, USA (Zuberer 1984). Mono-specific clustering of *L. hungarica* was present in the epidermal

depressions on the abaxial (lower) leaf surfaces, whilst *G. parvulum* and *Eunotia* spp. were found on *Lemna* roots.

Goldsborough (1993) speculated that the abundance of the aforementioned diatoms below the duckweed mat was likely due to decreased physical abrasion on the substratum by the mat, reduced herbivory, or an increase in nutrient availability. It was also observed that a rapid absorption of photosynthetically active radiation (PAR) in the upper few centimetres of the air/water interface produced a diurnal water temperature fluctuation of up to 15ºC, where the surface heat diffused slowly to the deeper strata. It was suggested that the thickness of the duckweed mats posed a barrier to atmospheric gas exchange with oxygen diffusion negligible through duckweed mats greater than 150g m^{-2} (Duffield 1981, Goldsborough 1993) resulting in anaerobiosis coupled with low/no oxygenic photosynthesis below the *Lemna* mat in summer (Goldsborough 1993). The role of PAR may be of particular significance for diatom communities associated with dense mat-forming duckweeds where Goldsborough and Robinson (1985) and Dale and Gillespie (1976) found that as little as 1% of the incident light intensity was transmitted through a dense *Lemna* mat.

For diatoms to thrive in the suboptimal environments of a thick duckweed mat Goldsborough (1993) suggested that they must employ a form of anoxygenic metabolism similar to that of photosynthetic bacteria. Alternatively they may be facultative or obligate heterotrophs, as this potential is found in a number of diatom species (Hellebust & Lewin 1977). Indeed such adaptations could explain the consistent abundance and dominance of *L. hungarica* in thick mats of duckweed, particularly in summer where light and nutrient conditions are least conducive to autotrophic productivity (Hustedt 1957, Goldsborough & Robinson 1985).

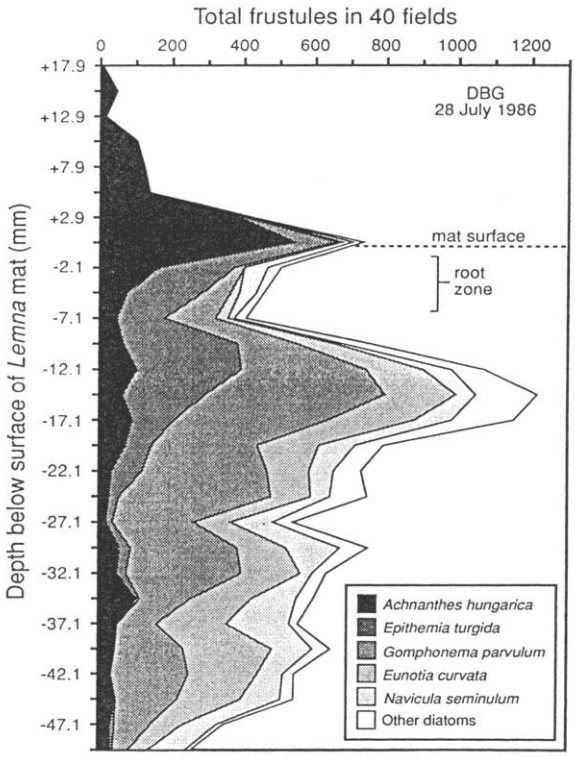


Figure 3.2. The spatial distribution of diatoms sampled from a vertical artificial substrate within the phyllosphere of *Lemna minor* (taken from Goldsborough 1993). Note that *Achnanthes hungarica = Lemnicola hungarica* and *Navicula seminulum = Sellaphora seminulum.*

Following Goldborough's pioneering work, a number of studies have investigated the consistency and robustness of diatom-duckweed relationships. In a diatom-substrate specificity study of herbarium specimens of Lemnaceae including *L. minor*, *Lemna gibba*, *Spirodela polyrhiza*, *L. trisulca* and *Wolffia arrhiza*, Buczkó (2007) found that *L. hungarica* dominated diatom assemblages attached to *L. minor*, *L. gibba*, *S. polyrhiza* and *W. arrhiza* in marked contrast to the assemblages found on *L. trisulca* which were dominated by *C. placentula.* Buczkó (2007) concluded that *L. hungarica* was tightly attached to well definable taxa of the family Lemnaceae. The micro-distribution of the epiphytic diatom assemblages of *L. minor* were different, with *L. hungarica* dominating the undersides of the fronds, *Gomphonema* spp. inhabiting the mid-surfaces of the roots and *Fragilaria* spp. occurring on the root tips. These findings concur with those of Goldsborough (1993) and Goldsborough and Robinson (1985) suggesting clear vertical structure to diatom assembly in surface mats of Lemnaceae.

Desianti (2012) also reported *L. hungarica* from *L. minor*, *S. polyrhiza* and *Wolffia* but did not find evidence of diatom assemblage differences between host-plant roots or fronds.

3.3 Aims and methods

3.3.1 Aims

The aforementioned research including contemporary field studies and studies of herbarium macrophytes has suggested a strong association between *L. hungarica* and the Lemnaceae. Nonetheless, these studies were conducted at a local level (lake regions) and hence cover a narrow range of host-plant species and environmental conditions. There have been few host-plant studies of *L. hungarica* conducted on other macrophyte species from different geographical sites and locations. In this chapter the results of an exploratory study aimed at determining the robustness of the *Lemna-L. hungarica* relationship on a global scale is presented. The study design negated any potential and local peculiarities and places the host-plant-diatom associations and the specific *L. hungarica*-duckweed association in a global context.

Furthermore, as a fundamental aim of this thesis is to determine whether the *Lemna-L. hungarica* association can be applied with statistical confidence to reliably infer past pond ecology, surface sediment samples from duckweed (*Lemna*) covered and from non-duckweed ponds were analysed for their diatom assemblages. This simple comparative study was designed to determine the potential of *L. hungarica* as a proxy indicator of past occurrences of *Lemna* which could later be applied in a palaeoecological study.

3.3.2 Methods

In order to assess the host macrophyte-diatom association for *Lemna*, 131 samples were taken from 39 different macrophyte species collected from sites around the world,

including North and South America, northern Europe, southern Africa, China, south-east Asia and Australia (Fig. 2.2, Table 2.1). See Chapter 2 for full methodological details of macrophyte sampling, diatom slide preparation and diatom counting and Appendix 1 for all the diatom taxa recorded.

The various macrophyte samples were grouped according to growth-form as: 'free-floating plants' (including *Lemna*, *Azolla*, *Riccia*, *Salvinia*, *Spirodela* and *Wolffia* spp.), 'attached-floating plants' (including *Trapa*, *Potamogeton*, *Nymphoides*, *Nuphar*, *Hydrocharis*, *Sparganium* and *Persicaria* spp.) and 'submerged plants' (including *Ceratophyllum*, *Callitriche*, *Hypericum*, *Utricularia*, *Myriophyllum*, *Hydrilla*, *Chara*, *Hippuris*, *Najas*, *Elodea* and *Littorella* spp.). As the majority of the previous studies have looked at epiphytic diatom assemblages on *L. minor* and as *L. minor* is a key species in this research (see Chapters 4, 5 and 6), it was decided to differentiate *L. minor* from the other free-floating macrophyte samples in the analyses presented here. Thus 'other free-floating plants' was used as a macrophyte group which included all free-floating species except for *L. minor*. Furthermore, *Lemna trisulca* was also separated from the 'other free-floating plants' category due to its different ecology. For example, *L. trisulca* is often found on the sediment surface or at mid-depth amongst the submerged macrophytes in ponds and lakes, and although it occurs at the water surface it is equally likely to be found throughout the water column. *L. trisulca* is known to rise to the air-water interface when it flowers (Greenhalgh & Ovenden 2007). Samples were collected from both the water surface and sub-surface in this investigation and therefore *L. trisulca* samples were not truly representative of either the 'other free-floating plants' or 'submerged plant' categories. On this basis *L. trisulca* was not selected for direct gradient analysis of the diatom data, but was included in the indirect gradient analysis and analysis of variance as an initial exploration of the diatom data.

3.4 Results

3.4.1 Freshwater macrophytes and associated epiphytic diatom floras

Relative abundance data for the dominant epiphytic diatom taxa (n=29) recorded from the 131 macrophyte samples are displayed in Fig. 3.3. A total of 272 diatom species were recorded, but as the focus of the *a priori* study was epiphytic diatoms, planktonic species were omitted together with taxa considered 'extremely rare' (defined as <2% relative abundance in all samples) resulting in a total of 217 epiphytic species. This preliminary data set of 217 species was explored with indirect analyses (DCA, CA) and is presented in Figures 3.4 and 3.5a respectively. Taxa that were considered to be 'rare' species (defined as <5% relative abundance in all samples) were also deleted from the data set prior to statistical analysis (CCA) resulting in 69 species (Fig. 3.6).

There were few clear indications of specific diatom taxa being solely associated with particular macrophyte growth forms (i.e. free-floating, attached-floating and submerged). Indeed, Figure 3.3 shows that most diatom taxa were likely to be found on all three macrophyte growth forms. The most notable of these more cosmopolitan species were *C. placentula*, *G. parvulum*, *Nitzschia palea*, *Amphora veneta*, *Synedra acus* var. *acus*, *Staurosira construens* var. *venter* and *Nitzschia frustulum*. There were a few taxa, however, that appeared to be strongly associated with free-floating macrophytes, namely *Lemnicola hungarica*, *Sellaphora seminulum* and *Nitzschia archibaldii*, although both *L. hungarica* and *S. seminulum* were also recorded, albeit in low abundances, from the other macrophyte growth forms. In addition, *N. archibaldii* was never very abundant and was only recorded from a few samples and consequently this species was not reliably tied to a particular macrophyte habitat.

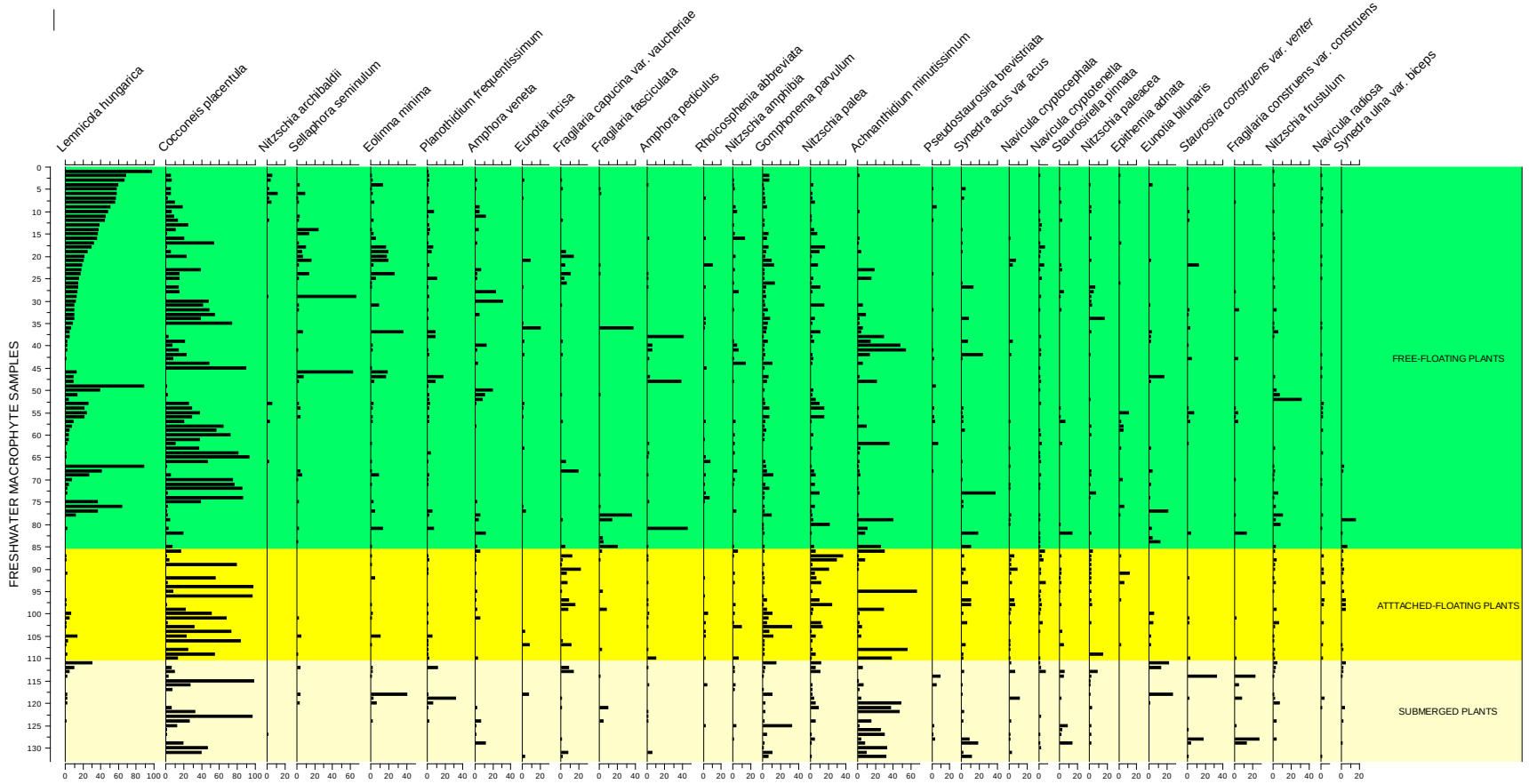


Fig 3.3. Dominant diatom taxa sampled from 131 freshwater macrophytes (macrophytes collected from January – November). Diatom taxa with <5% relative abundance in samples are omitted and rare diatom taxa represented in <5% of total macrophytes samples are also omitted. Different macrophyte groups are highlighted in different colour schemes. The *Lemna minor* samples are homogenized with the diatom data by organizing the samples by the descending relative abundances of *Lemnicola hungarica*. The other macrophyte samples are randomly ordered.

Samples: 1-45: *Lemna minor*; 46-48: *Lemna minuta*; 49-50: *Lemna gibba*; 51-53: *Lemna* cf. *aequinoctialis; 54*-66: *Lemna trisulca;* 67-74: *Spirodela polyrhiza;* 75: *Wolffia arrhiza;* 76-79: *Azolla filiculoides;* 80: *Azolla pinnata.;* 81: *Riccia fluitans*; 82-83: *Salvinia molesta*; 84: *Salvinia natans*; 85-89: *Trapa natans*; 90: *Potamogeton natans*.; 91: *Potamogeton crispus*.; 92: *Potamogeton pectinatus*; 93: *Potamogeton x nitens*; 94: *Potamogeton malaianus*; 95: *Potamogeton gramineus*; 96-98: *Nymphoides peltata*; 99-103: *Nuphar lutea*; 104-105: *Hydrocharis morsus-ranae*; 106-107: *Sparganium emersum*.; 108-109: *Persicaria amphibia*; 110: *Ceratophyllum submersum*; 111-116: *Ceratophyllum demersum;* 117- *Callitriche* sp.; 118: *Hypericum elodes*; 119: *Utricularia vulgaris*.; 120: *Myriophyllum verticillatum*.; 121: *Myriophyllum alterniflorum*; 122: *Myriophyllum spicatum*; 123: *Hydrilla verticillata;* 124-126: *Chara* spp.; 127: *Hippuris vulgaris*; 128: *Najas marina*; 129: *Elodea canadensis*; 130: *Elodea nuttallii*; 131: *Littorella uniflora*.

3.4.2 Relationships between contemporary epiphytic diatoms and macrophyte habitat

An initial exploratory DCA was performed primarily to establish whether diatom species responses were linear or unimodal. The analysis was performed on taxa that occurred at >2% relative abundance. This indirect gradient analysis provides a measure of beta diversity, or heterogeneity, in community composition (the extent of species turnover) which is given by the gradient length of the axes in the ordination diagram (i.e. measured as units of standard deviation – SD units). Rare taxa were down-weighted and detrending by segments was applied to the species data (Hill & Gauch 1980, Wartenberg *et al.*, 1987, Knox 1989). Figure 3.4 shows the results of the DCA analyses.

The gradient lengths of axes 1 and 2 were 4.431 SD and 4.066 SD respectively. As these are both greater than 4 SD units the use of unimodal methods was considered appropriate. *L. hungarica* is situated at the bottom left-hand side of the DCA biplot. The DCA demonstrated the presence of an outlier sample, this being *Salvinia molesta* which was collected from southern Thailand and the associated diatoms were: *Anomoeoneis vitrea*, *Eunotia bilunaris* var. *mucophila*, *Gomphonema clavatum*, *Navicula atomus*, *Nitzschia angustulata* and *Pinnularia maior*. Therefore, *S. molesta* was omitted from further analyses. The eigenvalues, measures of the explanatory power of the axes, of the first four axes explain 27% of the variability in the species data. The eigenvalues of the first two axes were 0.717 and 0.499 respectively, and explained 18.2% of cumulative species variation.

Following the DCA, CA was also performed on the 129 macrophyte samples and the remaining 217 diatom species (i.e. taxa with >2% relative abundance). CA summary statistics show that the eigenvalues of axes 1 and 2 were 0.521 and 0.466 respectively. The CA diagrams of both diatom species (a) and macrophyte samples (b) are shown in Figure 3.5.

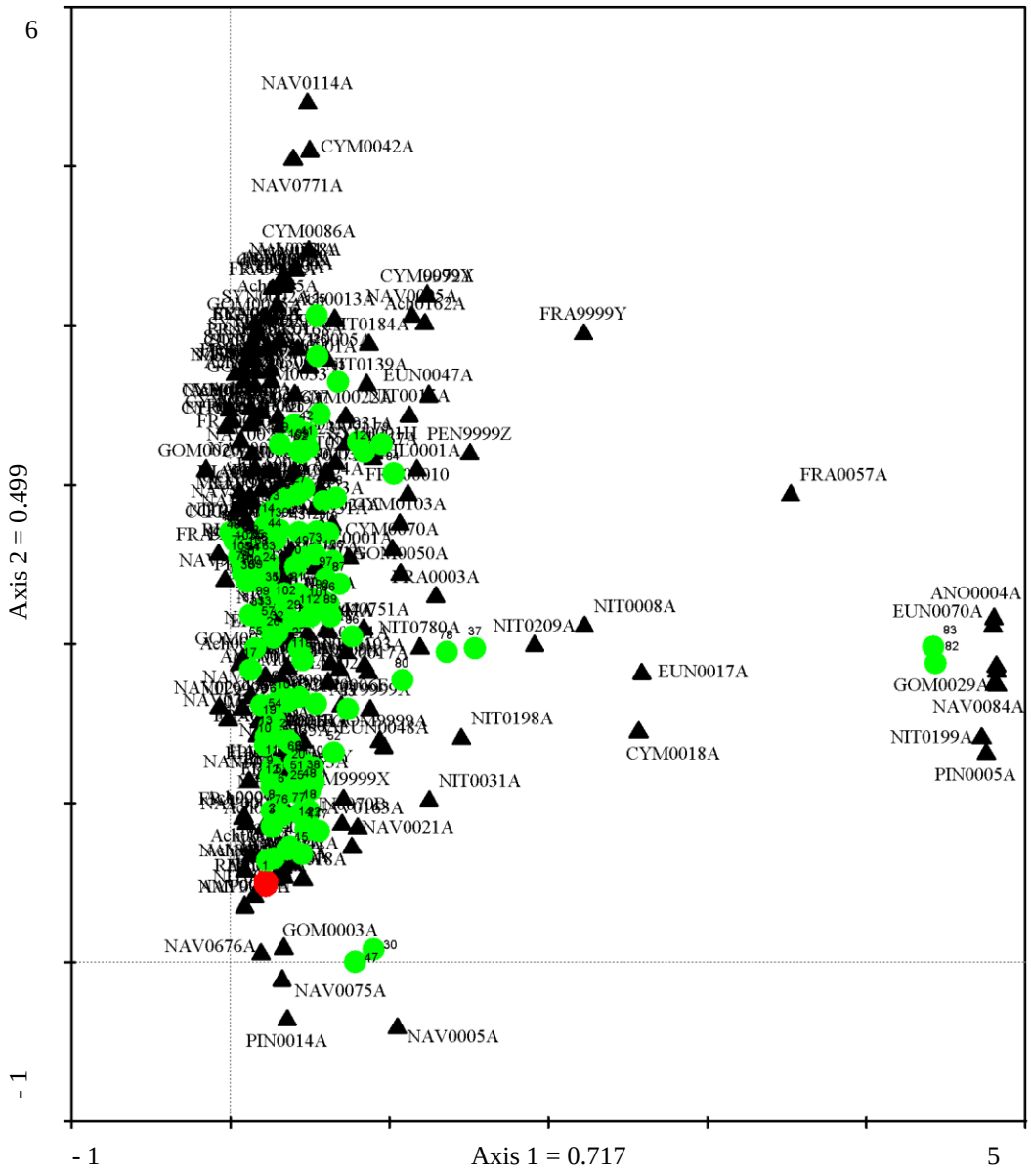


Figure 3.4. DCA scatterplot of the 217 diatom species (i.e. > 2%) recorded in the study, displaying ordination axes 1 and 2. Samples are presented as green circles and diatom species are represented as black triangles, except for *Lemnicola hungarica* which is represented as a red circle. Note the samples and associated diatom species outliers are positively associated with Axis 1. (See Appendix 1 for diatom species codes and names).

The first two axes explained 16.7% of the variance in the data. The first CA axis explains around 9% of total species variability (compared with 10.8% in the DCA analysis) which is high given the 200+ species in the data set. Despite vagaries of weather, differences in basin morphologies, varying physical and chemical

characteristics of the sampling sites and inherent contamination problems associated with macrophyte sampling from shallow water bodies, there were clear patterns in the data suggesting associations of diatom taxa with particular macrophyte growth forms (Fig. 3.5b). For example, samples from *L. minor* and 'other free-floating plants' broadly lie in close proximity within the ordination space with a cluster in the lower right-hand quadrant.

The epiphytic diatoms, *L. hungarica* and *S. seminulum* are located within the same ordination space as the free-floating plants and *L. minor* (Fig. 3.5). The submerged and attached-floating plants were located mainly in the upper left-hand quadrant and were associated with *Cymbella tumida, Navicula subrotunda* and *Navicula cryptotenelloides,* whilst most of the *L. trisulca* samples were located in the lower left-hand quadrant and were associated with *Psammothidium lauenburgianum, Gomphonema affine* and *Nitzschia intermedia*. Therefore, there is a reasonable separation of the free-floating plants, *L. trisulca,* attached-floating plants and the submerged plants within the ordination (Fig. 3.5b). This separation between the five *a priori* designated macrophyte groups was further explored with confirmatory data analysis by finding a configuration in the CA ordination space in which the distances between the macrophyte group samples best corresponded to dissimilarities of their epiphytic diatom compositions. To this end, NMDS was employed initially to configure the macrophyte groups in ordination space so that the distances between the samples (macrophyte groups) corresponded to dissimilarities, using Bray-Curtis dissimilarity coefficient distances, to represent the distance relationships among the macrophyte groups by preserving the rank-order of the distances (Fig.3.6a). Moreover, a 'stress' statistic was calculated to provide a measure of the 'lack of fit' between distances in ordination space (Lepš & Šmilauer 2003), in other words the 'stress' statistic measures the 'goodness-of-fit' of the solution in reduced ordination space (Birks *et al*., 2012).

A test of the homogeneity of the multivariate dispersion (HMD) was performed to assess the differences in dispersion (β-diversity) and to provide a clear visual presentation to determine if the macrophyte group centroids were in the same location

(Anderson 2006, Anderson *et al*., 2006). HMD analysis is a non-parametric method that compares variability of mean distance to centroid (dispersion) within groups versus variability in this distance among different groups (i.e. macrophyte groups). HMD is suitable for assessing the significance of compositional heterogeneity that is attributed to variation in the diatoms species relative abundances (Anderson 2006). Figures 3.6b and 3.7a show the HMD plot and the group centroid locations. The calculated mean centroid values for the macrophyte groups are given in Table 3.1a.

Although HMD analysis provides a robust measure of the compositional variability in terms of the average distances of dissimilarity to the centroid, it does not discriminate between samples (i.e. macrophyte groups) that differ in terms of diatom species composition (i.e. samples/groups could be equally homogeneous/heterogeneous but differ in their diatom species composition). As there were notable differences in the location and dispersion of the macrophyte sample groups (Fig. 3.6) a permutational multivariate analysis of variance using distance matrices (PerMANOVA) was performed employing ADONIS. ADONIS is a non-parametric method for multivariate analysis of variance that compares the variability among the other macrophyte groups; therefore the analysis enables assessments of the significance of the diatom compositional heterogeneity. ADONIS analysis was conducted on all the macrophyte groups and also separate pairwise comparisons between the groups were conducted (Table 3.1b). Finally, ANOSIM was also performed on the data which provided an *R* statistic (analogous to the *F*-ratio test in ANOVA) based on the difference of mean ranks both between and within the macrophyte groups to test if the diatom assemblage compositions varied across the macrophyte groups (Birks *et al*., 2012).

The NMDS plot (Fig. 3.6a) of the macrophyte groups broadly concurs with the CA ordination (Fig. 3.5b) and shows the group dissimilarities (separation between groups) with the Bray-Curtis dissimilarity index. The calculated 'stress' statistic (=0.265) demonstrates that there was a good representation in reduced dimensions as there was a reasonable goodness-of-fit of the solution in reduced ordination space. With HMD analysis (Fig. 3.6b) the dissimilarities were calculated using the Bray-Curtis index of

similarity with principle coordinate analysis (PCoA) (Anderson 2006). Figure 3.6a shows that there were significant differences in dispersion (β-diversity) of the epiphytic diatom assemblages between the macrophyte groups, as the group centroids were not in the same location in ordination space, which was also reflected in their differences in relation to their centroid values which are presented as boxplots (Fig. 3.7a). These differences in dispersion demonstrate that some macrophyte groups are more heterogeneous or homogeneous than other groups in terms of their diatom species occurrences and abundances (Table 3.1a). For example, the mean centroids for the macrophyte groups shows that the diatom assemblages of the 'other free-floating' group were more homogeneous (0.34) than the diatom assemblages associated with the 'submerged' group (0.54). Similarly, the *L. minor* diatom community assemblages were more homogeneous (0.46) than the diatom community assemblages associated with *L. trisulca* (0.51). Overall, the analyses revealed that the diatom assemblages associated with the different macrophyte groups were not only highly significantly different in their composition but also in their dispersion (β-diversity) across all the macrophyte groups (p=0.00002).

The *R* statistic (R=0.239, p=0.001 in ANOSIM) for all the macrophyte groups suggests that, in terms of dispersions, most of the diatom community assemblages were likely to be within the same macrophyte groups (Fig. 3.7b). However, even though the ANOSIM model showed that there were significant differences between the macrophyte groups and their respective diatom community assemblages, the statistical integrity of ANOSIM has recently been brought into question – indeed it is now recommended that ANOSIM models are analysed with ADONIS which seems to be a more robust alternative (Oksanen *et al*., 2011). When the data were analysed using ADONIS, there were significant differences between all five macrophyte groups *per se* and significant differences were evident between all five macrophyte groups and there were also significant differences when pairwise comparison tests were undertaken on the macrophyte groups, strongly suggesting significant differences in diatom community assemblages (Table 3.1b). It can be noted that ANOSIM and ADONIS analyses of the

macrophyte groups and their associated diatom community assemblages produced similar results.

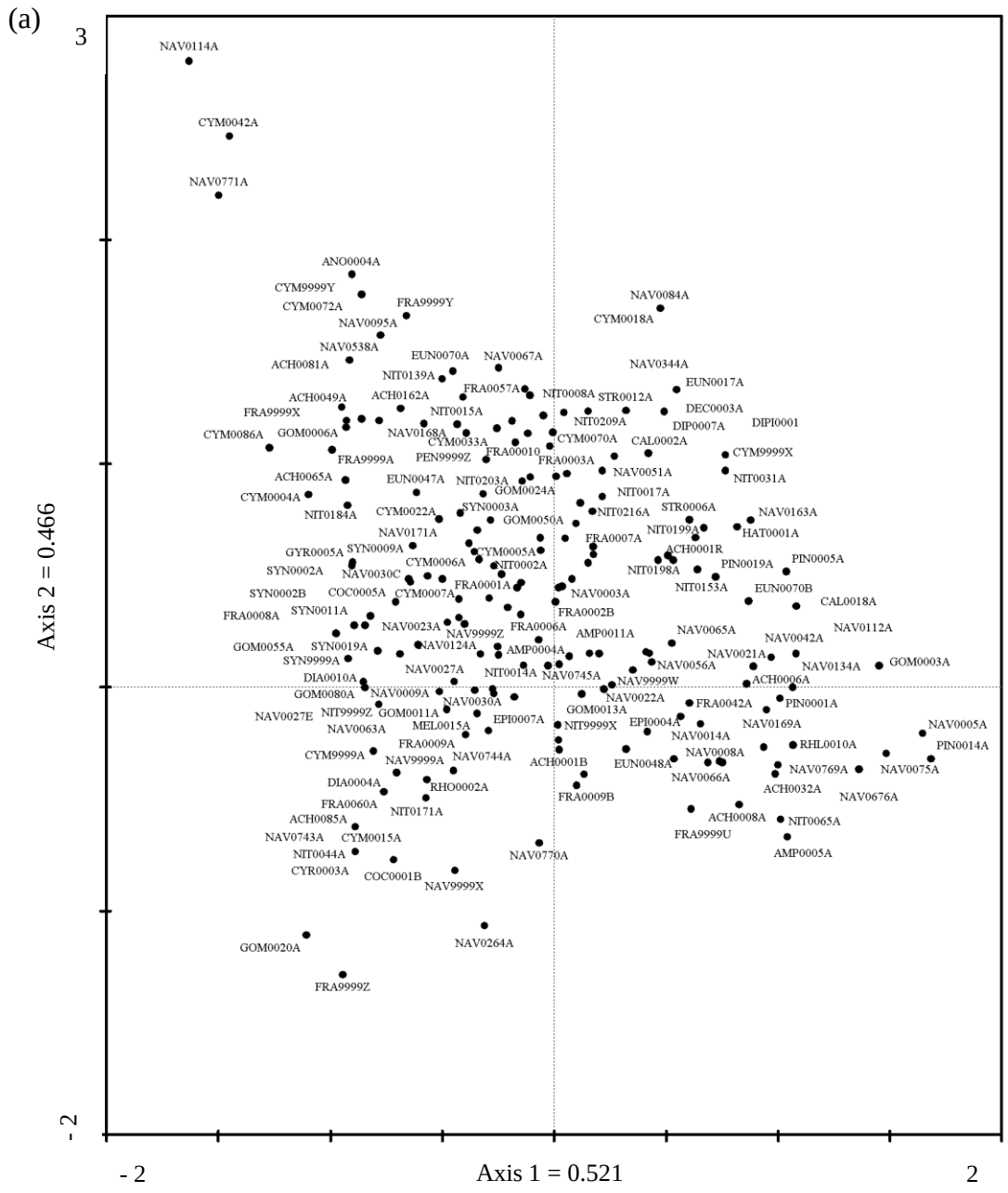


Figure 3.5a. CA plot of (a) the 217 common diatom taxa on axes 1 and 2. The eigenvalues are given for each axis. (See Appendix 1 for diatom codes).

(b)

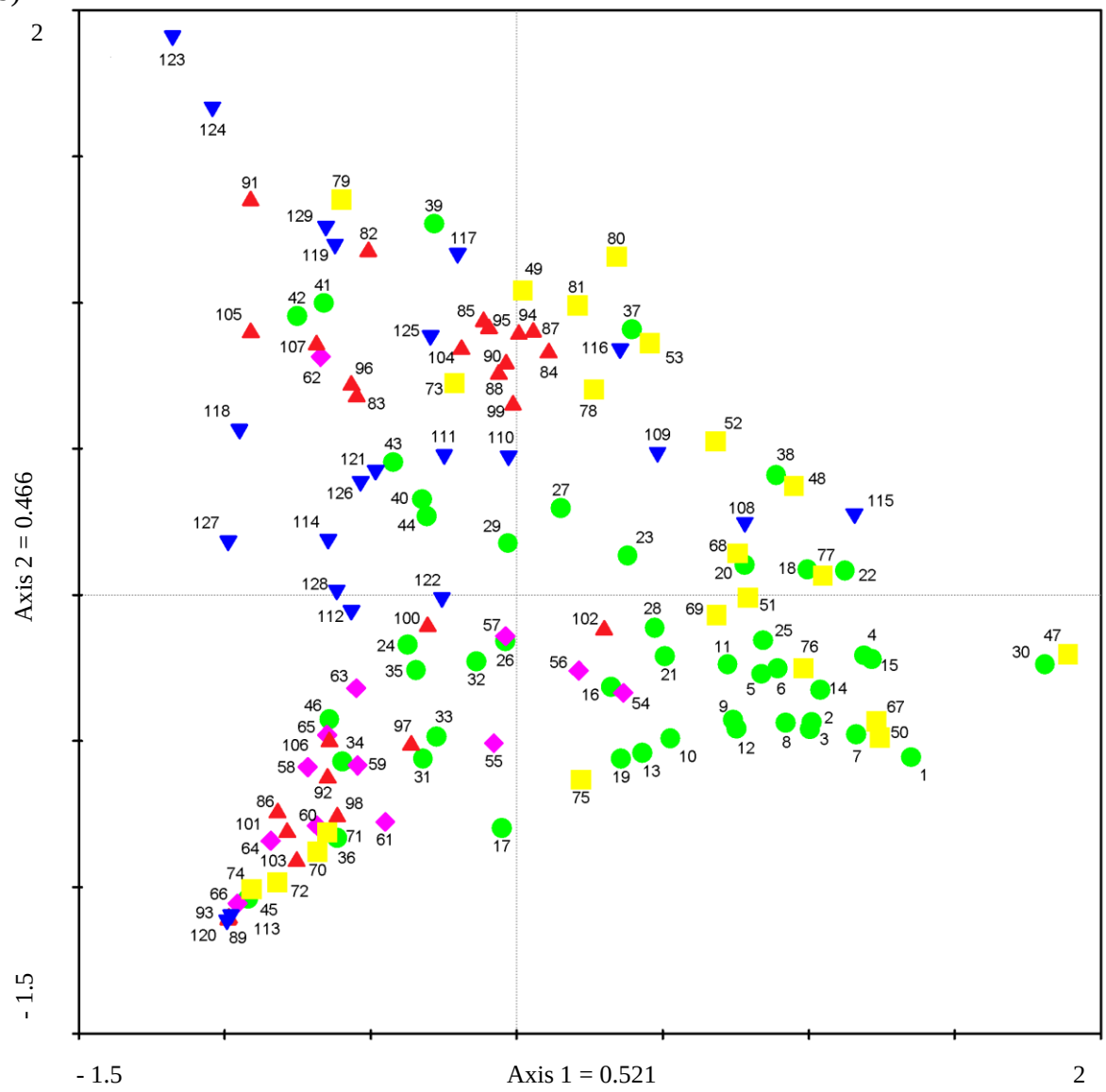


Figure 3.5b. CA plot of (b) the 129 freshwater macrophyte samples on axes 1 and 2. The eigenvalues are given for each axis. (See Fig 3.3 for sample numbers). Samples are colour coded based on the five macrophyte groups (green circles = *Lemna minor*; yellow squares = other free-floating plants; pink diamonds = *Lemna trisulca*; red up-triangles=- attached-floating plants; blue down-triangles = submerged plants.

(a) (b)

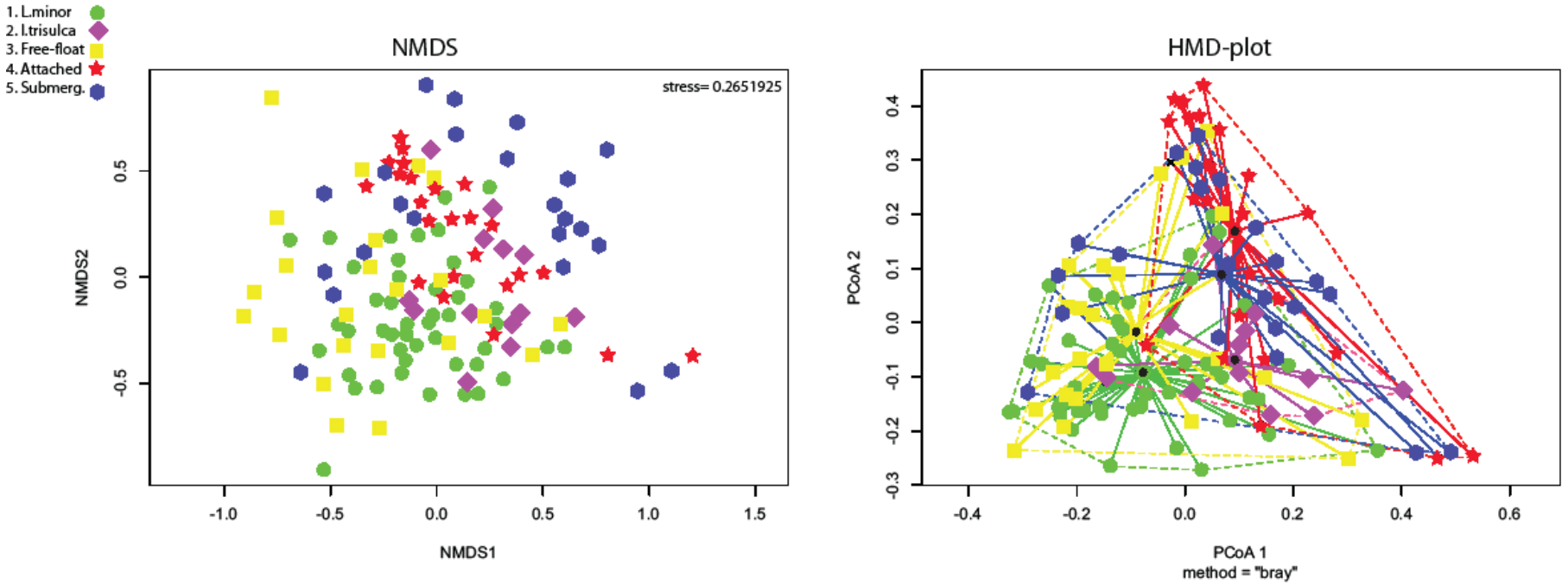


Figure 3.6. NonMetric Multidimensional Scaling (NMDS) plot (a) and Homogeneity Test of Multivariate Dispersion (HMD) plot (b) of the 129 freshwater macrophyte samples. The sample data were square-root and Wisconsin transformed and based on Bray-Curtis distances. NMDS plot (a) gives the stress (i.e. a measure of the goodness-of-fit) of the solution ordination in two-dimensional space. HMD plot (b) shows the location of the macrophyte group centroids (black dots). The key for the macrophyte samples is also given: green circles = *Lemna minor*; purple diamonds = *Lemna trisulca*; yellow squares = other free-floating plants; red stars = attached-floating plants; blue circles = submerged plants.

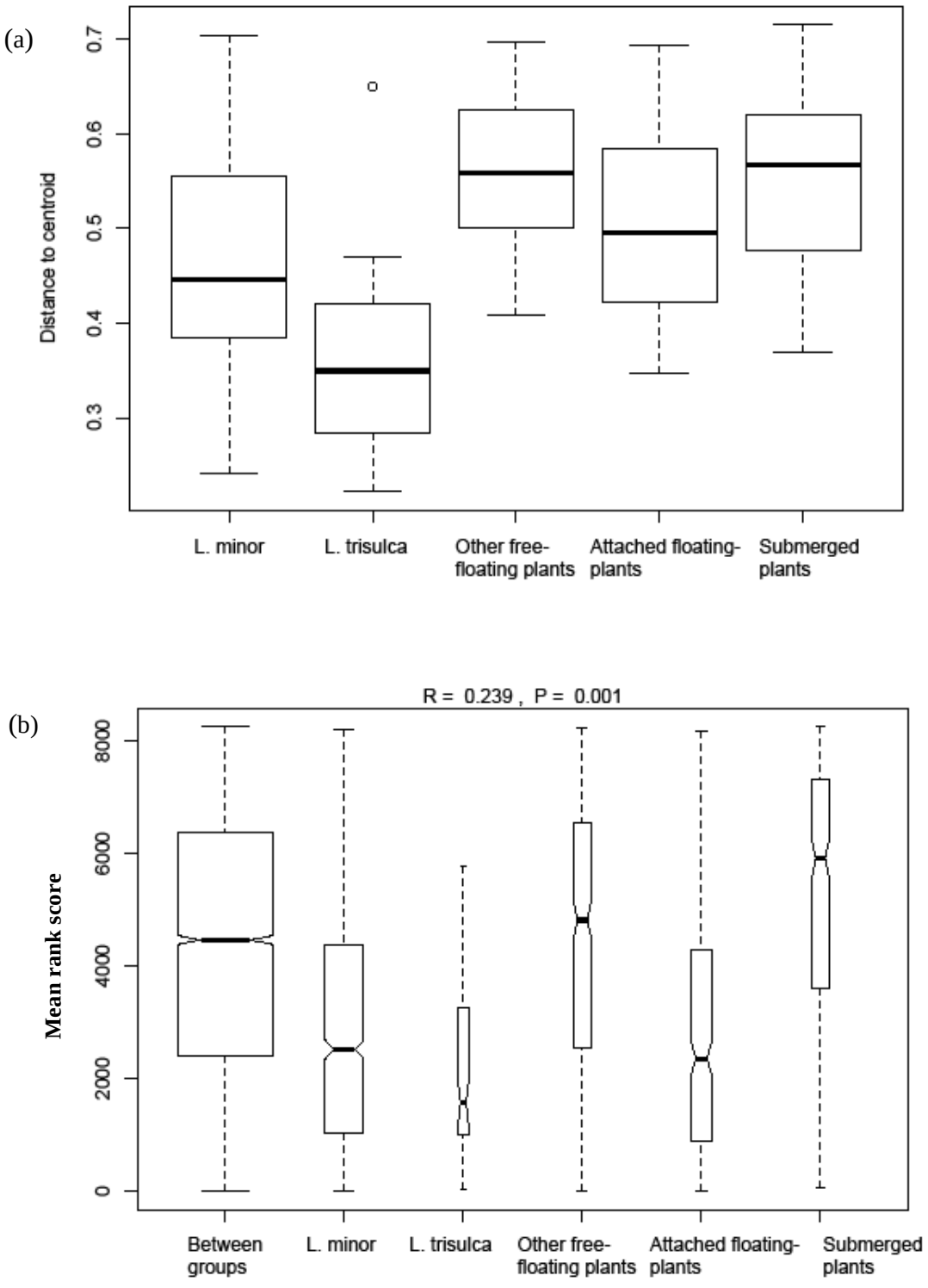


Figure 3.7. Homogeneity test of Multivariate Dispersion (HMD) box plots (a) and Analysis of Similarities (ANOSIM) boxplots (b) of the different macrophyte groups: *Lemna minor*, *Lemna trisulca*, other free-floating plants, attached floating plants, submerged plants. ANOSIM boxplot (b) gives the R statistic (R=0.239) and the statistical significance (P=0.001) between the five *a priori* selected macrophyte groups.

(a)

Macrophyte Group:	*L. minor*	Other free-floating	*L. trisulca*	Attached-floating	Submerged
Mean Centroid:	0.4612	0.3411	0.507	0.4534	0.5362

(b)

Macrophyte Group:	**DF**	**Sum Sq.**	**Mean Sq.**	**F. Model**	**R^2**	**p value**
All Groups	4	3.7160	0.92893	3.8175	0.10964	0.001
L. minor vs. *L .trisulca*	1	0.7373	0.73731	3.2953	0.05465	0.001
L. minor vs. Other free-floating	1	0.5748	0.57478	2.3621	0.03405	0.002
L. minor vs. Attached floating	1	1.5612	1 .56116	6.7019	0.08853	0.001
L. minor vs. Submerged	1	1.1820	1.18198	4.6090	0.06528	0.001
L. trisulca vs. Other free-floating	1	0.9370	0.93703	3.6751	0.09755	0.001
L. trisulca vs. Attached floating	1	0.7923	0.79231	3.4674	0.08785	0.001
L. trisulca vs. Submerged	1	0.7830	0.78698	2.8553	0.07963	0.001
Other free-floating vs. Attached floating	1	1.1517	1.15168	4.4933	0.08899	0.001
Other free-floating vs. Submerged	1	0.8448	0.84475	2.8390	0.06194	0.001
Attached floating vs. Submerged	1	0.4824	0.48244	1.7892	0.03824	0.044

Table 3.1. Homogeneity test of Multivariate Dispersion (HMD) analysis showing mean centroid values of the macrophyte groups (a) and PerMANOVA (ADONIS) analyses of dissimilarity between all macrophyte groups and pairwise comparison tests of significance between the macrophyte groups (b) based upon the epiphytic diatom assemblages.

3.4.3 Relationships between contemporary epiphytic diatoms, macrophyte hosts and water chemistry

Constrained ordinations (CCA) were carried out upon a reduced diatom sample data set with available water chemistry data (conductivity, pH, total phosphorus, and alkalinity). A total of 61 macrophyte samples, representing the main macrophyte growth forms, together with water chemistry data (excluding alkalinity) covering 37 different sites were analysed in the CCA. Moreover, CCA with forward selection was run to identify a subset of the environmental variables that explained statistically significant amounts of variation in the diatom species distributions. The main macrophyte growth forms (*L. minor*, other free-floating plants, attached-floating plants, submerged plants) were also employed as explanatory variables using their frequency data (i.e. nominal data). The resulting ordination diagram is shown in Figure 3.8.

The CCA (Fig. 3.8) graphically shows that the distribution of diatom species in ordination space is well correlated with the macrophyte and water chemistry variables (axis 1, r=0.857; axis 2, r=0.798). This suggests that a large proportion of the diatom-environmental relationships were explained by the measured environmental variables. The key explanatory environmental variables on axis 1 (eigenvalue: 0.161) were *L. minor* and other free-floating plants, whilst total phosphorous (TP) was the major explanatory variable on axis 2 (eigenvalue: 0.134). Axis 1 showed a statistical significance (p=0.002; F=3.799) suggesting a significant relationship between epiphytic diatom species associated with *L. minor* and other free-floating plants. It was interesting to note that *L. hungarica* was positively correlated with *L. minor,* other free-floating plants and TP, but negatively correlated with submerged plants. The first four axes explained over 79% of the variance in the data.

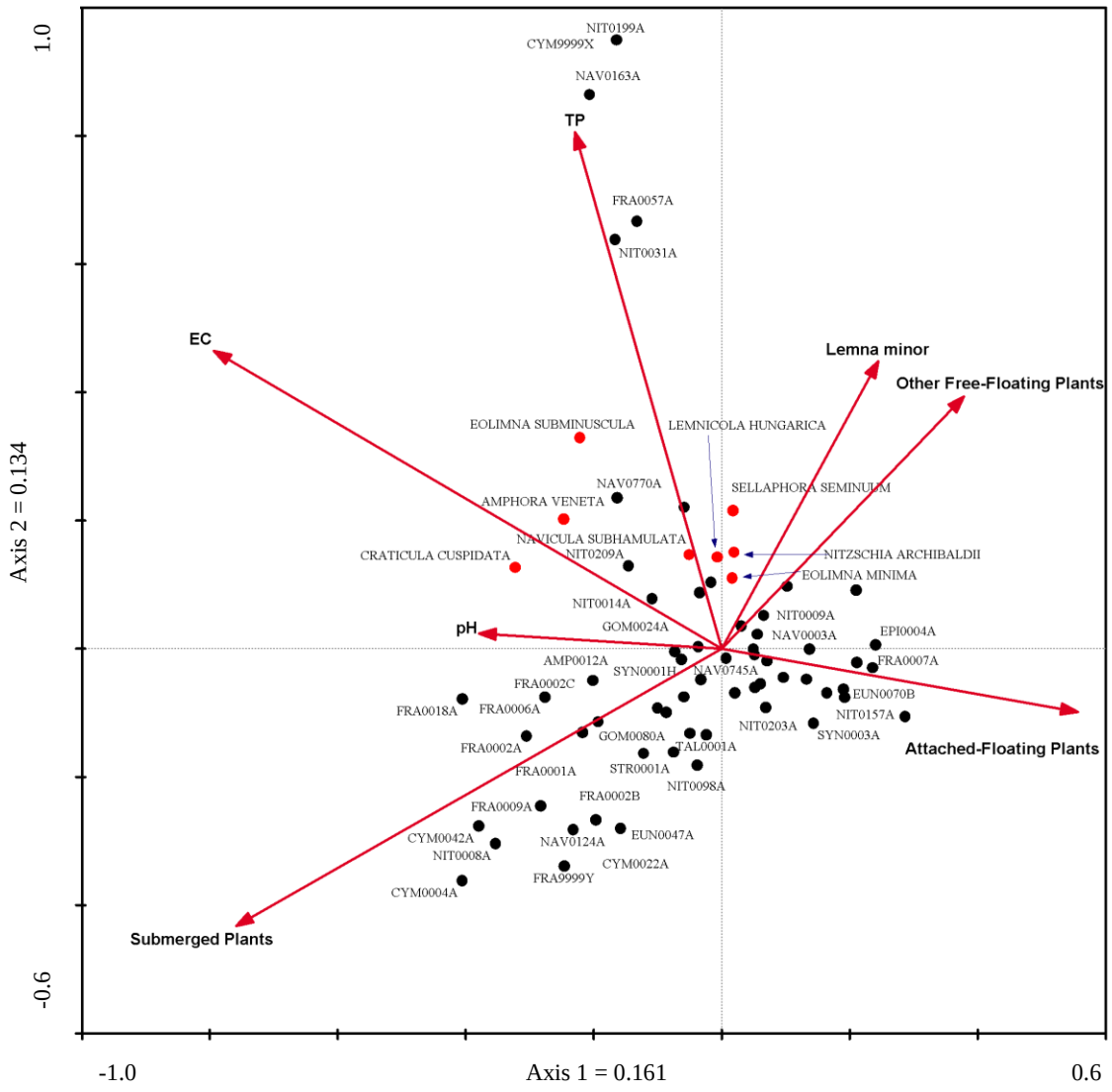


Figure 3.8. Canonical Correspondence Analysis (CCA) plot with forward selection of the 69 most common diatom taxa (i.e. diatom taxa with a minimum of 5% relative abundance) and significant environmental variables. Diatom taxa potentially associated with free-floating plants as identified from the Correspondence Analysis (CA) given in Fig. 3.5a are highlighted. (See Appendix 1 for diatom codes – species translation).

It is noticeable that the percentage variance explained by the first axis in CCA is close to that explained by the first axis in DCA (6.7 and 10.8 respectively). This suggests that the measured environmental variables explain a large amount of the variation in diatom species composition.

Linear regression analyses were performed to investigate the effects of water chemistry on the relative abundances of *L. hungarica* sampled from *L. minor* and other free-floating plants. There were no statistically significant correlations, except for *L. hungarica* sampled from *L. minor* with TP ($p=0.04$).

3.4.4 Evaluating the host macrophyte-diatom association hypothesis

Boxplots of relative abundance values of diatom taxa that exhibited relatively close proximity to *L. minor* in the CA (Fig. 3.5a) were produced for *L. hungarica, A. veneta, Eolimna minima, S. seminulum, N. archibaldii, Craticula cuspidata, Navicula subhamulata* and *Eolimna subminiscula* and compared across the five macrophyte groups: *L. minor*, other free-floating plants, *L. trisulca*, attached floating plants and submerged plants. The relative abundance boxplots of the taxa are given (Figs. 3.9a-h).

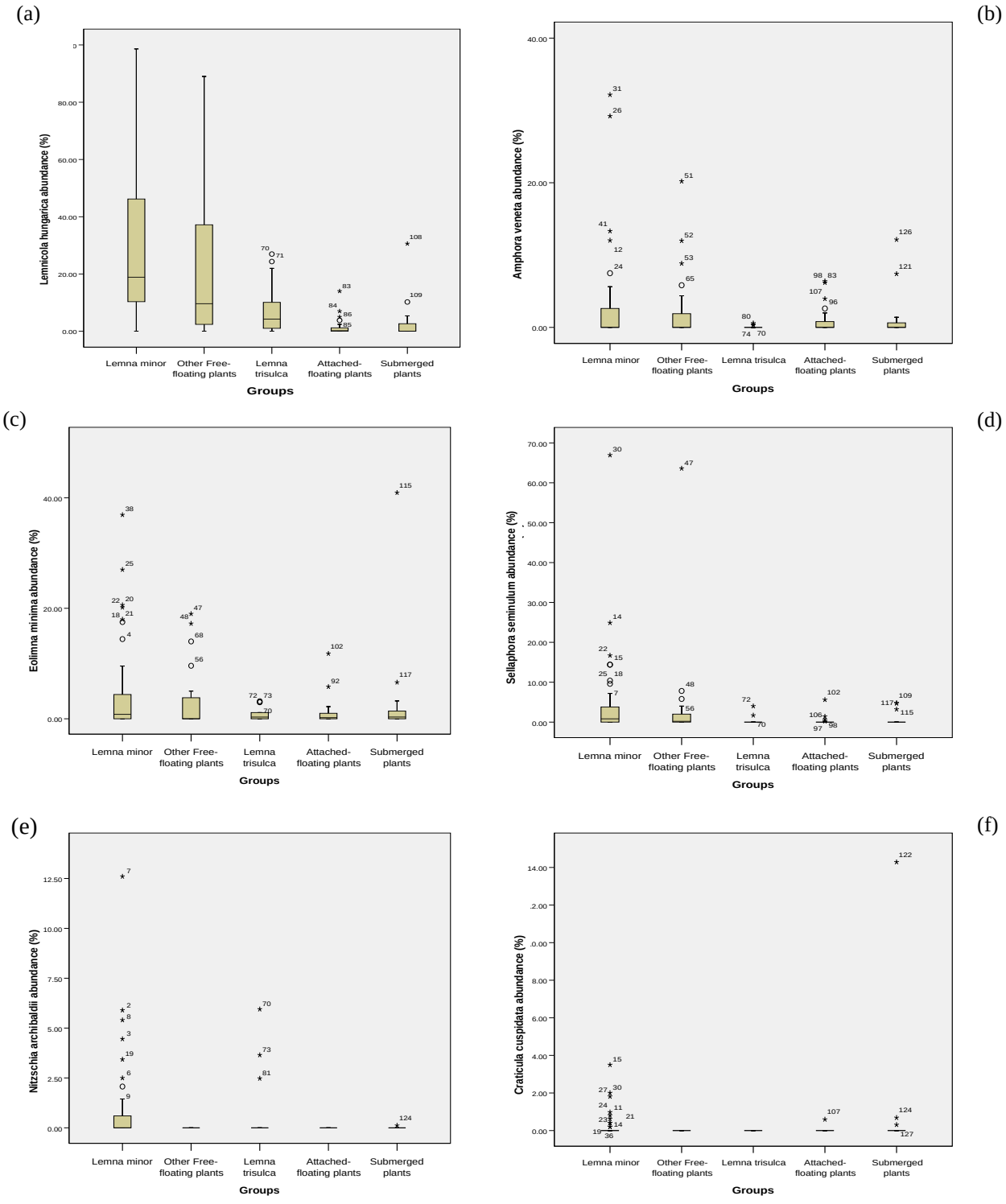
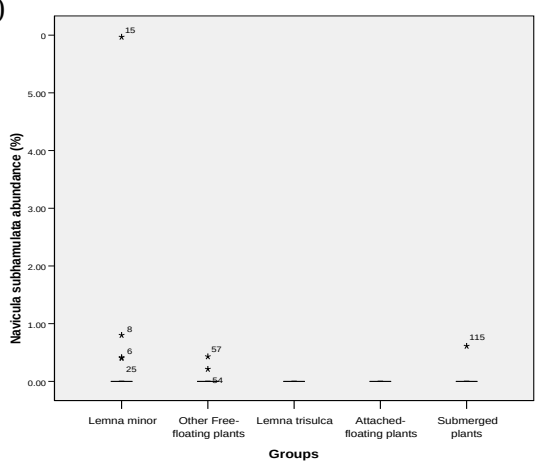


Figure 3.9. Boxplots of relative abundances of selected epiphytic diatoms associated with ***Lemna minor*** in comparison with other groups of freshwater macrophytes (see text). (a) ***Lemnicola hungarica,*** (b) ***Amphora veneta,*** (c) ***Eolimna minima***, (d) ***Sellaphora seminulum***, (e) ***Nitzschia archibaldii***, (f) ***Craticula cuspidata*** (expressed as medians, quartiles, extremes, outliers and mean values; 'outliers' are cases with values between 1.5-3 box lengths from the upper or lower edge of the box and 'extreme' cases have values >3 box lengths. Box length is the interquartile range).

(g)



(h)

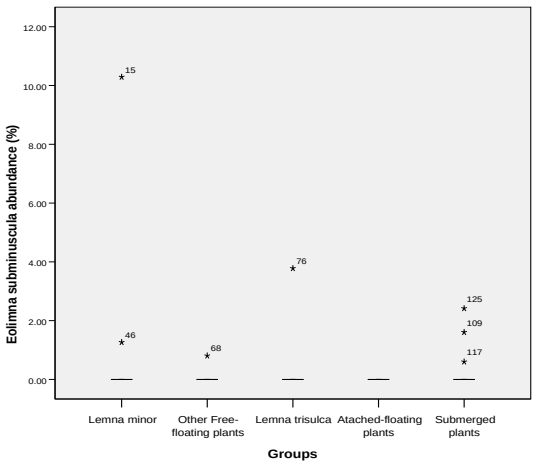


Figure 3.9 cntd. Boxplots of relative abundances of selected epiphytic diatoms associated with *Lemna minor* and other free-floating macrophytes in comparison with other groups of freshwater macrophytes (see text). (g) ***Navicula subhamulata*** and (h) ***Eolimna subminiscula.*** (expressed as medians, quartiles, extremes, outliers and mean values; 'outliers' are cases with values between 1.5-3 box lengths from the upper or lower edge of the box and 'extreme' cases have values >3 box lengths. Box length is the interquartile range).

Most of the selected diatom taxa did not reveal any particular associations with the macrophyte groups. *N. archibaldii* appeared to have an association with *L. minor* (Figs. 3.3, 3.5a & 3.8). However, the relative abundance (Fig. 3.9e) clearly showed that *N. archibaldii* was equally likely to be found as an epiphyte of *L. trisulca*. Similarly, although *C. cuspidata* showed an association with *L. minor*, it also had a high propensity to be associated with submerged macrophytes (Fig. 3.9f). Although *A. veneta* has been previously reported to be associated with *L. minor* (Goldsborough 1993) this study shows that, although often attached to *L. minor* and other free-floating macrophytes, *A. veneta* was also strongly associated with attached-floating and submerged macrophytes. Alternatively, *L. hungarica* (Fig. 3.9a) and *S. seminulum* (Fig. 3.9d) not only revealed associations with the free-floating macrophyte group *per se*, but were also strongly associated with *L. minor*. The strong association of *L. hungarica* with *L. minor* was particularly striking with respect to the large abundances recorded (Fig 3.9a), when compared with the other diatom taxa (Figs. 3.9b-h).

To determine the significance of the aforementioned host macrophyte-diatom associations, selected diatom data were further analysed by employing post hoc tests of

least-significant difference (LSD), a one-way ANOVA procedure, where a one-way analysis of variance of the quantitative dependent variable *L. hungarica* is compared with the independent variables represented by the various macrophyte groups. The mean diatom relative abundance data was also tested using Dunnett's t-tests where one of the macrophyte groups (i.e. submerged plants) were treated as a control and the other macrophyte groups were compared against it. A summary of the results of those diatom species which exhibited a statistically significant difference between the different macrophyte groups are presented in Tables 3.2-3.5.

	Macrophyte groups	Macrophyte groups	Sig. diff. (p =)
LSD	Lemna minor	Other Free-floating plants	.266
		Lemna trisulca	**.001**
		Attached-floating plants	**.000**
		Submerged plants	**.000**
	Other Free-floating plants	Lemna minor	.266
		Lemna trisulca	**.035**
		Attached-floating plants	**.000**
		Submerged plants	**.001**
	Lemna trisulca	Lemna minor	**.001**
		Other Free-floating plants	**.035**
		Attached-floating plants	.289
		Submerged plants	.392
	Attached-floating plants	Lemna minor	**.000**
		Other Free-floating plants	**.000**
		Lemna trisulca	.289
		Submerged plants	.829
	Submerged plants	Lemna minor	**.000**
		Other Free-floating plants	**.001**
		Lemna trisulca	.392
		Attached-floating plants	.829
Dunnett t (2-sided)	Lemna minor	Submerged plants	**.000**
	Other Free-floating plants	Submerged plants	**.003**
	Lemna trisulca	Submerged plants	.798
	Attached-floating plants	Submerged plants	.998

Table 3.2. Summary of the analysis of variance for ***Lemnicola hungarica*** abundance from the various macrophyte groups (significant differences at the 0.05 level between mean values are in bold).

	Macrophyte groups	Macrophyte groups	Sig. diff. (p =)
LSD	Lemna minor	Other Free-floating plants	.402
		Lemna trisulca	.081
		Attached-floating plants	**.035**
		Submerged plants	.295
	Other Free-floating plants	Lemna minor	.402
		Lemna trisulca	.333
		Attached-floating plants	.276
		Submerged plants	.838
	Lemna trisulca	Lemna minor	.081
		Other Free-floating plants	.333
		Attached-floating plants	.951
		Submerged plants	.436
	Attached-floating plants	Lemna minor	**.035**
		Other Free-floating plants	.276
		Lemna trisulca	.951
		Submerged plants	.392
	Submerged plants	Lemna minor	.295
		Other Free-floating plants	.838
		Lemna trisulca	.436
		Attached-floating plants	.392
Dunnett t (2-sided)	Lemna minor	Submerged plants	.665
	Other Free-floating plants	Submerged plants	.999
	Lemna trisulca	Submerged plants	.843
	Attached-floating plants	Submerged plants	.795

Table 3.3. Summary of the analysis of variance for ***Eolimna minima*** abundance from the various macrophyte groups (significant differences at the 0.05 level between mean values are in bold).

	Macrophyte groups	Macrophyte groups	Sig. diff. (p =)
LSD	Lemna minor	Other Free-floating plants	.759
		Lemna trisulca	.121
		Attached-floating plants	**.044**
		Submerged plants	.070
	Other Free-floating plants	Lemna minor	.759
		Lemna trisulca	.237
		Attached-floating plants	.142
		Submerged plants	.187
	Lemna trisulca	Lemna minor	.121
		Other Free-floating plants	.237
		Attached-floating plants	.966
		Submerged plants	.963
	Attached-floating plants	Lemna minor	**.044**
		Other Free-floating plants	.142
		Lemna trisulca	.966
		Submerged plants	.916
	Submerged plants	Lemna minor	.070
		Other Free-floating plants	.187
		Lemna trisulca	.963
		Attached-floating plants	.916
Dunnett t (2-sided)	Lemna minor	Submerged plants	.206
	Other Free-floating plants	Submerged plants	.475
	Lemna trisulca	Submerged plants	1.000
	Attached-floating plants	Submerged plants	1.000

Table 3.4. Summary of the analysis of variance for ***Sellaphora seminulum*** abundance from the various macrophyte groups (significant differences at the 0.05 level between mean values are in bold).

	Macrophyte groups	Macrophyte groups	Sig. diff. (p =)
LSD	Lemna minor	Other Free-floating plants	**.022**
		Lemna trisulca	.921
		Attached-floating plants	**.019**
		Submerged plants	**.025**
	Other Free-floating plants	Lemna minor	**.022**
		Lemna trisulca	.076
		Attached-floating plants	1.000
		Submerged plants	.991
	Lemna trisulca	Lemna minor	.921
		Other Free-floating plants	.076
		Attached-floating plants	.071
		Submerged plants	.080
	Attached-floating plants	Lemna minor	**.019**
		Other Free-floating plants	1.000
		Lemna trisulca	.071
		Submerged plants	.991
	Submerged plants	Lemna minor	**.025**
		Other Free-floating plants	.991
		Lemna trisulca	.080
		Attached-floating plants	.991
Dunnett t (2-sided)	Lemna minor	Submerged plants	.082
	Other Free-floating plants	Submerged plants	1.000
	Lemna trisulca	Submerged plants	.231
	Attached-floating plants	Submerged plants	1.000

Table 3.5. Summary of the analysis of variance for ***Nitzschia archibaldii*** abundance from the various macrophyte groups (significant differences at the 0.05 level between mean values are in bold).

Although *L. hungarica* (Table 3.2) did not show a significant difference in mean relative abundances when compared with *L. minor* and other free-floating plants (p= 0.266), it did exhibit highly significant differences when its mean relative abundances were compared between *L. minor* and *L. trisulca* (p=0.001), *L. minor* and attached-floating plants (p=0.0001) and between *L. minor* and submerged plants (p=0.0001).

Both *E. minima* (p=0.035) and *S. seminulum* (p=0.044) showed significant differences in their mean relative abundances between *L. minor* and attached-floating plants (Tables 3.3 and 3.4). Although this result signifies a preference of these diatoms for *L. minor*, these taxa did not show any significant differences in the multiple comparisons between the other macrophyte groups. *N. archibaldii* did show significant differences between *L. minor* and other free-floating plants (p=0.022), between *L. minor* and attached-floating plants (p=0.019), and also between *L. minor* and submerged plants (p=0.025). However, there were no significant differences in the mean relative abundances of this diatom found on *L. minor* when compared with *L. trisulca* (Table 3.5).

The diatom species, *A. veneta, C. cuspidata, N. subhamulata* and *E. subminiscula* did not show any significant differences in mean relative abundances for the multiple comparisons between the different macrophyte groups, suggesting a clear absence of host-macrophyte associations.

3.4.5 Indicator species analysis using TWINSPAN

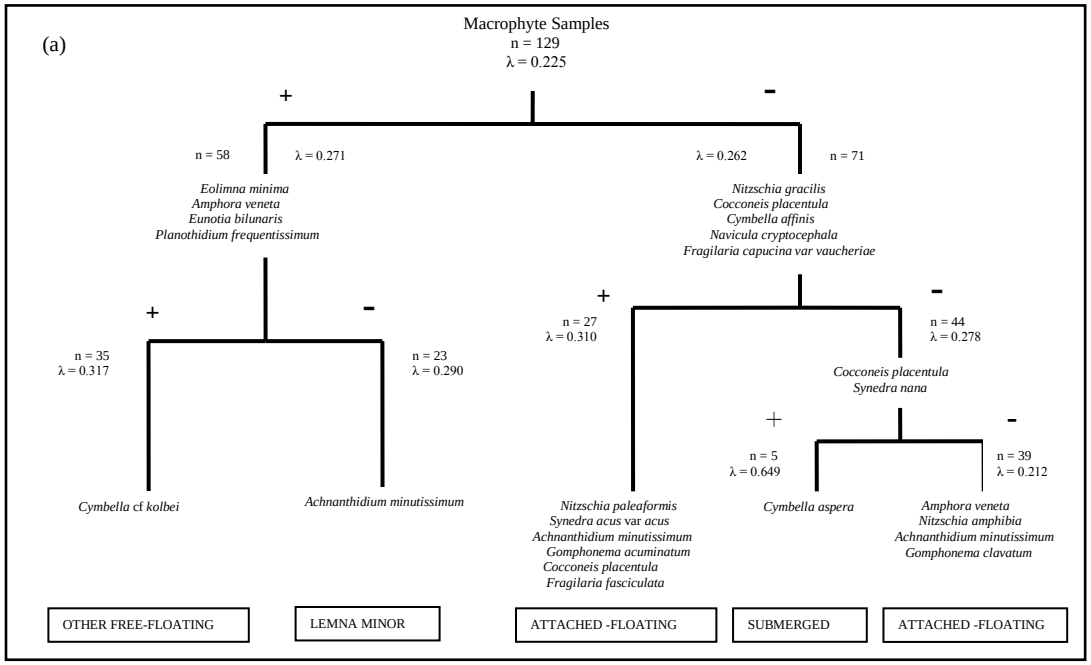
An indicator species is defined as "a species that is of narrow ecological amplitude with respect to one or more environmental factors and that is, when present, indicative of a particular condition or set of conditions" (Allaby, 1998). TWINSPAN is a classic method of finding indicator species in classified data and was applied to the data to see if *L. hungarica, S. seminulum* and other diatoms were indicators of free-floating plants.

To determine whether the diatoms *L. hungarica, E. minima, S. seminulum* and *N. archibaldii* might be used as indicator species to infer the past presence of free-floating

macrophytes generally and *L. minor* in particular, the diatom data were used in a TWINSPAN analysis. As indicator species analysis with TWINSPAN is based on qualitative data (Lepš & Šmilauer 2003) and in order not to lose potential information about species abundances (Hill *et al.*, 1975, Hill 1979), different pseudo-species cut levels were employed. Two dendrograms were produced (Fig. 3.10) using different arbitrary pseudo-species cut levels as follows: a = 0%, 2%, 5%, 10%, 20%; b = 0%, 5%, 10%, 20%, 40%.

The resulting end-groups in the TWINSPAN dendrograms were based *a priori* upon the main pre-defined macrophyte groups used in the previous analyses (i.e. '*L. minor*', 'other free-floating', 'attached-floating', and 'submerged'). The first dendrogram (Fig. 3.10a) did not give a clear classification of the diatom species with high affinities for particular macrophyte groups. For example, *Achnanthidium minutissimum* was given as an indicator species for attached-floating plants on both sides of the dichotomy together with being the sole indicator species for *L. minor*, whilst *Cymbella* cf. *kolbei* was the only indicator species for other free-floating plants. The submerged plant group was indicated solely by *Cymbella aspera*. The second dendrogram (Fig. 3.10b) also presented *C.* cf. *kolbei* as the sole indicator species for other free-floating plants, whilst *Eunotia arcubus* replaced *C. aspera* as an indicator species for submerged plants. Similarly, *A. minutissimum* was also listed as an indicator species for *L. minor*, but in this classification the diatoms *Planothidium frequentissimum*, *N. archibaldii*, *S. seminulum*, *Navicula radiosa*, *Navicula veneta* and *L. hungarica* were also indicator species for *L. minor*.

Clearly, the results of the indicator species analysis using TWINSPAN were ambiguous and potentially misleading with respect to defining diatom indicator species of macrophyte ecological groups. This ambiguity in defining indicator species maybe due to pre-defining the macrophyte end-groups *a priori* together with the use of pseudo-species in the analysis. Therefore, indicator species analysis using INDVAL was employed and as with TWINSPAN was also based on *a priori* pre-defined macrophyte groups.



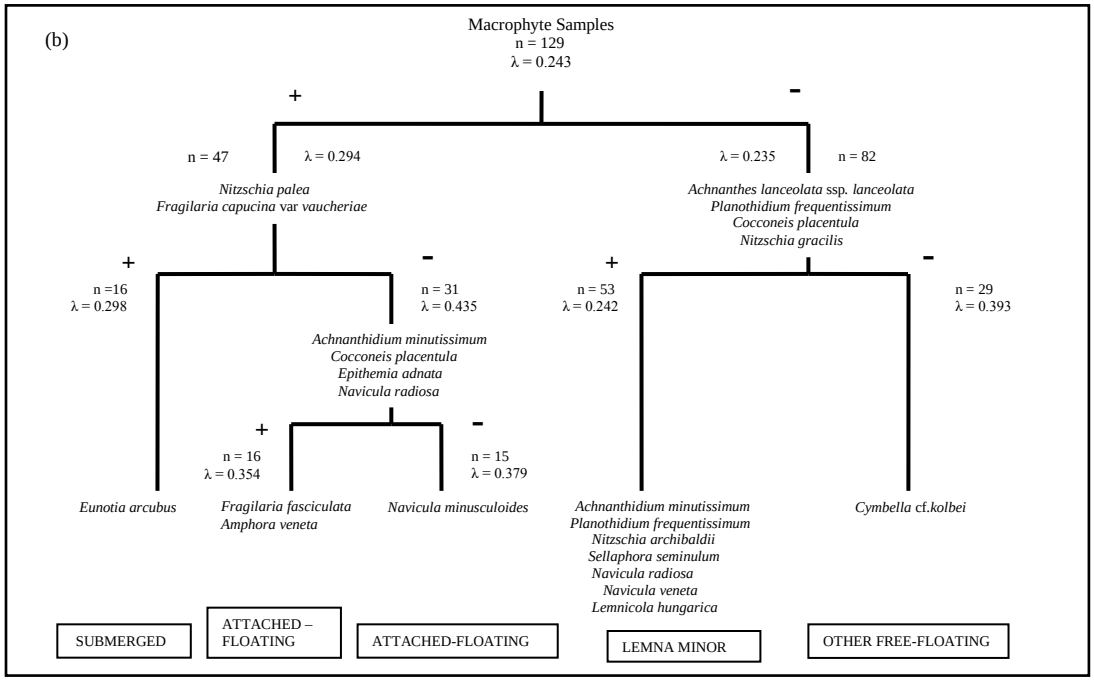


Figure 3.10. TWINSPAN classification dendrograms of epiphytic diatom species data for the 129 macrophyte samples. Indicator taxa identified, number of taxa, corresponding eigenvalues and associated ecological macrophyte groups are given for each TWINSPAN division. Cut-levels for (a): 0, 2, 5, 10, 20; and cut-levels for (b): 0, 5, 10, 20, 40.

3.4.6 Indicator species analysis using INDVAL

To further explore the potential of *L. hungarica*, *S. seminulum* and *Achnanthes exigua* var. *exigua* to be designated as 'Indicator Species' of *Lemna* the diatom data were subjected *a priori* to indicator species analysis with INDVAL. The statistical significance of the INDVAL analysis was tested using a Monte Carlo permutation test of significance (1000 random permutations) is presented in Table 3.6. The INDVAL analysis revealed that *L. hungarica* (p=0.001), *S. seminulum* (p=0.028) and *Achnanthes exigua* var. *exigua* (p=0.040) have a strong affinity or association with the macrophyte ecological group *L. minor* (i.e. Maxgroup 1 as defined by INDVAL) and can be statistically classified as indicator species for it. *L. hungarica* would appear to be more strongly associated with *L. minor* with the implication that this diatom could be used with a degree of confidence as an indicator species. *A. exigua* var. *exigua* was only found in seven *L. minor* samples and recorded at very low relative abundances (ranging from 0.2-0.7%). Consequently, more samples are needed to determine if *A. exigua* var. *exigua* can be classified as an indicator species for *L. minor.*

INDVAL classification did show statistical significances of diatom species associated with other free-floating plants (i.e. Maxgroup 2), namely *Nitzschia recta* (p=0.02), *Navicula minusculoides* (p=0.001), *Cymbella* sp. (p=0.02), *Nitzschia nana* (p=0.001) and *Nitzschia incognita* (p=0.007). Interestingly, these particular diatoms reflect the diatom taxa primarily associated with the macrophyte samples *Lemna* cf. *aequinoctialis*, *Azolla filiculoides* and *Azolla pinnata* but were not associated with *L. minor*.

Diatom Code	Diatom Species	Indicator Group (Maxgroup)	p value
ACH0165A	*Achnanthes catenata*	1	1.0000
ACH0016A	*Achnanthes delicatula*	1	1.0000
ACH0008A	***Achnanthes exigua var. exigua***	1	**0.0400***
ACH0032A	***Lemnicola hungarica***	1	**0.0010***
ACH0081A	*Achnanthes kolbei*	1	1.0000
ACH0001T	*Achnanthes lanceolata* spp. *robusta*	1	0.3720
ACH0085A	*Psammothidium lauenburgianum*	1	1.0000
AMP0005A	*Amphora normanii*	1	1.0000
AMP0001A	*Amphora ovalis*	1	0.3390
AMP0004A	*Amphora veneta*	1	0.4490
ANO0009A	*Anomoeoneis vitrea*	1	0.4050
CYM0015A	*Cymbella cesatii*	1	1.0000
DIA0004A	*Diatoma tenuis*	1	0.0920
EUN0017A	*Eunotia flexuosa*	1	1.0000
FRA0042A	*Fragilaria nitzschoides*	1	0.8450
GOM0004A	*Gomphonema gracile*	1	0.2890
GOM0013A	*Gomphonema parvulum*	1	0.7990
GOM9999A	*Gomphonema* sp.	1	0.4260
MER0001A	*Meridian circulare* var. *circulare*	1	1.0000
NAV0769A	*Navicula lundii*	1	1.0000
NAV0538A	*Navicula obdurata*	1	1.0000
NAV0743A	*Navicula subrhynchocephala*	1	1.0000
NAV9999U	*Mayamaea atomus* var. *alcimonica*	1	1.0000
NAV0066A	*Navicula capitata*	1	0.6790
NAV0745A	*Navicula capitatoradiata*	1	0.5110
NAV0344A	*Navicula eidrigiana*	1	1.0000
NAV0112A	*Navicula minuscula* var. *minuscula*	1	1.0000
NAV0065A	*Navicula gastrum*	1	1.0000
NAV0023A	*Navicula gregaria*	1	0.5820
NAV0042A	*Eolimna minima* var. *minima*	1	0.1130
NAV0014A	*Sellaphora pupula*	1	0.5120
NAV9999X	*Navicula raederiae*	1	1.0000
NAV0005A	***Sellaphora seminulum***	1	**0.0280***
NAV0075A	*Navicula subhamulata*	1	0.5250
NIT0199A	*Nitzschia angustulata*	1	0.6320
NIT0044A	*Nitzschia intermedia*	1	0.0960
NIT0171A	*Nitzschia subacicularis*	1	0.6420
PIN0001A	*Pinnularia gibba*	1	1.0000
RHL0001A	*Rhopalodia acuminata*	1	0.5890
STR9999A	*Stauroneis* sp.	1	0.4320
SUR0016A	*Suriella minuta*	1	0.1650

Table 3.6. Diatom taxa relationships with *L. minor* (i.e. Maxgroup 1) using INDVAL. The statistical significance of diatom relationships with *L. minor was* assessed using Monte Carlo permutation tests. **Note**: species highlighted with an asterisk (significance level: 0.05) are statistically significant indicator species of *L. minor* when compared with the other macrophyte ecological groups (see text).

3.4.7 Testing the use of *Lemnicola hungarica* and *Sellaphora seminulum* as *Lemna* indicators from surface sediments

L. hungarica and *S. seminulum* have been shown to have a strong and robust association with *L. minor*. To test the indicator species potential revealed from the INDVAL analysis for using these diatom taxa as proxy indicators of past *L. minor* in ponds, surface sediments from fourteen small freshwater ponds currently covered in extensive duckweed *(Lemna*) mats (25-100% surface cover) were sampled to determine their diatom community composition, and in particular the presence of both *L. hungarica* and *S. seminulum*. An analysis of surface sediments (see Chapter 2) would determine if the indicator status relationship of these diatoms can be successfully transferred to sediment. Samples were also collected from twelve similar sites that had no *Lemna* (duckweed) for comparison. The study sites are listed in Table 3.7. A comparison of the percentage relative abundances of *L. hungarica* and *S. seminulum* from the duckweed and non-duckweed sites is given in Figure 3.11.

The boxplots (Fig. 3.11) show that both *L. hungarica* (maximum=54%, minimum=5%, mean=16%) and *S. seminulum* (maximum=8%, minimum=1%, mean=3%) were recorded from the surface sediments of the *Lemna* sites. There was one *Lemna* site (Priory Pond 1) that did not record *L. hungarica* or *S. seminulum* and one other *Lemna* site (Church Farm Pond) that did not record *S. seminulum*. There was only one non-*Lemna* site (Sayer's Black Pit) which recorded *L. hungarica* but with a very low percentage relative abundance (0.003%); and there were three non-*Lemna* sites (Pond Farm Pond 2, Sayer's Black Pit and Otom Pit) which recorded *S. seminulum* but with very low percentage relative abundances (i.e. 0.002%, 0.01% and 0.006% respectively). Figure 3.12 shows photographs of two of the *Lemna*-covered sites (Saxlingham Road Pond and Priory Pond 1) used in the study.

Non-Duckweed Sites

Site	Site Code	Site No.	UK NGR
Pingo 37	PING37	1	TL93670962
Henry's Pit	HENR	2	TG06903245
Pond Farm Pond 2	POFA2	3	TG13203860
Bodham marl Pit	MARL	4	TG12703870
Salle Patch Pond	SALL	5	TG11052445
Bodham Rail Pit	RAIL	6	TG12353890
Pond Hills Pond	POHI	7	TG10336459
Bodham Mystery Pit	MYST	8	TG12603945
Sayer's Black Pit	SABA	9	TG12653960
Bullock Shed Pond 1	BULLS1	10	TG11302830
Kiosk Pit	KIOS	11	TG09402840
Cinders Hill Pond	CIND	12	TG10902880
Hempstead Rookery Pond	ROOK	13	TG10203745
Otom Pit	OTOM	14	TG09252750

Duckweed Sites

Site	Site Code	Site No.	UK NGR
Pingo 19	PING19	15	TL93852964
Roadside Pingo	PING999	16	TL94884934
Ramsgate Horse Pond	RAMS1	17	TG09353365
Pond farm Pond 1	POFA1	18	TG13203865
Church Farm Pond	CHFA1	19	TG10353670
Bullock Shed Pond 2	BULLS2	20	TG11102830
Aldersbrook Pond	ALDB	21	TQ42758633
Lower Farm Pond	LOFA1	22	TG13804025
Priory Pond 1	PRIO1	23	TG16754285
Saxlingham Road Pond	SAXR	24	TG02403955
Manor Farm Pond 29	WADD29	25	TG07153300
College Farm Pingo	PINGCF	26	TL93256962

Table 3.7. Non-duckweed and duckweed site characteristics used in the logistic regression analysis. Site numbers are those used in Fig. 3.11b.

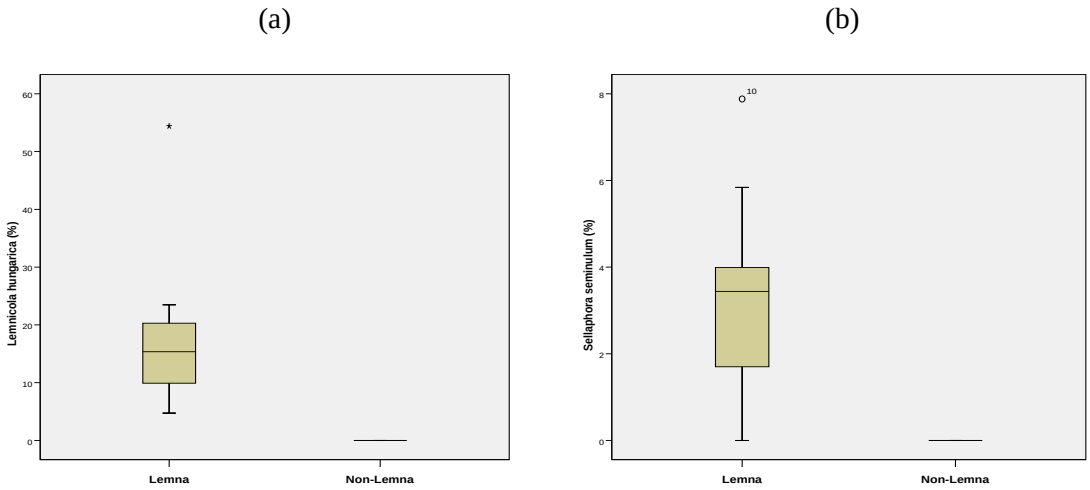


Figure 3.11. Percentage relative abundance of *Lemnicola hungarica* (a) and *Sellaphora seminulum* (b) in surface sediment samples from *Lemna*-covered (n=12) and Non-*Lemna* covered (n=14) ponds. Boxplots give medians, quartiles, outliers and box length gives the interquartile range.

3.4.7.1 Logistic regression analysis

Binomial logistic regression was used to determine whether the surface sediment diatom assemblages could confidently and faithfully predict (past) *Lemna* presence. The aim was to model the dependent categorical response variables (*L. hungarica* and *S. seminulum*) on a continuous predictor variable (duckweed cover).

The results of the logistic regression analysis of the dependent categorical variables *L. hungarica* and *S. seminulum* with the predictor variable (duckweed cover) were statistically significant. The regression model indicated that the duckweed-covered sites successfully predicted the presence of both *L. hungarica* ($p=0.0001$, $r^2=0.903$) and *S. seminulum* ($p=0.002$, $r^2=0.758$) confirming the validity of their indicator status. This predictive model was equally accurate for both the duckweed (93% correct) and non-duckweed sites (92% correct).

(a)

(b)

Figure 3.12. Photographs of duckweed covered ponds showing varying degrees of duckweed cover and the effects of riparian vegetation providing wind protection. Saxlingham Road Pond (a) with little riparian wind protection and Priory Pond 1 (b) with greater riparian wind protection. (Photographs: Carl Sayer).

3.4.7.2 Ordination analyses

Further to the logistic regression analysis of the surface sediment diatom assemblages between the duckweed vs. non-duckweed sites, the surface sediment diatom data were also explored with ordination methods. DCA of the diatom assemblage data from the duckweed and non-duckweed sites revealed that the gradient lengths of the first two axes were long at 3.46 and 3.37 SD units respectively. Consequently, it was decided that unimodal methods were most appropriate and CA of the diatom data was performed. The CA summary statistics show that the first two axes explained a large amount of the variance (15.4% and 26.7% respectively) similar to that in the DCA (15.4% and 24.7% respectively). The eigenvalues of the first two axes are shown in Fig. 3.13.

The diatom species ordination revealed that *L. hungarica* and *S. seminulum* were in close proximity within the ordination space graphically indicating their close association with duckweed (Fig. 3.13a). Moreover, the CA showed clear differentiation of the duckweed and non-duckweed samples (Fig. 3.13b). However, there appeared to be outliers amongst the non-duckweed sites (site 1, Pingo 37; site 7, Pond Hills Pond; site 12, Cinders Hill Pond) and one noticeable duckweed site outlier (site 23, Priory Pond 1). Site 1, Pingo 37 was dominated by small "*Fragilaria* spp.", namely, *Staurosira construens* var. *venter*, *S. construens* var. *construens*, *Staurosirella pinnata* and also *F. capucina* var. *capucina*. Site 7, Pond Hills Pond was dominated by *Stephanodiscus hantzschii*, *S. pinnata*, *S. construens* var. *construens* and *Amphora ovalis*. Site 12, Cinders Hill Pond was dominated by *S. pinnata*, *F. capucina* var. *capucina* and *Pinnularia maior* and site 23, Priory Pond 1 (duckweed site), was dominated by *Epithemia* spp. (namely, *E. sorex*, *E. turgida* and *E. adnata*). Interestingly, the three non-duckweed site outliers were all sites that had extensive riparian shading from trees and shrubs. The single duckweed site outlier (Priory Pond 1) is possibly explained by the fact that this particular duckweed site was the only site that did not record the two duckweed epiphytes *L. hungarica* and *S. seminulum* from the surface sediment sample.

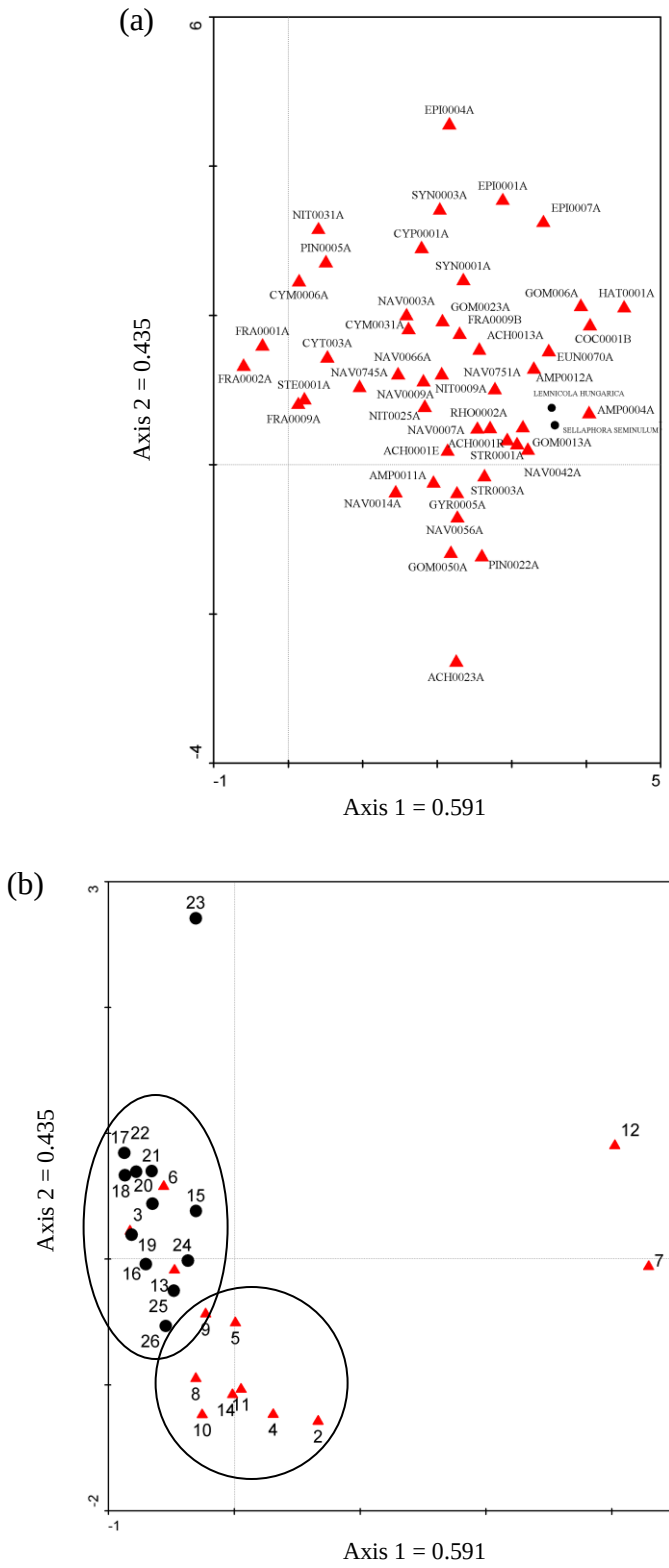


Figure 3.13.CA plot of all diatom taxa recorded from surface sediment samples on axes 1 and 2. *Lemnicola hungarica* and *Sellaphora seminulum* are highlighted (a). CA plot of the duckweed sites (circles) and non-duckweed sites (triangles) on axes 1 and 2. The duckweed and non-duckweed site groups in ordination space are highlighted. Note the site outliers (b). Sample numbers correspond to the specific site numbers (see Table 3.7; Appendix 1 for diatom codes).

3.5 Discussion

Several interesting and general observations on diatom-macrophyte relationships have come from this study. Firstly, concurring with other studies (Goldsborough & Robinson 1985, Goldsborough 1993 & 1994, Buczkó 2007) there was low species richness in the diatom assemblages found on free-floating macrophytes. This low species richness possibly reflects the peculiar nature of the water-surface zone and the specific ecological and biological requirements of epiphytic diatoms to tolerate and thrive in this habitat, characterised by high light intensities, wind disturbance and temperature fluctuations. Secondly, water chemistry did not play a major role in determining whether *L. hungarica* and *S. seminulum* were associated with duckweed. Indeed water chemistry was considered to be a secondary environmental variable in explaining epiphytic diatom community structure. The main ecological and biological driver in determining the *L. hungarica* and *S. seminulum* communities was the presence of free-floating plants.

3.5.1 Diatom indicators of duckweed

The existence of host-epiphyte relationships for macrophytes and algae has been a source of considerable and continued debate (Godward 1937, Prowse 1959, Allanson 1973, Gough & Woelkerling 1976, Moss 1976, Brown 1976, Cattaneo 1978, Cattaneo & Kalff 1979, Eminson & Moss 1980). Nonetheless, several previous studies on diatom-duckweed relationships have indicated that there is a strong association between *L. hungarica* and duckweed (Round 1973 & 1981, Patrick & Reimer 1966, Marvan & Komárek 1978, Bowker & Denny 1980, Germain 1981, Zuberer 1984, Goldsborough & Robinson 1985, Goldsborough 1993 & 1994, Round & Basson 1997, Buczkó 2007 and Desianti 2012).

The results of this ‘global’ diatom host-plant specificity study illustrates that most of the dominant diatom taxa recorded do not have a particular affinity for specific macrophyte species or growth forms. Indeed the data suggest that many taxa, most

notably the common species *C. placentula*, *A. minutissimum*, *N. palea* and *G. parvulum*, were observed to be cosmopolitan and found to live in a wide range of the sampled macrophyte species growth forms. Nevertheless, the dissimilarity and dispersion analyses suggested statistically significant differences in diatom community assemblage dispersion (β-diversity) and composition associated with the different macrophyte groups.

Other free-floating macrophytes from the 'global' study such as the water ferns, *Azolla filiculoides* and *Azolla pinnata* gave contrasting results. *A. filiculoides* samples (n=4) were dominated by several species including *L. hungarica*, *A. minutissimum*, *F. fasciculata* and *E. bilunaris* var. *mucophila*, whilst the diatom assemblage of *A. pinnata* (n=1) was dominated almost exclusively by *Nitzschia* spp., notably *N. nana*, *Nitzschia lacuum* and *Nitzschia paleacea.* Interestingly, the majority of the *A. filiculoides* samples were collected from sites that also had *Lemna* species present such as Murrurundi Billabong (Australia), Shaw Lake (Canada), Lake Titicaca (Peru) and Tai Hu Lake (China) albeit in different areas, but the *A. filiculoides* sample from Australia, dominated by *L. hungarica*, was a monocultural mat in the absence of free-floating lemnids (C. D. Sayer: pers. com.). It is interesting to speculate that perhaps *L. hungarica* has a habitat preference for species of the Lemnaceae, but is able to survive on other free-floating plants as a secondary and alternative habitat preference. Interestingly, from a fossil diatom study on riverine floodplain wetlands in south-east Australia, Gell *et al.*, (2005) found both *L. hungarica* and *S. seminulum* in a sediment core collected from Willsmere Billabong. The implication from the fossil diatom profile from Willsmere Billabong is that it is likely that free-floating plants, such as *A. filiculoides*, were present at this site.

Shallow water-bodies are readily mixed by wave and wind action effectively producing mixed macrophyte communities and, therefore, most diatom species can be found in more than one habitat in the natural environment (Lim *et al.*, 2001). These effects can 'mask' the potential to identify diatom species that faithfully indicate specific habitats. However, despite the vagaries of weather conditions together with the fact that samples

in this study were taken across a wide spectrum of water body sites and macrophyte growth forms, the analyses suggest that a relatively small number of taxa showed clear affinities with Lemnaceae namely, *L. hungarica*, *S. seminulum* and *N. archibaldii*. Although Goldsborough (1993) found *S. seminulum* on *L. minor*, this study is the first to demonstrate a statistically significant association between *S. seminulum* and free-floating plants, particularly *L. minor*, in comparison with attached-floating and submerged plants. *L. hungarica* clearly showed a strong affinity for this habitat type of free-floating plants and particularly *L. minor*. These observations broadly concur with other studies (Goldsborough 1993, Buczkó 2007) where *L. hungarica* was shown to dominate diatom assemblages of *L. minor*, but it was also found to be abundant on other duckweeds, namely *L. gibba*, *S. polyrhiza* and *W. arrhiza* (Buczkó 2007). This was in marked contrast to the diatom assemblages found on *L. trisulca* which were dominated by *C. placentula*. This study supports the findings of Buczkó (2007) as the samples of *L. trisulca* were also dominated by *C. placentula* (Fig. 3.3).

Interestingly, *N. archibaldii* (Table 3.8) was found to have a potential affinity with *L. minor*. However, this diatom was also shown to have an affinity with *L. trisulca* but it was only recorded at low percentage relative abundances and from just a few macrophyte samples and consequently *N. archibaldii* cannot be considered to be an indicator species for free-floating plants. From the previous studies by Goldsborough (1993) it was expected that *A. veneta* would show high relative abundances on *L. minor* when compared with the other macrophyte groups and growth forms. The INDVAL and other analyses did not support this assertion however, as *A. veneta* was found across a variety of macrophyte species and growth forms.

L. hungarica exhibited a clear preference for the Lemnaceae and was typically abundant on *Lemna* species. It was rarely found on attached-floating and submerged macrophytes. However, there were some inconsistencies within the data. Firstly, from a total of 45 *L. minor* samples *L. hungarica* and *S. seminulum* were absent from two of these samples, namely Bayfield Hall Lake and Upper Lough Erne. Both of these samples were dominated by *C. placentula* (48% and 91% respectively). It is reasonable

to surmise that *C. placentula* domination of these samples reflected a stochastic event whereby *C. placentula* colonised *L. minor* before *L. hungarica* and *S. seminulum* managed to get a 'foot-hold' or maybe *C. placentula* simply out-competed these diatoms. The consistent and widespread distribution of *C. placentula* across different macrophytes is indicative of a 'generalist' strategy and suggestive of a wide ecological and environmental tolerance. It is feasible that the physically stressful habitat upon free-floating plants is the 'fundamental niche' for both *L. hungarica* and *S. seminulum*, in the absence of competition from other adnate diatoms such as *C. placentula*, but in the presence of such interspecific competition these species may be restricted to a biologically stressful 'realised niche' on free-floating plants. This suggests that the ecological niche for *L. hungarica* and *S. seminulum* are free-floating plants, whether interspecific competition is present or not. *S. seminulum* has been recorded from the roots of *L. minor* as opposed to the fronds (Goldsborough 1993); however, both *L. hungarica* and *C. placentula* have been recorded from the fronds of lemnids (Buczkó 2007) where interspecific competition for frond occupancy would have a direct impact on the relative abundances of these motile adnate species (Fig. 3.3).

Secondly, there were three samples of the group '*L. trisulca*' (collected from the Danish shallow lakes: Denderup, En Sø and Døj Sø) which recorded unusually high abundances of *L. hungarica* (25%, 10% and 27%, respectively). In all three lakes, *L. trisulca* samples were collected from mixed surface mats of *L. trisulca* and *L. minor* (T. A. Davidson: pers. com.). In such a situation there would have been a high likelihood of 'cross contamination' of *L. trisulca* samples by *L. hungarica* from adjacent fronds of *L. minor*. That the other *L. trisulca* samples (n=10) did not record *L. hungarica* or *S. seminulum* supports the idea that these species are not indicator species of *L. trisulca* and that its different ecology does not suit these particular diatoms. Similar to *L. trisulca* there was a relatively high abundance of *L. hungarica* (14%) found on the attached-floating plant *Hydrocharis morsus-ranae* sample collected from Corracoash Lough (Northern Ireland), and a relative abundance of over 30% found on the submerged plant *Ceratophyllum demersum* which was collected from Tai Hu Lake (China). The *H. morsus-ranae* sample was again collected in close proximity to mats of

L. minor (B. J. Goldsmith: pers. com.) which had over 25% *L. hungarica*. The *C. demersum* sample was collected in close proximity to mats of *S. polyrhiza* which had 89% *L. hungarica*. Again, it is reasonable to surmise that the *H. morsus-ranae* and *C. demersum* samples could easily have been 'contaminated' with *L. hungarica* cells from the adjacent duckweed mats. These samples were found together with other macrophytes and clearly could not be classified as being monocultural mats. This situation demonstrates the potential difficulties of macrophyte collection techniques and in understanding different diatom micro-habitats from field studies (Round 1998).

3.5.2 Potential for diatom-duckweed indicator species in palaeoecological studies

In accordance with the contemporary macrophyte-diatom study the analysis of the surface sediment samples confirms the association between *L. hungarica* and *S. seminulum* and Lemnaceae. This tracking of *Lemna* in space augments and compliments the validity of the *Lemna*-epiphyte inference model to be able to confidently track *Lemna* dominance through time. The significant and reliable presence of these diatom taxa in the surface sediments of duckweed-covered sites suggests that the diatom-duckweed relationship is successfully transferred from the free-floating duckweed mats to the sediment. In turn this gives confidence in the ability of these two species to act as free-floating plant indicators in palaeoecological studies (see Chapter 5).

The TWINSPAN indicator species analysis was ambiguous and was likely due to the use of *a priori* pre-defined macrophyte end groups. However, the INDVAL results supported those of the earlier multivariate analyses demonstrating that the epiphytic diatoms, *L. hungarica* and *S. seminulum*, have a strong preference for the free-floating macrophyte habitat. Clearly, a critical evaluation of the two indicator species analyses (TWINSPAN and INDVAL) is needed. With the exception of Yang (2009) who employed INDVAL to identify indicator diatom species for epiphytic habitats based upon surface sediment diatom assemblages in the acidified Round Loch of Glenhead, Scotland, to date there are no similar studies concerning epiphytic diatom species and

their potential to be employed as indicator species of macrophyte 'habitats' or indeed other habitat affinities, in either contemporary or palaeoecological investigations from small ponds or lakes.

3.6 Conclusions

Both *L. hungarica* and *S. seminulum* can be confidently employed to identify past duckweed covered periods in ponds. Moreover, these two diatom species can now provide a robust palaeoecological tool to reconstruct ecological histories of ponds and shallow lakes. In turn these palaeoecological reconstructions could determine whether extensive duckweed mats are acting as physical ecosystem engineers upon the ecological structure and function of small freshwater bodies. The palaeoecological potential of *L. hungarica* and *S. seminulum* as indicator taxa will be further explored in a palaeolimnological investigation of the Bodham Rail Pit where duckweed phases or cycles have been observed over recent decades (see Chapter 5).

The nature of this host-plant association is poorly understood. For example, is the association due to the physical location of the duckweed at the water-surface interface (physical hypothesis) or is the association due to a biological interaction between the host duckweed and the diatoms (chemical hypothesis)? To this end, a simple laboratory experiment was undertaken in an attempt to elucidate the nature of this association by directly comparing the growth rates of *L. hungarica* upon different floating substrates (see Chapter 4).

Chapter 4. Is there a host-plant interaction between *Lemnicola hungarica* and *Lemna minor*?

4.1 Introduction

Quantitative sampling of periphytic algae can be problematic due to issues such as the inherent heterogeneity of algal communities, difficulties in precise measurements of the host surface, the actual physical removal of algal populations from the host plant (Hickman 1971) and the inherent analytical limitations in accurately locating organisms which may measure less than 10μm in length (Goldsborough 1989). Therefore, the use of artificial substrata has often been employed in periphyton ecological studies and, as a fresh surface is used each time, some control of experimental conditions is possible (Tippett 1970).

4.1.1 Epiphyton and artificial substrata

Artificial substrates have long been used in studies of periphyton community structure and productivity and authors have generally found that periphyton communities closely resemble that found on natural substrates (Castenholtz 1960, Sládecková 1962, Pieczynska & Spodniewska 1963, Dor 1970, Mason & Bryant 1975). On the other hand, other authors have reported significant differences in algal species diversity and abundance between artificial substrata and living aquatic macrophytes (Godward 1937, Foerster & Schlichting 1965, Tippett 1970, Brown 1976), the causes of which are unclear.

While artificial substrata should perhaps not be used in situations where they are intended to exactly mimic natural conditions, particularly in the estimation of biomass and productivity, they can be used successfully for investigating rates of colonisation, community interactions and the impact of environmental variables. Because

macrophytes provide substrata that are not inert, the use of artificial substrata for some investigations is questionable. Therefore, from an ecological perspective, artificial substrata should be used with caution and the collection of diatoms for ecological interpretation should ideally be sampled from natural habitats. Nevertheless, artificial substrata have clear advantages over natural substrates as they can be readily manipulated into different positions, can be adequately replicated, may be readily sampled, and there is an ease of determination of surface area. Possible contamination problems from host tissue are also eliminated, and composition analysis techniques can be employed without having to remove the periphyton from its substratum.

4.1.2 An experimental approach

To better understand the nature of the association between *Lemnicola hungarica* and *Lemna minor*, an experimental approach was developed. Field observations (see Chapter 3) indicate a strong association between *L. hungarica* and species in the Lemnaceae (duckweed). The specific aims of the experimental study were to investigate the physiological responses that may contribute to the survival and growth of *L. hungarica* on duckweed. Therefore, the nature of the relationship between *L. hungarica*, live and dead duckweed (*L. minor*) and an artificial substrata ('artificial duckweed') was investigated experimentally to try to elucidate if this taxon has any habitat preference between natural (duckweed) and artificial surfaces, to determine if *L. hungarica* gains an advantage living on duckweed due to exploiting nutrients leached from duckweed or is the association due to the physical location at the water-air interface and, therefore, to determine if artificial substrata can be used to aid our understanding of the ecology of *L. hungarica*. All the experimental surfaces were positioned at the interface of the air and the specific sterilised culture media. The surface materials used in the experiment included inert artificial surfaces and live biological samples. The valve counts of relative abundances of the *L. hungarica* populations on each substratum were analysed using light microscopy. Furthermore, different experimental substrates were investigated by scanning electron microscopy (SEM) at various time intervals to determine potential changes in the micro-niches

inhabited by *L. hungarica*. The latter was assessed by comparing diatom growth on *L. minor* frond surfaces and roots, and by determining any differences in abundance and growth rates between *L. minor* and its artificial surrogate.

4.1.3 Hypothesis testing

The Null hypothesis (i.e. 'physical hypothesis') states that there will be no statistically significant difference in *L. hungarica* relative abundances and the relative population growth rates between inert artificial surfaces and live biological samples. The alternative hypothesis (i.e. 'chemical hypothesis') states that there will be a statistically significant difference in the relative abundances and growth rates of *L. hungarica* between inert artificial surfaces and live biological samples with a greater abundance and, therefore, a higher relative growth rate on the live biological samples compared with the artificial surfaces. The testing of the hypothesis under controlled conditions should elucidate any causal relationships or refute any incorrect deductions (Cox 1993).

4.2 Methods and materials

4.2.1 Study samples

The live biological samples consisted of axenic samples of *L. minor*. The *L. minor* samples, together with associated *L. hungarica* epiphytes, were collected from two small freshwater ponds in the inner (HOME) and outer (RNOH) London area. The inner London pond was a garden pond in East London, England (UK NGR: TQ41614904) and the outer London pond was a fishing pond located in hospital grounds at Stanmore, Middlesex, England (UK NGR: TQ17359402). This approach was adopted in an attempt to negate any potential ecological peculiarities or distinct morphological and physiological characteristics of different *L. hungarica* and *L. minor* strains between the two sites. This approach of employing different duckweed strains from multiple sites was recommended by Hillman (1961).

The inert artificial surfaces employed in this experiment were designed to 'mimic' the size, growth form and architecture of natural *L. minor* samples whilst also standardising the experimental colonisation surfaces. A further criterion for the inert surfaces was that they allowed incident light transmission (within the wavelengths associated with photosynthetically active radiation [PAR]), whilst also providing a suitable and comparable surface for colonisation and growth of *L. hungarica*. This criterion was satisfied by employing clear polystyrene discs, approximately 5mm in diameter, which were cut from laboratory Petri-dishes using a heated 5mm metal borer. To simulate the single root of the *L. minor* samples, a 10mm length of nylon fishing line was attached to the abaxial, or under surface, of the disc by melting one end of the fishing line. This was achieved using chloroform delivered by a fine bore glass pipette. This method of attachment was used to simulate the natural root attachment (the prophyllum) of *L. minor* and also to negate any potential problems of contamination with the use of chemical adhesives. Furthermore, the effect of 'melting' the fishing line 'root' upon the underside of the discs produced a roughened surface, similar to the epidermal depressions on the undersides (abaxial) of the *L. minor* fronds, which could potentially provide micro-niches for diatom colonisation and growth.

The incident light transmission spectra through *L. minor* samples was determined using a spectrophotometer (HACH DR/400 OU). As the spectrophotometer sampling vial has the same characteristics as the inert artificial surfaces (i.e. clear polystyrene discs) this variable of light transmission could be ignored (confirmed by the negligent light absorption using blank clear sampling vials as controls). The light transmitted through glass was also determined as the culture vessels were composed of glass. Light and dark green tissue papers were placed within the vial in order to simulate and approximate the incident light transmission spectra through a *L. minor* frond. The resulting spectrograph is shown in Figure 4.1a which shows that neither the light or dark papers accurately compare with percentage light transmission through *L. minor* fronds over the measured wavelengths.

(a) (b)

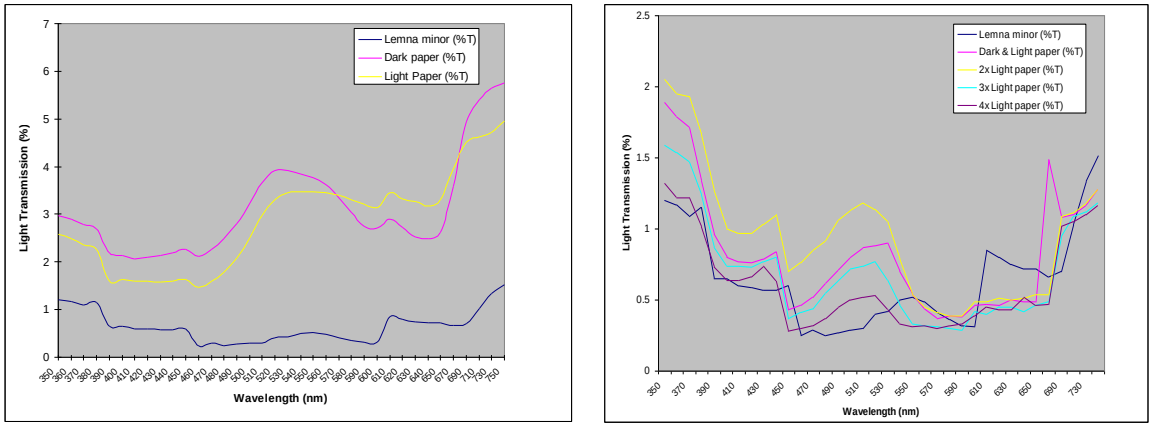


Figure 4.1. (a & **b)** Incident light transmission spectra (% transmission plotted against wavelength) comparing the spectrograms of *Lemna minor* fronds with light and dark coloured green tissue paper.

There are similarities in the pattern of the graph, particularly the percentage transmissions at lower and higher wavelengths. Nevertheless, the results were not deemed to be of sufficient accuracy for the experiment and, therefore, further combinations of light and dark coloured papers were trialled to simulate the percentage light transmission through *L. minor* fronds. The resulting spectrograph is shown in Figure 4.1b which shows that the percentage light transmission spectrograph obtained from of employing four layers of the light green paper was the most similar to the spectrograph of the *L. minor* fronds. Hence four layers of light green paper were used in the subsequent pilot study.

4.2.2 Culturing of *Lemnicola hungarica* and *Lemna minor*

The cells of the diatom *L. hungarica* consisted of two strains sampled from *L. minor* collected at the two study sites, RNOH and HOME. The *L. hungarica* cells were collected by simply shaking vigorously the collected *L. minor* fronds in distilled water (Goldsborough & Robinson 1985). Specimens of *L. hungarica* were carefully collected from the epiphytic diatom samples using a suction micro-pipette under inverted light microscopy. Subsequently the cells were inoculated into sterilised MBL diatom culture media (Nichols 1973), with pH adjusted to pH 7.2 by buffering, to provide pure strains of cultured *L. hungarica* cells. The two *L. hungarica* strains were placed within algal

culture cabinets (21ºC with full spectrum light at a light intensity of 120 µmol m^{-2} s^{-1}, following the natural summer diurnal cycle (16 hr light: 8 hr dark) and left to grow undisturbed.

The two strains of *L. minor* were isolated for sterilisation and culturing. Although several different sterilisation techniques have been used previously (Saeger 1930, Steinberg 1941, Landolt 1957, Hillman 1961) and various appropriate growth media, culture vessels and culture conditions have been described (Gorham 1945, 1950, Landolt 1957, Hewitt 1966, McLay 1976), several workers have experienced difficulties in cultivating sterile fronds of Lemnaceae (Bowker *et al.,* 1980). The sterilisation and cultivation of *L. minor* fronds employed in this study is a modification of the methodology described by Bowker and Denny (1980).

The temperature of the incubator was kept at a constant 21ºC and the *L. minor* samples were cultured with sterilised 20% Hutner's growth media (adjusted to pH 7.2), as higher temperatures (Landolt 1986) and increased phosphorus and nitrogen concentrations (Portielje & Roijackers 1995) would likely result in increased growth and vigour of the *L. minor* fronds. This would increase the difficulty and time associated with counting the diatom cells together with increasing the opportunity for bacterial and fungal contamination. Moreover, it was envisaged that there could be potential for bacterial and fungal contamination of the *L. minor* fronds as the samples would be removed from the incubator cabinet, and also from their culture vessels, to enable diatom counts to be made. Therefore, fresh *L. minor* and artificial *Lemna* samples and inoculated with fresh diatom cultures were made in preparation for SEM analysis.

4.2.3 Sample preparations

An essential criterion for the experiments was to control, as much as feasible, the variables of the experimental techniques particularly microbial contamination. Therefore, the culture vessels (Pyrex glass basins with Pyrex glass covers), the diatom culture media, MBL, (Nichols 1973) and the *L. minor* culture media, i.e. 20% Hutner's

media, (Landolt & Kandeler 1987, Szabó *et al.*, 2003) were sterilised by autoclaving at a temperature of 121ºC for 15 minutes before use. The chemical composition and the elemental concentrations of the growth media used are presented in Appendix 2.

The healthy *L. minor* fronds were washed in tap water to remove debris and invertebrates and were then inoculated into the 20% Hutner's growth media which was supplemented with 1.0g dm^{-3} sucrose and 500mg dm^{-3} soluble casein to encourage bacterial and fungal growth and germination of spores. This technique would facilitate the eradication of bacteria and fungi prior to sterilisation. About 500 fronds were selected from this enrichment culture and rinsed through distilled water. The fronds were transferred into aliquots of 4% sodium hypochlorite (Milton's solution) using a flamed nichrome wire loop within a laminar-flow sterile hood. The fronds were shaken until the marginal edges of the leaves began to bleach and after approximately 2 minutes the fronds were totally bleached. Thereafter each individual frond was aseptically removed from the sodium hypochlorite solution, rinsed through sterile distilled water and carefully inoculated into 40 x 15mm screw-top transparent plastic bottles containing Hutner's growth media after being sterilised by autoclaving. At daily intervals the cultures were shaken and inspected for microbial and algal contamination from chlorine-resistant cells, which presented primarily as a turbid suspension of *Chlorella* and desmid species, and any contaminated samples were discarded. It was necessary to discard all but about 6% of the treated fronds. The remaining uncontaminated fronds were placed within a culture cabinet in preparation for the experiment. The artificial '*L. minor'* substrata were also sterilised using 4% sodium hypochlorite (Milton's solution) under the same conditions as the sterilisation of the live *L. minor* fronds, and then subjected to several thorough rinses with distilled water.

The sides of the sterilised culture vessels were covered with black foil to prevent extraneous light from entering the vessels, thereby ensuring that the only source of light to enter the vessels was from above. This procedure was undertaken to prevent potential diatom colonisation on the sides of the glass experimental vessels and also to simulate, as far as possible, the natural field conditions of *L. minor* and the epiphytic *L.*

hungarica in freshwater ponds, thereby maintaining the primary source of light entering the *Lemna* mat directly from the surface. The glass covers of the vessels containing the artificial *Lemna* fronds were covered with four light green sheets of paper to simulate the transmitted light through the *Lemna* fronds. The samples were preincubated under experimental conditions for 8 days. The experiments were carried out in the Botany Laboratory at the Natural History Museum, London.

4.2.4 Pilot study

A preliminary pilot study was performed where several culture vessels containing 400ml of Hutner's growth solution were inoculated with either a single artificial *Lemna* frond or a single natural *L. minor* frond (from both RNOH and HOME sites), after being carefully 'seeded' with a single *L. hungarica* cell placed upon the under-surface of the fronds with the aid of a micro-suction pipette and inverted light microscopy at 400x magnification. The RNOH frond was 'seeded' with *L. hungarica* sampled from the HOME strain of *L. minor* to reduce potential bias from 'seeding' the same strain of *L. minor* with the same clonal strain of *L. hungarica* from the original samples, and likewise the HOME strain of *L. minor* was seeded with *L. hungarica* sampled from the RNOH *L. minor* strain. The artificial *Lemna* fronds were 'seeded' with both clonal strains of *L. hungarica* but were 'seeded' separately. The fronds were carefully inverted and placed upon the surface of the culture media within the vessels, with the glass covers replaced in situ, and then placed within the controlled environment of the algal culture cabinet. A comparative control was also set up which followed the criteria and methodology of the pilot study except that the control was not 'seeded' with cells of *L. hungarica.*

The first point of interest in the pilot study was that the collection of the diatom cells for transfer to the experimental surfaces, using a mouth-suction micro-pipette, proved to be very difficult as *L. hungarica* is an adnate species, and even though it is motile, the cells invariably became tightly attached to the surface of the container. A combination of different diatom sampling devices namely a suction micro-pipette and

the eyelash of a pig glued to a pencil was employed together with a sampling regime over different times of the day when the diatoms were motile to facilitate safe and secure sampling.

The second point of interest was that, after two weeks, inspection of the samples for growth of *L. hungarica* on the artificial *Lemna* fronds with the light green attenuation papers revealed that the diatoms had in fact died. This was attributed to the papers attenuating most of the light, resulting in less light availability for diatom photosynthesis. However, when the experiment was repeated without the light green paper covers, the diatom cells did grow and reproduce. Therefore, for the subsequent experiment the artificial *Lemna* fronds were treated under the same experimental conditions as the natural *L. minor* fronds, with no green paper for light attenuation. Light microscopy images of valves of *L. hungarica* taken from *L. minor* samples collected from the Bodham Rail Pit (Norfolk, E. England) are shown in Figure 4.2; the incubator cabinet and in situ experimental cultures are shown in Figure 4.3.

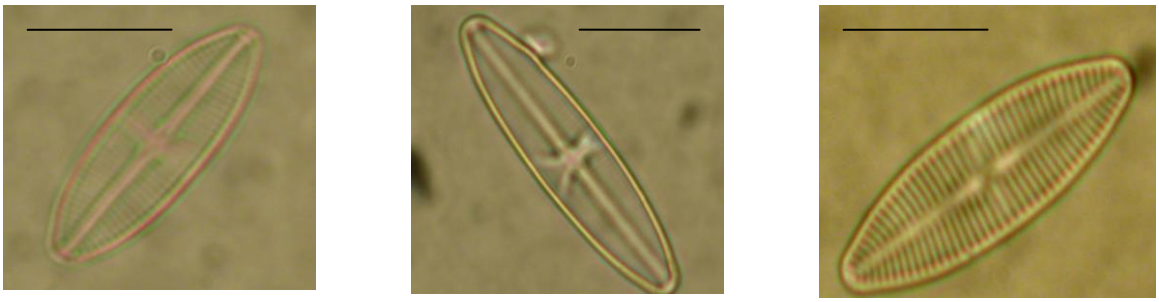


Figure 4.2. Light microscopy images of *L. hungarica* taken from L. minor samples, Bodham Rail Pit, E. England. Left and centre images show raphe view, right image shows non-raphe view. Scale bar = 10 μm. (Images by Dave Emson).

Figure 4.3. Incubator cabinet containing experimental culture vessels (left and top right) and *Lemna minor* specimens prior to axenic sterilisation (bottom right). (Photographs: Elliot Shubert).

4.2.5 *Lemna-Lemnicola* co-cultures

As well as experimentally comparing colonisation characteristics of the epiphytic diatom *L. hungarica* upon artificial and real *L. minor* fronds, a further experimental surface was used in the comparative growth rates. The dead and photosynthetically inert *L. minor* fronds that resulted in the sodium hypochlorite sterilisation technique were also 'seeded' with live *L. hungarica* cells. This potential surface for diatom colonisation was considered to be a 'half-way house' between live *L. minor* fronds and the artificial *Lemna* fronds as the surfaces are inert but nevertheless they are organic biological samples. Images showing artificial *L. minor* fronds used in the experiment are given in Figure 4.4 below.

Figure 4.4. Images of 'artificial *Lemna minor*'. Lateral view showing the 'artificial frond and root' floating in the culture vessel (top left), and the 'artificial frond' viewed from the surface (bottom left) prior to inoculation with *Lemnicola hungarica*. 'Artificial frond and root' (top right) and 'artificial frond' viewed from the surface (bottom right) after four weeks incubation with *Lemnicola hungarica.* (Photographs: Janet Hope; Dave Emson).

The pilot study highlighted the inherent problems associated with inoculating or seeding the experimental surfaces with an extremely 'stubborn' adnate diatom that consistently proved difficult to capture and manipulate with established techniques. Therefore, a very simple solution was to turn the practical logistics around and instead of attempting to inoculate the surfaces with the diatoms the experimental surfaces would be directly and carefully placed upon the *L. hungarica* cultures thereby facilitating the colonisation process under more amenable and natural conditions. The artificial *Lemna* fronds were inverted (i.e. the abaxial under-surface was placed upwards) and glass cover-slips that were colonised previously with *L. hungarica* were carefully placed over the under-surfaces to facilitate natural colonisation. The surfaces were regularly inspected for initial colonisation before the diatoms had time to

reproduce. It was found that the experimental surfaces were quickly colonised, usually within an hour of being set up. However, as the original samples were becoming contaminated with desmids and *Chlorella* species a fresh diatom sample batch was prepared for SEM analysis of the colonised substrates. The SEM revealed that these fresh samples were inoculated not only with *L. hungarica*, but had also been inadvertently inoculated with the other *Lemna* epiphyte, *S. seminulum* (see Figs. 4.8-4.12)

The individual culture vessel aquaria consisted of either a single and individual seeded artificial *Lemna* frond, or a live *L. minor* frond or a photosynthetically dead *L. minor* frond. These culture vessel aquaria were replicated with four vessels for each individual treatment using both the RNOH and HOME strains. Along with the control culture vessels, the aquaria were arranged randomly within the inoculation cabinets for a minimum of 14 days and a maximum of 49 days. Each individual experimental surface was examined after 7 days for diatom growth and abundance and immediately returned to the culture vessels to minimise contamination and disturbance. To take into account the diel cycle and diurnal pattern of cell division in the culture, sampling took place around the same time during each day. All observations and diatom counts were made using a Zeiss Axiovert 200 inverted light microscope at a magnification of 400x. The artificial and photosynthetically dead *L. minor* fronds were relatively transparent so that the diatoms could be readily observed and counted. Diatom colonisation and growth upon the live *L. minor* fronds was not as easy to observe, however, because of the *L. minor* chlorophyll pigmentation. Nonetheless, as the chloroplasts of the *L. hungarica* cells were a slightly different colour from the vivid green colour of the *L. minor* chloroplasts, possibly due to the pigments fucoxanthin and diatoxanthin, accurate counts of the cells could be made. Counting was made easier if *L. hungarica* colonisation was coincident with the more transparent aerenchyma air spaces that aid duckweed buoyancy, together with colonisation around the edges of the fronds. The technique for the direct microscopic diatom observations and counts upon *L. minor* was similar to the technique advocated by Carter (1982), except that staining of *L. minor* with Lugol's preservative was not deemed necessary. However, the accuracy of the

final count of the diatoms upon the live *L. minor* fronds was maximised by briefly and carefully bleaching the *L. minor* fronds with sodium hypochlorite to assist diatom observation.

4.3 Numerical analysis

As the experimental surfaces for colonisation were individually placed within their own individual culture vessels the initial and final populations can be ecologically classified as ‘closed populations’, with no immigration and emigration of diatoms affecting the size of the populations (Gotelli 1995). Although this situation is highly unlikely in nature it does facilitate focus upon traditional and established population growth models. It also lends itself to the application of standard mathematical models, allowing exponential population growth equations to be applied to the diatom data.

Various assumptions were made for the growing diatom populations: i) exponential growth models assume that population grows with constant birth and death rates and an unlimited supply of space and other resources, ii) they assume that all individuals in the population have the same birth and death rates, so there cannot be any underlying genetic variation in the population for these traits, iii) it is assumed that there are no differences in births and deaths due to age or body (cell) size, and iv) that there is continuous growth with no time lags (Gotelli 1995). Although, there is a violation of these conditions with respect to the limitations of growing space for both the artificial *Lemna* fronds and the dead *Lemna* fronds, this potential violation could be ignored as the short duration of the experiment was designed to accommodate such an assumption.

4.3.1 Calculating *Lemnicola hungarica* growth rates and doubling times

During exponential growth, the rate of increase of the diatom cells per unit time was proportional to the number of cells present at the start of the experiment. Therefore, the population growth follows the simple model of exponential population growth as given in equation 4.1.

$$\frac{dN}{dt} = rN \qquad \textit{Equation 4.1}$$

The population growth size prediction or projection is integrated from Equation 4.1, giving the solution equation as:

$$N_t = N_0 e^{rt} \qquad \textit{Equation 4.2}$$

Where N_0 is the population size at the beginning of the time interval, N_t is the population size at the end of the time interval and *r* is the constant instantaneous rate of population increase.

The 'instantaneous rate of population increase', *r*, (also called the 'intrinsic rate of increase' or the 'Malthusian parameter') can be determined from diatom population growth and therefore the various experimental surfaces can be directly compared. Solving equation 4.2 to determine *r*, gives:

$$r = \frac{\ln (N_t / N_0)}{t_1 - t_0} = \frac{\ln N_t - \ln N_0}{t_1 - t_0} \qquad \textit{Equation 4.3}$$

Another important feature of exponentially growing populations is that they exhibit a constant 'doubling time'. In other words, no matter what the size of the initial population, the population will always double in size after a fixed time period and *r* can be converted into the constant doubling time, which means that if the population has doubled in size, it will be twice as large as the initial population size. In this study the doubling time was estimated from equation 4.4.

$$t_{double} = \frac{\ln (2)}{r} \qquad \textit{Equation 4.4}$$

4.3.2 Statistical analysis

The significance levels of the growth parameters were evaluated by analysis of variance (ANOVA) using SPSS 13.0. The mean relative growth rates (*r*) of the two strains of *L. hungarica* (RNOH and HOME) upon the three different surface substrate types (Artificial, Dead and Live *Lemna*) are presented as boxplots (Fig. 4.7). The mean relative growth rates were further analysed by the post hoc test of least-significant difference (LSD) pairwise multiple comparison test, where a one-way analysis of variance of the quantitative dependent variable (*r*) was compared with the independent variables represented as the different surface substrate types. Furthermore, Dunnett's t-test was performed on the mean relative growth rate data for significance levels, by treating one of the surface types as a control (HOME Live) and comparing the mean relative growth rates of the surface substrates against this control group. The 'doubling times' of the two diatom strains growing upon the three different surface substrates were analysed using a single factor ANOVA test.

4.4 Results

4.4.1 Exponential population growth

The *L. minor* fronds and the seeded *L. hungarica* cells remained viable and healthy under the experimental conditions. The seeded cells of *L. hungarica* upon artificial and real *L. minor* fronds flourished under the prescribed conditions with steady-state growth observed during the exponential phase of growth. Exponential growth data of the two diatom strains from the various surfaces are presented in Figure 4.5. The exponential growth of the HOME dead, RNOH dead and the RNOH artificial samples was truncated as the surfaces became saturated by the colonisation of *L. hungarica*. This occurred as maximum carrying capacity was reached for these surfaces. The exponential growth of *L. hungarica* on the surface substratum HOME artificial was comparatively less marked than the other surfaces, but increased substantially after 42 days incubation. The exponential growth of both diatom strains on the live *L. minor*

surfaces was greater than exhibited on the other surfaces, particularly after 35 days incubation.

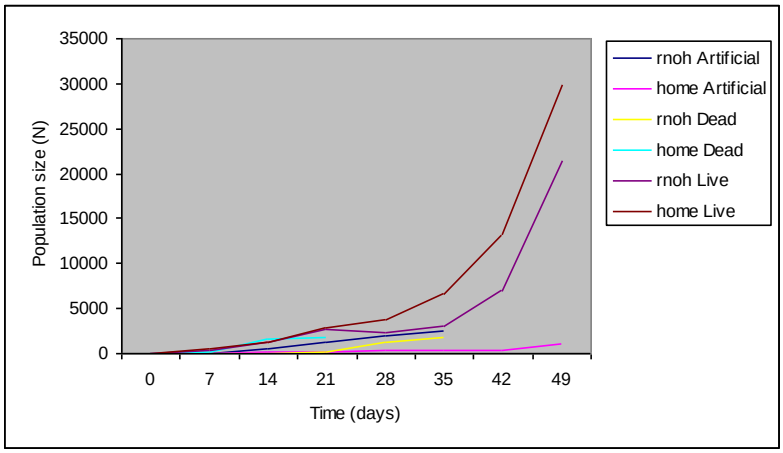


Figure 4.5. Trajectories of exponential population growth of the two strains (RNOH and HOME) of *Lemnicola hungarica* on the different surface substrate types (Artificial, Dead and Live).

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Although the exponential population growth of the two diatom strains provides information on the rate of growth and population size on the various colonising surfaces, a more meaningful interpretation is gained by measuring the per capitata (per individual) rate of population increase (i.e. '*r*') over a short time interval. This approach negates the effects of the different surface areas for potential diatom colonisation and growth. Therefore, individual values of the relative intrinsic rate of population increase '*r*' and 'doubling times' were calculated for the two diatom strains independently and also for the combination of the two strains for each surface substrate type (Table 4.1).

Diatom Strain	Substrate Type	Relative Growth Rate (*r*) Mean	Std. Err.	Std. Dev.	Doubling Time (Days)
RNOH	Artificial	0.222	0.047	0.189	3.122
	Dead	0.186	0.024	0.126	3.727
	Live	0.167	0.019	0.085	4.151
HOME	Artificial	0.113	0.043	0.162	6.134
	Dead	0.220	0.004	0.009	3.151
	Live	0.163	0.009	0.019	4.252
COMBINED	Artificial	0.115	0.024	0.160	6.027
	Dead	0.200	0.021	0.123	3.466
	Live	0.165	0.005	0.014	4.201

Table 4.1. Mean relative growth rates (*r* day^{-1}) and doubling times (day^{-1}) for the two strains of *Lemnicola hungarica*, separately and combined, from the three substrate types (i.e. Artificial, Dead and Live).

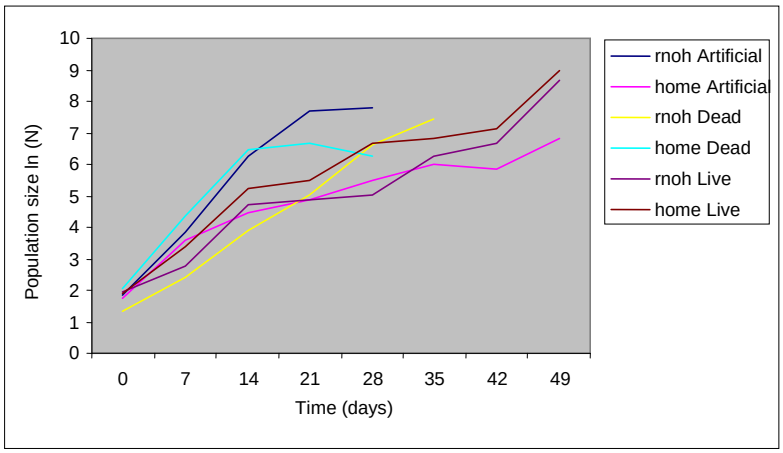


Figure 4.6. Trajectories of the mean intrinsic rate of increase '*r*' growth curves (ln N) for the two diatom strains recorded from the various surfaces (Artificial, Dead and Live).

L. hungarica showed substantial differences in '*r*' for the various experimental surfaces (Figs. 4.6, 4.7) with live surfaces showing greater population increases with time. However, the rate of growth data were further analysed for levels of statistical significance in a multiple comparison of the mean relative rates of growth (Table 4.2).

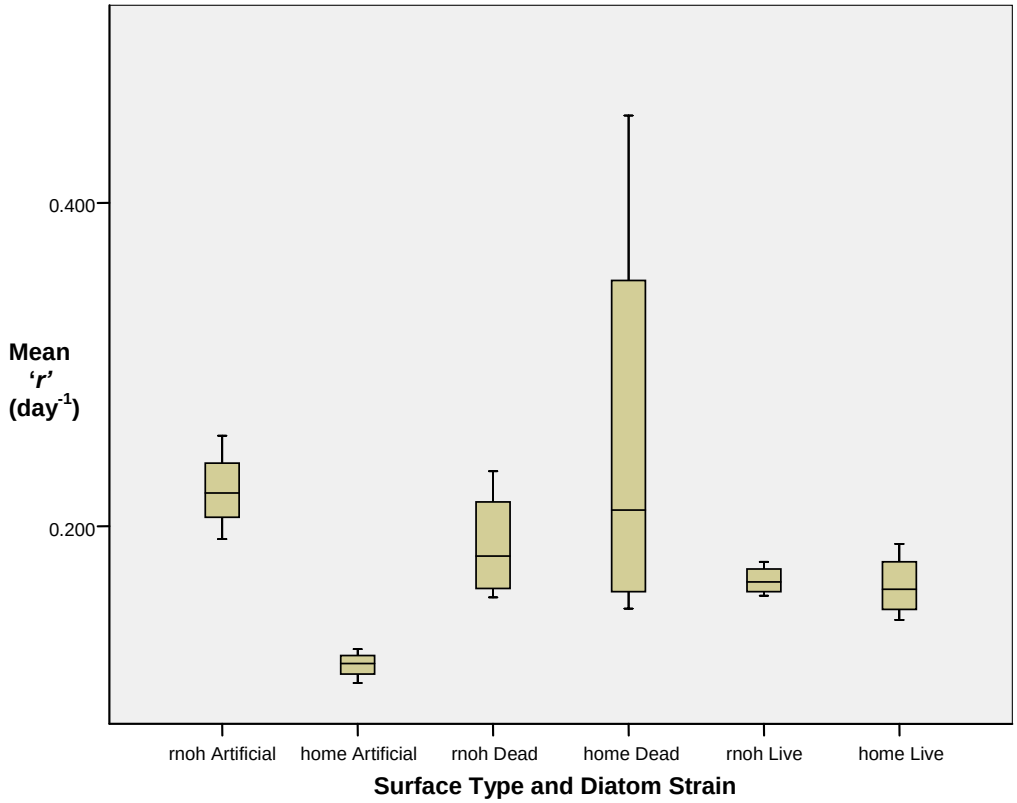


Figure 4.7. Boxplots of the mean relative growth rates (r day $^{-1}$) of the two diatom strains (RNOH and HOME) recorded from the various surface-types (Artificial, Dead and Live). (Boxplot expressed as medians and quartiles and box length is the interquartile range).

The multiple comparison tests (Table 4.2) show statistically significant differences in mean relative growth rates (r) between the two diatom strains growing on the artificial surfaces (p=0.021). The mean 'r' of the RNOH strain of *L. hungarica* was significantly greater than the mean 'r' of the HOME strain, which had the lowest mean 'r' compared with the other surfaces (Fig.4.7). Moreover, the mean 'r' of the HOME artificial strain was also significantly lower (p=0.004) than the mean 'r' of the same strain of diatom growing on the 'dead' surface, and the mean 'r' of the HOME strain growing on the 'dead' surface was also significantly greater (p=0.044) than the mean 'r' of the HOME strain growing on the 'live' surface. However, Dunnett t-tests, where the HOME live strain was used as a control to compare the mean 'r' with the other surfaces, did not reveal any significant differences (Table 4.2).

	Substrate Surface	Substrate Surface	Sig. diff. (p =)
LSD	rnoh Artificial	home Artificial	**.021**
		rnoh Dead	.436
		home Dead	.442
		rnoh Live	.208
		home Live	.183
	home Artificial	rnoh Artificial	**.021**
		rnoh Dead	.100
		home Dead	**.004**
		rnoh Live	.236
		home Live	.266
	rnoh Dead	rnoh Artificial	.436
		home Artificial	.100
		home Dead	.131
		rnoh Live	.616
		home Live	.565
	home Dead	rnoh Artificial	.442
		home Artificial	**.004**
		rnoh Dead	.131
		rnoh Live	.051
		home Live	**.044**
	rnoh Live	rnoh Artificial	.208
		home Artificial	.236
		rnoh Dead	.616
		home Dead	.051
		home Live	.940
	home Live	rnoh Artificial	.183
		home Artificial	.266
		rnoh Dead	.565
		home Dead	**.044**
		rnoh Live	.940
Dunnett t (2-sided)	rnoh Artificial	home Live	.520
	home Artificial	home Live	.677
	rnoh Dead	home Live	.964
	home Dead	home Live	.155
	rnoh Live	home Live	1.000

Table 4.2. Summary of multiple comparison tests of analysis of variance of the least significant difference (LSD) and Dunnett t-tests of the mean '*r*' of the two diatom strains growing on the different surfaces (significant differences at the 0.05 level between mean values are given in bold).

A single factor ANOVA test showed that there was no statistically significant difference in doubling times (p=0.61) between the two diatom strains regardless of the type of surface (Table 4.1). SEM images of the *Lemna* epiphytes *L. hungarica* and *S. seminulum* showing micro-distribution (surfaces and roots) upon the 'dead' fronds and artificial *Lemna* substrates are shown in Figures 4.9, 4.10, 4.11 and 4.12. The live *L. minor* substrates are not shown as, unfortunately, these substrates 'lost' their seeded diatoms during the intensive SEM sample preparations (Fig. 4.8b). Figure 4.9 shows both *L. hungarica* and *S. seminulum* after two weeks growth on the artificial *Lemna* substrate. However, despite employing aseptic techniques in the preparation of the samples, it was

noticeable that there was a degree of bacterial contamination of the samples (Fig. 4.9; 4.11b).

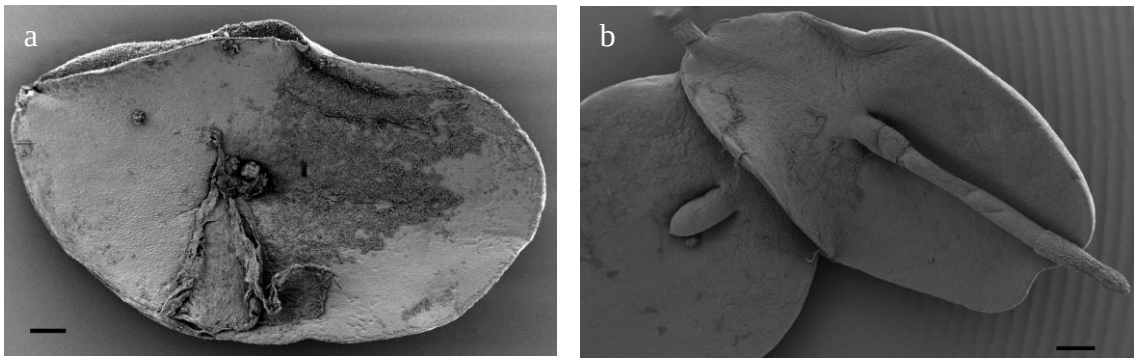


Figure 4.8. Scanning electron micrographs taken five weeks after diatom seeding. Dead *Lemna minor* frond showing colonisation of the adaxial surface spreading from the covered abaxial surface (a). Live *Lemna minor* frond (with budding daughter frond) with absence of colonising diatoms (b). Scale bars = 200 μm.

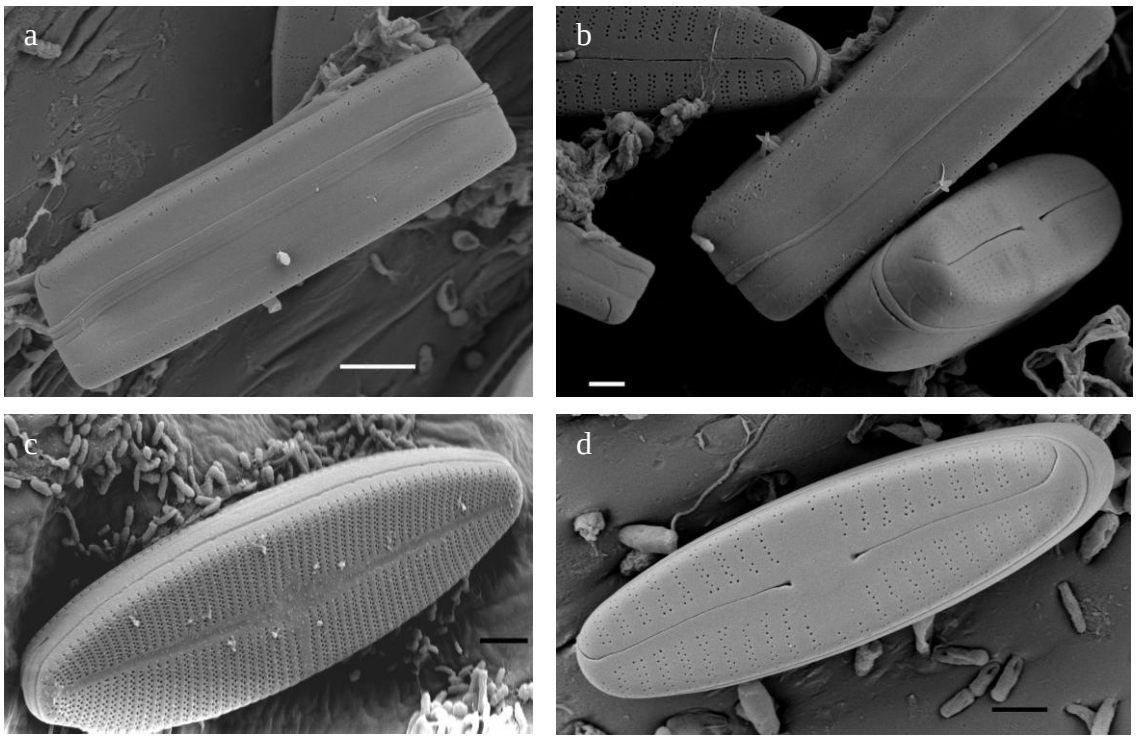


Figure 4.9. Scanning electron micrographs of *Lemnicola hungarica* and *Sellaphora seminulum* on an artificial *Lemna* frond (two weeks growth). *Sellaphora seminulum* girdle view (a), valve, girdle and apical view (b), valve view (d) and *Lemnicola hungarica* rapheless valve view (c). Scale bars = 1 μm.

Figure 4.10a, b and c shows the rapid colonisation by the *Lemna* epiphytic diatoms after five weeks growth on the artificial *Lemna* substrate: the surfaces became 'carpeted' as the diatoms spread out. It was noticeable that: i) they avoided the dark

raised edges that cast a shadow from the light source directly above the cultures (Fig. 4.10a, b and c), and ii) the motile diatoms also avoided the open stomata of the dead *Lemna* fronds (Fig. 4.10d).

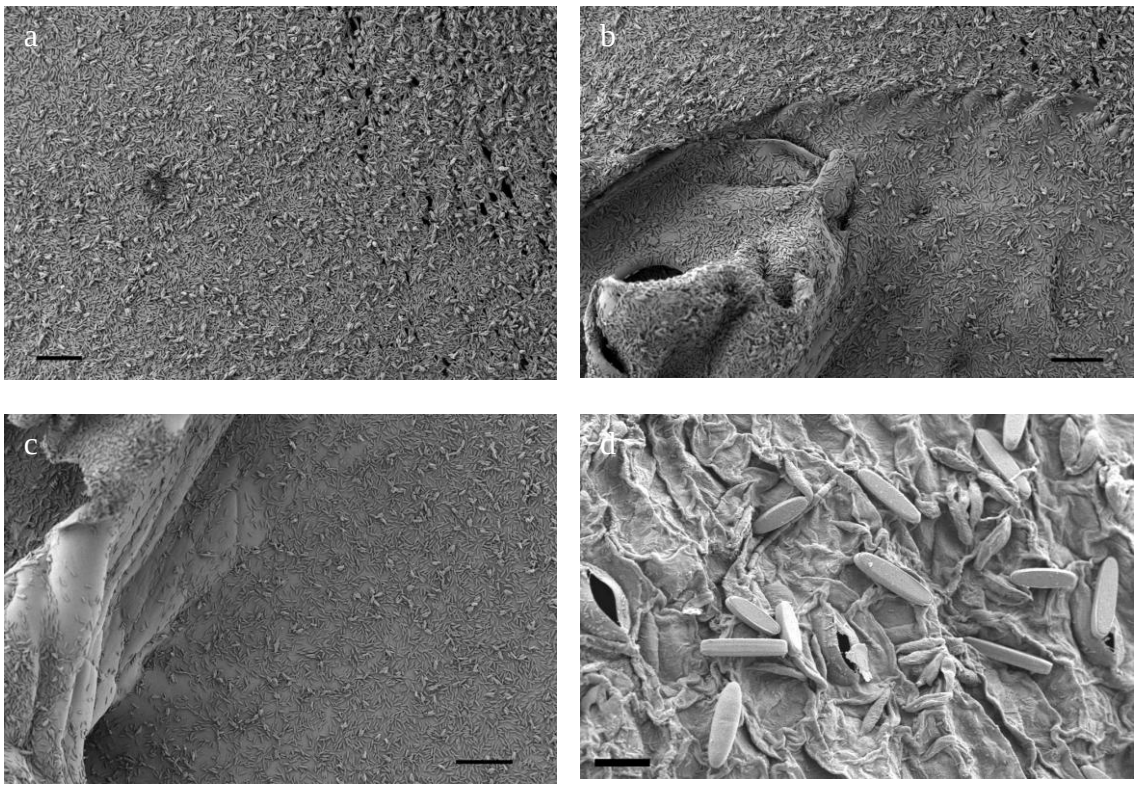


Figure 4.10. Scanning electron micrographs taken five weeks after diatom seeding on artificial *Lemna* fronds (a, b and c) and after two weeks on a dead *Lemna* frond (d). Complete covering of frond surface (a); lower diatom densities in recessed regions (b) and over rough raised edges (c); diatoms avoiding open stomata (d). Scale bars = 100 μm (a, b and c) and 10 μm (d).

SEM images (Fig. 4.11) of the diatom colonisation pathways on both real (natural) and artificial *Lemna* substrates showing 'optimal' positioning to exploit the above light source. Figure 4.11a shows *S. seminulum* living on the edge of frond life and Figure 4.11b shows diatoms navigating the frond edges via cyanobacterial 'highway'. The confirmed presence of the unidentified species of cyanobacteria (Elliot Shubert, pers. com) is a result of a degree of contamination of the samples. Both Figures 4.11c and 4.11d show diatoms avoiding dark recesses and the outer edges respectively. It should be noted that the high densities of diatoms seen are significantly higher than what is

seen in ‘field’ populations which is likely due to the absence of diatom intra-specific competition, and the absence of diatom grazers.

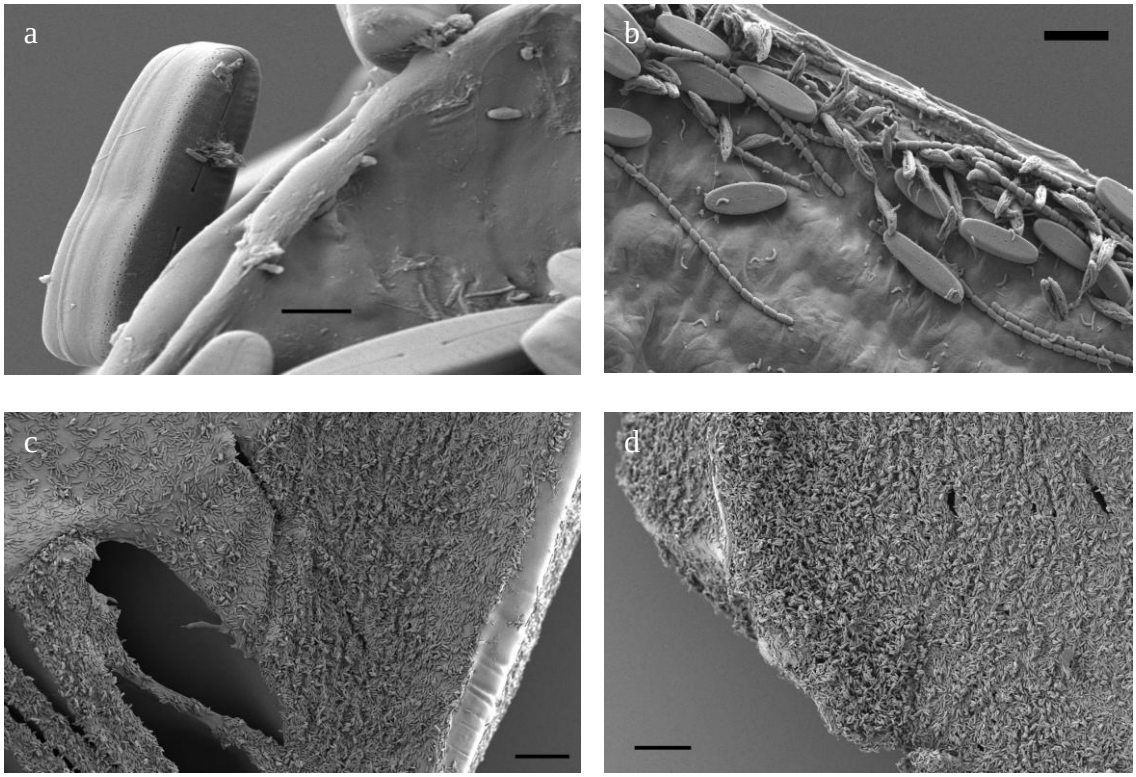


Figure 4.11. Scanning electron micrographs of diatom seeding on real and artificial *Lemna* fronds. *Sellaphora seminulum* cell seen actively moving away from edge of artificial frond (a); diatoms on the cyanobacterial ‘highway’ on dead *Lemna* (b); growth on artificial *Lemna* carpeting the frond surfaces but noticeably absent from the frond edge and the ‘artificial’ stomata (c and d). Scale bars = 100 µm (c & d), 10 µm (b) and 2 µm (a).

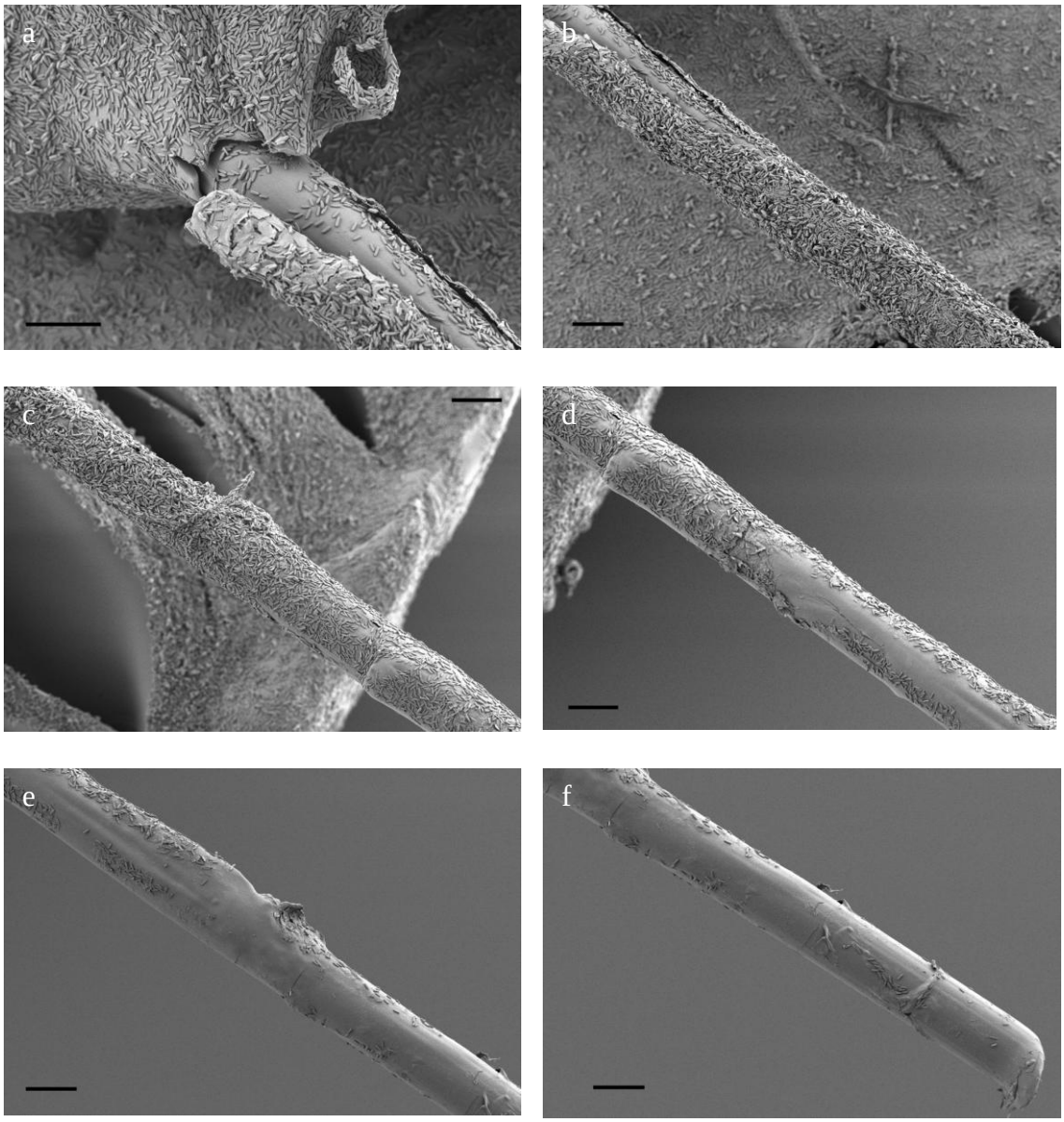


Figure 4.12. Scanning electron micrographs taken five weeks after diatom seeding on artificial *Lemna*. Images show the sequential colonisation route of the pioneering diatoms on the artificial root (from a - f). Colonisation from artificial root node and 'prophyllum' of both diatom species (a), along the proximal root (b), down the mid-root point (c, d and e) and reaching the distal root cap with *Sellaphora seminulum* (f). Scale bars = 100 µm.

The sequential route of diatom colonisation on artificial *Lemna* frond and root is shown in Figure 4.12. After the initial colonisation of the frond surface the diatoms spread

from the frond 'prophyllum' (Fig. 4.12 a) and tracked down the root surface (Figs. 4.12 b-f).

4.5 Discussion

The exponential population growth of the two strains of *L. hungarica* shows that both the RNOH live and the HOME live surfaces initially appeared to have a far greater population growth than the other surfaces, this being particularly evident after 35 days of incubation (Fig. 4.5). However, the timing of this enhanced growth coincided with increased growth of live *L. minor* fronds, providing an increase in surface area for diatom colonisation. Increased population growth on the live surfaces was concomitant with truncation of growth on the other surfaces which became saturated with diatoms as they reached individual carrying capacities between 21-35 incubation days. However, with the exception of HOME artificial, in the early stages of incubation the diatom population growth on the other surfaces tracked the population growth on the live surfaces. The exponential population growth on HOME artificial appeared to lag behind the other surfaces until the 42nd day of incubation when there was a rapid increase in diatom population growth. This apparent lag in growth cannot be adequately explained by the artificial nature of the surface because there was no apparent lag with the RNOH strain colonising the same type of substratum. The difference in exponential growth is reflected in the doubling time of RNOH artificial (3.122 day^{-1}) being almost half that of HOME artificial (6.134 day^{-1}) (Table 4.1). The only feasible explanation, given that the other experimental variables were controlled, is that the RNOH strain was simply more prolific in growth than the HOME strain.

The mean '*r*' of the two diatom strains (Table 4.1) and their time series trajectories (Fig. 4.5) show that there were similar rates of growth upon the three surfaces (range: 0.113-0.222 day^{-1}). Interestingly, the lowest mean '*r*' was recorded for HOME artificial (0.113 day^{-1}) whilst the highest '*r*' was found on RNOH artificial (0.222 day^{-1}). The mean '*r*' of both strains growing on the dead surfaces were relatively greater than the mean '*r*' for the other surfaces and, with the notable exception of RNOH artificial, the

corresponding doubling times were also relatively lower compared with the artificial and live surfaces (Table 4.1; Fig. 4.7). The two diatom strains had comparable mean '*r*' for the live surfaces (0.167 day^{-1} for RNOH; 0.163 day^{-1} for HOME strains).

When the mean '*r*' and the doubling times of the two diatom strains were combined for the three individual surfaces, it was found that the artificial surfaces had the lowest mean '*r*' (0.115 day^{-1}) with a concomitant higher doubling time (6.027 day^{-1}). The live surfaces had an intermediate mean '*r*' (0.165 day^{-1}) with a doubling time of 4.201 day^{-1}, whilst the dead surfaces gave the highest mean '*r*' (0.200 day^{-1}) and the lowest doubling time (3.466 day^{-1}). These data show that the growth of the combined diatom strains performed slightly better on natural substrates when directly compared with an artificial surrogate. Interestingly, the diatom strains growing on the live substrate had the lowest relative growth rate, and a concomitant higher doubling time, when compared with the dead surfaces. Even though diatom growth on the artificial substrate did not perform as well as on the natural substrates, nevertheless, there was colonisation by diatoms on the artificial substrates. This suggests that *L. hungarica* is able to colonise other types of substrates other than *L. minor* at the water-surface interface.

The multiple comparison analyses (ANOVA) of the mean '*r*' revealed that there were significant differences between RNOH artificial and HOME artificial ($p=0.021$); between HOME artificial and HOME dead ($p=0.004$) and between HOME dead and HOME live ($p=0.044$). However, when the two diatom strains and the three surfaces were directly compared against the HOME live control surface, no significant differences in the mean '*r*' were exhibited by either of the two strains regardless of surface type. Moreover, there were no statistically significant differences ($p=0.61$) in the doubling times of both diatom strains growing upon any of the three different surfaces. These results suggest that, perhaps, the 'make-up' of the substrates (i.e. artificial or natural) is secondary to the actual location of the substrates at the water-surface interface. The diatoms did not appear to be gaining any benefit in resources from the natural substrates.

Scanning electron microscopy of the artificial *Lemna* substrates (Figs. 4.8-4.12) may suggest a degree of niche differentiation between *L. hungarica* and *S. seminulum* as seemingly *L. hungarica* preferred the frond surfaces whilst *S. seminulum* was seen to colonise along the length of the roots (Fig. 4.12). This observation corroborates the findings of Goldsborough 1993 (see Chapter 3, Fig. 3.1). Interesting observations were that: i) the *L. hungarica* cells appeared to use the strands of cyanobacteria as 'highways and byways' to facilitate movement across the surfaces after the surfaces became contaminated by colonising cyanobacteria towards the end of the growth experiments (as the surfaces were removed several times from their sterile incubation vessels for diatom counts) and ii) *L. hungarica* cells were often located in the depressions between duckweed cells, resulting in less protrusion of the diatom cells. This may suggest an adaptation against grazing loss in natural filed conditions. Indeed a similar phenomenon was also seen with the other *L. minor* epiphyte *S. seminulum* (Fig. 4.11). These observations would appear to support the proposal by Wotton & Preston (2005) that the complex micro-architecture of surface films can provide an excellent locomotory substratum for gliding and crawling micro-organisms.

It can be speculated that *L. hungarica* has a narrow ecological optimum, requiring high light intensities for optimum growth as demonstrated by the death of all the *L. hungarica* cells growing in culture vessels covered by green paper in the pilot study. In an analysis of diatom spatial micro-distribution on an artificial substratum positioned vertically through mats of *L. minor* Goldsborough (1993) found that *L. hungarica* was the only diatom occurring among the *Lemna* fronds wrapping the substratum above the waterline and comprised >90% of total diatoms at the air/water interface. Goldsborough (1993) also noted a decrease in *L. hungarica* below these surface layers in the root zone, as it was replaced by other diatom species such as *S. seminulum*. These purported requirements of high light conditions at the water-substrate interface are adequately supplied by the reduced morphological characteristics of the small fronds of floating Lemnids, as they are effectively able to maximise photosynthesis capabilities and to out-compete other macrophytes. Indeed *L. hungarica* is rarely found on the larger and

thicker leaves of other floating macrophytes such as the Potamogetonaceae, the Nymphaeaceae or the Hydrocharitaceae (see Chapter 3).

The surface film, where free-floating plants occur, is exposed to intense solar radiation and physico-chemical interactions, yet despite these apparently harsh conditions a community of organisms thrives in these surface films. This prompts the question whether organisms found here are specialists or generalists. The results of both the 'global' pilot study (see Chapter 3) and this laboratory experiment on *L. hungarica,* would suggest that this species can be classified as a specialist adapted to survive and thrive at the water-air interface. These surface film environments have often been over-looked by researchers studying water bodies, and their importance is only now being recognised (Wotton & Preston 2005). Indeed the complex of organic material (chemicals and micro-organisms) that accumulates at the water-air interface, including free-floating plants, forms a surface film that is a highly dynamic environment. These surface films resemble the biofilms characteristic of benthic substrata and are likely to play a similarly important role in the biology of water bodies, so much so that the energy and organic matter flux of water-bodies should refer to the benthic-pelagic-surface coupling (Wotton & Preston 2005). This study also supports this assertion where ponds seem to have a strong and close coupling between these three components.

In a similar ecological-growth study Desianti (2012) found that *L. hungarica* occurred in higher abundances on artificial substrates when enriched with additional phosphorus and, moreover, that *L. hungarica* was limited by high light levels. It was concluded that there was a nutrient interaction between *L. hungarica* and duckweeds. These results and conclusions are contrary to the findings of this study. However, this apparent discrepancy could be related to the phosphorus enrichment of the culture media and the provision of shade over the culture vessels as was the case in the Desianti (2012) study. This study did not enrich the culture media with added phosphorus and did not incorporate shading as an additional variable. As *L. hungarica* is epiphytic on *Lemna* and as *Lemna* shows prolific growth in habitats with high nutrients, then perhaps *L. hungarica* (and possibly *S. seminulum*) requires both floating mats of *Lemna* and high

nutrient status of the water column for optimal population growth. Clearly, further experiments are needed to elucidate the autecology of both *L. hungarica* and *S. seminulum*.

4.6 Conclusions

This simple autecological experiment on the nature of the *L. hungarica*-duckweed relationship demonstrates that this diatom does not have a clear preference for colonisation and growth upon any of the three experimental surfaces. This broadly concurs with Desianti (2012), although in this study *L. hungarica* colonised artificial plastic substrates when the growth media was enriched with phosphorus. There were no statistically significant differences in the mean '*r*' or in the doubling times of either strains of the diatom from the artificial, dead or live surfaces. It would be reasonable to assume that both the mean '*r*' and the doubling times for both diatom strains would be higher and lower, respectively, for colonisation and growth upon the live biological *L. minor* surface as this is the natural surface on which *L. hungarica* has been universally recorded. However, this was not demonstrated and the results suggest that *L. hungarica* does not gain any specific advantage, with respect to '*r*' and doubling times, from the live biological *L. minor* host. Under the prescribed experimental conditions there is no demonstrable biological interaction between *L. hungarica* and its apparent host. Therefore, the biological interactive chemical hypothesis is rejected and the null hypothesis of a simple physical surface effect of the floating substrata is postulated as the most likely explanation for the *L. hungarica* association with duckweed.

This study suggests that artificial substrates can be readily utilised in experimental studies of *L. hungarica.* Further experiments controlling and manipulating the light, culture media concentration and temperature regimes would provide further valuable information on the autecology of *L. hungarica* by defining the ecological optima characteristics of the diatom in its natural environment. Finally, it would be interesting to perform the experiments not only with *L. hungarica* but also with *S. seminulum*, as

this species was also identified by INDVAL (Species Indicator Analysis) as an indicator of *L. minor* and other free-floating plants in Chapter 3

Chapter 5. Inferring past *Lemna* dominance from diatom records: a test of the validity of the *L. hungarica* / *S. seminulum* – *Lemna* indicator model

5.1 Introduction

Diatom analysis is a widely established technique for tracking environmental change using lake sediments (Stoermer & Smol 1999, Battarbee *et al.*, 2001). However, although there are exceptions (e.g. Håkansson & Regnéll 1993), there have been few diatom-based palaeolimnological investigations of small freshwater ponds with most studies focussing on deep and shallow lakes.

Dense free-floating mats of *Lemna* are a common occurrence in ponds but, to date, our knowledge of how *Lemna* (duckweed) influences pond ecosystems in the long term is poor. This lack of understanding is partly due to the fact that *Lemna* fronds do not preserve well in sediments and so have only rarely been exploited in palaeolimnological studies. To the knowledge of the author the only palaeolimnological study where *Lemna* has left a direct macrofossil signature is for Edku Lake, Egypt, where low concentrations of *Lemna minor* fronds were found in recent sediments (Birks *et al.*, 2001, Birks 2002). Lemnaceae rarely produce flowers (Hillman 1961, Landolt 1986) and therefore there is a lack of *Lemna* pollen and seeds present in sediment cores.

The association of the epiphytic diatoms *Lemnicola hungarica* and *Sellaphora seminulum* with the Lemnaceae potentially affords a robust indirect means of inferring past *Lemna* abundance in the palaeolimnological record. Previous studies (e.g. Goldsborough & Robinson 1985, Goldsborough 1993 & 1994, Round & Basson 1997, Buczkó 2007) have acknowledged this association, but the strength of the association

has been little tested in both space and time nor has the association's potential been studied or tested in a palaeolimnological investigation. This thesis has revealed a robust association between the Lemnaceae and *L. hungarica* and *S. seminulum* (see Chapter 3). Moreover, it has also been demonstrated that these diatom species can be utilised to track *Lemna* (duckweed) in surface sediment samples collected from duckweed-dominated and non-duckweed sites. However, the association has never been tested in time using a palaeolimnological approach.

This chapter focuses on the Bodham Rail Pit, Norfolk, England where periods of *Lemna* dominance are known to have occurred in the recent past. In this study any past periods of *Lemna* and their timing will be investigated using the sedimentary diatom record and evidence for cyclicity in *Lemna* dominance will be sought. Importantly, a comparison of the fossil diatom record with the observed historical record of *Lemna* occurrence at the site will be made as a means of validating the diatom-duckweed model developed in Chapters 3 and 4.

5.2 Study site and characteristics

The Bodham Rail Pit, Norfolk, eastern England (52º 54′ 20. 62″N; 1º 09′ 21. 23″E) is a small (0.1ha, 1000m^2), shallow (mean depth [April 2010] = 103cm) pond surrounded by a 10m grassland buffer zone and is set in arable farmland (Fig. 5.1). The pond is primarily fed by ground-water but also receives surface run-off via a cut channel from the adjacent road located in the SE corner (Fig. 5.1c). The water-level of the Rail Pit fluctuates seasonally by about one metre.

The pond was likely formed from past marl excavation. The practice of 'marling' in Norfolk can be traced back to the mid 13th century, where the calcareous marl was used to correct the acidity or to improve the texture of agricultural soils (Prince 1964). The Bodham Rail Pit (hereafter referred to as the Rail Pit) was likely formed by flooding from groundwater after marl extraction ceased. There are no known historical records of the Rail Pit and therefore little concrete information on its formation and age.

Nevertheless, a tithe map for the locality was produced by surveyor James Wright in 1841, which shows the existence of the Rail Pit (www.historic-maps.norfolk.gov.uk/tithe.aspx). The tithe map dating to 1841 (Fig. 5.1) shows that, as well as the Rail Pit, there were several other ponds in the locality in the early 19th century, but some of these were in-filled in the last century. A map of the area from 1886-1891 shows a smaller pond to the immediate east of the Rail Pit (Fig. 5.2b). This pond ('Son of Rail') was also recorded on later maps of the area for approximately 100 years, but by the 1970s it was also filled-in and reclaimed as farm land. Interestingly, at around the same time, the boundary between fields 167 and 162 was removed, and the Rail Pit no longer became the demarcation boundary between the two fields (see Appendix 4).

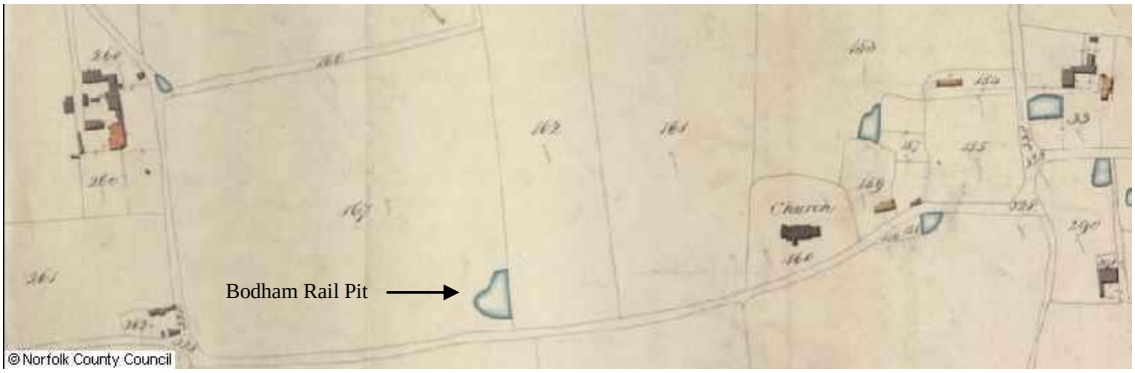


Figure 5.1. Location of the Bodham Rail Pit in 1841 from the original tithe map of Bodham, North Norfolk, England. The Bodham Rail Pit is situated at the bottom right corner of field 167 (arrowed). Interestingly, the tithe map predates 'Son of Rail' pond seen in maps from the 1880s (Fig. 5.2b).

There is an established oak tree (*Quercus robur*) growing within an old species-rich hedge along the eastern edge of the pond which possibly coincides with the original excavation of the pit (Fig. 5.1d). The complete perimeter of the steep riparian banks is dominated by mature trees and shrubs such as Ash (*Fraxinus*), Silver Birch (*Betula pendula*), Sycamore (*Acer pseudoplatanus*), Blackthorn (*Prunus spinosa*), Hawthorn (*Crataegus monogyna*) and Bramble (*Rubus fruticosus*) (Fig. 5.1d). The pond supports populations of Smooth Newt (*Lissotriton vulgaris*), Great Crested Newt (*Triturus cristatus*) and Crucian Carp (*Carassius carassius*). The Crucian Carp is designated as a

Biodiversity Action Plan (BAP) species for the county of Norfolk (Copp & Sayer 2010, Sayer *et al.*, 2011).

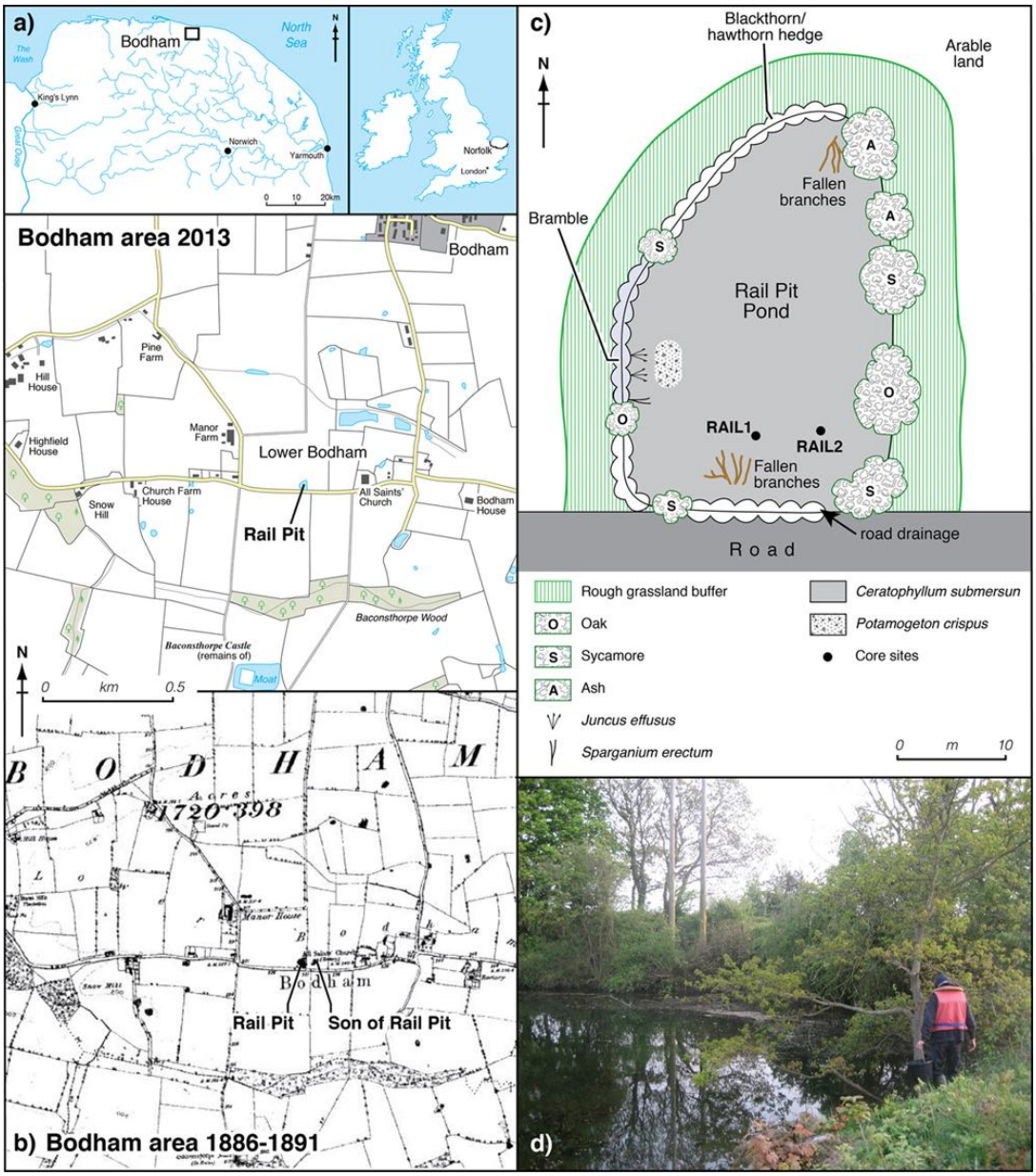


Figure 5.2. Location of Bodham Rail Pit today (a), and in the late 19th Century (b) showing the second pond (now a 'ghost pond') the 'Son of Rail Pit', (c) detailed site description showing distribution of contemporary aquatic, emergent and riparian vegetation and core locations, and contemporary photograph of the site from the west bank (d).

The water chemistry of the Rail Pit has been studied as part of a long-term monitoring programme (2010-2014). The data recorded from the time of the 'Big Ben' coring (April 2010) was: chlorophyll-*a* (Chl-*a*)=20.3 µg L^{-1}; total phosphorus (TP)=211 µg L^{-1}; soluble reactive phosphorus (SRP)=89 µg L^{-1}; and nitrate nitrogen (NO_3^- -N)=0.019 mg L^{-1}.

A summary of the water chemistry for three years of monitoring (2010-2013) is given in Table 5.1 and Figure 5.3.

	Chla	TP	SRP	NO_3^- -N
Mean	29.4	351	138	0.012
Minimum	5.6	164	36	0.008
Maximum	74.6	856	465	0.054

Table 5.1. Summary of mean, minimum and maximum values for key chemical variables measured in the Bodham Rail Pit from 2010-2013. Chla (chlorophyll-a - µg L^{-1}), TP (total phosphorus - µg L^{-1}), SRP (soluble reactive phosphorus - µg L^{-1}), NO_3^- -N (nitrate-nitrogen - mg L^{-1}).

There was considerable seasonal variation in the nutrient data as indicated by substantial differences between maximum and minimum values. High mean values of the key nutrients suggest that the Rail Pit is a eutrophic-hypereutrophic pond likely due to nutrient inputs from surrounding arable land and also from road run-off (containing sediments from adjacent arable fields). There were pronounced changes down the water depth profile (Fig. 5.3) in: i) light (surface=410 µmols, bottom=16 µmols), ii) dissolved oxygen (surface=10.3 mg L^{-1}, bottom = 1.4 mg L^{-1}), iii) pH (surface=7.82, bottom 6.88), iv) water temperature (surface=8.5^{0}C, bottom=7.7^{0}C), and v) electrical conductivity (surface=405 µS cm^{-3}, bottom=695 µS cm^{-3}).

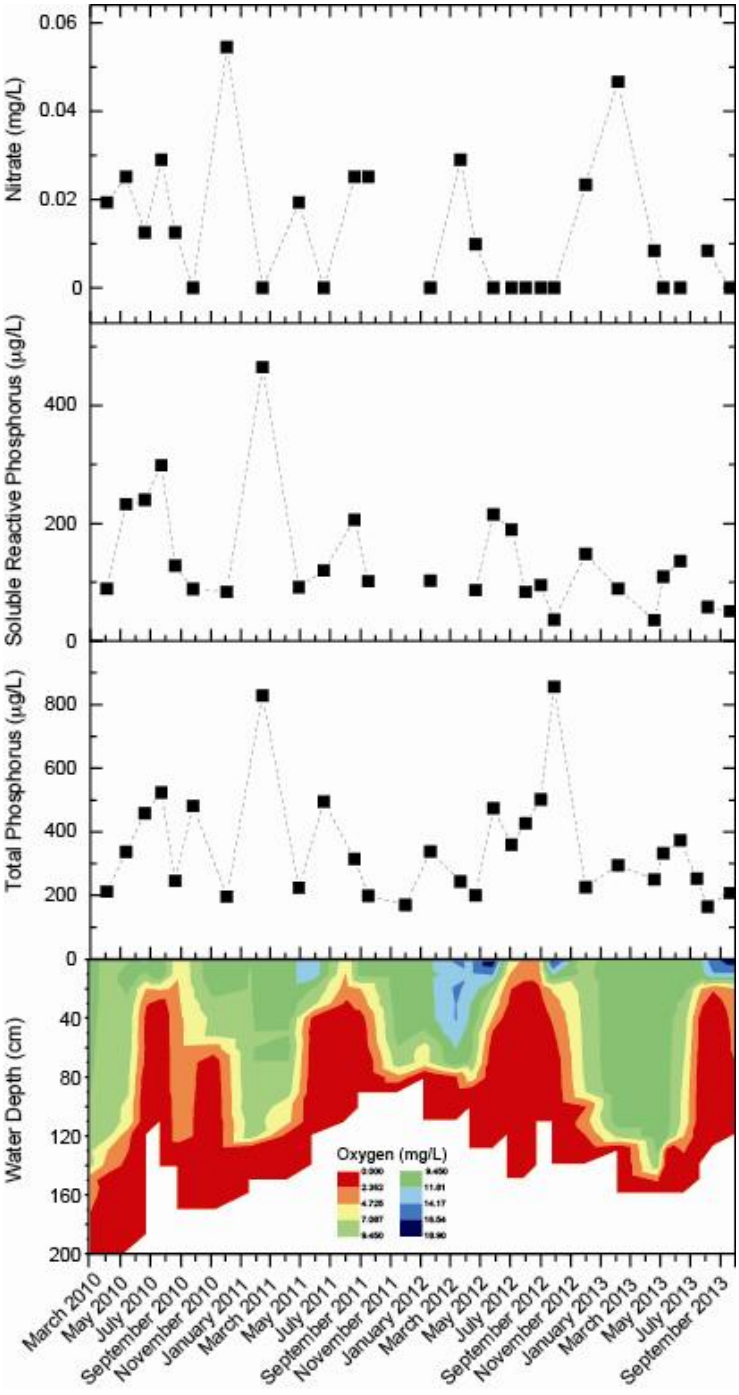


Figure 5.3. Depth profiles for Nitrate (nitrate-mg L^{-1}), SRP (soluble reactive phosphorus-µg L^{-1}), TP (total phosphorus-µg L^{-1}) and O_2 (dissolved oxygen-mg L^{-1}) for the Rail Pit. (Greaves *et al.*, unpublished data).

An ongoing seasonal monitoring programme indicates that this chemically enhanced density stratification appears as an almost permanent feature of the Bodham Rail Pit to the extent that it could be classified as an endogenic, or even a biogenic type of water-body (Wetzel 1983). Stratification is likely due to biological and decomposition processes (with accumulations of bicarbonate in the lower stratum) together with a general lack of wind-mixing owing to its small size and sheltered situation.

5.2.1 Recent macrophyte history

Observations of aquatic plants found at the Rail Pit have been derived from the field notebook of C. D. Sayer and cover the last 30 years. During this time the site has supported few aquatic plant species, with just six species present: *Ceratophyllum submersum, Potamogeton natans, Potamogeton crispus, Lemna minor, Lemna minuta* and *Lemna trisulca* post 1995. Plants were recorded using the DAFOR scale (Dominant=5, Abundant=4, Frequent=3, Occasional=2, Rare=1), but before this (1979-1995) information is derived from casual observations. Table 5.2 summarises the Rail Pit plant data and shows cyclical shifts between submerged macrophytes and lemnid dominance. During the periods of dominance (>90% surface cover) by lemnids the growth of submerged macrophytes seems to have been prevented until after *Lemna* die-back. *C. submersum* and *P. natans* were abundant during years of low *Lemna* abundance (1994-1998) but then disappeared when lemnids become dominant (1999-2005). Then following the abrupt decline of *Lemna* dominance (2005), the Rail Pit was described as looking like a 'bacterial soup' (C.D Sayer, pers.com) until *C. submersum* reappeared as the dominant macrophyte (DAFOR=4 in 2008), in addition to *P. crispus* (DAFOR=3 in 2009). During this second phase of submerged plant dominance, *P. natans* was absent. In 2010 *L. trisulca* was recorded for the first time as a rare species (DAFOR=1) and *Cladophora* sp. also became more prevalent (DAFOR=3).

At the time of core collection (April 2010), the only macrophytes present were *C. submersum* (Abundant), *L. minor* (Rare), and *L. minuta* (Rare). However, the following month (May 2010) saw a change in DAFOR status for these species in conjunction with

the appearance of other macrophytes (Table 5.2). Marginal plants recorded were *Alisma plantago-aquatica, Solanum dulcamara, Epilobium hirsutum, Hypericum tetragonum,* and *Ranunculus sceleratus.*

Year	*Ceratophyllum submersum*	*Potamogeton natans*	*Potamogeton crispus*	*Lemna trisulca*	*Lemna minor*	*Lemna minuta*	*Cladophora* spp.
2014	5	0	0	1	1	1 <1	0
2013	5	0	0	0	1	2 <1	4
2012	5	0	2	0	1	2 <5	5
2011	5	0	1	0	1	2 <5	3
2010	5	0	2	1	1	1 <1	3
2009	5	0	3	0	0	0 0	0
2008	4	0	0	0	0	1 <1	0
2007	0	0	0	0	0	1 <1	1
2006	0	0	0	0	0	1 <1	0
2005	0	0	–	–	*	5 90	–
2004	0	0	–	–	*	5 95	–
2003	0	0	–	–	*	5 100	–
2002	0	0	–	–	*	5 95	–
2001	0	0	–	–	*	5 90	–
2000	0	0	–	–	*	5 60	–
1999	2	3	–	–	*	5 90	–
1998	4	4	–	–	*	1 <5	–
1997	4	4	–	–	*	1 <1	–
1996	4	4	–	–	*	1 <1	–
1995	3 or 4	3 or 4	–	–	–	Non-*Lemna*	–
1994	3 or 4	3 or 4	–	–	–	Non-*Lemna*	–
1986-early 1990s	–	–	–	–	–	*Lemna*	–
1979-1985	–	–	–	–	–	Non-*Lemna*	–

Table 5.2. Recent history of aquatic macrophytes in the Bodham Rail Pit. Macrophyte abundances are presented using the DAFOR scale (D: Dominant [5], A: Abundant [4], F: Frequent [3], O: Occasional [2], R: Rare [1]). Highlighted areas denote periods of free-floating plant dominance. *Lemna minuta* abundances are also presented as percentage coverage of the water surface. From 2010 onwards annual estimates of DAFOR are based on two summer surveys. * *Lemna minor* was likely co-dominant with *Lemna minuta* in these years; – data unavailable.

(a)

(b)

Figure 5.4. Photographs of the Bodham Rail Pit looking in a NW direction showing *Lemna* dominance in 2003 (a) and *Lemna* absence in 2008 (b). Note some clearance of trees in 2008 for electricity power line access; Over-hanging branches from an old oak tree (*Quercus robur* L.) are seen near right. (Photographs: Carl Sayer).

5.3 Aims

The initial aim of this study was to apply the diatom-duckweed indicators, *L. hungarica* and *S. seminulum*, to a sediment core from the Rail Pit to see if abundances of these two

diatoms match temporally with known periods of *Lemna* dominance (Table 5.2). Assuming that the diatom-duckweed indicators successfully track known periods of *Lemna* dominance a second aim was to extend the diatom-duckweed indicator model back in time to determine periods of *Lemna* dominance prior to the observational record (i.e. before 1979).

5.4 Methods

To date, with the exception of Håkansson and Regnéll (1993) who investigated land use change in Lake Bussjösjön (southern Sweden) using fossil diatoms and pollen, this is the first study to use palaeoecological techniques in small farmland ponds. Although the palaeoecological assessment focused strongly upon the diatom history of the Rail Pit (this chapter), other key biological taxa were analysed in an attempt to infer changes in ecological structure and function as a direct consequence of past duckweed (*Lemna*) cover using plant and animal macrofossils and fossil pigments (see Chapter 6).

5.4.1 Sediment core extraction

A 118cm sediment core (RAIL1) was collected using a 'Big Ben' wide-bore piston corer from a southern central location of the Rail Pit from a depth of 108cm on 3 April 2010 (Fig. 5.5). The 'Big Ben' corer has an internal diameter of 140mm and therefore a 1cm sediment slice contain approximately 150cm^3 of wet sediment (Patmore *et al.*, 2014). On collection the uppermost 10-20cm of sediment was highly flocculated. After time for the sediment to settle the core was sliced on site at 1cm intervals and the samples were subsequently stored in sealed whirl-pak bags at 4°C in the dark. On extrusion the core length was reduced to 75cm. It is likely that this reduction in core length was due to compaction of the very soft upper sediments.

Given the fluid upper section of RAIL1 and the key aim of tracking recent duckweed (*Lemna*) coverage, a second short (22cm) core was collected from an adjacent location using a Glew gravity corer (Glew 1993) on 20 August 2010. It was expected that this

core would span the time period of recent duckweed cover from the 1980s to the mid 2000s (Fig. 5.4). This core (RAIL2) was sliced at 0.5cm intervals. The sediment samples from both cores were transported back to University College London and stored together in a refrigerated store room at 4°C prior to analysis.

Figure 5.5. Left: Core RAIL1 collected from the Bodham Rail Pit using the 'Big Ben' piston corer in April 2010. Note the dark brown/black organic silt and lighter grey marl at the base of the core. Right: close-up of Core RAIL2 collected in August 2010 using a Glew gravity corer. (Photographs: Carl Sayer).

5.4.2 Radiometric analysis

Dried sediment samples from cores RAIL1 and RAIL2 were analysed for ^{210}Pb, ^{226}Ra, ^{137}Cs and ^{241}Am by direct gamma assay at the Bloomsbury Environmental Isotope Facility (BEIF) at University College London, using an ORTEC HPGe GWL series well-type coaxial low background intrinsic germanium detector. ^{210}Pb was determined via its gamma emissions at 46.5 keV, and ^{226}Ra using the 295 keV and 352 keV gamma rays emitted by its daughter isotope ^{214}Pb following three weeks storage in sealed

containers to allow radioactive equilibration. ^{137}Cs and ^{241}Am were measured by their emissions at 662 keV and 59.5 keV respectively (Appleby *et al.*, 1986). The absolute efficiencies of the detector were determined using calibrated sources and sediment samples of known activity. Corrections were made for the effect of self absorption of low energy gamma rays within each sample (Appleby *et al.*, 1992). ^{210}Pb chronologies were calculated using the constant rate of ^{210}Pb supply (CRS) model (Appleby & Oldfield 1978). Because of irregular declines in unsupported ^{210}Pb activities resulting in a non-monotonic feature in the ^{210}Pb profile of both the RAIL1 and RAIL2 cores, the use of the constant initial ^{210}Pb concentration (CIC) model was precluded (Appleby & Oldfield 1978).

5.4.3 Lithostratigraphy: loss-on-ignition and carbonate content

Water content of the sediment samples was calculated by drying a known weight of sediment overnight in an oven at 105^{o}C. Loss on ignition (LOI) measurements were made after the dried samples were combusted in a muffle furnace at 550^{o}C for 2 hours and cooled to room temperature in a dessicator before reweighing. This gave the percentage of dry weight lost on ignition, a crude measure of sediment organic content. The remaining ash sample was heated in a muffle furnace to 950^{o}C for 4 hours, cooled in a dessicator and then re-weighed to determine carbonate content. The difference between the ash weight and the weight lost at 950^{o}C was multiplied by 1.36 (the difference between the molecular weights of CO_2 and CO_3) to derive carbonate content, expressed as a percentage of dry weight (Dean 1974).

5.4.4 Diatom analysis

A total of 48 sediment samples were analysed for diatoms from core RAIL1. The uppermost 10-20cm of the core was analysed at contiguous 1cm levels and below this samples were analysed at 2cm intervals. A total of 44 contiguous samples were analysed for diatoms in core RAIL2 at 0.5cm intervals covering the full length of the 22cm core.

Samples were prepared for diatom analysis using standard methods (Battarbee 1986, Battarbee *et al.,* 2001), see Chapter 2. As the aim of diatom analysis was to see how well the diatom stratigraphically tracked *Lemna*, it was necessary to explore diatom responses using percentage and concentration data, therefore large diatom counts (> 500 per sample) were undertaken. All samples were mounted on microscope slides using Naphrax^{TM} and absolute numbers of diatoms present in 0.1g of sediment were counted using a light microscope at x1000 magnification. Diatom counts were expressed as numbers of diatoms per 0.1 gram wet weight of sediment (Fig. 5.6).

Diatom silica dissolution can lead to poor preservation, breakage and fragmentation of valves in freshwater systems (Barker 1992, Gasse *et al.,* 1997, Ryves *et al.,* 2003) and the effects can be differential between species (Barker *et al.,* 1994, Ryves *et al.,* 2001, Battarbee *et al.,* 2005). Indeed, sometimes dissolution can result in the complete destruction of the diatom silica record, while partial diatom dissolution can bias diatom assemblages towards more resistant taxa with profound implications for reconstructing environmental and ecological change (Ryves *et al.,* 2006). Poor preservation and diatom dissolution is a particular feature of high alkalinity waters, especially marl lakes (Round 1964, Flower 1993), although anecdotal evidence exists for both good and poor preservation in alkaline systems (Hecky & Kilham 1973).

To assess the extent of dissolution problems for the RAIL1 and RAIL2 cores, several diatom 'test slides' were made at regular intervals throughout the length of the cores. Preliminary observations revealed that overall there was minimal dissolution of the frustules and therefore the diatom counts could be undertaken with confidence, and without the need to apply corrective indices e.g. the Diatom Dissolution Index (Ryves *et al.,* 2001). Analysis of the 'test slides' of cores RAIL1 and RAIL2 revealed that the numbers of diatoms found on the slides were such that it was possible to count all diatom frustules present, therefore, enabling diatom concentration to be calculated from the original weight of sediment used. The numbers of diatom valves counted per sample ranged from 500-4700, but only 104 valves were present at the 54cm level in RAIL1. Despite the relative paucity of diatoms in this sample there was no evidence of

either fragmentation or even partial dissolution of the diatom frustules in core RAIL1 or RAIL2. Thus the fossil diatom record was used with relative confidence to reconstruct environmental and ecological changes throughout the natural history of the Rail Pit. The absolute diatom counts for core RAIL1 profile are presented (Fig. 5.6).

The diatom data for core RAIL1 were expressed as concentrations (Fig. 5.15a) and also as % relative abundances (Fig. 5.15b & Fig. 5.16). For both the RAIL1 and RAIL2 cores the diatoms *L. hungarica* and *S. seminulum*, i.e. species associated with *Lemna*, were combined to form a '*Lemna* Indicator Metric' from the summation of their % relative abundances. It was envisaged that this '*Lemna* Indicator Metric' could identify any past phases of *Lemna*-dominance in the Rail Pit.

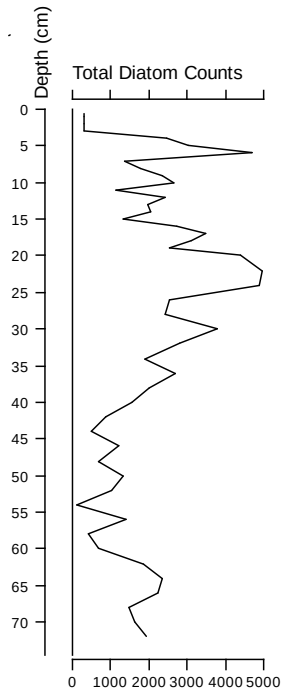


Figure 5.6. The total number of diatoms counted at each sediment level of RAIL1. Note the erratic nature of the counts, the low numbers and concentration of recorded diatoms at the 54 cm level and the relatively high counts at the base of the core. Diatom counts are per 0.1 g sediment.

5.4.5 Data manipulation and analysis

The fossil diatom data for both the RAIL1 and RAIL2 cores were analysed with ordination methods using indirect and direct gradient techniques, constrained cluster analysis (CONISS), linear regression and correlation coefficient analyses. All core diagrams were generated using the programs Tilia (Version 1.7.16), Tiliagraph (Grimm 1991a, b), TGView (Grimm 2002) and C2 (Juggins 2007).

An initial exploratory Detrended Correspondence Analysis (DCA) was performed on the diatom data of both cores (Hill 1973, Hill & Gauch 1980) using CANOCO 4.5 (ter Braak & Šmilauer 2002), primarily to establish whether diatom species responses were linear or unimodal. DCA provides a measure of beta diversity (the extent of species turnover) in community composition which is given by the gradient length of the axes in the ordination diagram (i.e. measured as units of standard deviation). Rare taxa were not down-weighted thereby leading to a more robust estimate of compositional turnover (Birks 2012). Species data were detrended by segments (Hill & Gauch 1980, Wartenberg *et al.*, 1987) and species and samples were standardised by the weighted averaging algorithm (Lepš & Šmilauer 2003). For correspondence analysis (CA) and principle components analysis (PCA) the data were square-root transformed and the axes scaling was focused on inter-species distances.

The RAIL2 diatom data were further investigated using redundancy analysis (RDA), a constrained form of PCA and a constrained form of multivariate multiple regression (ter Braak & Prentice 1988). In the RDA, explanatory variables (predictors or independent variables) were employed to predict the values of the response variables (diatom abundances) by modelling diatom responses on the explanatory variables of the categorical factors of *Lemna* dominance and *Lemna* non-dominance. These predictors of *Lemna* and non-*Lemna* dominance were re-coded into so-called 'dummy' environmental variables (i.e. indicator or binary variables) before performing the RDA. The data were constrained by the two environmental 'dummy' variables of (i) *Lemna* dominance and (ii) *Lemna* non-dominance taking the form of binary values of either 1

(indicating the presence of diatom taxa associated *Lemna* dominance) or 0 (indicating the presence of diatom taxa associated with *Lemna* non-dominance). These environmental variables were represented by triangular symbols that were placed at the centroids of the scores for samples that have a value of 1 or 0 for the particular dummy variable. In other words, the centroid score for the dummy *Lemna* or non-*Lemna* variables represents the average of the scores of samples belonging to that class (i.e. *Lemna* or non-*Lemna* environmental variables).

In the RDA biplot the distance between the centroids of the *Lemna* and non-*Lemna* variables approximated the dissimilarity of their diatom species composition (expressed using Euclidian distance). The distances between these two dummy environmental centroids allowed a prediction to be made of membership of the samples, where a sample has the highest probability of belonging to the class with its centroid closest to that sample point. That is to say, that the distance between the diatom species points and those of the *Lemna* and non-*Lemna* dummy environmental variables approximate the relative total abundances of the diatom species in the samples of that class (Lepš & Šmilauer 2003). The ordination axes of all the diatom species (response variables) were constrained to be linear combinations of the dummy predictor variables (Birks 2012). Species were centred and scaling of scores focused on inter-species correlations (Fig. 5.23). As the upper sections of RAIL1 were highly flocculant a similar constrained RDA of the RAIL1 diatom data was not performed.

5.4.6 RAIL1 and RAIL2 core correlation

There are several numerical approaches to sediment core correlation which attempt to provide a quantitative measure of the degree of reproducibility between cores (Thompson 1991, Birks *et al.,* 2012). However, while numerical approaches are less subjective than simply 'eyeballing', they are often unable to make use of the full range of stratigraphical information and can, therefore, generate inappropriate correlations (Birks *et al.,* 2012). Hence simple visual or graphical approaches are still widely applied (Shaw & Cubitt 1979, Shaw 1982, Edwards 1984).

In this study the diatom and lithostratigraphic data sets were divided so that the sum of variation was minimized and zones were determined by the sum-of-squares method, dividing the data sets into successively smaller groups by splitting existing zones (Gordon & Birks 1972, Birks & Gordon 1985). An agglomerative clustering technique was applied to the lithostratigraphic (Figs. 5.11 & 5.12) and diatom data (Figs. 5.15 & 5.17) with the constraint that clusters are based on the agglomeration of stratigraphically adjacent samples (Birks & Gordon 1985). The constrained incremental sum-of-squares cluster analysis (CONISS) with the measure of dissimilarity being the squared Euclidian distance (i.e. equivalent to total within group sum-of-squares) was applied to both cores to aid correlation (Grimm 1987).

The Pearson correlation coefficient was calculated to provide an independent numerical measure of the amount of association between the two diatom species data sets of *L. hungarica* and *S. seminulum* (i.e. species similarity based on quantitative data) and to provide a measure of the statistical significance of the correlation coefficient of the diatom data in core RAIL1 (Fig 5.13).

5.5 Results

5.5.1 Core stratigraphies

The 75cm long sediment core RAIL1 was comprised of grey/brown silt (recent sediments), very dark brown silt (middle sediments) and lighter grey clay marl at the core base, indicating that it likely covered the whole history of the Rail Pit. The 22cm long core RAIL2 was composed entirely of grey/brown silt (Fig.5.5).

5.5.2 Chronologies for cores RAIL1 and RAIL2

For core RAIL1 total ^{210}Pb activity reached an equilibrium depth with supporting ^{210}Pb at 38cm (Fig. 5.7a). Unsupported ^{210}Pb activities, calculated by subtracting the supporting ^{210}Pb activity from the total ^{210}Pb, decline irregularly with depth. In the top

17cm of the core, ^{210}Pb activities decline more or less exponentially with depth, indicating a relatively uniform sediment accumulation rate. The ^{210}Pb profile shows a trough at 34.5cm. This suggests an increase in the sediment accumulation rate possibly due to a sediment slumping event (Fig. 5.7b). Use of the CIC model was precluded because of the non-monotonic feature in the ^{210}Pb profile.

The ^{137}Cs activity versus depth profile (Fig. 5.7c) has a well resolved peak at 26.5cm. The slow decline of ^{137}Cs activity in the sediment above this peak may imply incomplete sediment mixing (Berner 1980). However, the ^{137}Cs peak almost certainly records the 1963 fallout maximum from atmospheric testing of nuclear weapons and therefore sediment mixing can be ruled out. The simple CRS model places the 1963 layer at 23.5cm, slightly above the ^{137}Cs peak (26.5 cm) in the core. A final chronology and sediment accumulation rate was calculated using the CRS dating model with reference to the 1963 layer identified by the ^{137}Cs record (Table 5.3, Fig. 5.8).

There was a sudden and dramatic increase in sedimentation rate at a depth of 34.5cm (1940s) with a rate of 0.47g cm^{-2} yr^{-1} which may be due to the sediment slumping (Fig. 5.8). This is followed by a relatively uniform period covering the 1950s to the 1970s of 0.04-0.07g cm^{-2} yr^{-1} and an increased relatively uniform sedimentation rate of 0.08-0.1g cm^{-2} yr^{-1} for the last 30 years.

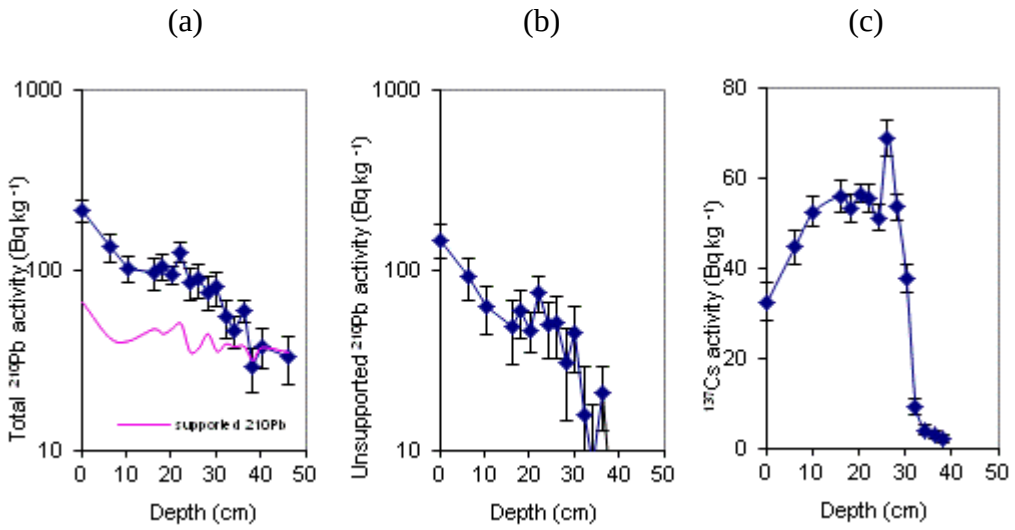


Figure 5.7. Radionuclide fallout concentrations in the RAIL1 core taken from the Bodham Rail Pit, Norfolk, showing (a) total ^{210}Pb, (b) unsupported ^{210}Pb, and (c) ^{137}Cs concentrations versus depth.

Depth	Dry mass	Chronology			Sedimentation rate		
		Date	Age				
cm	g cm^{-2}	AD	Yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2010	0		-	-	-
0.50	0.028	2010	0	2	0.07	0.54	23.2
6.50	0.7598	1999	11	2	0.08	0.69	27.4
10.50	1.1283	1995	15	3	0.1	0.94	30.6
16.50	1.7859	1988	22	3	0.1	0.87	39.3
18.50	2.0421	1985	25	3	0.08	0.53	30.6
20.50	2.3566	1981	29	3	0.09	0.51	26.3
22.50	2.7152	1975	35	3	0.04	0.26	23.2
24.50	3.0418	1968	42	4	0.05	0.38	34.7
26.50	**3.2859**	**1963**	**47**	**4**	**0.04**	**0.39**	**39.0**
28.50	3.4969	1959	51	4	0.07	0.62	53.4
30.50	3.7086	1955	55	5	0.04	0.31	41.5
32.50	4.0044	1950	60	7	0.1	0.49	86.7
34.50	**4.488**	**1949**	**61**	**8**	**0.47**	**1.7**	**115.0**
36.50	5.12	1939	71	11	0.05	0.16	51.3

Table 5.3. ^{210}Pb chronology of core RAIL1 taken from the Bodham Rail Pit, Norfolk. Note that data highlighted in bold likely correspond to the fallout radionuclide maximum of 1963 (26.5 cm depth) and the increased sedimentation rate (0.47 g cm^{-2} yr^{-1}) recorded at 34.5 cm depth.

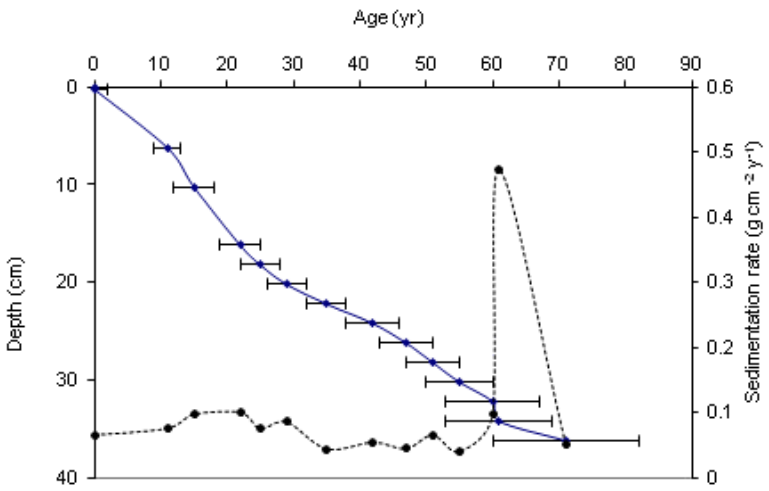


Figure 5.8. Chronology of core RAIL1 taken from the Bodham Rail Pit, Norfolk, showing CRS model of ^{210}Pb dates and sedimentation rates. Note the solid lines shows the chronological age with depth, whilst the dashed line indicates the sedimentation rate.

For core RAIL2 equilibrium depth of total ^{210}Pb activity with supporting ^{210}Pb occurs at 19cm and, as in core RAIL1, unsupported ^{210}Pb activities decline irregularly with depth. The sudden decline of total and unsupported ^{210}Pb activity at 18.5cm (i.e. 1940s) may imply a non-continuous sedimentation process (Figs. 5.9a & 5.9b). The ^{137}Cs versus depth profile (Fig. 5.9c) has a well resolved peak at 16.5cm. This is almost certainly derived from the 1963 atomic weapons fallout maximum; an interpretation supported by the detection of tracers of ^{241}Am over 15.5-18.5cm. A poorly resolved ^{137}Cs peak at 10.5cm may be derived from the 1986 Chernobyl accident fallout, but this is a tentative suggestion at best.

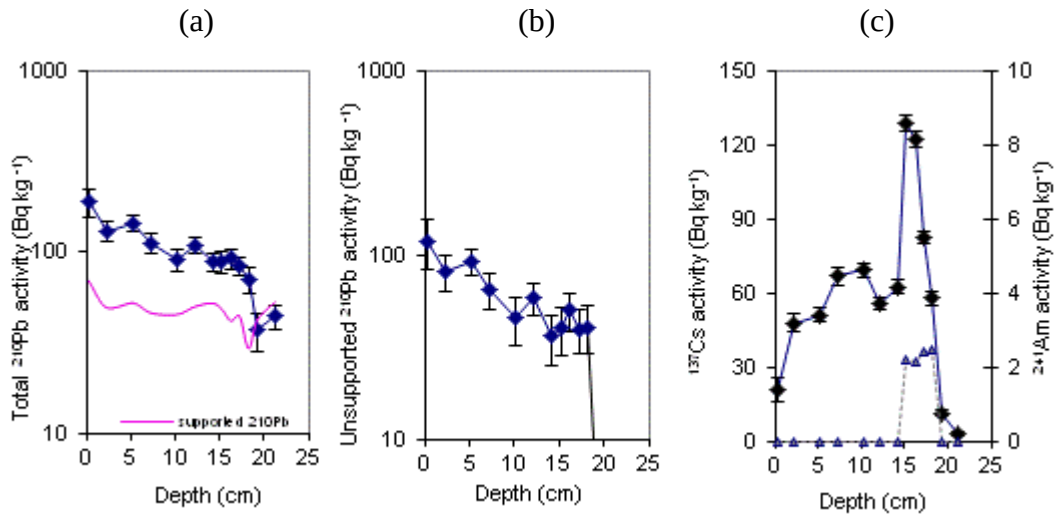


Figure 5.9. Fallout radionuclide concentrations in the core RAIL2 taken from the Bodham Rail Pit, Norfolk, showing (a) total ^{210}Pb, (b) unsupported ^{210}Pb, and (c) ^{137}Cs and ^{241}Am concentrations versus depth.

As for core RAIL1, use of the CIC models was precluded by the irregular decline in unsupported ^{210}Pb activity (Appleby & Oldfield 1978, Appleby 2001) and consequently the CRS model was used (Appleby & Oldfield 1978). The simple CRS dating model places 1963 at 12.5cm, just above that suggested by the ^{137}Cs at ^{241}Am records at 15.5cm. This may be due to non-continuous sedimentation process in the later sediment record (i.e. before 1963). The chronologies of the core were calculated using the CRS model with reference to the 1963 layer identified by the ^{137}Cs record (Table 5.4). These calculations indicate that sediment accumulation rates gradually increased from 0.02g cm^{-2} yr^{-1} in the 1940s to 0.06g cm^{-2} yr^{-1} since the 1980s (Fig. 5.10).

Depth	Dry mass	Chronology			Sedimentation rate		
		Date	Age				
cm	G cm^{-2}	AD	Yr	±	g cm^{-2} yr^{-1}	cm yr^{-1}	± %
0	0	2011	0		-	-	-
0.50	0.02	2011	0	2	0.056	0.442	31.0
2.50	0.285	2006	5	2	0.072	0.519	23.2
5.50	0.714	1999	12	2	0.051	0.323	19.2
7.50	1.071	1993	18	3	0.059	0.308	24.8
10.50	1.67	1983	28	3	0.062	0.325	31.1
12.50	2.025	1976	35	4	0.039	0.211	24.6
14.50	2.402	1967	44	5	0.047	0.265	35.2
15.50	**2.559**	**1963**	**48**	**6**	**0.038**	**0.248**	**35.3**
16.50	2.707	1958	53	7	0.026	0.175	32.0
17.50	2.856	1953	58	8	0.028	0.198	37.7
18.50	2.987	1948	63	10	0.023	0.161	38.1

Table 5.4. ^{210}Pb chronology of core RAIL2 taken from the Bodham Rail Pit, Norfolk. Note that the data highlighted in bold refer to the fallout radionuclide maximum of 1963 (15.5 cm depth).

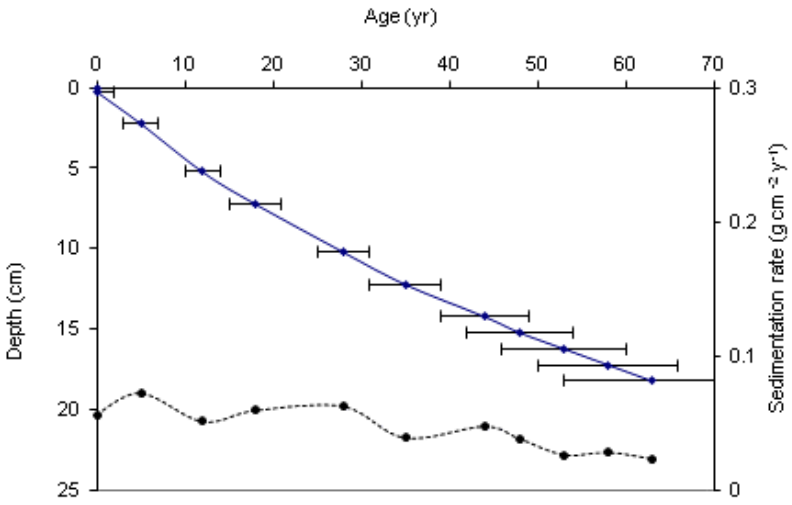


Figure 5.10. Chronology of core RAIL2 taken from the Bodham Rail Pit, Norfolk, showing CRS model of ^{210}Pb dates and sedimentation rates. Note the solid lines shows the chronological age with depth, whilst the dashed line indicates the sedimentation rate.

5.5.3 Lithostratigraphy of cores RAIL1 and RAIL2

There were marked changes in percentage loss on ignition (%LOI) values throughout RAIL1 (Fig. 5.11a). The base of the sequence had a relatively low organic content (<10%) which steadily increased to 30% at 44cm and then declined sharply to less than 20% at 40cm before increasing gradually to 35% at 32cm. Radiometric dating suggests

that this second increase in %LOI occurred c. 1940s and coincided with the distinct increase in sedimentation rate at the 34.5cm level (Table 5.3, Fig. 5.8). The LOI values then fluctuated at around 30-35% towards the top of the core with a notable spike of 38% at the 14cm level. Percentage carbonate content (%carbonate) of the core was wholly different to that exhibited by the organic matter. As the Rail Pit was likely a marl pit excavated for calcareous clays, high carbonate content was observed at the base of the core (60%), but at around 60cm level the percentage carbonate was reduced to 17%. There were two subsequent peaks in the %carbonate at 50cm (37%) and at 38cm (23%). The increase in %carbonate at this latter level broadly mirrors the sudden and dramatic decrease in the organic matter at 44-32cm. From about the 32cm level %carbonate gradually decreased to a value of 6% at the top of the core (Figs. 5.11a, 5.12a).

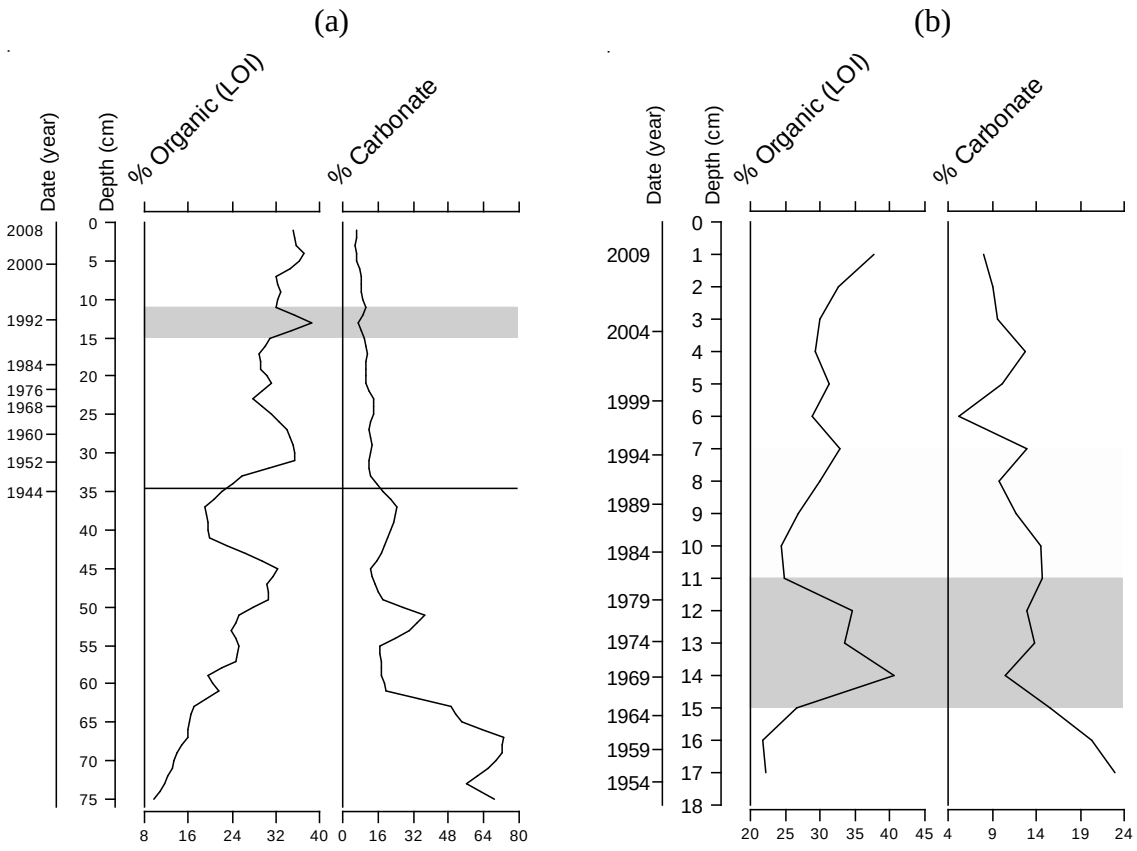


Figure 5.11. Lithostratigraphy of cores RAIL1 (a) and RAIL2 (b). The grey highlighted areas show the %LOI peak, and the single black line marks the timing of the sudden increase in sediment accumulation at 34.5 cm in RAIL1 (b).

The most striking feature in the lithostratigraphy of core RAIL2 is the rapid increase in %LOI from the core base reaching a maximum of 40% at the 14cm level (Figs. 5.11b, 5.12b). This peak likely corresponds to a similar feature in RAIL1 (40%) at 12cm (Figs. 5.11a, 5.12a). Interestingly the peak in %LOI in RAIL1 occurs just after the documented *Lemna* phase of 1986 to early 1990s, but this %LOI peak occurs just before this *Lemna* phase in RAIL2. Radiometric dating of RAIL2 places the onset of the increase in %LOI at the mid 1960s and the end of the peak in the early 1980s (11cm), whereas radiometric dating of RAIL1 places the onset of the %LOI peak at the late 1980s and the end of the peak in the early 1990s. Although there is some discrepancy in the radiometric dates of this %LOI peak between both cores, the consistent patterns in organic matter give an initial basis for correlating the RAIL1 and RAIL2 cores (see Figs. 5.12a, 5.12b). It was noticeable that the marked decrease of sediment organic matter at 45-32cm level in RAIL1 (and a concomitant increase in carbonate) occurred between *Lemna* Phase 1 and *Lemna* Phase 2 indicated by the diatom stratigraphies (see Figs. 5.15 & 5.16).

5.5.4 Correlation of cores RAIL1 and RAIL2

Although many palaeoecological studies of lakes are based upon a single core taken from the deepest part of a lake, invariably from a central location, multi-core studies have shown the complexities of sediment (and microfossil) deposition in lake basins (e.g. Battarbee 1978, Anderson 1986, Anderson 1990), in addition to spatio-temporal variations in the sediment accumulation rate and in sediment composition (e.g. Yang *et al*., 2002, Punning *et al*., 2004). Various factors such as lake-basin morphology and topography, allochthonous sediment discharge, autochthonous primary production, sediment resuspension and lake level fluctuations impact upon the accumulation of sediments and the resulting sedimentary signal (Håkanson & Jansson 1983, Blais & Kalff 1995, Weyhenmeyer *et al*., 1997). Moreover, water level fluctuations (which the Rail Pit is known to experience) have been shown to have significant effects upon sediment characteristics and changes in aquatic vegetation communities (Tarras-Wahlberg *et al*., 2002). Due to these factors, it is recognized that single cores are

unlikely to be representative of whole lakes (Anderson 1986). As a consequence, multi-core approaches have become increasingly more common (see Davidson et al., 2005, Thompson *et al*., 2012), and with this a requirement for core cross correlation so that sedimentary profiles can be matched (Thompson *et al*., 2012).

To provide sufficient material for multi-proxy studies several cores can be collected and combined (Birks and Birks 2006). However, with the employ of the use of the recently developed 'Big Ben' large-diameter corer, together with the study site being a small pond (as opposed to a large lake) it was envisaged that one centrally located core (RAIL1) would be sufficient for a multi-proxy study (Zhao *et al*., 2005). A second short Glew core (RAIL2) was collected due to the flocculant sediment of the upper section of the main core (RAIL1), therefore it was necessary to correlate the two cores. There are several approaches to the correlation of multiple cores using various parameters such as diatom biostratigraphy, lithostratigraphy and radiometric dating.

The radiometric dating of cores RAIL1 and RAIL2 suggests that sediment accumulation rates (SAR) differ between the two cores. For example, the SAR of the late 1950s for core RAIL1 was >0.6cm yr^{-1} whilst for core RAIL2 the SAR was <0.2cm yr^{-1} for the same time period. There were, however, several features in common in the organic matter and carbonate profiles which allowed the cores to be correlated (Fig. 5.12). A notable feature of both RAIL1 and RAIL2 was a distinct and rapid increase in the organic content (i.e. high %LOI) and a concomitant decrease in the amount of carbonate immediately after the termination of the dense *Lemna* mats at approximately 15-11cm depth in the core profiles (i.e. late 1980s to early 1990s for RAIL1; late 1960s to early 1980s for RAIL2). It seems likely that this sudden increase in organic matter arises directly from the termination of the *Lemna* phase, with the senescent *Lemna* biomass being assimilated into the surface sediment.

Given the above discrepancies in the dating of major LOI changes further efforts at core cross-correlation are needed, with the diatoms affording possibilities in this respect. The diatom biostratigraphies of cores RAIL1 and RAIL2 (Figs. 5.15, 5.17

respectively) showed a degree of concordance in terms of diatom relative abundance changes with similar stratigraphic profiles for *Epithemia adnata*, *Epithemia turgida*, *Pinnularia maior*, *Gyrosigma acuminatum* and *Amphora veneta* which showed similar dates between both RAIL1 and RAIL2 cores. Notably concordance was also evident for the *Lemna*-indicators of *Lemnicola hungarica* and *Sellaphora seminulum* where these taxa showed remarkably similar timing in both cores, which were dated at 1999-2005 (Figs. 5.16, 5.18).

Although there was concordance for the aforementioned diatoms, there were also several diatom compositional differences between the two cores. For example *Cyclostephanos invisitatus* was present in core RAIL2 but was not present in core RAIL1, and *Encyonema minuta* appeared in the core profile of RAIL1 before the most recent *Lemna* phase but the timing was not replicated in the core profile of RAIL2 where *E. minuta* only appeared after termination of the most recent *Lemna* phase.

Differences in sediment accumulation rates (SAR) between two cores collected just a few metres apart suggests complex processes of sedimentation likely linked to localized leaf fall and a lack of water and sediment mixing. This lack of mixing in a steep-sided pit where diatoms may live in distinct micro-niches may also be responsible for small-scale variation in the diatom stratigraphies. A strong habitat-dependent spatial variability in the distribution of microfossils in lake sediments has been demonstrated elsewhere (Dixit & Evans 1986) and is worthy of further investigation in small ponds.

Despite the above issues, the fossil diatom records and particularly the lithostratigraphic data can provide a reasonable basis for correlation of the two cores. In order to facilitate this, the diatom and lithostratigraphic data of the two cores were divided into characteristic ‘zones’ using the CONISS method. The resulting dendrograms showed overall good agreement in the positions of the zones for the two cores (see Appendix 4 for zonation diagrams of diatom and lithostratigraphic data and Fig. 6.18 in Chapter 6). Therefore, the correlation of cores RAIL1 and RAIL2 was based upon the diatom and lithostratigraphic records in combination.

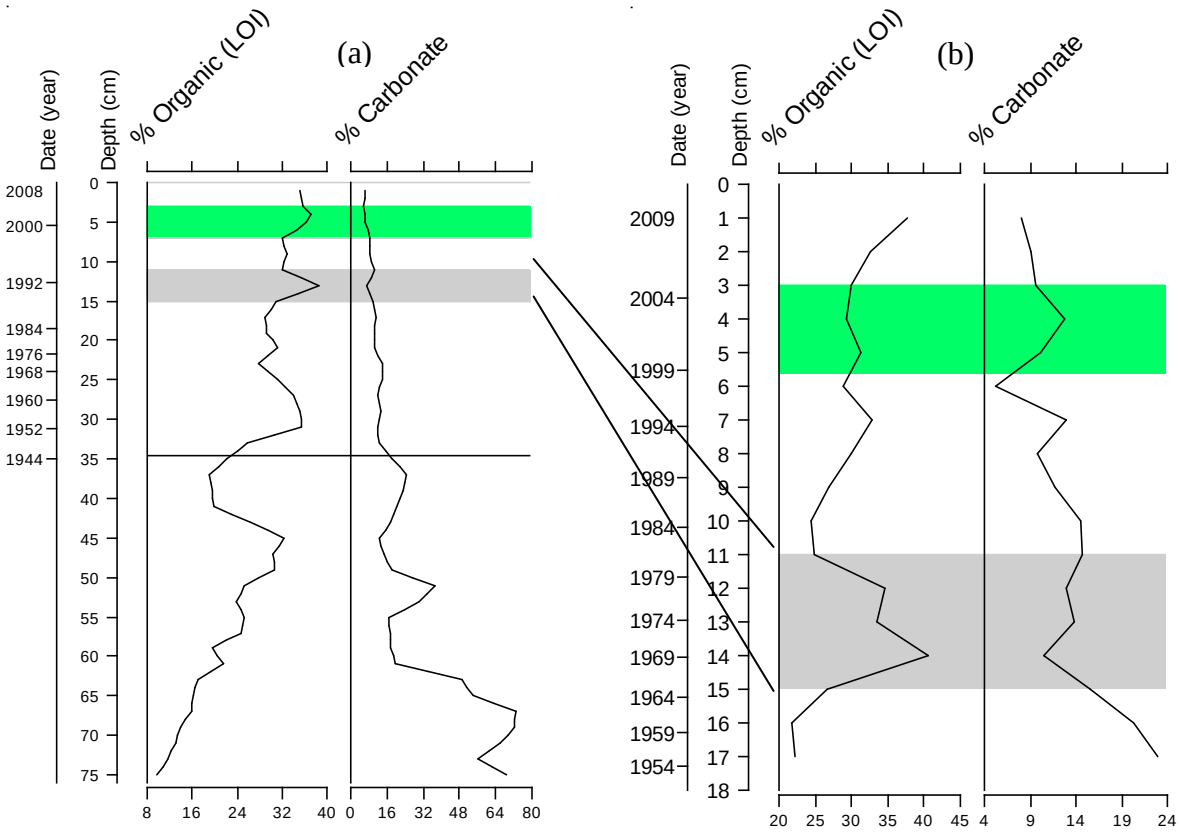


Figure 5.12. Lithostratigraphy showing % loss on ignition (LOI) and % carbonate of RAIL1 (a) and RAIL2 (b). The green highlighted areas denote most recent *Lemna* dominance phase dates (1999-2005) based on observational data (see Table 5.2). The grey highlighted areas denote the correlation point of the two cores based upon %LOI and % carbonate data. The black bold line highlights the peak in the sedimentation rate of RAIL1 (a). The connecting lines denote suggested points of core correlation based on %LOI and % carbonate.

5.5.5 The relationship between the fossil *Lemna*-associated diatoms, *Lemnicola hungarica* and *Sellaphora seminulum*

The bivariate relationship between the two diatom taxa *L.hungarica* and *S. seminulum*, which were identified as having an association with free-floating macrophytes per se and *L. minor* in particular (see Chapter 3), was further examined with exploratory data analysis. Bivariate statistics were employed to quantify the relationship between the two diatom taxa by estimating their covariance to provide a numerical estimation of the bivariate relationship. Pearson's correlation coefficient was employed to determine if there was a negative or a positive relationship between the two diatom taxa, and to

provide a numerical statistic of the relationship. A scatter-plot of the two diatom variables was produced to graphically display the bivariate diatom data (Fig. 5.13).

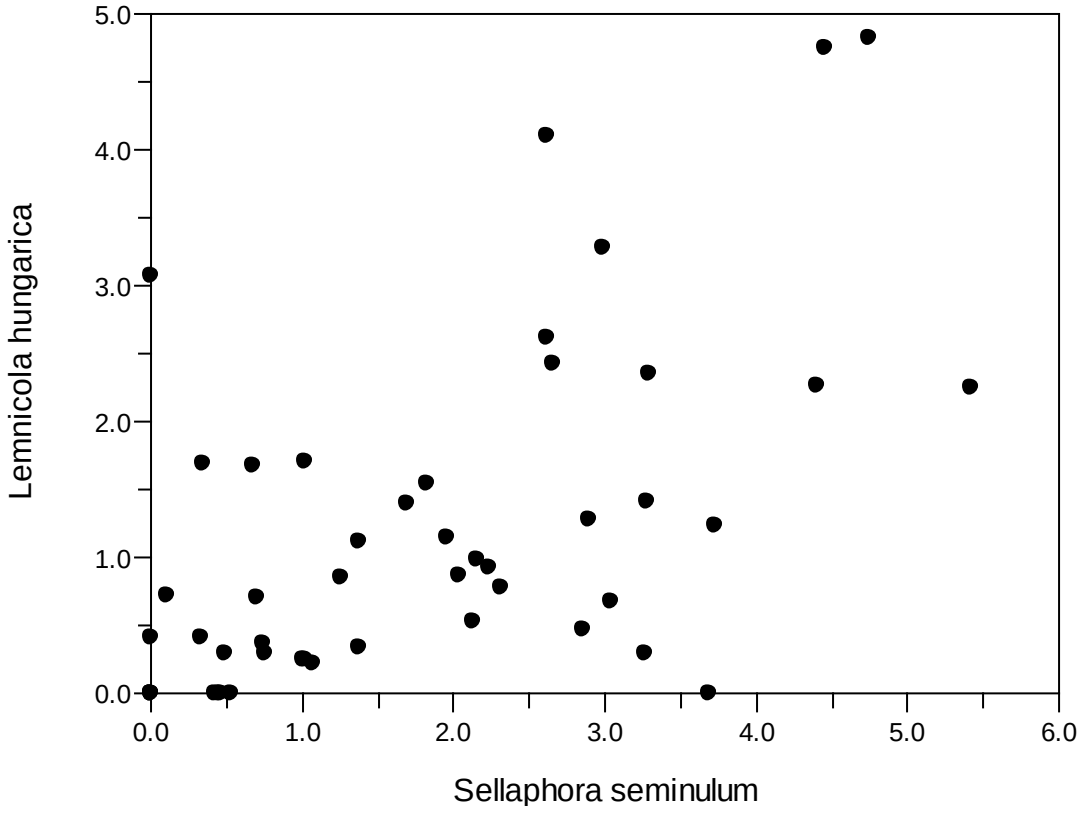


Figure 5.13. Scatter-plot of the relationship between *Lemnicola hungarica* and *Sellaphora seminulum* concentrations for RAIL1. Diatom concentrations are: log cells per 0.1 g wet wt^{-1}.

There was a positive linear relationship between the two diatom variables, although the sample points were fairly scattered. The Pearson correlation coefficient (r) provides a numerical measure of the amount of association between the two diatom scores. There was a significant positive relationship between *L. hungarica* and *S. seminulum* abundances ($r=0.57$, $DF=46$, $p<0.001$) for RAIL1. As Pearson's correlation coefficient is sensitive to outliers and non-linearity such as skewed data (Juggins & Telford 2012) the data were also examined using the Spearman's rank test. This is less affected by outliers and skewed data as the correlation coefficient ranks the data in numerical size.

As with the Pearson's correlation coefficient there was a highly significant positive relationship between *L. hungarica* and *S. seminulum* abundances ($r=0.54$, $DF=46$, $p<0.001$).

5.5.6 Diatom stratigraphies

5.5.6.1 Comparison of *Lemna* indicator diatom records of RAIL1 and RAIL2 with observed periods of *Lemna* dominance

The core stratigraphies of *L. hungarica* and *S. seminulum* were examined to see if changes in the abundances of these species match with the known timing of *Lemna* dominance from observational records (Table 5.2). Figure 5.14 shows relatively high degree of concordance between both *L. hungarica* and *S. seminulum* and the most recently observed *Lemna* period of 1999-2005 in cores RAIL1 and RAIL2. However, there was little concordance between *L. hungarica* and *S. seminulum* with the observed earlier *Lemna* period of 1986 to early 1990s in core RAIL1. This discrepancy in concordance between the diatom data and the radiometric dating (RAIL1) is approximately ten years and is likely to be due to errors in the radiometric dating. There was, nevertheless, a reasonable degree of concordance between *L. hungarica* and *S. seminulum* with the observed *Lemna* period of 1986 to early 1990s in core RAIL2.

As with RAIL1 *L. hungarica* and *S. seminulum* followed a clear pattern in the diatom record of RAIL2: i) well established during the first recorded *Lemna* phase albeit at lower concentrations than the second *Lemna* phase, ii) reduction in concentrations after the first *Lemna* phase ended, iii) immediately increased with the onset of the second *Lemna* phase and iv) reduced concentrations following the ending of the second *Lemna* phase. The population dynamics of both species, therefore, appeared to reasonably track the timing of the most recent observed period of *Lemna* dominance in 1999-2005 but clearly was unable to track the timing of the purported observed period of *Lemna* dominance of 1986 to early 1990s. Figure 5.14 graphically illustrates the concordance between the observed *Lemna* dominance period of 199-2005 with the diatom record,

and the lack of concordance between the observed *Lemna* dominance of 1986 to early 1990s with the diatom record of both RAIL 1 and RAIL2 cores. However, because of the discrepancy in concordance between the *Lemna*-indicator diatoms and radiometric dating, and the uncertainty in the exact timing of the observed *Lemna* period of 1986 to early 1990s, it was decided to omit this earlier *Lemna* period from both the RAIL1 and RAIL2 core stratigraphic diagrams (Figs. 5.15-5.19).

In addition to the absolute diatom counts, ranging from 500-4700 counts per sample (RAIL1: Fig. 5.15a; RAIL2 Fig. 5.17a), the diatom data were expressed as percentage relative abundances (RAIL1: Fig. 5.15b; RAIL2: Fig. 5.17b) to better show the diatom patterns. The resulting diagrams (Figs. 5.16, 5.18) more clearly defines the rare and common taxa but also demonstrates the potential for employing a *Lemna* indicator metric based on the sum of the two duckweed-associated epiphytic diatoms *L. hungarica* and *S. seminulum*. In RAIL1 these two taxa appeared in high absolute abundances in the section 7-3cm (= *Lemna* Phase 4) as clearly recorded by field observations (Table 5.2, Fig. 5.4a). At the onset of this *Lemna* phase, both *L. hungarica* and *S. seminulum* increased in absolute numbers (Fig. 5.15). This match between the *Lemna* indicator diatoms and the timing of the most recently observed *Lemna* phase strongly suggests that the *L .hungarica*/*S. seminulum Lemna*-indicator model has great potential. This validation means that the model can be faithfully applied to the lowermost section of the core profile to infer the timings of the presence of *Lemna* in the Rail Pit.

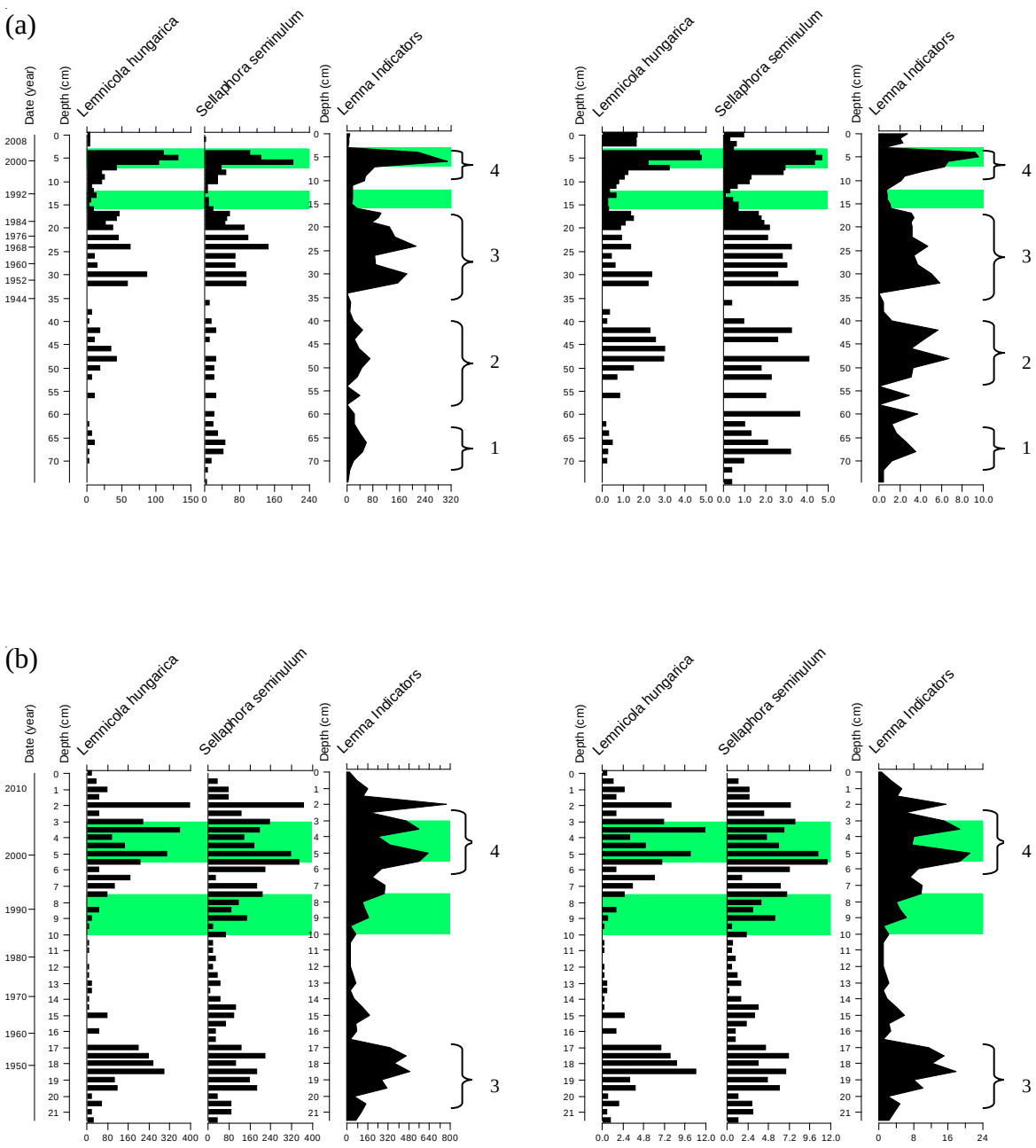


Figure 5.14. Stratigraphy of the *Lemna* associated diatoms *Lemnicola hungarica* and *Sellaphora seminulum* recorded from cores RAIL1 (a) and RAIL2 (b). L/H stratigraphs denote absolute abundances; R/H stratigraphs denote % relative abundances. *Lemna* Indicators (i.e. summation of *L. hungarica* and *S. seminulum* abundances) are also presented. The green highlighted areas denote the timing of *Lemna*-dominance from observation (Table 5.2). Note that the observed *Lemna* dominance of 1999-2005 tallies well with the diatom record, but there is little concordance of the diatom record with the observed *Lemna* dominance of 1986 to early 1990s. Core RAIL1 (a) shows the most recent observed *Lemna* phase (i.e. 4 = Phase 4) and the diatom derived *Lemna* phases (i.e. 3 = Phase 3; 2 = Phase 2; 1 = Phase 1). Core RAIL2 (b) shows corresponding Phase 4 and Phase 3. The radiometric dates and depths are also presented.

5.5.6.2 The diatom record of RAIL1

A total of sixty diatom species were recorded from core RAIL1 which most likely covers the entire history of the Rail Pit (see Appendix 1 for a full species list). The resulting diatom stratigraphy (Fig. 5.15a) suggests two to perhaps three time periods when the surface of the pond was covered and dominated by floating duckweed mats (Phases 2-4). One *Lemna* phase inferred from the *Lemna*-indicator diatoms covered the pond from the early 1950s to the mid 1980s (Phase 3) whilst another *Lemna* phase was observed from late 1990s to mid 2000s (Phase 4). A third, earlier *Lemna* period (Phase 2) can also be inferred and is more readily indicated by the percentage relative abundance data than the absolute data (Fig. 5.15b). It was not possible to give an indication of the timing of this *Lemna* period from the radiometric dating, but it almost certainly predates the war years. A fourth, still earlier *Lemna* phase can also be identified (Phase 1: 70-65cm). However, the relatively lower abundances of the *Lemna*-indicator diatoms in this section compared with Phases 2 to 4 suggest *Lemna* presence rather than *Lemna* dominance. The diatom record of RAIL1 has been divided into four major zones based on the cluster analysis, with Zone 4 being divided into three sub-zones.

Zone 1 (75-62cm)

The earliest 'pioneering' diatom assemblages (Zone 1: 75-62cm) were dominated by the pennate diatoms, *Achnanthes ingratiformis*, *Cymbella tumida*, *Staurosira elliptica*, *Amphipleura pellucida*, *Psammothidium lauenburgianum*, *Cymbella caespitosa*, *Fragilaria capucina* var. *mesolepta*, *Amphora inariensis* and *Epithemia* spp. (*E. adnata*, *E. sorex* and *E. turgida*). These species suddenly disappeared at the 56cm level with the exception of the three *Epithemia* spp. The *Lemna*-indicator diatoms *L. hungarica* and *S. seminulum* are present in Zone 1 inferring that *Lemna* was also likely present, with *S. seminulum* obtaining relatively high percentages (Fig. 5.14).

Zone 2 (62-32cm)

Both *E. adnata* and *E. sorex* rapidly declined in this zone and at the 34cm level they were absent. Other diatom species that were relatively abundant in this zone were *Navicula radiosa, Navicula cryptotenella, Achnanthidium minutissimum, Sellaphora pupula, Encyonema minuta* and *Amphora pediculus*. These species also declined above 56cm, but at 40cm they underwent a relatively sudden increase in abundance. At 40cm there appeared to be a relative explosion of diatoms in terms of numbers of species recorded and absolute densities, with species such as *Planothidium frequentissimum, Gomphonema parvulum, Amphora libyca, Gomphonema truncatum* var. *truncatum, Eolimna minima, Craticula cuspidata* and *Rhoicosphenia abbreviata* all increasing. Zone 2 sees the first *Lemna* dominance phase (Phase 2) inferred by the high percentages of both the *Lemna*-indicator diatoms over 54-42 cm (Figs. 5.14 & 5.15).

Zone 3 (32-17cm; c. early 1950s-mid 1980s)

Eunotia bilunaris was abundant in the early history of the Rail Pit but together with the other dominant 'pioneering' species rapidly declined in population size. However, as with the diatom species associated with Zone 2 such as *Gomphonema acuminatum* and the three *Epithemia* species, *E. bilunaris* underwent a sudden rapid reappearance (32cm) but increased in concentration at the onset of the inferred *Lemna* dominance phase (Phase 3) by the *Lemna*-indicator model which characterises Zone 3. Although not very abundant, the two recorded species from the genus *Pinnularia* (= *P. maior* and *P. subcapitata*) exhibited contrasting patterns of onset and timing of appearance in the fossil record. Following a relatively prolonged appearance in the fossil record (58-20cm) *P. maior* then declined rapidly and completely disappeared with the onset of the second (Phase 3) *Lemna* dominance phase (17 cm, Zone 3). Conversely, *P. subcapitata* was absent from the fossil record until an abrupt appearance at 24cm level and then persisted through the second *Lemna* dominance phase (Phase 3) in Zone 3. There were large increases in abundances of *P. frequentissimum, Cocconeis placentula, G. parvulum* and *Fragilaria capucina* var. *capucina* in Zone 3. Notably, the absolute

numbers of *Navicula radiosa*, *Navicula cryptotenella*, *A. minutissimum*, *S. pupula*, *E. minuta* and *Amphora pediculus* declined at the onset of the phase of *Lemna* dominance at the top of the zone (17cm).

Zone 4 (17-0cm; c. 1986-2010)

Zone 4 was divided into three sub-zones, namely Zone 4a, 4b and 4c.

The hiatus between the two phases of *Lemna* dominance characterises Zone 4a and saw a marked reduction in abundances in the *Lemna*-indicator diatoms. There were increases in the planktonic diatoms *C. meneghiniana* and *S. parvus*, where maximum abundances occurred, but both *S. hantzschii* and *C. pseudostelligera* were absent. Zone 4a was dominated by *C. placentula*, *G. parvulum*, *N. cryptotenella*, *E. minima*, *Achnanthes conspicua*, *Synedra acus* var. *acus*, *P. frequentissimum* and *F. capucina* var. *capucina.* There was a sudden explosion in abundances of some diatom species particularly *Navicula rhyncocephala*, *Fragilaria fasciculata*, *C. cuspidata*, *E. minuta* and *Gomphonema acuminatum*, and *Gyrosigma acuminatum* suddenly returned to the fossil record in Zone 4a.

Zone 4b is characterised as a *Lemna*-dominance phase (Phase 4) which was derived from the *Lemna*-indicator model. The planktonic diatoms *C. meneghiniana* and *S. parvus* were dominant but there was a decline in their abundances; small numbers of *S. hantzschii* appeared towards the latter part of Zone 4b but *C. pseudostelligera* was absent. *N. rhyncocephala*, *S. pupula* and *Synedra ulna* abruptly disappeared with the onset of this *Lemna*-dominance phase. It was noticeable that diatom species that were previously dominant before this *Lemna* phase (Phase 4) such as *C. placentula*, *G. parvulum*, *N. cryptotenella* and *A. conspicua* decreased in abundances during Zone 4b and the absolute numbers of *N. radiosa*, *N. cryptotenella*, *A. minutissimum*, *S. pupula*, *E. minuta* and *A. pediculus* declined at the onset of *Lemna* dominance. Similarly, *Diatoma tenuis*, *Nitzschia palea*, *F. capucina* var. *capucina*, *F. fasciculata*, *Stauroneis anceps*, *Hantzschia amphioxys* var. a*mphioxys*, *G. acuminatum*, *Navicula lanceolata*

and *Neidium ampliatum* were present throughout the sediment record but had markedly declined during the latter stage of this phase of *Lemna*-dominance (Phase 4).

Zone 4c marks the end of *Lemna*-dominance (Phase 4) where there was a sudden decrease in the *Lemna*-indicator diatoms. There was an immediate increase in abundances of the planktonic diatoms *C. meneghiniana* and *S. parvus* and there was a return to the fossil record by *S. hantzschii* and *C. pseudostelligera*. *Nitzschia* species, particularly *Nitzschia palea*, also increased in numbers. Many diatom species that were abundant before *Lemna*-dominance Phase 4, such as *F. fasciculata*, *C. placentula* and *P. frequentissimum*, returned to their former high abundances in Zone 4c. Other species that had previously been recorded in earlier core sequences, such as *Amphora libyca* and *E. bilunaris*, suddenly returned to the fossil record in Zone 4c.

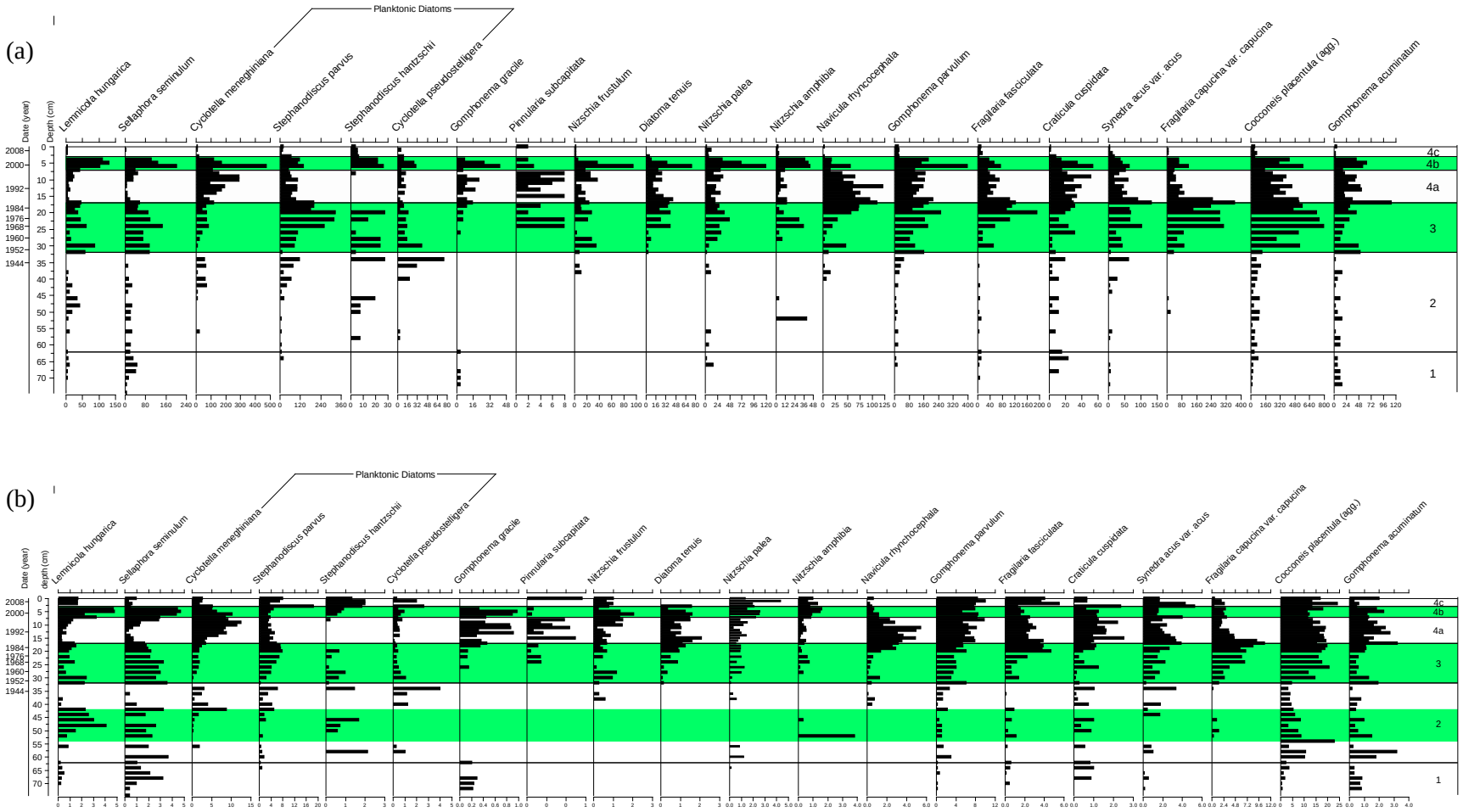


Figure 5.15. Stratigraphs showing the fossil diatom (cells per 0.1 g sediment) record of RAIL1 (a) and % relative abundance (b). The diatoms *Lemnicola hungarica* and *Sellaphora seminulum* associated with *Lemna* are presented on the far left-hand side of the diagrams. The planktonic diatoms *Cyclotella meneghiniana*, *Stephanodiscus parvus*, *Stephanodiscus hantzschii* and *Cyclotella pseudostelligera* are presented together as a group. The other diatoms are presented in order of their chronologies. The green bands (both diagrams) denote inferred periods of duckweed dominance (uppermost band is based upon recorded observations and the *Lemna* indicator metric [Phase 4]; lower bands are based upon the *Lemna* indicator metric [Phases 3 & 2]). The zones derived from the diatom data are presented. Both depth (cm) and radiometric dates (year) are presented on the y axis.

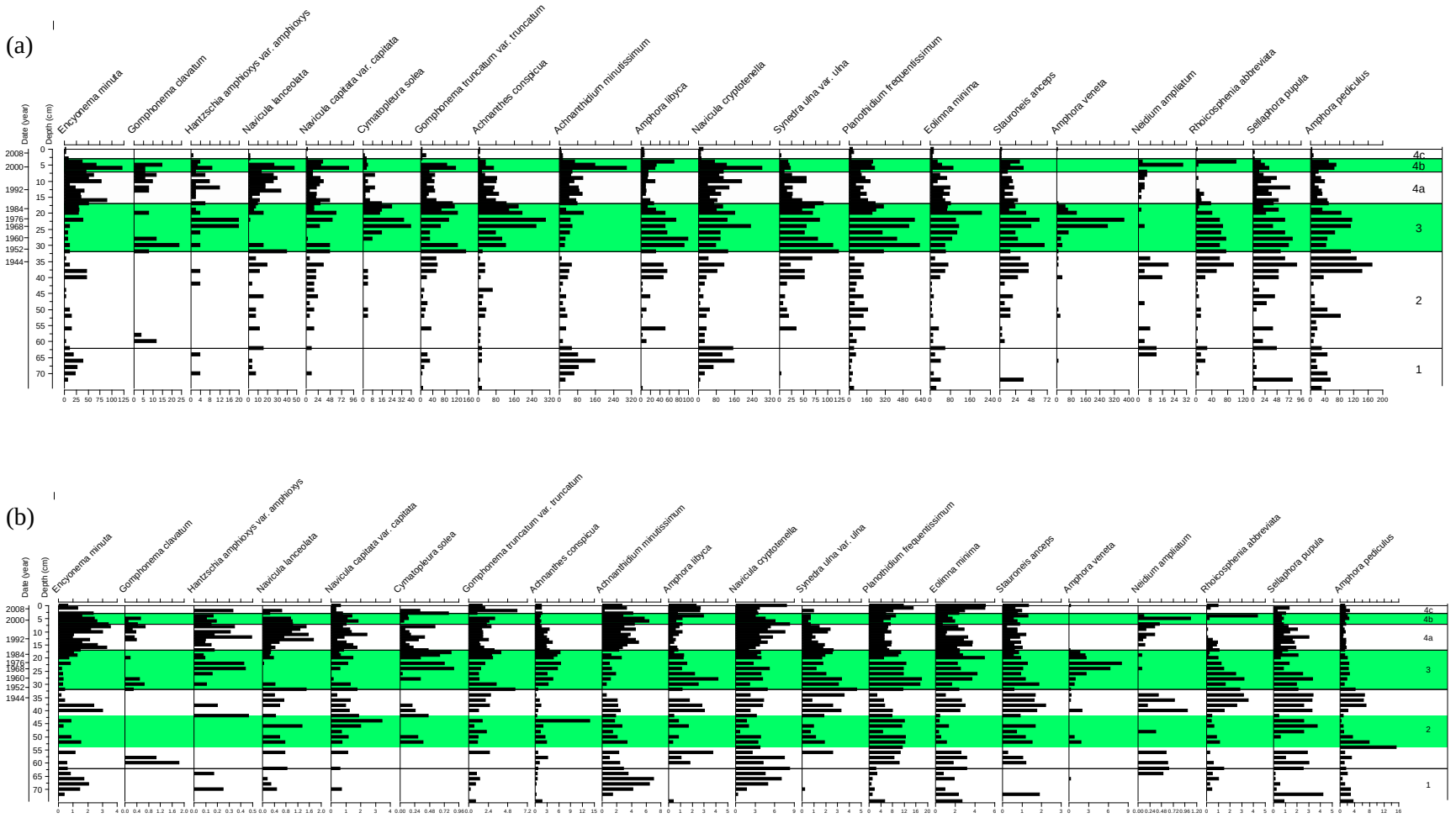


Figure 5.15 cntd. Stratigraphs showing the fossil diatom (cells per 0.1 g sediment) record of RAIL1 (a) and % relative abundance (b). Both depth (cm) and radiometric dates (year) are presented on the y axis.

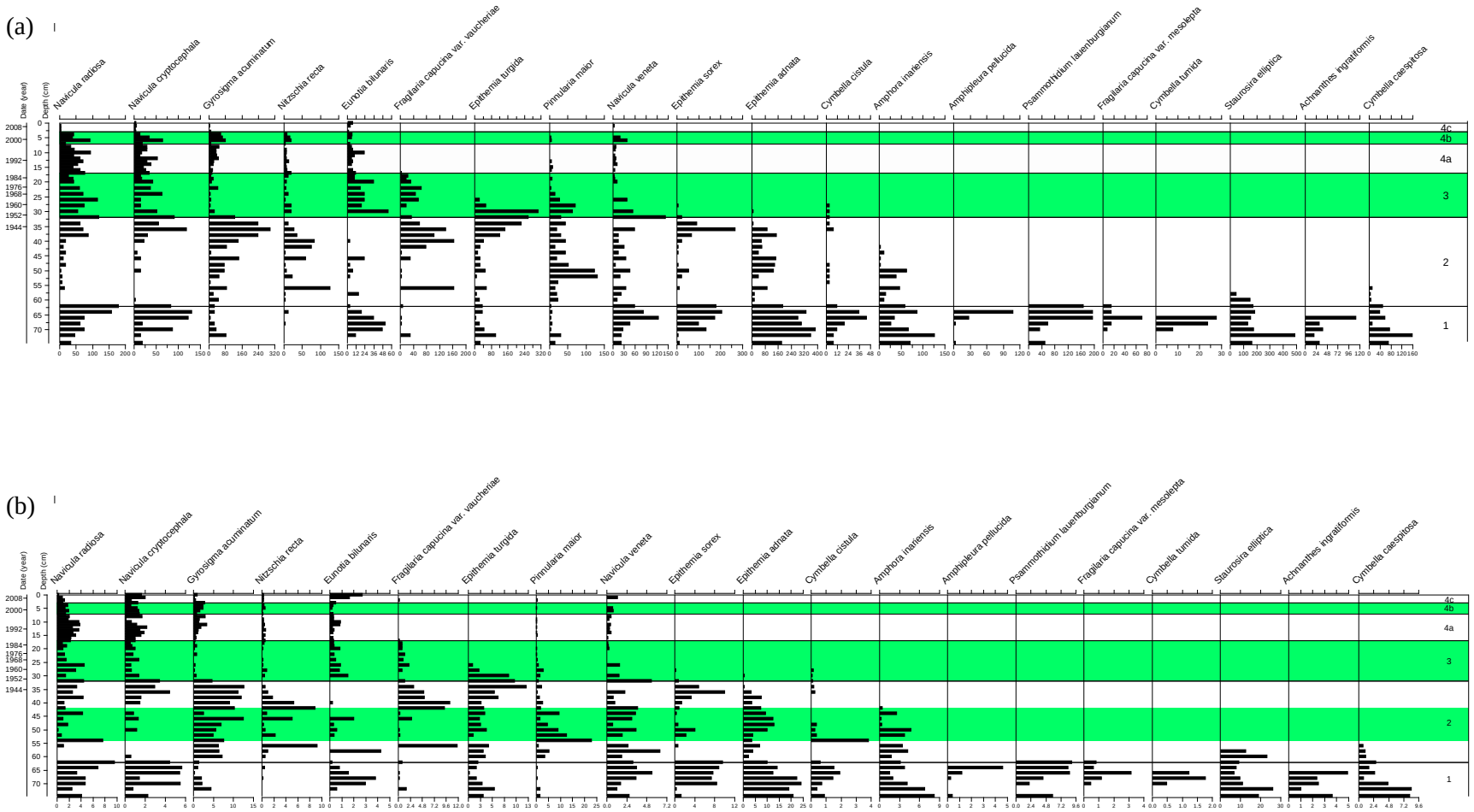


Figure 5.15 cntd. Stratigraphs showing the fossil diatom (cells per 0.1 g sediment) record of RAIL1 (a) and % relative abundance (b). Both depth (cm) and radiometric dates (year) are also presented on the y axis.

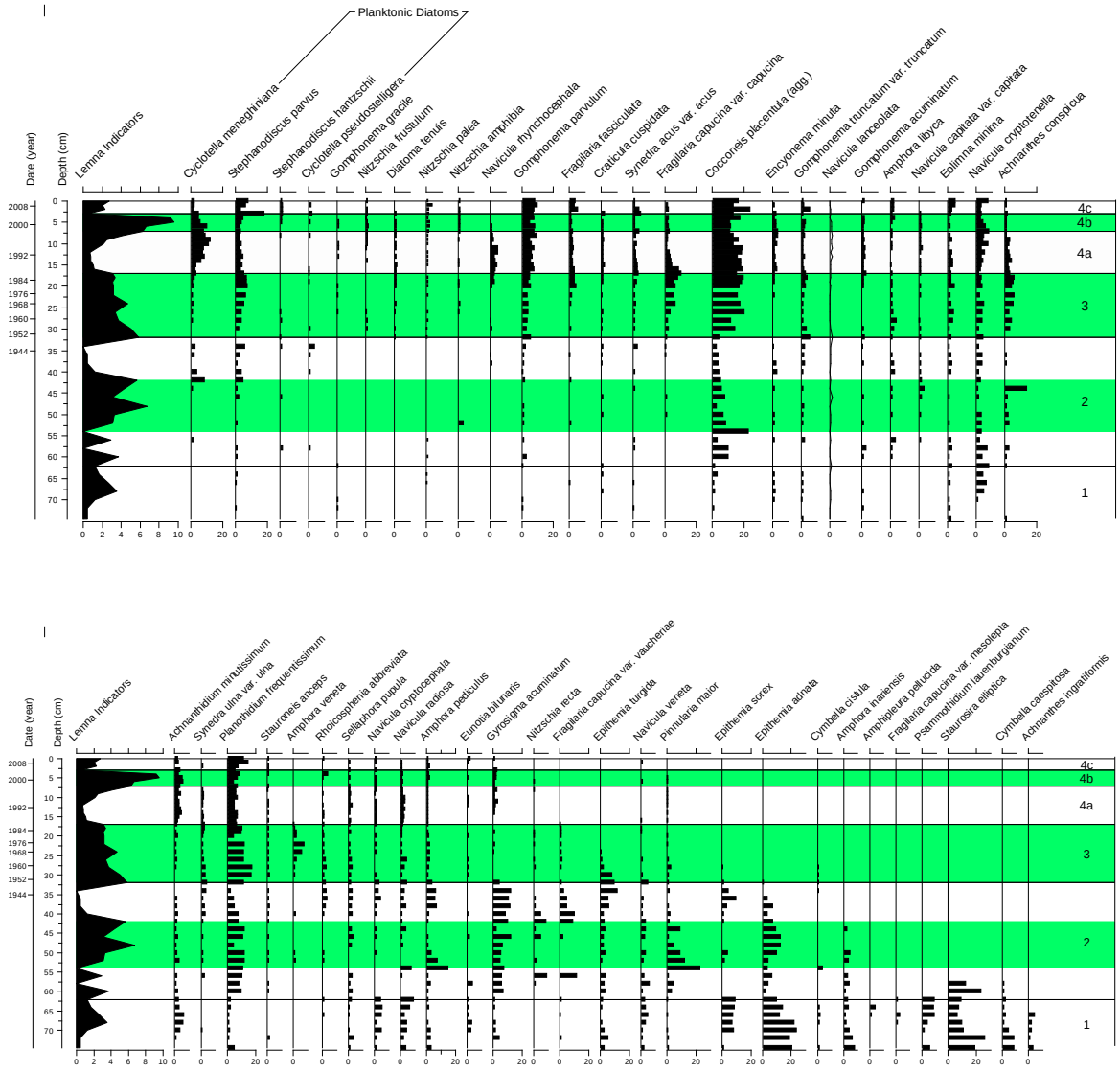


Figure 5.16. Diatom stratigraphy of core RAIL1 expressed as % relative abundances. The individual fossil diatom profiles are presented in order of their chronological zonation. The *Lemna* (duckweed) indicator metric derived from the summation of the *Lemna* epiphytes (*L. hungarica* and *S. seminulum*) is shown at the far left-hand side of the diagrams. The green bands are *Lemna* phases inferred from observations and the *Lemna* indicator metric (Phase 4: upper band) and the *Lemna* indicator metric (Phases 3 & 2: lower bands). Both depth (cm) and radiometric dates (year) are also presented on the y axis.

5.5.6.3 The diatom record of RAIL2

Interestingly, a total of 72 diatom species were recorded from the RAIL2 core compared to just 60 species in RAIL1 core (see Appendix 1 for full species list). The resulting diatom stratigraphy (Figs. 5.17, 5.18) shows the concentrations of the species recorded and suggests two *Lemna* phases from 22-15cm and from 5.5-3cm. There were several 'pioneering' diatom species recorded from the lowermost section of RAIL1, such as *A. ingratiformis, C. tumida, A. pellucida, P. lauenburgianum, F. capucina* var. *mesolepta, C. caespitosa, S. elliptica* and *A. inariensis* that were not recorded in RAIL2. The diatom record has been divided into three major zones based on the cluster analysis, with Zone 3 being divided into two sub-zones.

Zone 1 (21-15cm; c. late 1940s-mid 1960s)

Zone 1 of RAIL2 is characterised as a *Lemna*-dominance phase derived from the *Lemna*-indicator diatoms (*L. hungarica* and *S. seminulum*) which equates to Phase 3 of RAIL1. Together with the *Lemna*-indicator diatoms, Zone 1 was co-dominated by *C. placentula* and *P. frequentissimum*. Several diatom species were found in Zone 1 but also disappeared from the fossil record during this zone, notably *G. acuminatum, P. maior, S. pupula, Gomphonema augur, N. ampliatum* and the three *Epithemia* spp. (*E. sorex, E. turgida* and *E. adnata*). Planktonic taxa were also present, particularly *C. meneghiniana* and *S. parvus*.

Zone 2 (15-5.5cm; late 1960s-mid 1990s)

Zone 2 represents the period between the two *Lemna* phases and was characterised by the loss of many diatom species (e.g. *N. ampliatum, G. augur, E. turgida, E. sorex, Nitzschia constricta*) and concomitant increases in species such as, *N. rhyncocephala, C. cuspidata, F. exigua, F. fasciculata, F. capucina* var. *capucina, N. palea* and *Nitzschia dissipata*. Notably, there were large decreases in the *Lemna*-indicator diatoms. Other species, such as *A. veneta* and *A. conspicua*, recorded their highest

densities during Zone 2 before disappearing with the onset of the second *Lemna* phase. *Cymatopleura solea, Nitzschia hungarica, Suriella minima, Diatoma tenuis* and *Achnanthes coarctica* were only recorded from Zone 2. There were losses seen in the planktonic diatoms *C. pseudostelligera*, *Cyclostephanos invisitatus* and *S. hantzschii*, whereas *C. meneghiniana* persisted and *S. parvus* became the dominant planktonic diatom.

Zone 3 (5.5-0cm; c. 2005-2010)

Zone 3 was divided into two sub-zones based on cluster analysis, with Zone 3a covering the second *Lemna* phase (equivalent to Phase 4 in RAIL1) and Zone 3b the post-*Lemna* phase. Many diatom species disappeared from the fossil diatom record with the onset of Zone 3a such as *Cymbella cistula, Navicula cryptocephala, Nitzschia fonticola, P. gibba* and *P. borealis*. Zone 3b was characterised by a general increase in planktonic diatom abundances (*C. meneghiniana, S. hantzschii* and *S. parvis*) but *C. pseudostelligera* and *C. invisitatus* had disappeared. There were two diatom species recorded for the first time namely, *Synedra biceps* and *Staurosira construens* var. *venter* in Zone 3b. *E. bilunaris* was recorded in low abundances in Zone 3a, but showed signs of recovery after termination of the *Lemna* phase (Zone 3b).

As with RAIL1, the diatom data are presented as absolute diatom counts (Fig. 5.17a) and as percentage relative abundances (Fig. 5.17b, Fig. 5.18) to better show the diatom patterns especially in the rare and common taxa

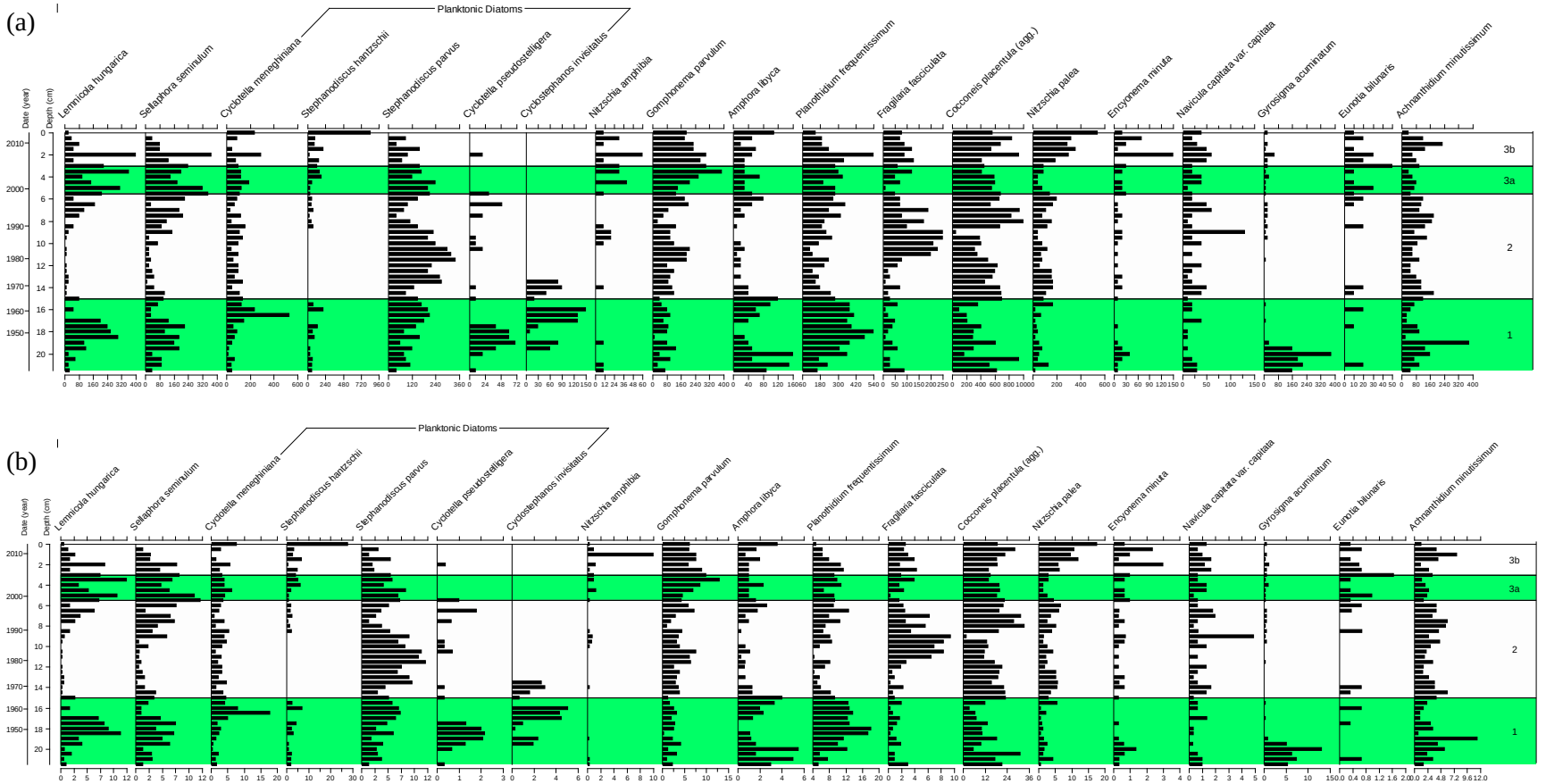


Figure 5.17. Stratigraphs showing the fossil diatom (cells per 0.1 g sediment) record of RAIL2 (a) and % relative abundance (b). The diatoms *Lemnicola hungarica* and *Sellaphora seminulum* associated with *Lemna* are presented on the far left-hand side of the diagrams. The planktonic diatoms *Cyclotella meneghiniana*, *Stephanodiscus hantzschii*, *Stephanodiscus parvus*, *Cyclotella pseudostelligera* and *Cyclostephanos invisitatus* are presented together as a group. The other diatoms are presented in order of their chronologies. The green bands (both diagrams) denote inferred periods of duckweed dominance (uppermost band in upper diagram [a] is based upon recorded observations and the *Lemna* indicator metric [Phase 4]; lower band is based upon the *Lemna* indicator metric [Phase 3]). The zones derived from the diatom data are presented. Both depth (cm) and radiometric dates (year) are presented on the y axis.

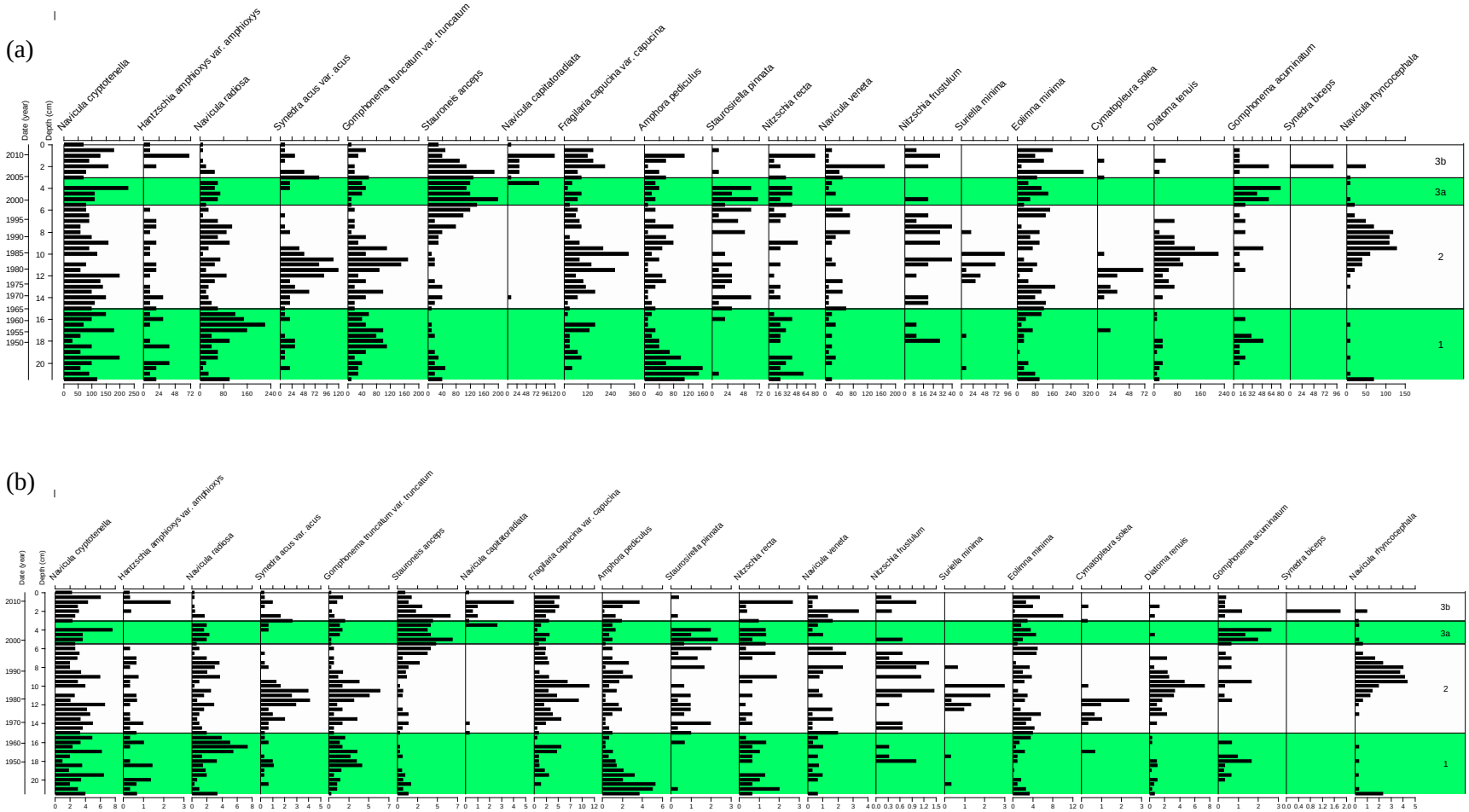


Figure 5.17 cntd. Stratigraph showing the fossil diatom (cells per 0.1 g sediment) record of RAIL2 (a) and % relative abundance (b). The two green bands show the periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and the *Lemna* indicator metric [Phase 4]; lower band is based upon the *Lemna* indicator metric [Phase 3]). The stratigraph also shows the zones which were derived from the diatom data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

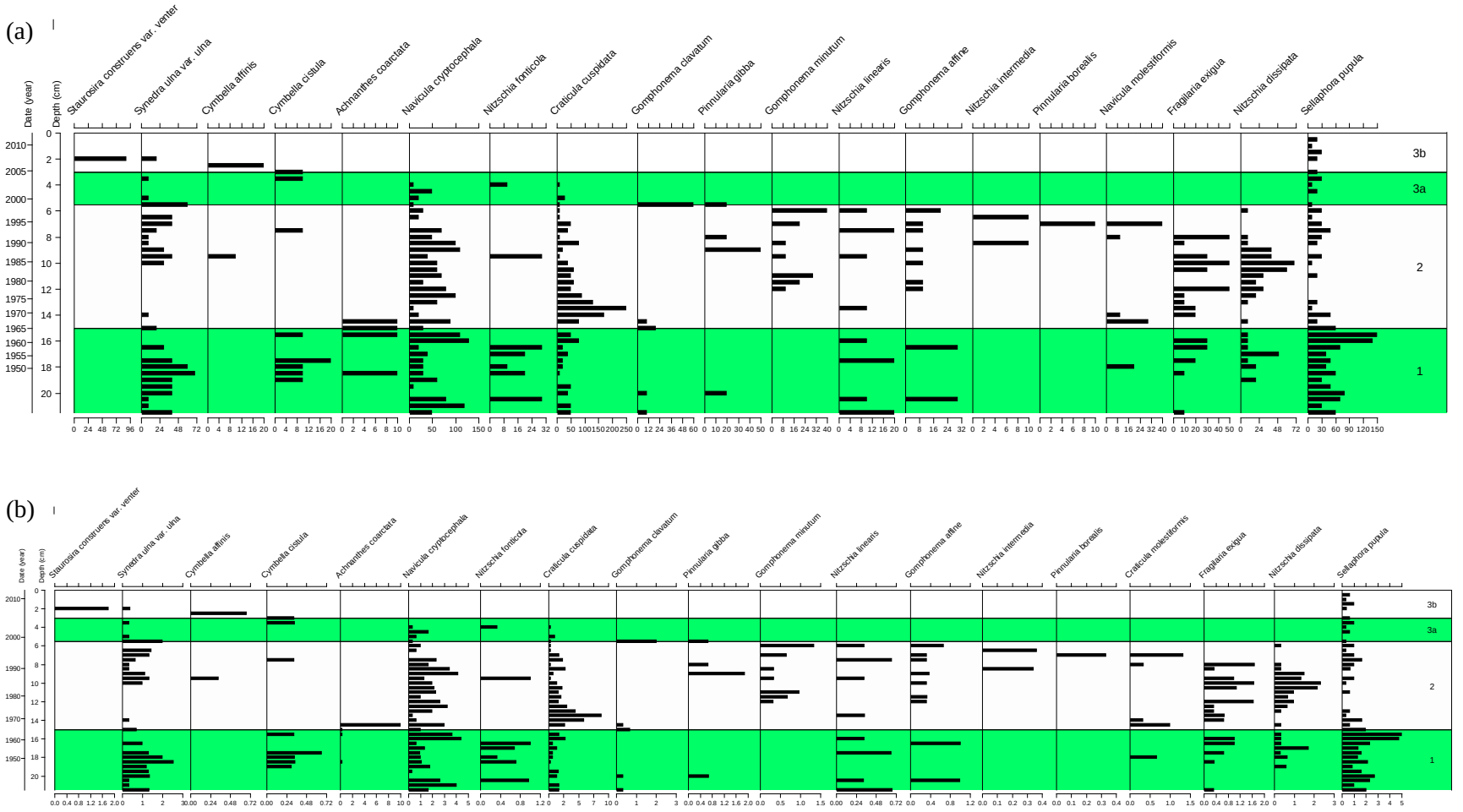


Figure 5.17 cntd. Stratigraph showing the fossil diatom (cells per 0.1 g sediment) record of RAIL2 (a) and % relative abundance (b). The two green bands show the periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and the *Lemna* indicator metric [Phase 4]; lower band is based upon the *Lemna* indicator metric [Phase 3]). The stratigraph also shows the zones which were derived from the diatom data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

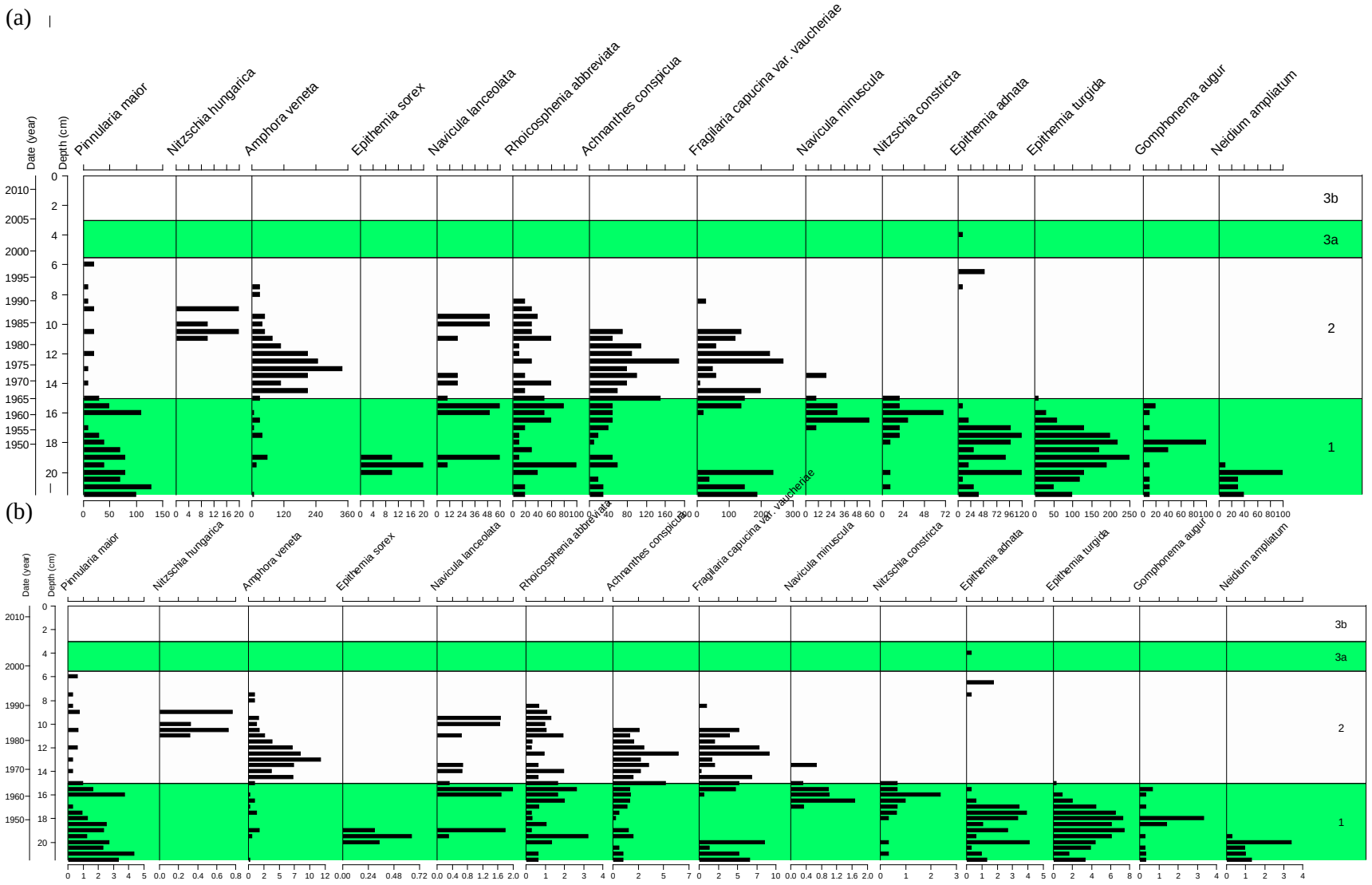


Figure 5.17 cntd. Stratigraph showing the fossil diatom (cells per 0.1 g sediment) record of RAIL2 (a) and % relative abundance (b). See legend above for description.

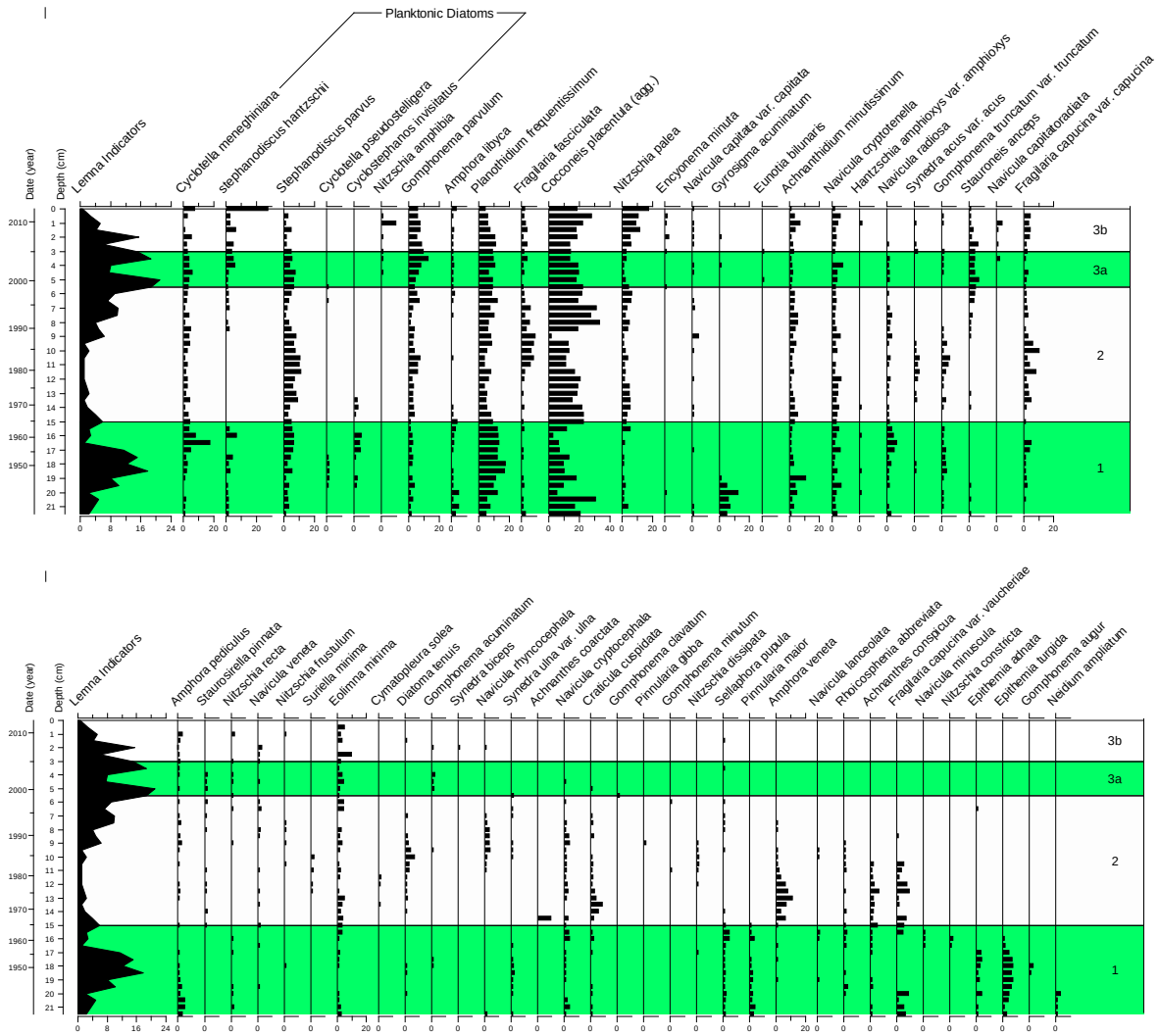


Figure 5.18. Diatom stratigraphy of core RAIL2 expressed as % relative abundance. The individual fossil diatom profiles are presented in order of their chronologies. The *Lemna* (duckweed) indicator metric derived from the summation of the *Lemna* epiphytes (*L. hungarica* and *S. seminulum*) is shown at the far left-hand side of the diagrams. The green bands denote the periods of duckweed (*Lemna*) dominance (uppermost band based upon recorded observation and the *Lemna* indicator metric [Phase 4]; lower band is based upon the *Lemna* indicator metric [Phase 3]). The stratigraph also shows the zones derived from the diatom data. Both depth (cm) and radiometric dates (year) are also presented on the y axis.

5.5.7 A comparison of the diatom records of RAIL1 and RAIL2

There were considerable similarities in the benthic and epiphytic diatom flora assemblages seen in the two cores (Table 5.5) such as the relatively high abundances towards the uppermost sections of *N. palea*, *C. placentula*, *P. frequentissimum*, *G. parvulum*, *N. cryptotenella* and *A. minutissimum* which are species tolerant of heavy organic pollution (Slàdeček 1986, Van Dam *et al.*, 1994). There were also similarities in the planktonic species. In both RAIL1 and RAIL2 cores, *S. parvus* and *C. meneghiniana* persisted through the recent *Lemna* phases with *C. meneghiniana* even showing signs of increasing population densities during both *Lemna* phases. *S. hantzschii* and *C. pseudostelligera* densities were negatively affected by the *Lemna* phases, but, as with the other planktonic diatoms, they appeared to temporarily increase during the 'hiatus' between the two *Lemna* phases (RAIL2, Zone 2). *C. invisitatus* was recorded only from the RAIL2 core and appeared to be negatively correlated with the timing of *L. hungarica* and *S. seminulum* in the core sequence and then completely disappeared from the fossil record before the onset of the recent *Lemna* phases.

The disappearance of *E. turgida* and *E. sorex* in the fossil diatom record was well correlated between the two cores (Figs. 5.15 and 5.17) as these species were seemingly negatively affected by the *Lemna* phase in the c. mid 1960s for *E. turgida* (RAIL1 & RAIL2), and in the c. late 1940s for *E. sorex* (RAIL1 & RAIL2). Although there was a slight disparity in the timing of the disappearance of *E. adnata* from the diatom fossil records between the two cores (i.e. c. early 1940s in RAIL2 and the c. mid 1960s in RAIL1) this could possibly be explained by the inherent margins of error in radiometric dating (Figs. 5.7 & 5.9). It is interesting to note that *A. veneta* was effectively absent from the historical diatom record, but suddenly appeared (28-17cm) as a distinct assemblage at the same time as *L. hungarica*-*S. seminulum* Phase 3. *A. veneta* population also dramatically disappeared at the 17cm level in RAIL1. It was also interesting to note that *C. placentula* appeared to track the presence of both the documented and inferred *Lemna* phases, mirroring the timing of the *L. hungarica*-*S. seminulum* assemblages, which could suggest an indirect *Lemna* influence.

Although RAIL2 represented a shorter time period than RAIL1, there were more diatom species recorded and many of these species had greater concentrations in the former. It is reasonable to assume that the higher species diversity and species concentrations reflects i) the finer resolution of the 0.5cm core slices, ii) an increase in primary production as a direct response to increased eutrophication, and iii) the possible and likely spatial heterogeneity ('patchiness') within the Rail Pit. The increase in primary productivity is shown by the lithostratigraphic analysis, where there was an increase in the organic content of the sediment (%LOI). The organic content increased from 26% to over 40% after the first *Lemna* phase (Zone 1) and increased from 32% to 40% (Zone 3a) after the second *Lemna* phase (Zone 3a). These increases are also broadly seen in the organic content (%LOI) in RAIL 1 (Fig. 5.11a).

The timing of the two most recent phases of *Lemna* dominance indicated by CONISS for both RAIL1 and RAIL2 cores was generally in good agreement with historical observations (Table 5.2). The first observed *Lemna* phase of 1986 to early 1990s was, however, not clearly defined from CONISS zonation or the radiometric dating between the two cores. The second observed *Lemna* phase of 1999-2005 was indeed indicated by CONISS zonation and was in good agreement with the radiometric dating for both RAIL1 and RAIL2 cores.

Species change	RAIL1	RAIL2
Gyrosigma acuminatum decline	c. late 1940s (*) (depth = 32 cm)	c. late 1940s (*) (depth = 19 cm)
Epithemia turgida decline	c. early 1960s (*) (depth = 26 cm)	c. late 1950s (*) (depth = 17 cm)
Amphora veneta peak	c. early 1960s-early 1980s (depth = 26-17 cm)	c. mid 1960s-early 1980s (depth = 15-10 cm)
Pinnularia maior decline	c. early 1960s (*) (depth = 26 cm)	mid 1960s (*) (depth = 15 cm)
Navicula rhyncocephala peak	c. mid 1980s-late 1990s (depth = 19-7 cm)	c. early 1980s-mid 1990s (depth = 14-7 cm)
Fragilaria capucina var. capucina decline	c. early 1950s (*) (depth = 32 cm)	c. early 1980s (*) (depth = 11 cm)
Lemnicola hungarica & *Sellaphora seminulum* increase	c. early 1990s (*) (depth = 10 cm)	c. early 1990s (*) (depth = 8 cm)
Lemnicola hungarica & *Sellaphora seminulum* decline	c. 2005 (depth = 3 cm)	c. 2006 (depth = 3 cm)

Table 5.5. Comparison of the correlations of selected diatom species changes between cores RAIL1 and RAIL2. (*) denotes decline in diatom species abundances coincident with *Lemna*-dominance phases.

5.5.7.1 Diatom chronological responses

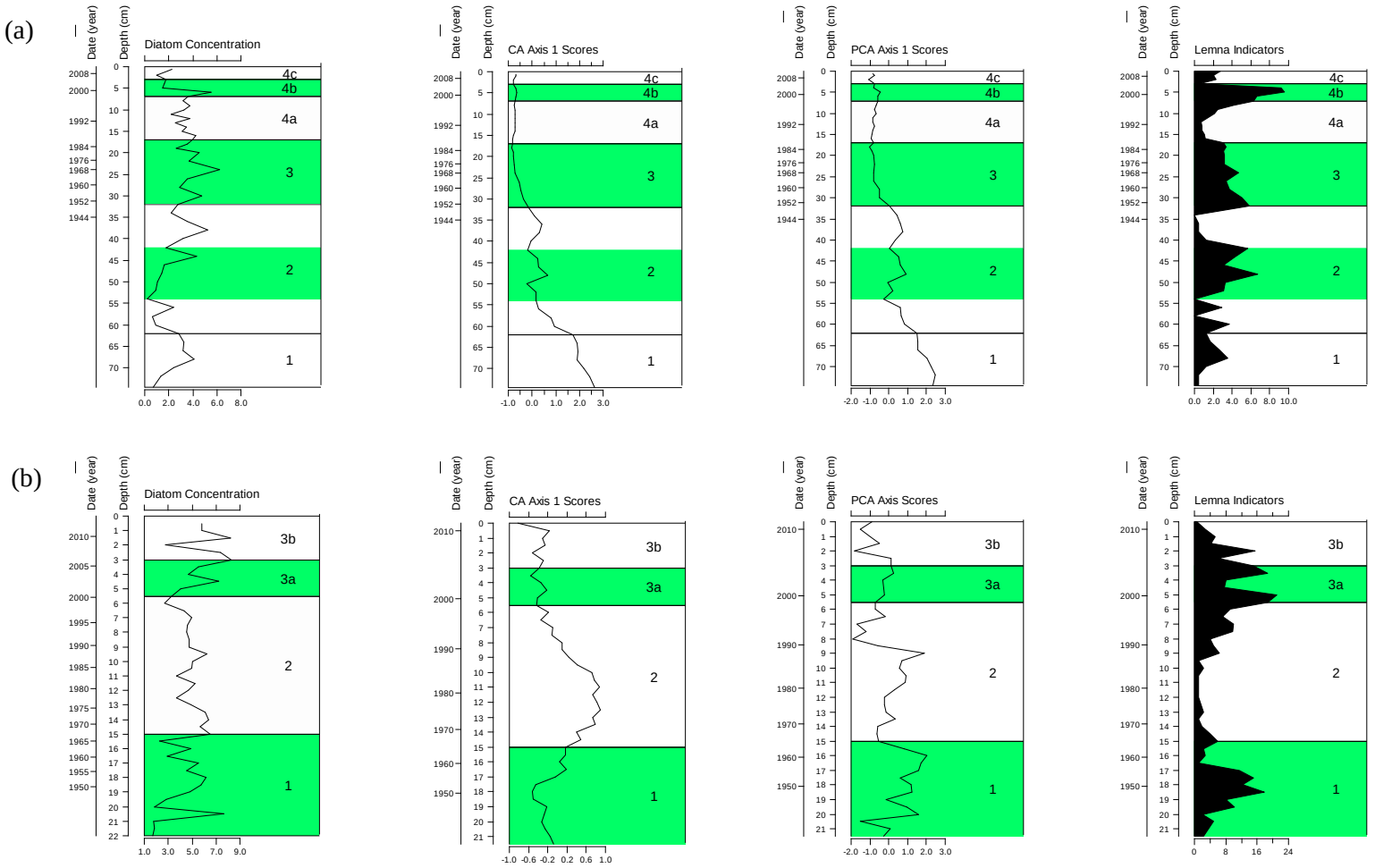


Figure 5.19. Summary diagram of the diatom responses for cores RAIL1 (a) and RAIL2 (b). L-R: diatom concentrations showing the total number of diatoms per sample estimated from the microsphere method (x 10^6 cells g wet wt^{-1}); CA axis 1 sample scores; PCA axis 1 sample scores summarising the species compositional and ecological changes and *Lemna* indicator metric (summation of *L. hungarica* and *S. seminulum*). Zonation (based upon the cluster analysis of all of the diatom assemblages) is shown. Shaded areas/zones indicate periods of *Lemna* dominance zones inferred from the *Lemna* indicator metric.

Figure 5.19 shows that the diatom concentrations fluctuated throughout the core profiles ranging from 1-6 $x10^6$ cells g wet wt^{-1} for RAIL1 and 1.5-8 $x10^6$ cells g wet wt^{-1} for RAIL2. Both the CA and PCA axis 1 scores for RAIL1 are strikingly similar with scores of + 2.5 at the base of the core but gradually decrease to – 0.5 at the top of the core. The axis 1 scores abruptly increased during *Lemna* dominance Phase 2 (i.e. 54-42cm), and again between *Lemna* dominance Phases 2 and 3 (i.e. 42-32cm). These changes in the CA and PCA axis 1 sample scores were mirrored in core RAIL2.

In RAIL1 there was a rapid increase in diatom concentrations and diatom diversity (Zone 1) followed by a decrease at 62cm (Fig. 5.19), which coincided with a rapid decrease in carbonate (see Fig. 5.11 above). After a second rapid increase in concentration and axis 1 scores there follows a sudden and drastic decrease at 54cm (2.4×10^6 cells 0.1g wet wt^{-1} to $<0.2 \times 10^6$ cells 0.1g wet wt^{-1}). Thereafter follows another increase in diatom concentration and compositional change (Zone 2) which is characterised by oscillations in diatom concentrations (Fig. 5.19a). This trend continued until the c. early 1950s. Zone 3 marks the beginning of *Lemna* dominance Phase 3 which sees a rapid and distinct reduction in diatom composition on the one hand, but on the other sees the largest increase in diatom concentrations (6.2×10^6 cells 0.1g wet wt^{-1}). This reflects the appearance of the *Lemna* epiphytes *L. hungarica* and *S. seminulum* in the post war years. Conversely, after termination of this *Lemna* phase, Zone 4a sees an increase in diatom community composition with the expansion of planktonic and benthic species, even though there was a decrease in overall diatom concentrations. The patterns seen during *Lemna* dominance Phase 3 are mirrored in *Lemna* dominance Phase 4 (Zone 4b) in the late 1990s, where concentrations and species composition are abruptly reduced. Immediately after the cessation of this *Lemna* phase both the concentrations and diatom axis 1 scores begin to increase once more.

This pattern of diatom species compositional and ecological change reflects i) the establishment of benthic and epipelic communities in the early history of the Rail Pit then ii) a marked reduction in these communities followed by iii) an increase in epiphytic communities and the *Lemna* indicator diatoms *L. hungarica* and *S. seminulum*

in particular, iv) the appearance of planktonic communities, most notably between the *Lemna* phases and v) the development of co-dominance of benthic, epiphytic and planktonic communities as a response to increasing eutrophication and dominant *Lemna* phases.

5.5.8 Exploratory data analysis (RAIL1)

5.5.8.1 Ordination analyses of changes in community composition

The gradient lengths of DCA axis 1 and 2 were 3.935 SD and 3.389 SD respectively, and both axes 3 and 4 were also similar in length (3.915 and 3.547 respectively). As the axes lengths were all approaching 4.0 SD the use of both unimodal and linear methods could be considered appropriate (Lepš & Šmilauer 2003). However, it was decided to initially employ unimodal methods as it was assumed that the diatom species data were heterogeneous and symmetrical around the species optimum. An indirect unimodal ordination analysis of the diatom data using correspondence analysis (CA) was performed on diatom species and samples (Fig 5.20). The first two axes explained most of the variance with 30.8% and 43.1% explained by axis 1 and 2, respectively, which was similar to that of the DCA (35.2% and 42.9%).

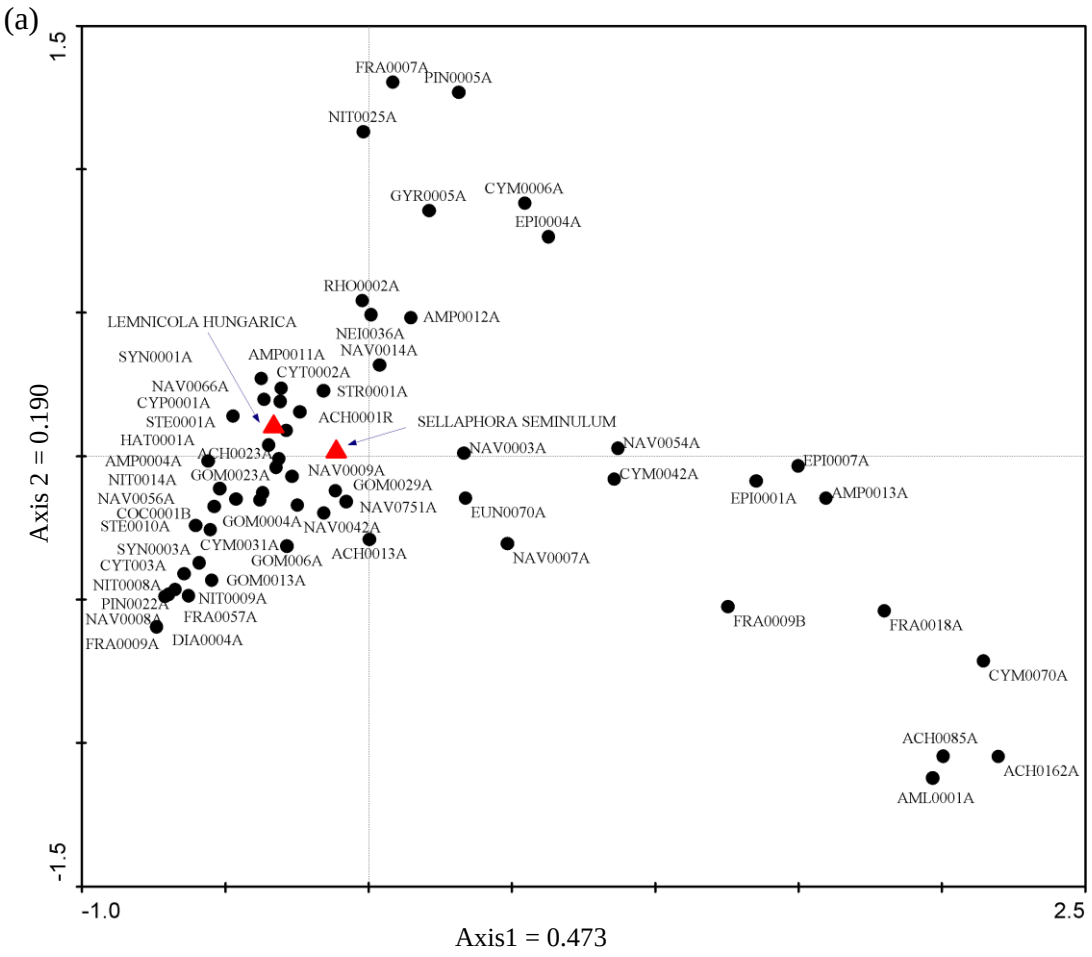


Figure 5.20a. CA plot on axes 1 and 2 for RAIL1 of the species scores of the 60 diatom taxa recorded. The epiphytic diatoms, *Lemnicola hungarica* and *Sellaphora seminulum*, are highlighted. (See Appendix 1 for diatom codes).

The scatter of the samples in the CA biplot (Fig. 5.20b) clearly reveals the so-called 'arch effect' of the sample positions on the first two axes. The positions of the samples on the second (vertical) axis were strongly dependent on their positions on the first (horizontal) axis. This 'arch' effect can be interpreted as a limitation of the method as the consecutive axes are made mutually independent as only linear independence is sought, or the effect can be a consequence of the projection of the non-linear relations of the response variables to the underlying gradients into a linear Euclidian drawing space (Legendre & Legendre 1998).

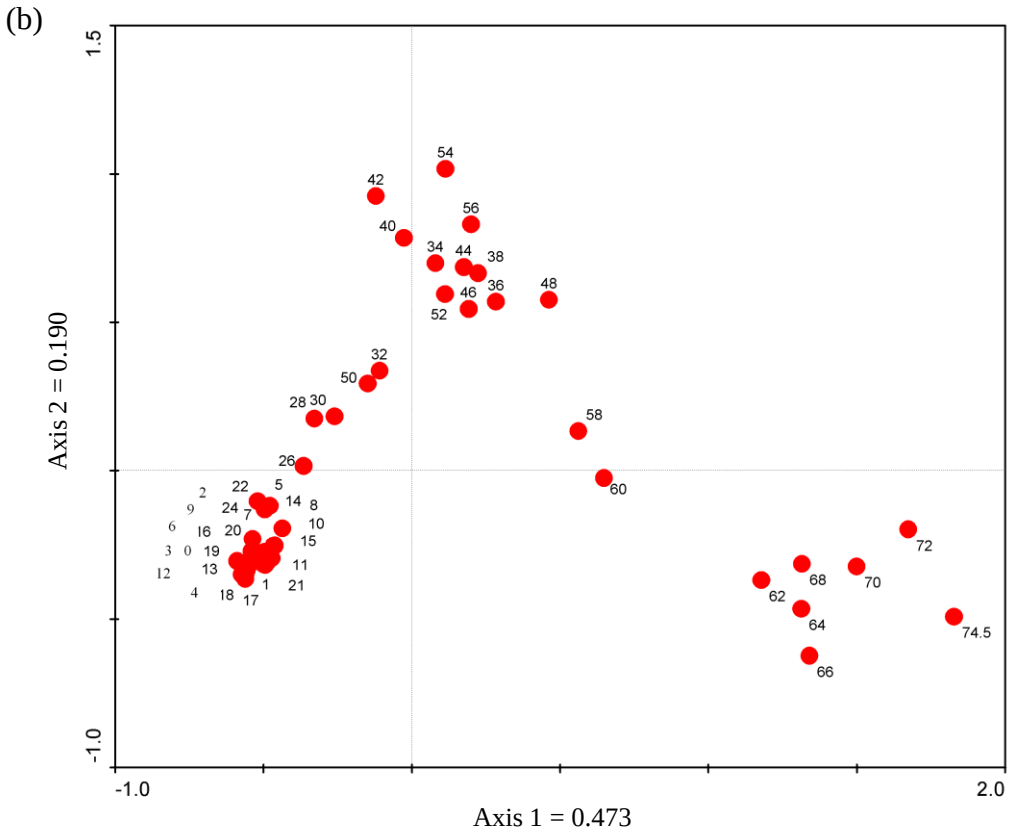
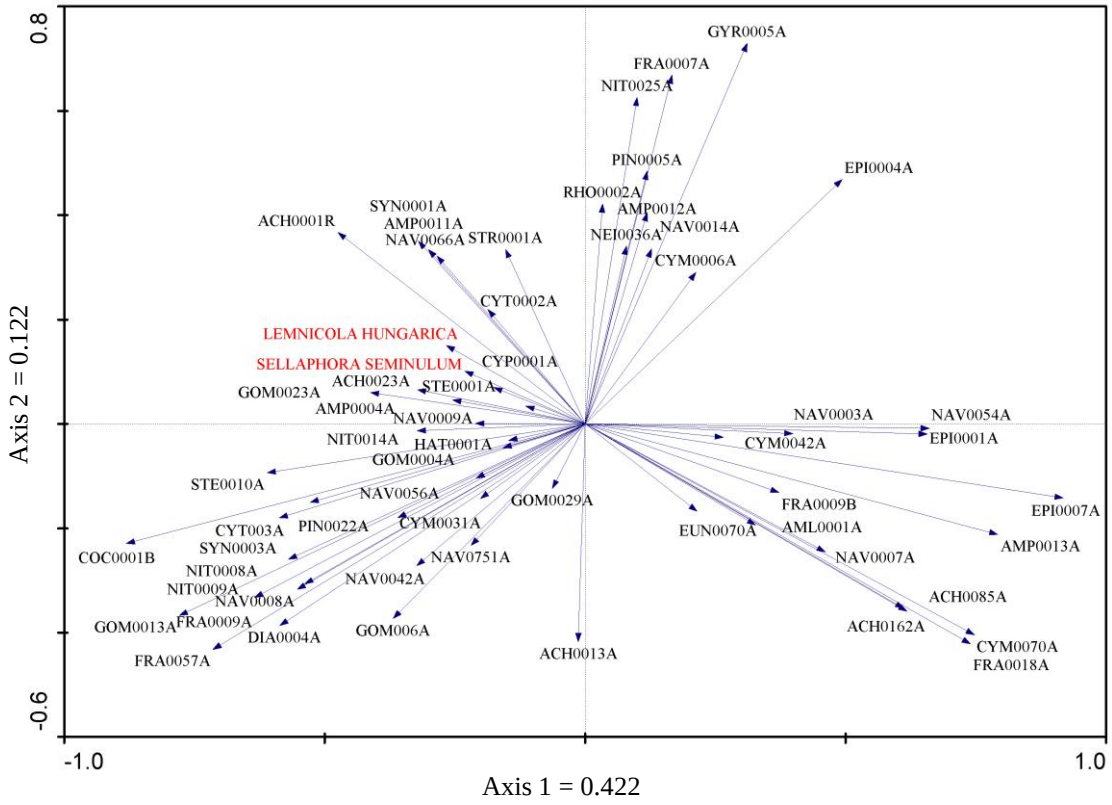


Figure 5.20b. CA plot on axes 1 and 2 for RAIL1of the sample scores for the 48 samples analysed. The sample numbers denote the sample depths in the core.

Although detrending by segments (Hill & Gauch 1980) is usually employed for making the recovered compositional gradient straight or linear (Lepš & Šmilauer 2003), the method lacks a convincing theoretical basis and is even considered as being inappropriate by some authors (Knox 1989, Wartenberg *et al.*, 1987). Therefore, as the gradient lengths given by the DCA indicate that both types of ordination methods could be employed (Lepš & Šmilauer 2003) it was decided to use the linear ordination method, principle components analysis (PCA), to explore the compositional changes (Fig. 5.21). PCA axes 1 and 2 explained over 54% of the total species variation and furthermore, demonstrated that the species recorded from the more recent history (e.g. *P. subcapitata*, *N. frustulum*, *N. palea* and *D. tenuis*) were correlated with axis 2 (vertical axis) whereas the early 'pioneer' species were correlated with axis 1 (horizontal axis). The ordination demonstrates the high correlation of the two identified *Lemna* epiphytes, *L. hungarica* and *S. seminulum* (Figs. 5.20a, 5.21a).

(a)



(b)

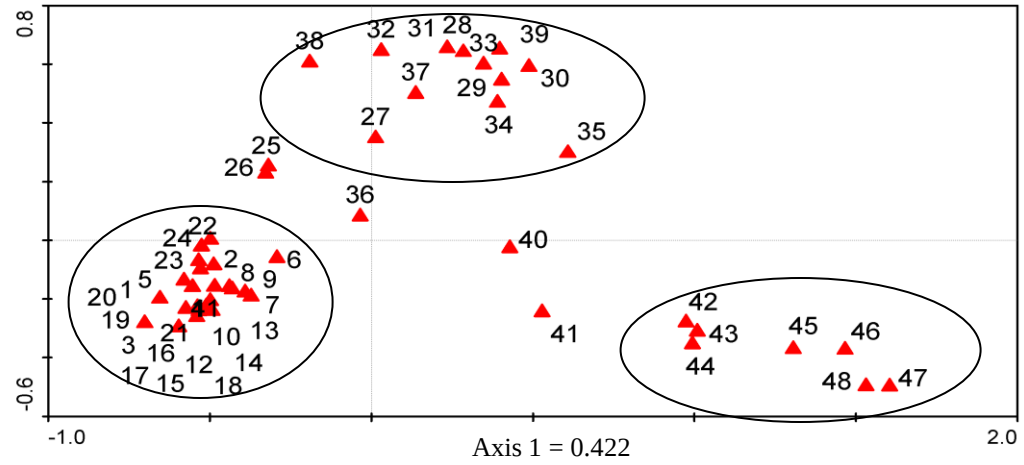


Figure 5.21. PCA plot on axes 1 and 2 for RAIL1 of (a) the species scores of the 60 diatom taxa recorded and (b) the sample scores for the 48 samples analysed. The sample numbers in (b) denote the sample depths in the core. The epiphytic diatoms, *Lemnicola hungarica* and *Sellaphora seminulum*, are highlighted in (a). Circles denote approximations of sample groups. (See Appendix 1 for diatom codes).

The species biplots produced by both CA and PCA show that there was considerable species turnover (Beta diversity) throughout the history of the Rail Pit (Figs. 5.20, 5.21). There appears to be at least three relatively distinct 'areas' within the ordination space that are characterised by particular suites of diatom communities with no obvious outlier samples (Fig. 5.21b). There is a distinct suite of species from the oldest samples (bottom R/H quadrant) such as *A. pellucida*, *P. lauenburgianum*, *A. ingratiformis*, *A. inariensis*, *C. caespitosa*, *E. sorex*, *E. adnata* and *S. elliptica*. It was noticeable that these 'pioneer' species were all recorded from samples from around 60cm down to the base of the core (75cm). This corresponds well with Zone 1 in the diatom stratigraphic zonation (Fig. 5.15) and is characterised by a sudden drop in carbonate (i.e. from over 70% to less than 20%) at the 62-60cm level. Positioned between these 'pioneer' (Zone 1) and the more recent diatom communities (Zones 3 & 4) was a suite of 'intermediate' diatoms namely *F. capucina* var. *vaucheriae*, *E. turgida*, *G. acuminatum*, *N. recta* and *P. maior* which were most abundant in Zone 2. The 'intermediate' suite of diatoms is positioned in the upper quadrants. These three distinct communities in the RAIL1 core were clearly visible in the PCA biplot (Fig. 5.21a). The 'intermediate suite' of species (upper quadrants) correlate well with the middle section of the core and are characterised by *F. capucina* var. *vaucheriae*, *E. turgida*, *C. cistula*, *G. acuminatum*, *N. recta* and *P. maior*. The third suite of species (lower L/H quadrants) is the largest single 'group' and comprises species that are normally associated with more eutrophic conditions and includes the *Lemna* epiphytes, *L. hungarica* and *S. seminulum*, which are found in close proximity within the ordination space (Figs. 5.20a & 5.21a). It is interesting to note that this recent period coincides with the dominance of *Lemna* mats at the Rail Pit (Table 5.2 and Figs. 5.15 & 5.17). Figure 5.21b shows the approximations of the three groups of samples.

5.5.9 Exploratory data analysis (RAIL2)

5.5.9.1 Ordination analyses of changes in community composition

As with RAIL1 an initial exploratory DCA was performed on the RAIL2 diatom data to establish whether the diatom species responses were linear or unimodal. The gradient lengths of axis 1 and 2 were remarkably similar at 1.606 SD and 1.605 SD respectively, and both axes 3 and 4 were also similar in length at 1.372 and 1.014 respectively. As the axes lengths were all just over 1.0 SD the use of linear methods was considered appropriate to explore the diatom data. The eigenvalues of the first four DCA axes explain over a third of the variability in the species data, with axis 1 and 2 explaining most (i.e. around 30%) of the cumulative species variation.

As for RAIL1, the linear unconstrained method of PCA was employed to explore patterns in the species dataset. The summary statistics of the PCA of the RAIL2 diatom data show that the first two axes explain over 44% of the variance of the species data. The main patterns in the diatom data are similar to the PCA of RAIL1. For example, *L. hungarica* and *S. seminulum* are correlated with axis 2 and are within close proximity in ordination space together with *P. frequentissimum*, whereas the more ubiquitous *C. placentula* was clearly correlated with axis 1. Interestingly, *E. bilunaris* was found in close proximity to *L. hungarica* and *S. seminulum* in the PCA of RAIL2 but this pattern was not seen in the PCA of RAIL1. The PCA biplots of axis 1 and 2 are presented in Figure 5.22. However, unlike RAIL1, there were no distinct groups of samples seen in the PCA biplot of RAIL2 and, furthermore, there was an obvious outlier sample (upper L/H quadrant) seen in the RAIL2 biplot (Fig. 5.22b).

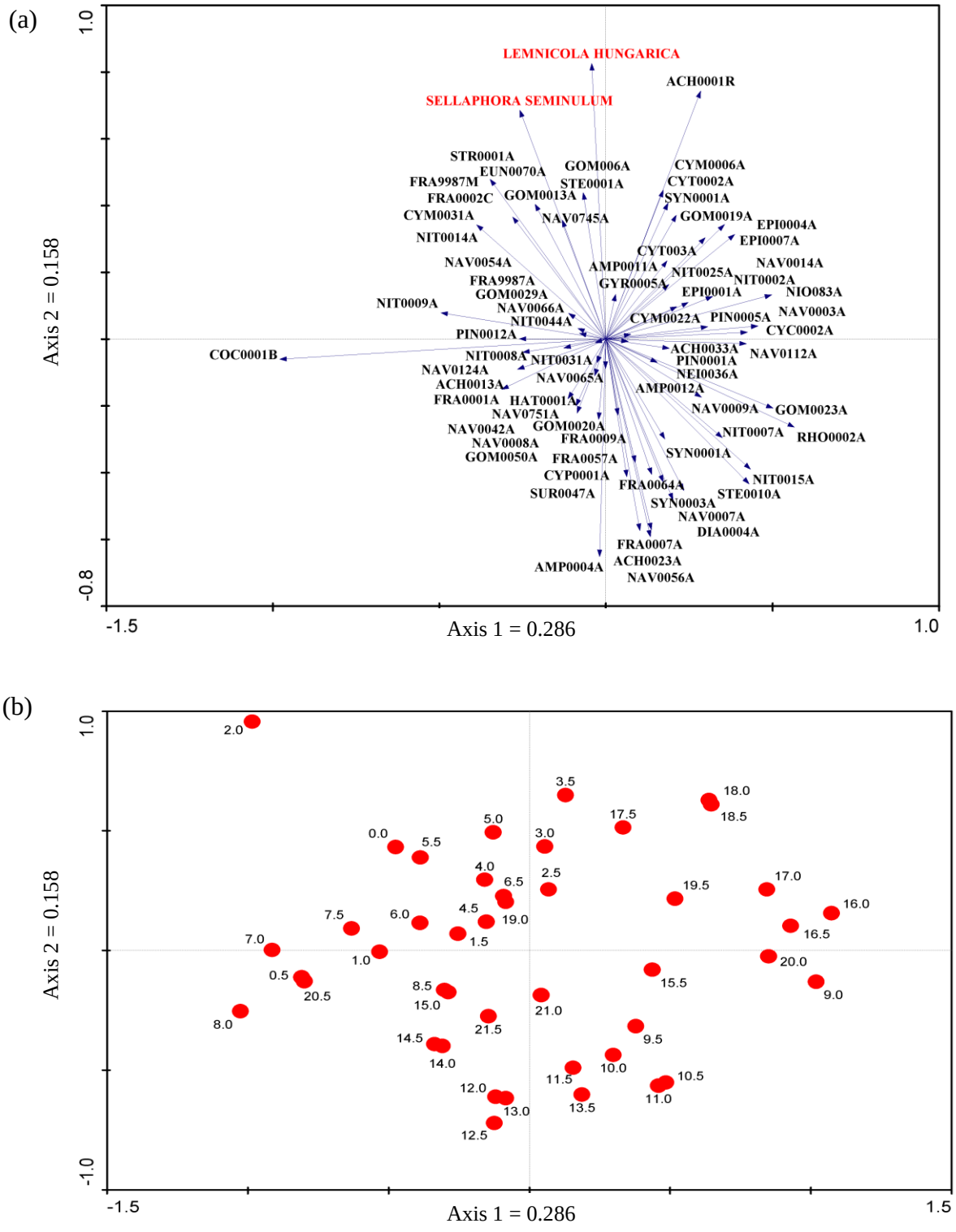


Figure 5.22. PCA plot on axes 1 and 2 for RAIL2 of (a) the species scores of the 72 diatom taxa recorded and (b) the sample scores for the 44 samples analysed. The sample numbers in (b) denote the sample depths in the core. The epiphytic diatoms, *Lemnicola hungarica* and *Sellaphora seminulum*, are highlighted in (a). (See Appendix 1 for diatom codes).

A redundancy analysis (RDA) was performed on the species data from RAIL2 and was constrained by the '*Lemna*' and 'No-*Lemna*' dummy environmental variables. The summary statistics of the RDA of all the diatom species and samples show that axes 1 and 2 explain over 36% of the variance of the species data. The resulting biplot (Fig 5.23) displays the main pattern in the correlation coefficients between the response variables and the '*Lemna*' and 'No-*Lemna*' dummy environmental predictor variables. The centroid scores (triangles) for the dummy variables represent the average scores of species belonging to that particular class.

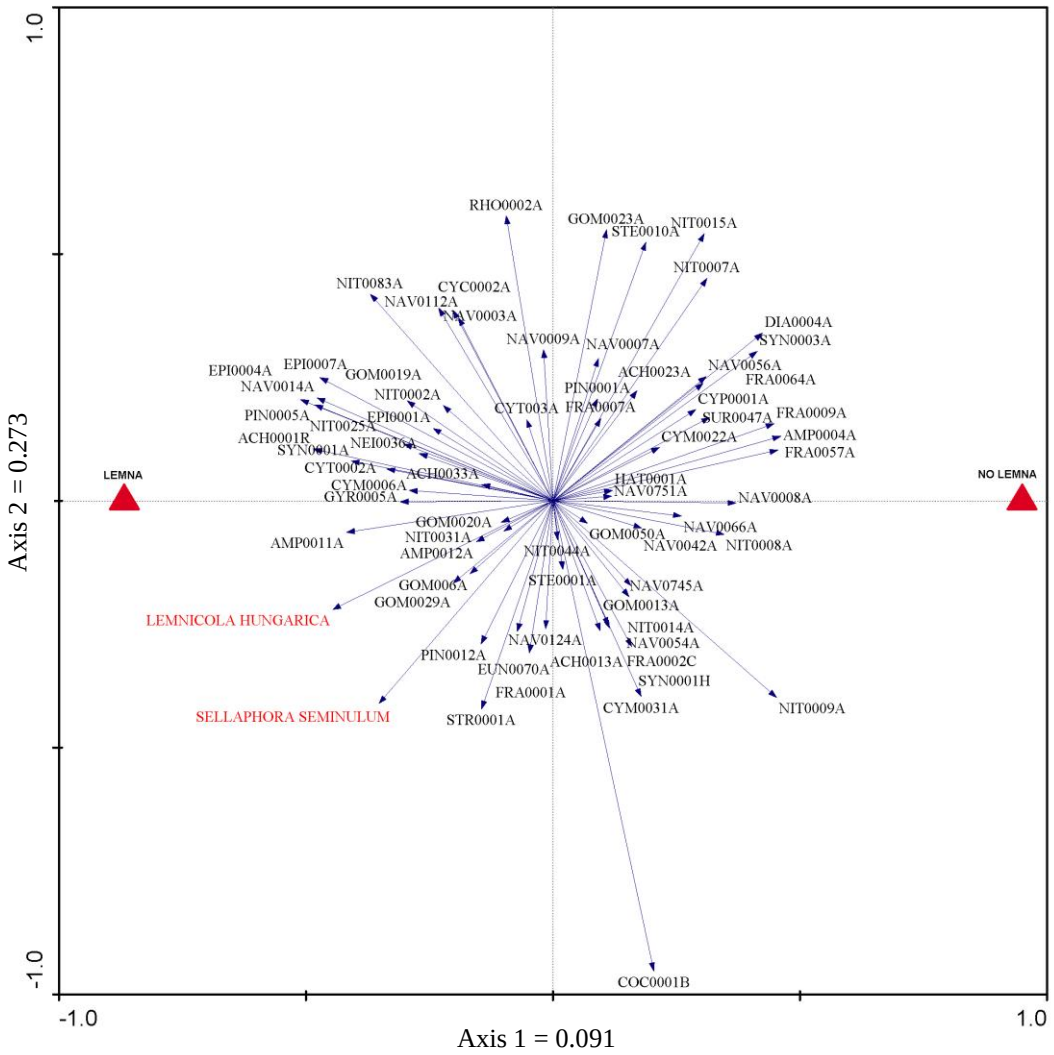


Figure 5.23. RDA biplot of the 72 diatom taxa recorded from the sediment samples on axes 1 and 2 for RAIL2. Diatom species, the environmental variables *Lemna* and No-*Lemna* i.e. the 'dummy' environmental variables (centroids) are also shown (triangles). The epiphytic diatoms, *Lemnicola hungarica* and *Sellaphora seminulum*, are highlighted. (See Appendix 1 for diatom codes).

The relationship of all diatom species recorded from RAIL2 with the '*Lemna'* and 'No-*Lemna'* environmental variables was statistically tested using a Monte Carlo permutation test. There was found to be a high statistical significance between the response of the diatom communities and the two dummy environmental predictor variables (r=0.749, F-ratio=4.187, p=0.002). There was close proximity of the diatom species scores for *L. hungarica* and *S. seminulum* (highlighted in Fig. 5.23) with the quantitative environmental variable centroid (*Lemna* dominance). The ordination biplot shows that *C. placentula* was 'intermediate' between the two *Lemna* and non-*Lemna* environmental predictor variables, demonstrating the ecological 'generalist' nature of this particular diatom. However, other species such as *N. hungarica*, *A. veneta*, *D. tenuis* and *S. acus* var. *acus* showed an association with the No-*Lemna* dummy environmental variable (Fig. 5.23).

5.6 Discussion

5.6.1 Core chronologies and correlation

The high percentage of carbonate and concomitant low organic matter at the base of the RAIL1 core strongly suggests that the whole sediment sequence was collected covering the entire history of the Rail Pit (Fig. 5.5). As the upper 20cm of the RAIL1 core was highly flocculant upon collection, a second complimentary core (RAIL2) was collected specifically to record the most recent history as accurately as possible and this appears to have been achieved (Fig. 5.5). The use of more than one core reduces the bias caused by between-core variability in diatom accumulation rates, and although more time consuming, can provide supplementary information not offered by traditional approaches (Anderson 1989).

The radiometric dating of the RAIL1 and RAIL2 core sequences was not possible using the CIC models as there was an irregular decline in the unsupported ^{210}Pb activities resulting in a non-monotonic feature in the ^{210}Pb profiles. However, the chronology over the last 70 years at least appears to be reliable (RAIL1) showing a small but

significant rise in sediment accumulation rates over this time frame, except for the notable increase at the 34.5cm level (c. 1949). This increase could be the result of a 'drying out' and a consequent slumping event, as the lithostratigraphic analysis (i.e. %dry weight, %LOI, %carbonate) does not seem to indicate a change in autochthonous primary production at this point even though there were marked changes in the lithostratigraphy throughout the core (Figs. 5.11a & 5.12a). Sediment slumping is a well known phenomenon in deep lakes that exhibit steep slopes in their sediment profiles (e.g. Dong, 2010) but little is known about sediment slumping in shallow ponds and, therefore, more work is needed to explore this potential phenomenon as sediment slumping can be problematic in palaeolimnological studies. Although there were notable differences in the sediment accumulation rates between the two cores, suggesting spatial patchiness of sediment accumulation (Tables 5.3 & 5.4), the sedimentation data (and the continuous presence of fish, see Chapter 6) together with observations indicate that, even though the Rail Pit experienced a 'drying out' event covering large areas of the Rail Pit during the extremely hot and dry summer of 1976 and in the early 1990s, it did not completely 'dry up'. Therefore, it is unlikely that the Rail Pit experienced episodes of completely 'drying out' and it is unlikely that the Rail Pit experienced sediment slumping in the past.

5.6.2 Tracking environmental change using diatoms

5.6.2.1 Interpretation of the Rail Pit diatom record

The Rail Pit is a small shallow water body with an extensive littoral zone relative to the pelagic zone thereby providing a range of habitats that are more conducive to benthic algal growth and diversity than to plankton (Wetzel 1983, 2001). However, phytoplankton species were recorded from both RAIL1 and RAIL2 cores, namely *C. meneghiniana*, *C. pseudostelligera*, *S. parvus*, *S. hantzschii* (RAIL1 and RAIL2) and *C. invisitatus* (RAIL2).

As the origins of the Rail Pit were founded from the extraction of marl (thereby creating a marl pit) it was expected that the 'pioneering' diatom species found in Zone 1 at the base of RAIL1 would consist of alkaliphilous species, such as *S. elliptica, F. capucina* var. *mesolepta, Cymbella* and *Epithemia* species (Battarbee *et al.,* 2012). However, also found within this diatom community were, unpredictably, the acidophilous diatom *E. bilunaris*. It is reasonable to assume that filamentous algae were also present at this time as *E. bilunaris* (Hindák & Hindáková 2003), together with the alkaliphilous *Epithemia,* are frequently epiphytes of filamentous algae (Power *et al*., 2009). The presence of aerophilous diatom taxa, such as *A. inariensis*, *N. ampliatum*, *Encyonema minuta* and *H. amphioxys* var. *amphioxys* together with the *Epithemia* species, which can have N-fixing blue-green endosymbionts (DeYoe *et al.,* 1992) within the community of 'pioneer' species (Zone 1) suggests that the early Rail Pit could have patches exposed at the surface, that filamentous algae were likely to have been present and it was likely to be nutrient poor, particularly with respect to nitrogen.

Zone 2 of RAIL1 incorporates the first *Lemna* dominance phase (i.e. Phase 2) and saw extinctions of 'pioneering' species such as *E. adnata* and *E. sorex*. Other diatom species such as *N. radiosa, N. cryptotenella, A. minutissimum, Sellaphora pupula, Encyonema minuta* and *A. pediculus* also declined during this era. However, after the termination of the *Lemna* phase these species underwent a relatively sudden increase and largely persisted through the sediment record. Moreover, the ending of this first *Lemna* dominance phase (Phase 2) witnessed a relative explosion of the numbers and densities of diatom species, namely *Planothidium frequentissimum, G. parvulum, Amphora libyca, G. truncatum* var. *truncatum*, *Eolimna minima*, *Craticula cuspidata* and *R. abbreviata* (Fig. 5.15). This rapid increase, particularly at the latter part of Zone 2, is likely reflecting the onset of eutrophication and was further explored with pigment and macrofossil analyses (see Chapter 6).

The dominance of the second *Lemna* dominance phase (i.e. Phase 3) for the most part constitutes and defines Zone 3 in core RAIL1. As with the first *Lemna* dominance phase (Phase 2) there were changes in the diatom communities. For example, after a

relatively prolonged appearance in the fossil record *P. maior* completely disappeared with the onset of the second *Lemna* dominance phase (Phase 3). Conversely, *Pinnularia subcapitata* was absent from the fossil record until an abrupt appearance and persistence during *Lemna* Phase 3. A similar pattern of appearance and abundance to *P. subcapitata* was seen with *A. conspicua, Diatoma tenuis, G. acuminatum, N. palea, F. capucina* var. *capucina, F. fasciculata, Stauroneis anceps* and *Hantzschia amphioxys* var. *amphioxys*. *G. acuminatum, N. lanceolata* and *N. ampliatum*. Zone 3 saw large increases in the epiphytic diatoms *C. placentula* and *G. parvulum* and persisted into Zone 4. It is likely that these taxa colonised the *Lemna* mats (see Chapter 3) but were also likely colonising the submerged macrophyte *Ceratophyllum submersum*, which first appeared in the fossil record at precisely this time (see Chapter 6).

Zone 4 is comprised of three sub-zones where the third and final *Lemna* dominance phase (Phase 4, Zone 4b) is 'sandwiched' between the two non-*Lemna* zones (Zones 4a and 4c). This zone saw a sudden increase in the abundances of the planktonic diatoms before (Zone 4a) and after (Zone 4c) the *Lemna* dominance Phase 4. A similar pattern was also seen with several benthic diatom taxa such as *N. rhynchocephala, F. fasciculata, S. acus* var. *acus, A. libyca* and *F. capucina* var. *capucina*. These species are all indicative of highly eutrophic conditions and provide evidence of nutrient enrichment during the recent history of the Rail Pit (c. late 1940s/early 1950s to present). *S. parvus, S. hantzschii* and *C. meneghiniana* increased in abundance during and after the *Lemna* dominance phases, but *C. invisitatus* and *C. pseudostelligera* declined after the first *Lemna* dominance phase (Phase 2). The pattern seen in the changes in the diatom communities in the most recent history of the Rail Pit are likely to be driven by increased nutrient loading from the adjacent arable fields as they coincide with the timing of the intensive use of agricultural fertilizers in lowland Great Britain from the 1940s (Robinson & Sutherland, 2002).

5.6.2.2 The relationship between *Lemna* and *L. hungarica* and *S. seminulum*

One of the key aims of the sedimentary diatom analysis was to investigate further the relationship between *Lemna* and the two *Lemna* epiphytes, *L. hungarica* and *S. seminulum*. This palaeoecological investigation is complimentary to the analyses carried out in earlier investigations (see Chapters 3 & 4 above) in assessing the strength of this epiphytic association and ultimately to determine whether these particular diatoms can be utilised as biological proxies to model past trends of *Lemna* abundances (e.g. boom-bust *Lemna* cycles). The regression analysis (Fig. 5.13) showed a significant positive linear relationship between the two *Lemna* epiphytes despite the potential impacts of various biological, chemical and taphonomic processes. These results clearly corroborate the idea that *L. hungarica* and *S. seminulum* both provide evidence of *Lemna* abundances.

The first four explanatory axes of the RAIL2 PCA explained over two thirds of the species variability (cf. one third with the DCA) with axis 1 alone explaining over 28% and axes 1 and 2 explaining over 44%. The PCAs of RAIL1 and RAIL2 revealed that the *Lemna* epiphytes, *L. hungarica* and *S. seminulum*, had a positive correlation.

The RDA analysis of RAIL2 clearly shows that, as well as the fitted '*Lemna'* and 'No-*Lemna*' dummy environmental variables, there were other latent environmental variables influencing diatom compositional variation. Although the first RDA axis explained only 9.1% of the species variation (cf. 28.6% in the PCA) the Monte Carlo permutation test demonstrated that the RDA constrained by the '*Lemna'* and 'No-*Lemna*' environmental variables was statistically significant (r=0.749, F-ratio=4.187, p=0.002). The relatively long lengths of the *L. hungarica* and *S. seminulum* arrows suggest that the dummy environmental variable '*Lemna*' had a large effect upon these particular species. The '*Lemna*' and 'No-*Lemna*' dummy environmental variables were responsible for a significant proportion of total diatom variability. The RDA biplot (Fig. 5.23) graphically illustrates that the abundance of the two *Lemna* epiphytes was

constrained and explained by the presence of *Lemna* dominance. This suggests that the *Lemna*-epiphyte inference model can be utilised with confidence to track past *Lemna* phases and ultimately to investigate potential ecological engineering effects of past *Lemna* dominance on the Rail Pit ecosystem.

5.6.3 Comparison of *Lemna* indicators and *Lemna* history

The diatom stratigraphies of RAIL1 and RAIL2 were generally very similar. The consistent pattern of inferred *Lemna*-dominance in both the RAIL1 and RAIL2 cores implies distinct *Lemna* 'on-off' phases. This consistent pattern was highlighted when the absolute diatom counts (RAIL1) were explored further by examining the data in terms of i) relative percentage abundances of the diatom taxa (Fig. 5.15b) and ii) the *Lemna*-indicator metric (Fig. 5.16).

The timing of the presence and termination of the dense *Lemna* mats is in general agreement with historical observations. Although these historical observations were not dedicated detailed surveys (Table 5.2) it was noted that there were two *Lemna* periods in the recent history of the Rail Pit (i.e. first period: 1986 to early 1990s; second period: 1999-2005). There was, however, some discrepancy in the relationship between the *Lemna*-epiphyte model and historical observations as the model was unable to clearly differentiate between the two recently observed *Lemna* periods (Table 5.2). This lack of demarcation shown by the inference model is probably due to the relatively close timing between the two *Lemna* periods, and that the first *Lemna* period was not a phase of such strong *Lemna* dominance as indicated by the relatively lower abundances of the *Lemna*-epiphytes (Fig. 5.14). However, the second observed *Lemna* period was considered to be a phase of *Lemna* dominance and corresponded remarkably well with *Lemna*-dominance Phase 4 in the fossil record (i.e. Zone 4b in RAIL1; Zone 3a in RAIL2).

The first of the more recent *Lemna* periods in RAIL1 (Phase 3) occurred at 32-17cm, i.e. c. early 1950s to 1986, whilst the first *Lemna* period in RAIL2 was at 22-15cm i.e.

c. early 1950s to 1966. The difference in the timing of this *Lemna* phase between the two cores is likely to be because the RAIL2 core was truncated and terminated at 22cm, whereas the RAIL1 core extended beyond this level to the base of the sediment profile at 75cm. It may also be a result of the flocculent nature of the most upper section of the sediment profile, the differences in the sediment accumulation rates, and the inherent spatial heterogeneity between coring sites. Nonetheless, a much stronger agreement in the two cores was seen in the timing of the most recent *Lemna* phase (Phase 4). This occurred at 7-3cm, c. 1999-2005 in RAIL1 and at 5.5-3cm, c. 2000-2006 in RAIL2. Therefore, in summary the *Lemna*-epiphytic inference model based on *L. hungarica* and *S. seminulum*, was able to infer the presence of distinct phases of *Lemna* dominance in the Rail Pit (Fig. 5.14), which was in agreement with the observed recent history of *Lemna* dominance over the last few decades. Application of the inference model to the lower core sections of RAIL1 indicated that there has been an earlier phase of *Lemna* dominance (Phase 2, 54-42cm). However, despite slightly elevated numbers of *L. hungarica* and *S. seminulum* in the sections 72-58cm and 42-37cm, the density of the indicator taxa were not of sufficient magnitude to constitute *Lemna* dominance. Nevertheless, the RAIL1 diatom stratigraphy demonstrated that (for the Rail Pit at least) *Lemna* are not a constant established feature in the macrophyte 'pondscape', but appear to be cyclical.

The *Lemna*-epiphyte inference model was unable to directly identify the recent arrival in Britain in the 1970s (Landolt 1979) of the alien, highly invasive and aggressive *Lemna minuta* (Walker 2007, Willby 2007). *L. minuta* was observed to be co-dominant with *L. minor* in the third *Lemna* phase of the late 1990s to mid 2000s. It is because of this co-dominance, and seemingly tenuous co-existence, of the two *Lemna* species that it was not possible to derive a *L. minuta* signal separate from that of *L. minor* in the sediment record.

The recent macrophyte history of the Rail Pit shows that *L. minuta* was first recorded in 1996 (but could potentially have been present in the 1980s) and it was co-dominant with the native *L. minor*. This co-dominance meant that i) it was not possible to clearly

identify the exact timing of the arrival of *L. minuta* at the Rail Pit and, therefore, ii) it was not feasible to isolate and to specifically quantify the role of *L. minuta* in the *Lemna*-epiphyte inference model. However, the previous 'global' study on macrophyte-epiphytic relationships (see Chapter 3; Fig. 3.2) revealed that the two *Lemna*-indicator diatoms *L. hungarica* and *S. seminulum* were also recorded as epiphytes from *L. minuta*. The growth of *L. minuta* had rendered native *L. minor* as a rare species and was now the dominant Lemnid in the Rail Pit (see Chapter 6).

The diatom data suggest that *Lemna* dominance is cyclical in nature but because of their unique growth form (i.e. free-floating and rapid vegetative growth rates) *Lemna* spp. react and respond relatively quickly to eutrophication, particularly *L. minuta*, thereby giving them a competitive advantage over other macrophytes.

5.6.3.1 *Lemna* cyclicity as a driver of diatom community change

It was interesting to identify the indirect effects of *Lemna* dominance upon diatom compositional change in the Rail Pit (RAIL1), by simply comparing the diatom record before and after the phases of *Lemna* dominance. The six diatom species that apparently disappeared from the community after the first (Phase 2) and second (Phase 3) inferred *Lemna* dominance phases (i.e. were not present in Zones 3, 4a, 4b & 4c) were: *Cymbella cistula*, *E. adnata*, *E. sorex*, *E. turgida*, *F. capucina* var. *vaucheriae* and *A. veneta*. Furthermore, fifteen diatom species apparently disappeared from the community after the third (Phase 4) *Lemna* dominance phase (i.e. were not present in Zone 4c) such as: *P. maior*, *N. recta*, *G. acuminatum* and *R. abbreviata*. *Lemna* Phase 4 negatively impacted upon the abundances, and presence, of both the planktonic and benthic diatom communities. There were no new diatom species that occurred after the completion of the *Lemna* dominance Phases 3 and 4 (i.e. present in the surface sediments of Zone 4c). The *Lemna* phases (i.e. Phases 2-4) appear to have negatively impacted upon the already 'established' diatom community composition which resulted in 35% of the diatom species being lost from the diatom community. This high species

turnover is reflected in the long DCA gradient lengths, where the first four axes explained over 50% of species variability.

Although RAIL2 only covered the recent history of the Rail Pit, this core recorded more diatom species (72 species) than RAIL1 (60 species) which sampled the entire history of the Rail Pit. This was probably a reflection of the higher sampling resolution as RAIL2 was sliced at 0.5cm intervals (cf. 1.0cm slices in RAIL1) and the high diatom counts made which reduced the degree of uncertainty and counting errors (Maher *et al.*, 2011). As RAIL2 core was sampled at a high resolution it was possible to confidently identify the indirect effects of *Lemna* dominance upon the diatom compositional change in the recent history of the Rail Pit, by simply comparing the species presence-absence before and after the phases of *Lemna* dominance. As seen in RAIL1, the presence of the *Lemna* dominance phases negatively impacted upon the 'established' diatom community composition resulting in nearly 42% of the diatom species being lost from the diatom community in core RAIL2. The six diatom species that disappeared from the community after *Lemna* dominance Phase 3 (i.e. were not present in Zones 2, 3a & 3b) were: *N. ampliatum*, *Gomphonema augur*, *E. turgida*, *Nitzschia constricta*, *Navicula minuscula* and *E. sorex*. There were twenty four diatom species that apparently disappeared from the community after the observed *Lemna* dominance Phase 4 (i.e. were not present in Zone 3b) such as: *E. adnata*, *F. capucina* var. *vaucheriae* and *A. conspicua*. There were just three species that were only found after the completion of both *Lemna* dominance phases (i.e. present in the surface sediments of Zone 3b), these were: *Synedra biceps*, *Navicula capitatoradiata*, *S. construens* var. *venter* and also *Cymbella affinis* which was also present in low densities in Zone 2, i.e. the brief hiatus between the two *Lemna* dominance Phases 3 and 4.

Interestingly, *C. meneghiniana* despite blooming in the late summer-autumn, was clearly unaffected by the *Lemna* dominance cover in late summer. *C. meneghiniana* is a common dominant diatom in eutrophic shallow lakes (Brugam 1983) being able to adapt to a wide range of environmental conditions, and was the most abundant and dominant of the planktonic species in the Rail Pit. It is known to have strong

heterotrophic capabilities (Hellebust & Lewin 1977), and therefore perhaps this metabolic adaptation facilitated and promoted the growth of this diatom in the dark environment beneath the *Lemna* mats.

The overall effect of the *Lemna* phases on the diatom communities was an initial increase and then a decrease in accumulation rates whilst there was an overall decrease in species diversity. The *Lemna* phases reflected an increase in the *Lemna* epiphytes *L. hungarica* and *S. seminulum* seemingly at the expense of overall diatom species diversity, even though the presence of other 'non-*Lemna* associated diatoms' suggest that other diatom niches such as plankton and epipelon were still present. It could be argued that the impact on the diatom communities by these *Lemna* phases had a strong impact on diatom community structure and diversity.

5.7 Conclusions

The diatom assemblages of both RAIL1 and RAIL2 cores were diverse with no particular species dominating, and many taxa were consistently recorded throughout the profiles. The large diatom counts (mean: 1925 per sample for RAIL1; mean: 525 per sample for RAIL2) enabled subtle shifts in species composition and abundance to be detected and allowed rarer diatom taxa to be enumerated thereby maximising the potential to extract ecological information from the diatom data. These large diatom counts directly resulted in showing the coherence between the *Lemna*-epiphyte inference model and past *Lemna* phases or cycles, which would not have been so clear if the standard 300 diatom valve count approach was used. Perhaps it might be scientifically prudent if future pond/shallow lake workers attempt large diatom counts to ensure that any potential ecological signals can be confidently identified from rare diatom species.

The study identified a strong association between *Lemna* occurrence and *L. hungarica* and *S. seminulum* which was used as the basis for the *Lemna*-epiphyte inference model. When the inference model was applied to the Rail Pit cores the timing of the *Lemna*

phases agreed reasonably well with that documented by historical observations. The *Lemna* phases were identified as discrete zones and correlated well with both the diatom and lithostratigraphic records. The application of the model to the Rail Pit demonstrated the reliability of this novel technique for identifying past *Lemna* abundances and the cyclical nature of these past occurrences. Indeed, these data support the notion that the *Lemna* phases can be confidently classified as distinct *Lemna* cycles.

The diatom records of core RAIL1 suggest that *Lemna* dominance presented as three clear phases throughout the history of the Rail Pit with a period of early *Lemna* presence. The first period of *Lemna* presence (Phase 1, 72-58cm) and an early *Lemna* dominance phase (Phase 2, 54-42cm) were identified from the inference model. Another *Lemna* dominance phase (Phase 3) occurred from c. late 1940s to c. early 1950s and terminated in the c. mid 1980s in RAIL1 although it was estimated to terminate somewhat earlier in the c. mid 1960s in RAIL2. However, the timing of a further *Lemna* dominance phase (Phase 4) was very similar in both cores (c. 2000 to c. mid 2000s). The sedimentary diatom data suggest that *Lemna* dominance is cyclical in nature.

The extent of the impact on the diatom communities of the recent *Lemna* phases observed in this study suggests that ecologically the *Lemna* mats cause a 'perturbation event'. Hence it is reasonable to suggest that they are acting as physical ecological engineers upon the structure and diversity of the diatom communities. It begs the question of whether dense *Lemna* mats were capable of bringing about catastrophic regime shifts or alternative stable states throughout the natural history of the Rail Pit by their potential ecological engineering affects. The impact of *Lemna* dominance on the wider ecological system of the Rail Pit is investigated by analysis of fossil plant pigments and macrofossils (see Chapter 6).

Chapter 6. Long-term changes in pond ecology in response to eutrophication and *Lemna* invasion: a multi-proxy study

6.1 Introduction

Regime shifts between clear-water and turbid conditions are now recognised as intrinsic features of many shallow lake ecosystems (Scheffer *et al.*, 2001). However, to date, there has been relatively little attention given to studying these ecological characteristics in ponds. Moreover, the evidence for regime shifts is primarily derived from short-term experiments and contemporary sampling such that their long term manifestation is not known. The only way to study ecosystem state changes over ecologically relevant timescales is with palaeoecological analyses (McGowan *et al.*, 2005). Lake and pond sediments can capture and integrate structural changes of entire water bodies across multiple components of the food web (Leavitt & Findlay 1994, Sayer *et al.*, 2010a).

In addition to clear-water and phytoplankton dominated states, Scheffer *et al.,* (2003) demonstrated that free-floating plant dominance can also be a self-stabilising ecological state in small freshwater ecosystems. Further, there is strong potential for free-floating plants (e.g. *Lemna*), when at high abundances, to function as ecological engineers in ponds primarily by decreasing light penetration which can be reduced by up to 99% (Lewis & Bender 1961, Landolt 1986, Goldsborough 1993, 1994), reducing oxygen production by phytoplankton leading to an increase in anaerobic decomposition and accumulation of organic matter, and lowering water temperatures (Pokornŷ & Rejmánková 1983, Landolt 1986, Portielje & Roijackers 1995). Using a multi-proxy palaeolimnological approach, this chapter investigates long-term changes in the biological structure and ecology of a pond (Bodham Rail Pit) that has experienced

dense blooms of duckweed (*Lemna*) over the last few decades. Fossil pigments as biomarkers of the phototrophic community, together with macrofossils (plant and animal) are used to compliment the fossil diatom analysis undertaken in Chapter 5. Reconstructions of ecological change in the Bodham Rail Pit are examined within the context of regime shifts and ecological engineering by free-floating *Lemna* dominance. More specifically the following questions are addressed:

1. Is there any evidence that explosive blooms of *Lemna* are a consequence of eutrophication?

2. What is the ecological impact of dense mats of *Lemna* on a small farmland pond? Specifically, is *Lemna* functioning as an ecological engineer on the structure and function of the plant and animal communities in a small farmland pond?

3. What are the potential ramifications for the management of small farmland ponds with respect to maintaining aquatic species richness and diversity?

The site information and history of the Bodham Rail Pit are given in Chapter 5 which shows that the Rail Pit has recently experienced several phases of free-floating *Lemna* dominance.

6.2 Methods

This study employed a multi-proxy palaeoecological approach including diatoms (see Chapter 5), algal (and higher plant) pigments, and plant and animal macrofossils. This chapter focuses on two cores, namely RAIL1 and RAIL2, with respect to fossil pigment analyses, and RAIL1 with respect to macrofossil analysis. Details of the methodology regarding core collection, core chronologies, lithostratigraphic analyses and diatom analysis are given in Chapter 5.

6.2.1 Sedimentary pigment analysis

Sedimentary pigments have been widely used to measure environmental change in lakes, such as eutrophication, by quantifying historical changes in algal and plant community composition and abundances (Hall *et al.,* 1997). Furthermore, the controls on algal abundance and community compositional change have been specifically investigated to elucidate ecosystem state changes (McGowan *et al.,* 2005). Fossil pigment analyses could provide insights into ecosystem changes but at smaller ecological scales. Thus, palaeoecological techniques have huge potential for detecting such ecological changes in ponds.

Pigments from algae, phototrophic bacteria and higher plants often preserve for long periods in the sediment record and have been recorded throughout the Holocene (Sanger 1988). Their faithful preservation is due to the water-insoluble nature of lipophilic molecules and the widespread occurrence of suitable sedimentary environments for preservation such as organic, anoxic and aphotic conditions (Leavitt & Hodgson 2001). Analyses of fossil pigment records have become widely used to indicate algal and bacterial community composition (Züllig 1981, Yacobi *et al.,* 1990), food-web interactions (Leavitt *et al.,* 1989, 1994a, b) and past UV radiation environments (Leavitt *et al.,* 1993, 1999). In sediment cores pigments have also been used as indicators of anthropogenic impacts on aquatic ecosystems ranging from eutrophication to climate change (Leavitt *et al.,* 1994c, Hall *et al.,* 1999).

Pigments are present in all photosynthetic organisms where they harvest light for photosynthesis and afford photo-protection (Porra *et al.,* 1997). As they are produced from a whole range of photosynthesising organisms they potentially represent the entire phototrophic community, and overall primary production. They also differ widely in their taxonomic specificity (Leavitt & Hodgson 2001) and are specific to particular photosynthetic groups (Jeffrey *et al.,* 1997) (see Table 6.1). Therefore, because of pigment taxonomic specificity, sedimentary pigment records can be used as biomarkers to reconstruct past phototrophic communities. It was hoped that any potential periods of

past anoxia resulting from dense *Lemna* mats could be identified from bacterio-chlorophyll and carotenoids from anaerobic phototrophic bacteria, as they have been successfully used as biomarkers for anoxic events and state changes in lake systems in the past (Squier *et al.*, 2002).

Pigment	Affinity (taxonomic groups)	Source	Stability
Chlorophylls			
* Chl a	Higher plants, Algae	P, L	3
* Chl b	Higher plants, Chlorophyta, Euglenophyta	P, L	2
Chl c	Dinophyta, Diatoms, Chrysophyta	P, l	4
Carotenoids			
* β-carotene	Higher plants, Algae	P, L, t	1
α-carotene	Cryptophyta, Chrysophyta, Dinophyta Some Chlorophyta	P, l	2
* Alloxanthin	Cryptophyta	P	1
Fucoxanthin	Dinophyta, Diatoms, Chrysophyta	P, L	2
* Diatoxanthin	Dinophyta, Diatoms, Chrysophyta	P, L, s	2
Diadinoxanthin	Dinophyta, Diatoms, Chrysophyta, Cryptophyta	P, L, s	3
Peridinin	Dinophyta	P	4
Echinenone	Cyanobacteria	P, l	1
* Zeaxanthin	Cyanobacteria	P, l	1
Canthaxanthin	colonial Cyanobacteria	P, l	1
Myxoxanthophyll	colonial Cyanobacteria	P, l	2
Oscillaxanthin	Cyanobacteria (Oscillatoriaceae)	P, l	2
* Lutein	Chlorophyta, Higher plants, Euglenophyta	P, L, t	1
Neoxanthin	Chlorophyta, Higher plants, Euglenophyta	l	4
Violaxanthin	Chlorophyta, Higher plants, Euglenophyta	l	4
Okenone	Purple sulphur bacteria	P	1
Chlorophyll Degradation products			
* Pheophytin a	Chl a derivative (general)	P, L, t, s	1
* Pheophytin b	Chl b derivative (general)	P, L, t, s	2
Pheophorbide a	Chl a derivative (senescent diatoms)	P, l, s	3

Table 6.1. Summary of pigments recovered from lake sediments and their taxonomic affinities. The predominant sources are identified as planktonic (P), littoral (L), terrestrial (T) and sedimentary (S) where upper case letters indicate the quantitatively more important pigment sources. The relative degree of chemical stability and preservation is ranked from most (1) to the least (4) stable. Note: pigments with the least stability are rarely found in the sediment record. * indicates pigments found at the Bodham Rail Pit. (Modified from Leavitt & Hodgson 2001).

A fossil pigment analysis of cores RAIL1 and RAIL2 from the Rail Pit was undertaken to provide palaeoecological information on the pond's ecological evolution, changing patterns of historical aquatic primary production, and to potentially elucidate the engineering effects of free-floating *Lemna* dominance on the phototrophic community and wider ecosystem structure and functionality.

Sediment samples for pigment analysis were taken in the field from both cores RAIL1 and RAIL2. Samples were taken at 1cm intervals for RAIL1 (75 samples in total), and approximately at 1cm intervals for RAIL2 (17 samples in total). Because of the labile nature of pigments, sub-samples were immediately placed into black, air-tight bags and frozen to prevent pigment degradation from light, heat and oxygen (Leavitt & Hodgson 2001). The samples were kept frozen (<-20°C) as raw samples and then freeze-dried with an Edwards Modulyo 4k freeze-drier just prior to pigment extraction and analysis, as freeze-drying (lyophilizing) has been shown to improve pigment extraction (Louda *et al.,* 2000). The freeze-dried samples were then transported (within black bags and wrapped in foil to prevent photodegradation) using a cool-box containing ice-blocks to the HPLC Laboratory at the School of Geography, University of Nottingham.

6.2.1.1 Sedimentary pigment extraction, separation and identification

Pigments were extracted using a solvent of acetone (80%), methanol (15%) and deionised water (5%), a method known to be suitable for freeze-dried samples from freshwater sediments (Leavitt & Hodgson 2001). The extraction solvents were degassed by sonication (Decon R FS200b sonicator). Pigment samples were extracted overnight at 4°C, then filtered with a 0.22µm PTFE filter, dried under nitrogen gas and redissolved in a 70: 25: 5 mixture of acetone, ion pairing reagent (IPR 0.75 g tetrabutyl ammonium acetate and 7.7g ammonium acetate in 100ml deionised water) and methanol. The samples were analysed using an Agilent Technologies 1200 Series High Performance Liquid Chromatograph (HPLC) fitted with a Thermo Scientific ODS Hypersil reverse column (205 x 4.6mm, 5µm particle size). Between 0.1-0.2g of freeze-dried samples was required to obtain sufficient colour for successful pigment analysis,

and an injection volume of 100µl was used for the run sequences. All extraction runs were compared with a green standard (fresh ground grass leaves) in order to show the main pigment retention times, and were calibrated against commercial standards to enable the conversion of pigment peak areas to concentration data. The sample runs were undertaken in random order under ambient conditions of safety lighting throughout the analysis. The pigment separation analysis was performed using the methodology of Chen *et al.*, (2001) with a modification of the separation conditions (Table 6.2). All samples were kept under low intensity safety lights throughout the procedure.

Time (mins)	**A**	**B**	**C**	**Flow** (ml min^{-1})
0	100	0	0	1
4	0	100	0	1
38	0	25	75	1
39	0	25	75	1
43	100	0	0	1
52	100	0	0	1

Table 6.2. Separation conditions of the HPLC solvents (modification of Chen *et al.*, 2001). A: 80% methanol, 20% 0.5 mol ammonium acetate; B: 90% acetonitrile, 10% deionised water; C: HPLC grade ethyl acetate.

Pigments were identified by comparing their absorption spectra characteristics and elution times with known standards. Following isolation and separation of the chlorophyll and catotenoid pigments, their abundances were quantified and identified from respective chromatograms based upon peak retention times and peak areas of absorbance spectra, and compared to authentic standards of individual pigments. These spectra often present a large number of peaks including pigment degradation products, potential coelution of pigments and unknown pigments. Examining individual pigment concentrations in the context of their historical maxima is suggested to be the most reliable method of interpreting the sediment pigment record (Leavitt 1993). Furthermore, Leavitt (1993) advocates normalizing pigment concentrations to the

organic carbon pool which can partly compensate for bias in pigment degradation under different preservation conditions.

6.2.1.2 Pigment data formulation and analyses

The pigment data produced from the individual sample levels from cores RAIL1 and RAIL2 were quantified by linking the area of individual peaks to a concentration for individual pigments based on standard protocols (Chen *et al.,* 2001). The pigment concentrations are presented as an expression of the amount of pigment per gram of organic matter (i.e. nmol g^{-1} organic matter). In conjunction with determining the concentrations of individual pigments, a further determination of the degree of degradation was performed by comparing the ratios of both chlorophyll *a* and chlorophyll *b* with their respective pheophytin *a* and pheophytin *b* degradation products. The ratios indicate the degree of chlorophyll pigment preservation, with higher ratios indicating greater preservation. Furthermore, the specific ultraviolet radiation pigment (UVR) was also used to provide a UVR index, which scales the amount of UVR screening compound giving an estimation and an index of algal UVR protection (Leavitt *et al.,* 1997). The UVR Index is an index of the scaling of the amount of the UVR screening compound/pigment for the amount of algae present which gives an index of UVR protection, and ultimately an indirect method for determining water clarity (Leavitt *et al.,* 1997).

The individual pigments identified from the analyses were sorted by their affinities and placed within specific taxonomic groups. Chlorophyll *b* (and degradation product pheophytin *b*) and lutein are found in green algae (but not all algae) and higher plants, chlorophyll *a* (and degradation product pheophytin *a*), and β-carotene are ubiquitous across all types of algae and higher plants (including *Lemna*) and therefore, cannot be differentiated from the actual source of the pigments (i.e. from *Lemna* or other plants/algae). However, it was decided to group these particular pigments in an attempt to facilitate ecological interpretation of the pigment stratigraphies. This was particularly useful when cross-referencing with the timing of *Lemna* dominance as the implication

would be that dense *Lemna* mats would likely be a major source of these pigments. The expression, '*Lemna* marker' pigments was therefore loosely applied to the pigment data. Cryptophytes (alloxanthin), diatoms (diatoxanthin) and cyanobacteria (zeaxanthin) were also grouped by their taxonomic affinities (Leavitt & Hodgson 2001; McGowan *et al.,* 2005). Their pigment chromatograms consistently revealed small but distinct peaks of an unknown carotenoid pigment at 22.907 minutes retention time, which was located intermediately between the pigments zeaxanthin (16.597 minutes) and Chlorophyll *b* (24.644 minutes). This unknown carotenoid pigment is associated with purple sulphur bacteria and is referred to as 'Carotenoid PSB' in sedimentary pigment analyses (S. McGowan, pers. com.).

6.2.2 Macrofossil analysis

Sedimentary plant macrofossils such as diaspores (seeds, fruits, spores, oospores) and vegetative parts (leaves, leaf spines, roots, tissue and woody fragments) have been used in palaeolimnological reconstructions of past vegetation and climate change (Birks 1980, Birks & Birks 1980, Wasylikova 1986). In addition, plant macrofossils are widely used to infer aquatic vegetation composition in the past (Davidson *et al*., 2005, Birks & Birks, 2006, Sayer *et al*., 2010a). In this study plant macrofossils were used to reconstruct the past aquatic vegetation of the Rail Pit. Furthermore, various animal macrofossils (e.g. ostracod shells, trichopteran frontal clypeal plates, *Chaoborus* mandibles, *Sialis lutaria* mandibles, *Plumatella* statoblasts, fish scales, and cladoceran ephippia) were also enumerated to provide additional insights into whole-ecosystem change.

Cladocerans have a long history of being employed in a wide range of lake studies such as trophic state changes (Hofmann 1996), changes in predation pressure (Kerfoot 1981, Leavitt *et al.,* 1989) and changes in macrophyte abundance (Thoms *et al.,* 1999, Johansson *et al.,* 2005, Davidson *et al.,* 2010a). These investigations are a result of cladocerans being at the centre of food-webs as they include both benthic and pelagic taxa which makes them sensitive to both bottom-up and top-down structuring forces

and shifts in the balance of benthic and pelagic productivity (Davidson *et al.*, 2010a, 2010b). Cladocerans are considered to be strong candidates as the single best indicator of palaeoecological conditions related to changing trophic status and any alterations in food-web structure in shallow lakes and ponds (Davidson 2010a, 2011, Jeppesen 2011). Macrofossils of invertebrate taxa other than cladocerans are rarely enumerated from sediment records, but there is huge potential in their application in the reconstructions of past aquatic ecosystems.

A total of 21 sediment samples were enumerated at 4cm intervals for core RAIL1 only. An additional sediment interval (54cm) was also analysed as the diatom analysis revealed it to be an 'anomalous' sample. The procedure used for macrofossil analysis follows the preparation method modified from Birks (2001). Sediment samples of between 25cm^3 and 60cm^3 were used, with the exact sample volumes determined by water displacement. The samples were soaked in 10% potassium hydroxide (KOH) overnight to disaggregate and disperse the sediment. Sediment was then carefully sieved through 355μm and then sieved through 125μm meshes to separate course and fine fractions. The separate residue fractions were then transferred to separate lidded containers and kept cool (4^{0}C) prior to examination. The entire residue on both the 355 and 125μm sieve fractions were examined. However, due to the large volumes of sediment retained in the 355μm sieve, sub-samples of approximately 25% volume were analysed for terrestrial and aquatic macrophyte leaves to ascertain a terrestrialisation index and an index of aquatic macrophyte representation respectively.

Sieved material was systematically examined under a stereo-microscope using bright-lights at a magnification of 10-40x and all plant and animal macro-remains were isolated, identified and enumerated by comparison to a substantial modern reference collection and relevant taxonomic keys and various reference publications held at the ECRC, UCL and the Natural History Museum, London. Trichopteran fronto-clypei were identified by Paul Wood and Lynda Howard at the Department of Geography, Loughborough University and aquatic bryophytes were identified by Pauline Lang at the Scottish Environmental Protection Agency (SEPA). Problematic seeds were

identified with the assistance of Hilary Birks (University of Bergen). All plant and animal macrofossil data were standardized as the number of fossils per 100cm^3 wet sediment and, to aid interpretation of the stratigraphs, plant and animal macrofossils were plotted separately. The duckweed (*Lemna*) dominance phases, highlighted as green bands in the stratigraphs, are based upon recorded observations and from the *Lemna* indicator metric (upper band, Phase 4), whilst the lower bands (Phases 2 and 3) are based solely upon the *Lemna* indicator metric.

Because of taxonomic limitations for charophyte (*Chara* and *Nitella*) oospores, species-level identification was not possible. There were four *Potamogeton* species identified from both seeds and leaf fragments but there were also many small leaf fragments of *Potamogeton* that could not be confidently identified to species-level and were, therefore, summed by wet weight to represent a crude stratigraphic change of overall Potamogetonaceae representation. Furthermore, the many small leaf fragments of terrestrial plants (mostly tree species) were also summed by wet weight to present a crude representation of the degree of terrestrialisation and riparian growth. These fossil *Potamogeton* and terrestrial leaf representations would provide indices to which a direct stratigraphic comparison could be made to assess changes in the relative importance of aquatic macrophyte production and terrestrialisation with time.

6.2.3 Data manipulation and numerical analyses

The data produced from both the pigment and macrofossil analyses are presented as stratigraphs in the first instance to facilitate interpretation and analysis. The *Lemna* indicator metric, obtained from the diatom analyses, was incorporated into the plant and animal macrofossil stratigraphs to highlight past *Lemna* cycles and, therefore, to ascertain any potential effects upon the flora and fauna of the pond. An initial exploratory DCA was performed (Hill 1973, Hill & Gauch 1980) using the program CANOCO 4.5 (ter Braak & Šmilauer 2002), primarily to establish whether pigment and plant/animal macrofossil species responses were linear or unimodal. The rare animal species or aggregates were not down-weighted (Birks 2012). Species data were

detrended by segments (Hill & Gauch 1980, Wartenberg *et al.*, 1987) and species and samples were standardised by the weighted averaging algorithm (Lepš & Šmilauer 2003).

PCA and RDA were performed on the pigment data as the DCA indicated that pigment responses were linear. The data were log (x +1) transformed to normalize the data and the axes scaling was focused on inter-species (i.e. pigment) distance. DCA and PCA were performed on the macrofossil data as their responses were linear. Further, individual plots of PCA axes 1 scores to enable an examination of compositional and ecological changes were performed on the pigment and macrofossil data, in conjunction with the diatom data.

Numerical zonation using constrained cluster analyses (CONISS) was performed on the pigment, macrofossil and diatom data to identify the timing of potential aquatic ecosystem state changes. Zonation of core RAIL1 was performed solely on the aquatic plant macrofossil data with the riparian macrofossils passively included in the plant stratigraphy. The core diagrams were generated using the programs Tilia (version 1.7.16), Tiliagraph (Grimm 1991a, b) and TGView (Grimm 2002) to provide zonation of the macrofossil data, and C2 (Juggins 2007) to provide stratigraphs of the macrofossil data.

6.3 Results

The radiometric dating results, lithostratigraphies, recent historical macrophyte observations and descriptive data of both RAIL1 and RAIL2 cores are presented in Chapter 5 (see sections: 5.2.2, 5.4.2, 5.4.2, & 5.4.3).

The results of the diatom analyses (see Chapter 5) indicated that there were four *Lemna* phases in RAIL1: Phase 1 (72-58cm), Phase 2 (54-42cm), Phase 3 (32-17cm) and Phase 4 (7-3cm). However, the diatom analyses also indicated that Phase 1 (72-58cm) could not be classified as a dominant *Lemna* phase, but was simply recording the presence of

Lemna. Therefore, it was decided to omit *Lemna* Phase 1 from the stratigraphic diagrams. Nevertheless, the greater abundances of *Lemna*-indicator diatoms recorded from the other *Lemna* phases (i.e. Phases 2, 3 and 4) suggested that these could indeed be classified as dominant *Lemna* phases. As with the diatom stratigraphic diagrams (see Chapter 5) it is the dominant *Lemna* Phases 2, 3 and 4 that are shown in the pigment and macrofossil stratigraphic diagrams.

6.3.1 Pigment preservation of cores RAIL1 and RAIL2

Initial pigment chromatographs for RAIL1 revealed that there was no coelution between the isomeric carotenoids lutein and zeaxanthin and, therefore, these pigments were expressed and plotted separately. Although overall pigment preservation was satisfactory throughout core RAIL1 (and RAIL2), it was noticeable, however, that there were erratic and sporadic sequential occurrences of some of the more labile xanthophyll pigments especially fucoxanthin, neoxanthin, canthaxanthin and also echinenone. Therefore, in order not to bias the overall fossil pigment stratigraphies and to minimize the influence of pigment degradation, these pigments were excluded from the zonation analyses (McGowan *et al.*, 2005). Their profiles were, however, noted and included in the overall interpretation of the fossil pigment data. Down-core pigment concentrations were interpreted as being relatively independent of degradation or preservation effects as there was a degree of stability between chlorophyll *a* and chlorophyll *b* and for the ratios of their respective degradation products pheophytin *a* and pheophytin *b* (Fig. 6.1) This degree of stability was evident in both cores RAIL1 and RAIL2.

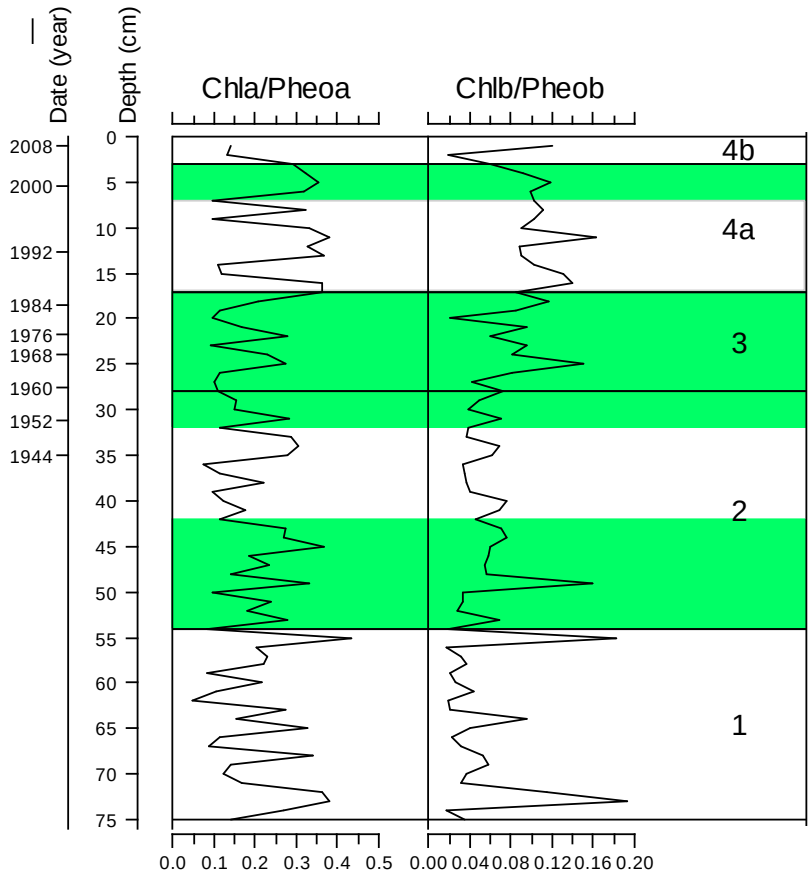


Figure 6.1. Ratios of fossil Chlorophyll *a* and Chlorophyll *b* and their degradation products of Pheophytin *a* and Pheophytin *b* in core RAIL1. The green bands show periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and from the *Lemna* indicator metric [Phase 4]; lower bands are based upon the *Lemna* indicator metric [Phases 3 & 2]). The stratigraph also shows the zones derived from the pigment data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

From the pigment chromatographs (not shown) it was noticeable that RAIL1 contained relatively large amounts of chlorophyll *a*' (i.e. the oxidative degradation product divinyl chlorophyll *a*) throughout the core profile, most notably during periods where there was no *Lemna* dominance, and also with the onset of the termination of the final *Lemna* dominance Phase 4. Consequently, it was decided to sum the degradation products of chlorophyll *a* (i.e. pheophytin *a*, chlorophyll *a*') and to compare the ratio of the proportions of the summed degradation products with the proportion of chlorophyll *a* (Fig. 6.2a). Furthermore, to aid interpretation of the fossil pigment record within the

context of potential ecological engineering effects of duckweed, a UVR index was calculated using the chronological ratio between the UVR absorbing compound/pigment and the summation of the carotenoid pigments alloxanthin, diatoxanthin, lutein and zeaxanthin (Leavitt *et al.*, 1997). The UVR Index is an index of benthic algal UVR protection and is presented in Figure 6.2b.

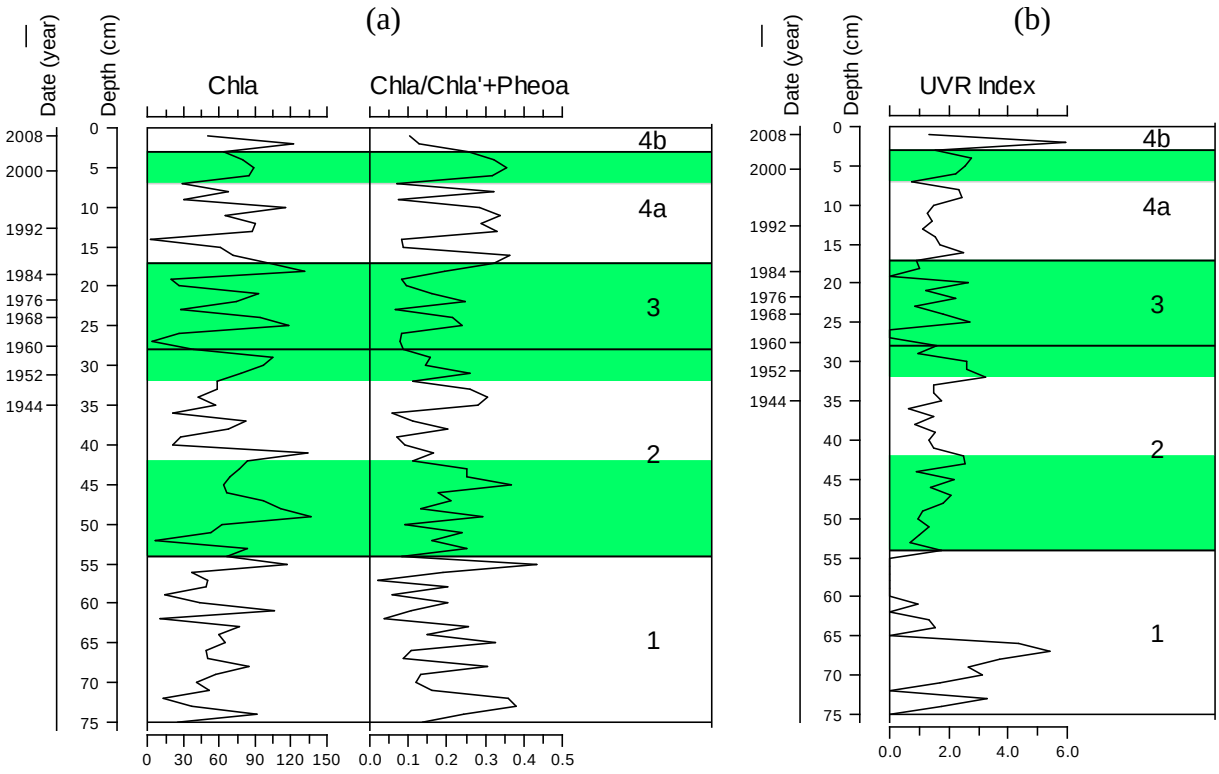


Figure 6.2. (a) Fossil Chlorophyll *a* and Chlorophyll *a* degradation product ratios; and (b) the fossil pigment UVR Index (i.e. an index of UVR protection) of RAIL1. The green bands show periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and from the *Lemna* indicator metric [Phase 4]; lower bands are based upon the *Lemna* indicator metric [Phases 3 & 2). The stratigraph also shows the zones derived from the pigment data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

In RAIL2 as with RAIL1, lutein and zeaxanthin eluted separately and were chromatically expressed separately. Overall, the preservation of fossil pigments of RAIL2 showed better preservation (i.e. less pigment degradation products) than RAIL1. This enhanced preservation is demonstrated by the fact that there was relatively less chlorophyll *a*' (an oxidative degradation product of chlorophyll *a*) seen in core RAIL2

(Fig. 6.3a). An index of UVR protection was also performed (Leavitt *et al.*, 1997) for RAIL2 (Figure 6.3b).

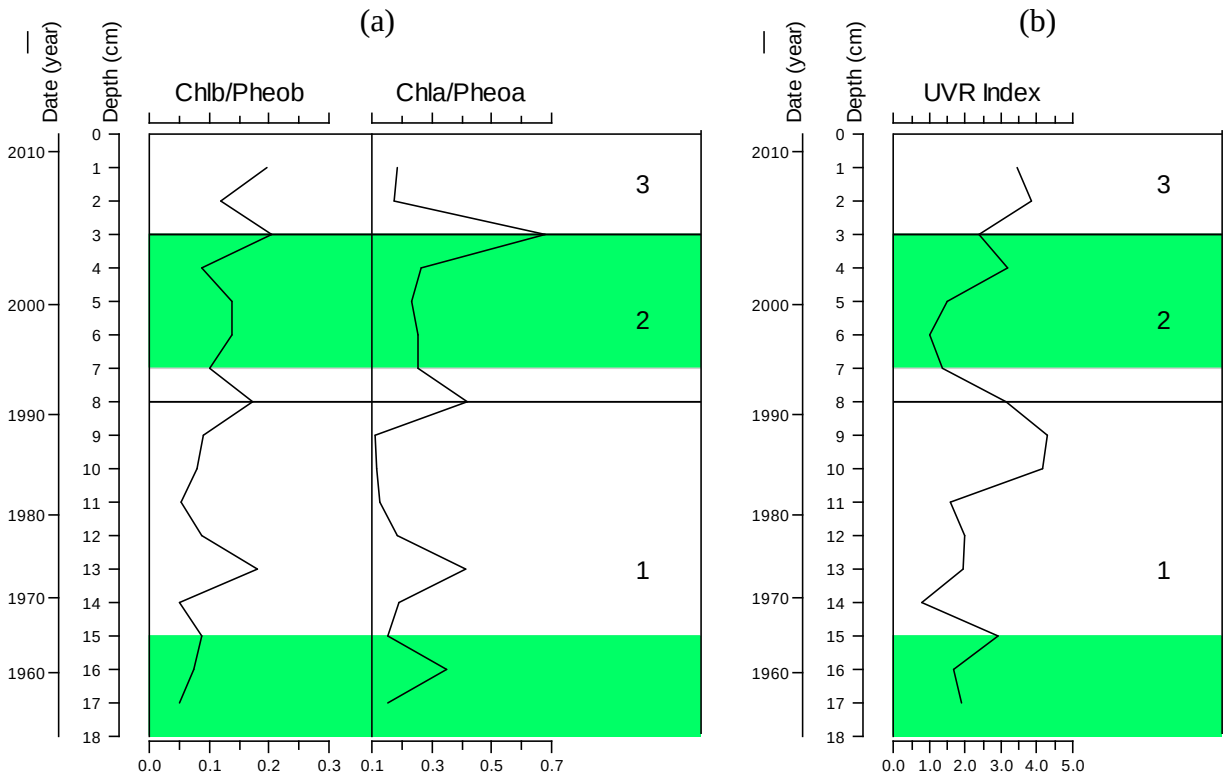


Figure 6.3. (a) Fossil Chlorophyll *a* and Chlorophyll *a* degradation product ratios; and (b) the fossil pigment UVR Index (i.e. an index of UVR protection) of RAIL2. The two green bands show periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and from the *Lemna* indicator metric [Phase 4]; lower band is based upon the *Lemna* indicator metric [Phase 3]). The stratigraph also shows the zones derived from the pigment data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

Overall, total pigment concentrations were relatively stable throughout the core profiles except for a few notable declines at 54cm, 22cm and 14cm (RAIL1) (Figs. 6.1, 6.2). Factors that promote preservation of pigments include low oxygen concentrations, high sedimentation rates, cold water conditions and low light conditions (Sanger 1988, Leavitt 1993, Leavitt & Hodgson 2001). These factors appeared to be evident at the Rail Pit as shown by the stable pigment concentrations and the results of on-going monitoring of key physical and chemical variables (see Fig. 5.3 Chapter 5), especially during periods of *Lemna* dominance. This may be due to a number of factors such as i) high sedimentation rates, particularly over the last 60-70 years, and increased organic

matter accumulation (see Figs. 5.6, 5.8, and Tables 5.2, 5.3 Chapter 5), ii) surveys have revealed permanent chemical stratification of the water column creating cooler conditions towards the pond bed, (see Table 5.1a Chapter 5), and iii) *Lemna* dominance promoted low light (PAR) and low oxygen concentrations (see Fig. 5.3, Table 5.1a and Appendix 5).

6.3.2 Pigment stratigraphies

6.3.2.1 Core RAIL1

Cluster analysis (CONISS) of the sedimentary pigment data revealed four major zones for RAIL1 (Fig. 6.4 and see Appendix 4).

Zone 1 (75-54cm)

This zone is characterised lithostratigraphically by a gradual increase in sedimentary organic matter and a concomitant decrease in carbonate. There is a steady increase in the pigments associated with plants and algae (i.e. chlorophyll *b*, pheophytin *b*, lutein, chlorophyll *a*, pheophytin *a*, β-Carotene) these being the aforementioned '*Lemna* marker' pigments. At 68cm there is a substantial increase in sedimentary concentrations of the UVR absorbing pigment reflected in the high UVR Index (Fig. 6.2b), and there is also a gradual increase in the bacterial derived pigment Carotenoid PSB. The pigment chlorophyll *a'* (formed by the oxidative degradation of chlorophyll *a)* is present at relatively low concentrations, as are the carotenoid pigments alloxanthin, diatoxanthin and zeaxanthin. Interestingly, at 60-55cm there are increasing concentrations seen in all sedimentary pigments, with the notable exception of the UVR absorbing pigment which disappears from the record during this phase (Figs. 6.2b, 6.3). The non-dominant *Lemna* phase (i.e. Phase 1) is present within Zone 1.

Zone 2 (54-28cm)

The so-called '*Lemna* marker' pigments all show sudden and dramatic spikes in sedimentary concentrations with the onset of Zone 2 (54cm) which is coincident with the start of the *Lemna* Phase 2. At 55-54cm there are sudden and large spikes in the concentrations of the other sedimentary pigments which coincide with the onset of a pronounced peak in carbonate (16% to 40%). However, at the height of this carbonate peak (54-52cm) there is an equally sudden and pronounced trough in the '*Lemna* marker' pigments. Thereafter there are peaks and troughs of the '*Lemna* marker' pigments. Towards the mid-section of Zone 2 (42-40cm) there is a reduction in organic matter together with a concomitant increase in carbonate, which sees further increases in the '*Lemna* marker' pigments. There are also large increases in sedimentary pigment concentrations of the UVR absorbing pigment, alloxanthin, diatoxanthin, zeaxanthin and carotenoid PSB. The dominant *Lemna* Phase 2 is present in Zone 2. This zone terminates at 28cm, which is within *Lemna* dominance Phase 3 (*Lemna* Phase 3 begins at 32cm) and is characterised by sudden reductions in concentrations of all sedimentary pigments.

Zone 3 (28-17cm, c. 1958-1988)

This zone effectively encompasses *Lemna* Phase 3 and sees increases in sediment concentrations of the '*Lemna* marker' pigments. This zone witnesses rapid peaks and troughs of the other carotenoid pigments (alloxanthin, diatoxanthin, zeaxanthin and carotenoid PSB), but also reveals fluctuating concentrations of the UVR absorbing pigment. There are two sediment levels (19cm and 27cm) where the UVR absorbing pigment disappears from the sediment profile.

Zone 4a (17-7cm, c. 1988-2005)

Zone 4a sees substantial increases in all of the sedimentary chlorophyll and carotenoid pigments and also the pigment UVR absorbing pigment and carotenoid PSB. There is a

particularly large spike of the UVR absorbing pigment at 15cm which is also reflected in the high UVR Index (Fig. 6.2b). Zone 4a also sees a sudden spike (12cm) in sedimentary organic matter.

Zone 4b (7-0cm, c. 2005-2010)

This zone sees a sudden increase in the '*Lemna* marker' pigments, the chlorophyll and carotenoid pigments and the specific pigment derivatives from algae (β-carotene) and bacteria (carotenoid PSB). The highest concentration of sedimentary UVR absorbing pigment is seen at the onset of this zone.

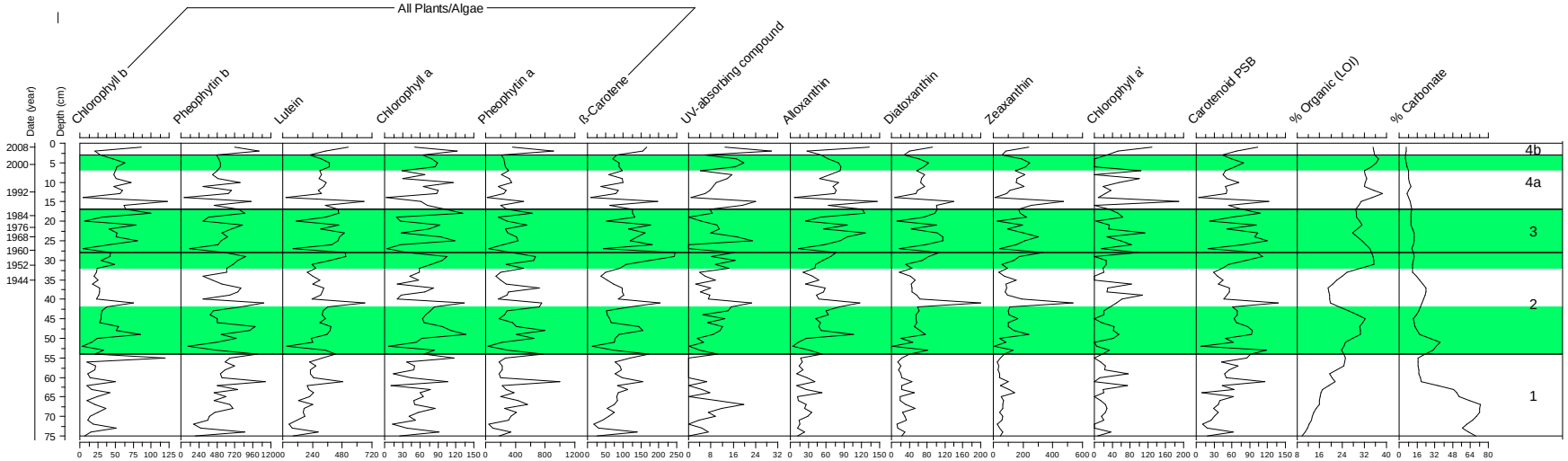


Figure 6.4. Stratigraph showing the fossil pigments (nmol g^{-1} organic matter) of RAIL1. The group 'All plants/algae' includes pigments associated with *Lemna*. The UVR-absorbing compound/pigment can be used to derive a UVR Index (a measure of water clarity). Alloxanthin (cryptophytes), Diatoxanthin (diatoms), Zeaxanthin (cyanobacteria), Chlorophyll *a*' (oxidative degradation product), Carotenoid PSB (purple sulphur bacteria) and lithostratigraphic data (% organic matter, % carbonate) are shown. The green bands show periods of duckweed (*Lemna*) dominance: upper band based upon recorded observations and the *Lemna* indicator metric (Phase 4); lower bands are based upon the *Lemna* indicator metric (Phases 3 & 2). The stratigraph also shows the zones derived from the pigment data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

6.3.2.2 Core RAIL2

Cluster analysis (CONISS) of the sedimentary pigment data revealed three zones for RAIL2 (Fig. 6.5 and see Appendix 4).

Zone 1 (18-8cm, c. 1950-1992)

The '*Lemna* marker' pigments along with the other chlorophyll and carotenoid pigments, including the algal (β-carotene) and bacterial (carotenoid PSB) derivatives all show increasing concentrations in this zone. A sudden increase in organic matter at 14-12cm is mirrored by increases in the key chlorophyll and carotenoid pigments, most notably chlorophyll *b*, chlorophyll *a*, lutein and β-carotene. This overall pattern in sedimentary pigment concentrations is seemingly reversed from the mid section (11cm) until the end of Zone 1 (8cm) as the key chlorophyll and carotenoid pigments ('*Lemna* markers') decrease, as do alloxanthin, diatoxanthin, zeaxanthin and carotenoid PSB. These distinct trends track a sudden decrease in sedimentary organic matter. However, the opposite trend is seen with the chlorophyll *a*', which actually increases. At the transition of Zone 1 and Zone 2 (8cm) the key '*Lemna* marker' pigments significantly increase, in particular the UVR absorbing pigment, but chlorophyll *a*' gradually declines after reaching its maxima at 10cm. Also seen at approximately 10cm in Zone 1 are increases in sediment concentrations of the UVR absorbing pigment, alloxanthin, diatoxanthin, zeaxanthin and carotenoid PSB. However, there is a marked reduction in the chlorophyll pigments and their degradation products in the upper part of this zone, before they increase at the Zone 1/2 transition.

Zone 2 (8-3cm, c. 1992-2005)

Zone 2 starts just prior to the beginning of *Lemna* dominance Phase 4 which starts at 7cm. The '*Lemna* marker' pigments are at their highest concentrations between 8-5cm, and then decline towards the latter section of the zone between 5-3cm. The carotenoids alloxanthin, diatoxanthin, zeaxanthin and carotenoid PSB stabilize and maintain

relatively high concentrations. However, there were marked reductions in both chlorophyll *a*' and the UVR absorbing pigment.

Zone 3 (3-0cm, c. 2005-2010)

Zone 3 commences at the termination of the third *Lemna* dominance cycle (Phase 4) and is characterised by sharp reductions in concentrations of all the sedimentary pigments, especially the '*Lemna* marker' pigments and chlorophyll *a*'.

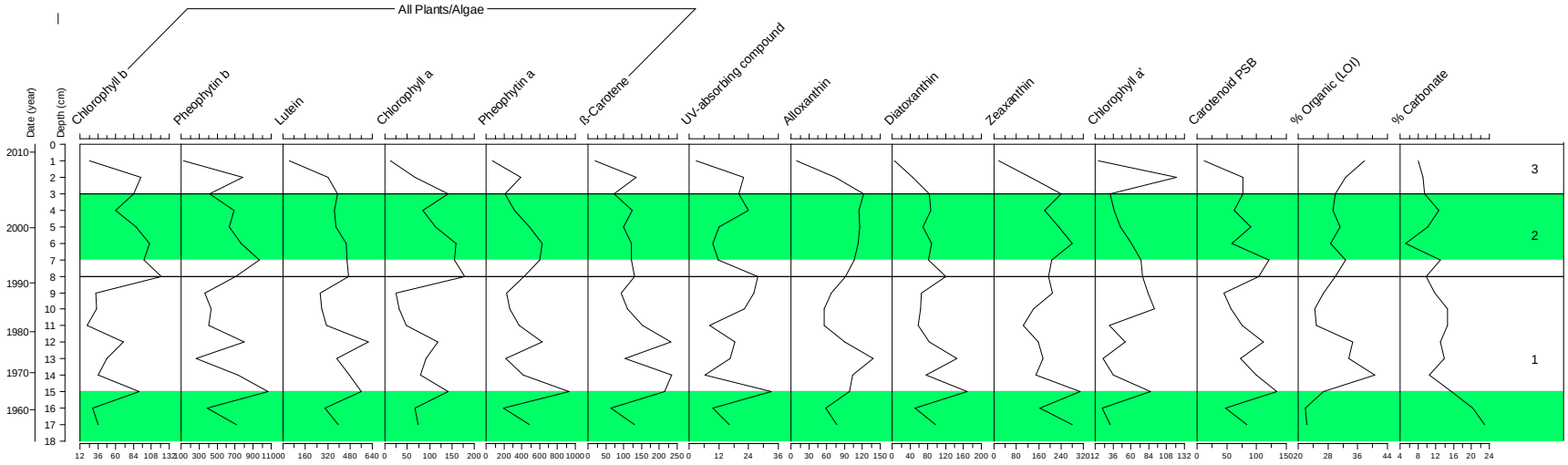


Figure 6.5. Stratigraph showing the fossil pigments (nmol g^{-1} organic matter) of RAIL2. The group 'All plants/algae' includes pigments associated with *Lemna*. The UVR-absorbing compound/pigment can be used to derive a UVR Index (a measure of water clarity). Alloxanthin (cryptophytes), Diatoxanthin (diatoms), Zeaxanthin (cyanobacteria), Chlorophyll *a*' (oxidative degradation product), Carotenoid PSB (purple sulphur bacteria) and lithostratigraphic data (% organic matter, % carbonate) are shown. The two green bands show periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and from the *Lemna* indicator metric [Phase 4]; lower band is based upon the *Lemna* indicator metric [Phase 3]). The stratigraph also shows the zones derived from the pigment data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

6.3.3 Pigment Data Analysis

6.3.3.1 RAIL1: ordination analyses of changes in fossil pigment composition

An exploratory DCA showed that the gradients of pigment response were short with gradient lengths of axes 1 and 2 of 0.549 SD and 0.461 SD respectively. Hence, the linear method of PCA was performed on the fossil pigment data (Lepš & Šmilauer 2003). PCA axes 1 and 2 explained nearly 80% of the variance in the species data. The PCA biplot of axes 1 and 2 shows that the main sedimentary pigments were associated with axis 2 and grouped together in the top L/H quadrant (Fig. 6.6).

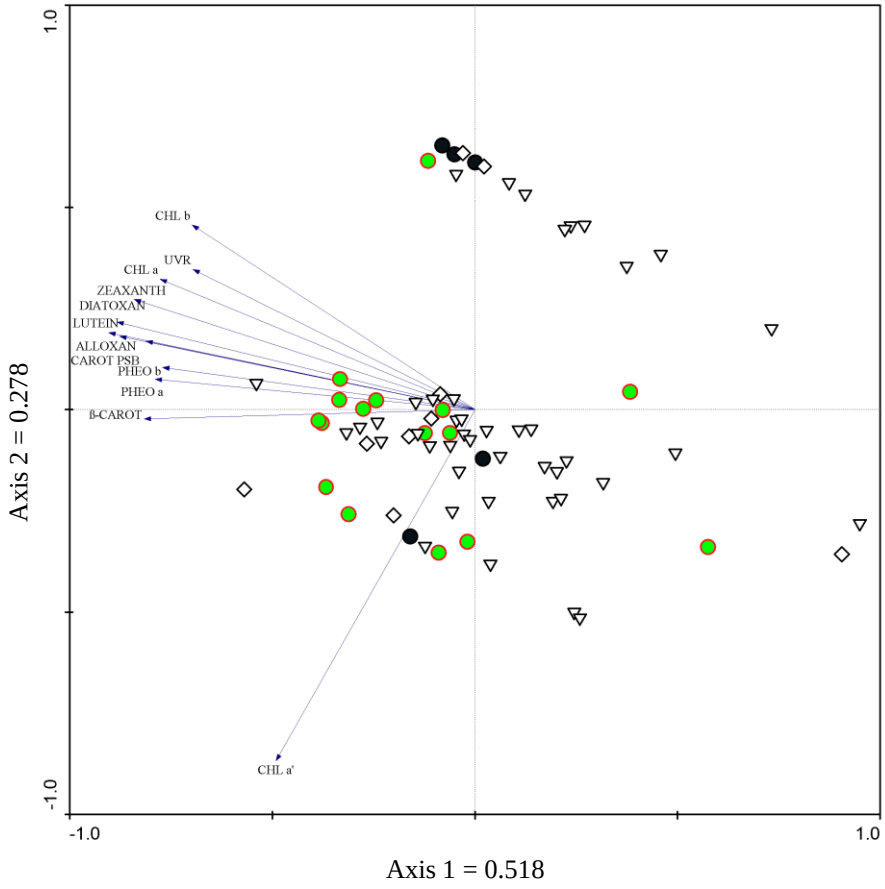


Figure 6.6. PCA biplot on axes 1 and 2 of the sedimentary pigment concentration scores and the sample scores for RAIL1. Chlorophyll *a* (CHL *a*), Chlorophyll *b* (CHL *b*), UVR-absorbing pigment (UVR), Zeaxanthin (Zeaxanth), Diatoxanthin (Diatoxan), Lutein, Alloxanthin (Alloxan), Carotenoid PSB (Carot PSB), Pheophytin *a* (Pheo *a*), Pheophytin *b* (Pheo *b*), Chlorophyll *a*' (CHL *a*') and β-Carotene (β-Carot). Samples: *Lemna* Phase 4 in Zone 4a (solid black circle); between *Lemna* Phases 4 & 3 (Zone 4a), i.e. No *Lemna* (diamond); *Lemna* Phase 2 (Zone 2) (solid green circle); Bottom of core (Zone 1) No *Lemna* (down triangle).

Chlorophyll *a*' (the oxidative degradation of chlorophyll *a*) was strongly associated with axis 1 and was located in the bottom L/H quadrant. The *Lemna*-associated samples in the upper core, i.e. Phase 4, Zone 4 b, denoted by solid black circles (Fig. 6.6) were primarily associated with high concentrations of the main sedimentary pigments; whilst the bottom of core 'no *Lemna'* samples, denoted by down triangles (Zone 1) were associated with relatively low concentrations of the main sedimentary pigments. The PCA biplot shows that chlorophyll *a*' was almost entirely driving the ordination by forcing all the samples containing no chlorophyll a' to the upper quadrants of the ordination space. The pigment data were further explored with PCA axis 1 scores (Fig. 6.7a) which gives an indication of pigment compositional changes (Birks 1987) and total sedimentary pigment concentrations were also calculated (Fig. 6.7b).

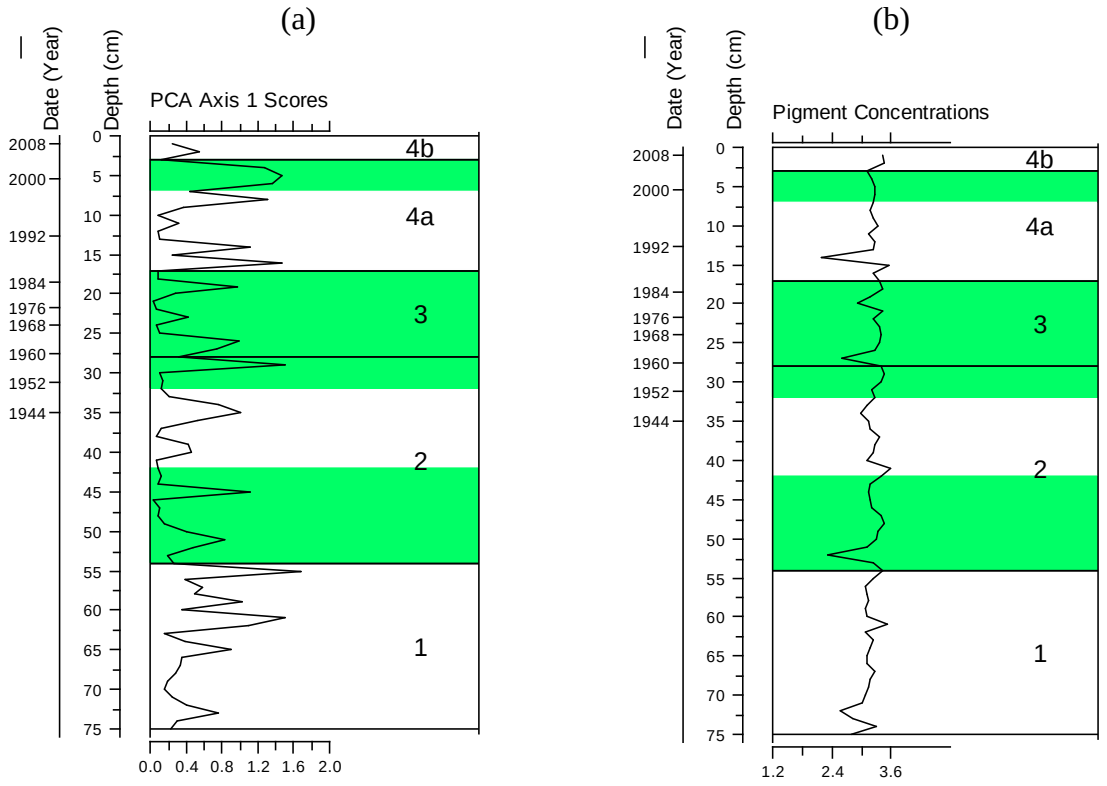


Figure 6.7. Summary diagram of pigment responses for core RAIL1. (a) PCA axis 1 scores of pigment compositional and ecological changes. (b) Total pigment concentration (nmol g^{-1} organic matter) Pigment concentrations were log (x+1) transformed. Zonation (based on cluster analysis of all pigment concentrations) is shown. Shaded areas indicate *Lemna* dominance phases derived from diatom data.

PCA axis 1 scores of the pigment compositional data varied considerably throughout the RAIL1 core with notable peaks and troughs. The distinct peak at 62-61cm is characterised by increases in pigments associated with algae and higher plants (including *Lemna*) with a concomitant increase in sedimentary pigment concentration (Fig. 6.7b). This pattern is repeated with the second distinct peak (56-55cm) and the trough seen immediately after this peak marks the transition between Zones 1 and 2 (Fig. 6.7a) and coincides with the onset of *Lemna* Phase 2. This decrease in PCA axis 1 scores is mirrored by the pigment concentrations (Fig. 6.7b). However, at 41-40cm there is a sudden increase in UVR absorbing pigment and also sudden increases in cryptophytes, diatoms, cyanobacteria and purple sulphur bacteria, reflecting a rapid ecological change in the phototrophic communities. The sudden and marked increase in these pigments coincides with the termination of *Lemna* dominance Phase 2 (Fig. 6.3), and is reflected in a spike in pigment concentrations (Fig. 6.7b).

The peak in PCA axis 1 scores at 29cm coincides with the start of *Lemna* dominance Phase 3 (in Zone 3); the pigment stratigraphy (Fig. 6.3) shows that this is associated with increases in algal and higher plant pigments (including *Lemna*) and also with cyanobacteria, cryptophytes and particularly diatoms and purple sulphur bacteria. It is noticeable that the UVR absorbing pigment becomes virtually extinct at this time, recovers slightly and then is again virtually extinct at the 19cm level, before dramatically increasing immediately after the termination of *Lemna* dominance Phase 3. There is a large trough at 13cm in all of the sedimentary pigment concentrations, followed by sudden increases in concentrations which are reflected in the pronounced spike in sedimentary organic matter at 12cm level (Fig. 6.3).

Interestingly, total pigment concentrations remain fairly constant throughout the core profile, except for 2-3 distinct troughs (Fig. 6.7b). The fossil pigment concentrations of core RAIL1 were further explored by RDA, constrained by the dummy environmental variables '*Lemna*' and 'No-*Lemna*' to determine any potential effects of *Lemna* mats on pigment concentrations (Fig. 6.8). RDA axes 1 and 2 explained over 77% of the variance in the RAIL1 species data.

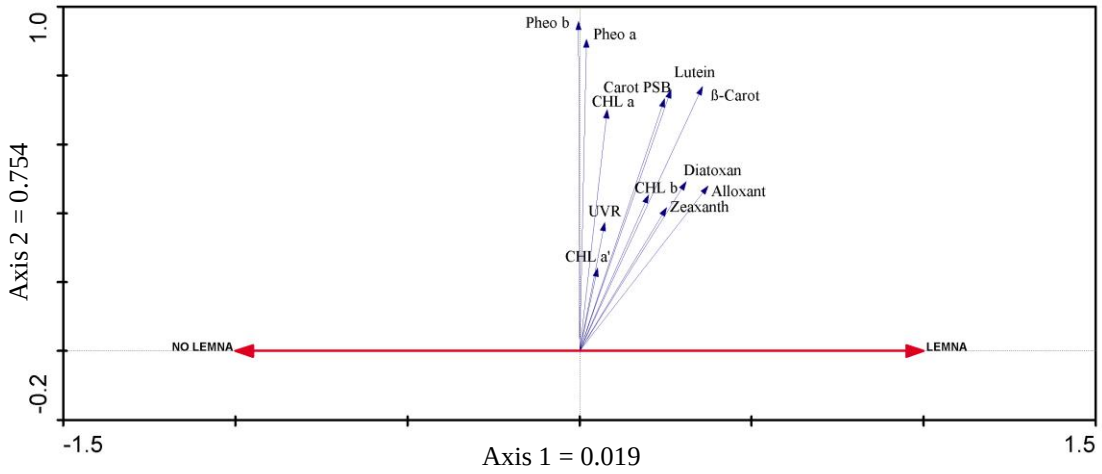


Figure 6.8. RDA plot on axes 1 and 2 of the sedimentary pigment scores for RAIL1. Chlorophyll *a* (CHL *a*), Chlorophyll *b* (CHL *b*), UVR-absorbing pigment (UVR), Zeaxanthin (Zeaxanth), Diatoxanthin (Diatoxan), Lutein, Alloxanthin (Alloxan), Carotenoid PSB (Carot PSB), Pheophytin *a* (Pheo *a*), Pheophytin *b* (Pheo *b*), Chlorophyll *a*' (CHL *a*') and β-Carotene (β-Carot). The constraining environmental variables '*Lemna* and No *Lemna*' are also shown.

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The RDA biplot shows that the pigments were not correlated with the '*Lemna'* or 'No-*Lemna'* variables and appeared to be constrained by other factors. The relationship of the sedimentary pigments recorded from RAIL1 with the '*Lemna'* and 'No-*Lemna'* environmental variables was statistically tested using a Monte Carlo permutation test and no statistical significance was found (p=0.256, F-ratio=1.43, 499 permutations). Overall, there was low correlation between the '*Lemna'* and 'No-*Lemna'* variables and pigment concentrations, suggesting that other phototrophic communities and macrophytes other than *Lemna* were influencing the pigment ordination.

6.3.3.2 RAIL2: ordination analyses of changes in fossil pigment composition

As with RAIL1 an initial exploratory DCA was performed on the sedimentary pigment concentration data to determine gradient length. The short gradient lengths of DCA axis 1 and 2 of 0.549 SD and 0.461 SD, respectively, suggested that linear methods were appropriate for exploring the data further. Hence, an unconstrained PCA was performed on the sedimentary fossil pigment data. PCA axes 1 and 2 explain nearly 80% of the

variance in the species data. The PCA biplot of axes 1 and 2 shows that the main sedimentary pigments were grouped together in the top R/H quadrant (Fig. 6.9).

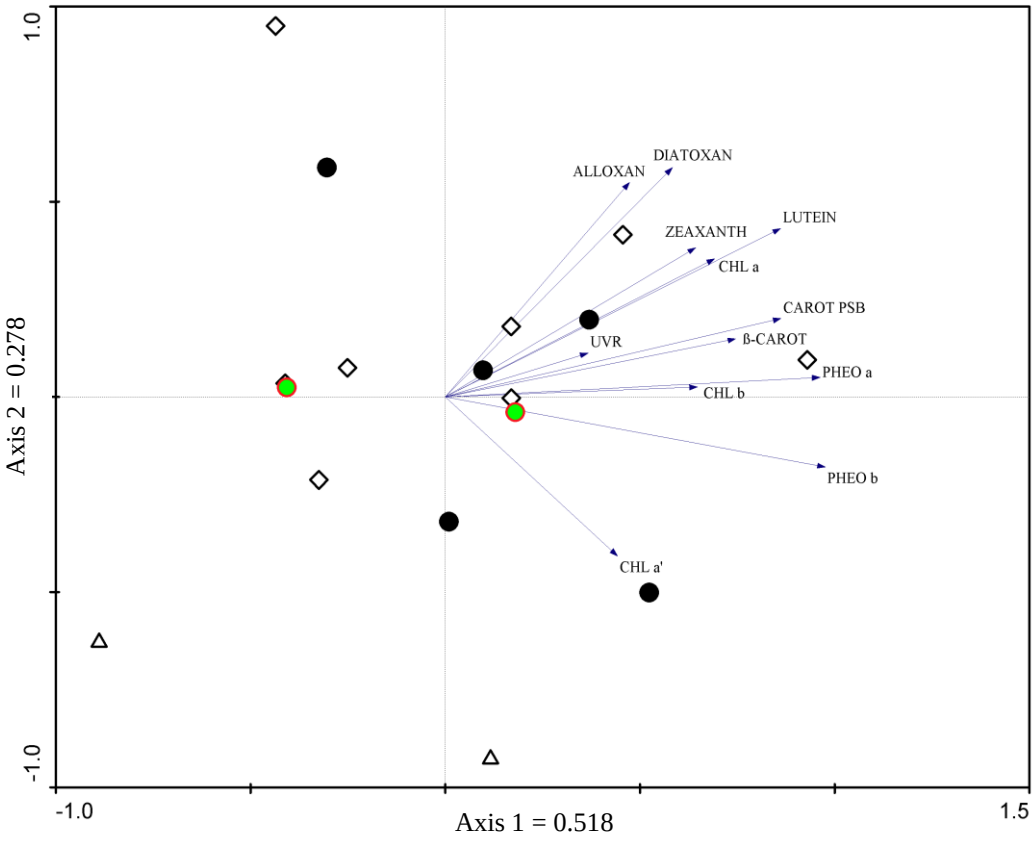

Figure 6.9. PCA biplot on axes 1 and 2 of the sedimentary pigment concentration scores and the sample scores for RAIL2. Chlorophyll *a* (CHL *a*), Chlorophyll *b* (CHL *b*), UVR-absorbing pigment (UVR), Zeaxanthin (Zeaxanth), Diatoxanthin (Diatoxan), Lutein, Alloxanthin (Alloxan), Carotenoid PSB (Carot PSB), Pheophytin *a* (Pheo *a*), Pheophytin *b* (Pheo *b*), Chlorophyll *a*' (CHL *a*') and β-Carotene (β-Carot). Samples: upper core (Zone 3) No *Lemna* (up triangle), *Lemna* Phase 4 in Zone 2 (solid black circle), between *Lemna* Phases 4 & 3 (upper Zone 1) No *Lemna* (diamond), *Lemna* Phase 3 in lower Zone 1 (solid green circle).

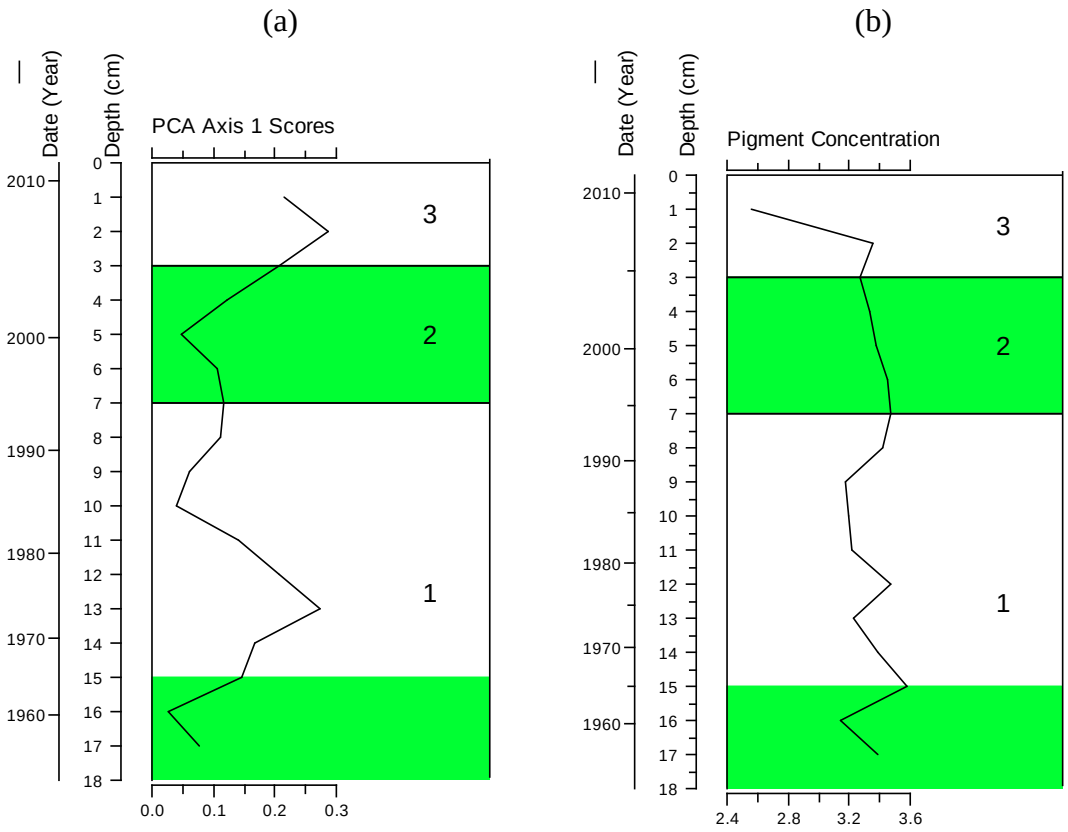

Figure 6.10. Summary diagram of pigment responses for core RAIL2. (a) PCA axis 1 scores of pigment compositional and ecological changes. (b) Total pigment concentration (nmol g^{-1} organic matter). Pigment concentrations were log (x+1) transformed. Zonation (based on cluster analysis of all pigment concentrations) is shown. Shaded areas indicate *Lemna* dominance phases derived from the diatom data.

The PCA axis 1 scores of the pigment compositional data (Fig. 6.10a) show that there was a noticeable compositional change in Zone 1 at 15-10cm which coincided with the ending of *Lemna* Phase 3. The other noticeable compositional change was seen in Zone 2, which was reflected in an increase in pigment concentrations (Fig. 6.10b). This change was associated with increases in cryptophytes, diatoms, cyanobacteria and purple sulphur bacteria, but was also associated with marked decreases in pigments from algae and higher plants (Fig. 6.5). There were also notable increases in the UVR absorbing pigment and chlorophyll *a*' between *Lemna* Phases 3 and 4. The increase in compositional change seen in *Lemna* Phase 4 (Zone 2) reflects the decrease in the UVR absorbing pigment but also increases in diatoms and cyanobacteria (Fig. 6.5).

As with RAIL1, fossil pigment concentrations for core RAIL2 were further explored by RDA, constrained by the dummy environmental variables '*Lemna'* and 'No-*Lemna*' (Fig. 6.11). RDA axes 1 and 2 explain over 25% of the variance in the RAIL2 species data, and the first four axes explain 93% of the variance.

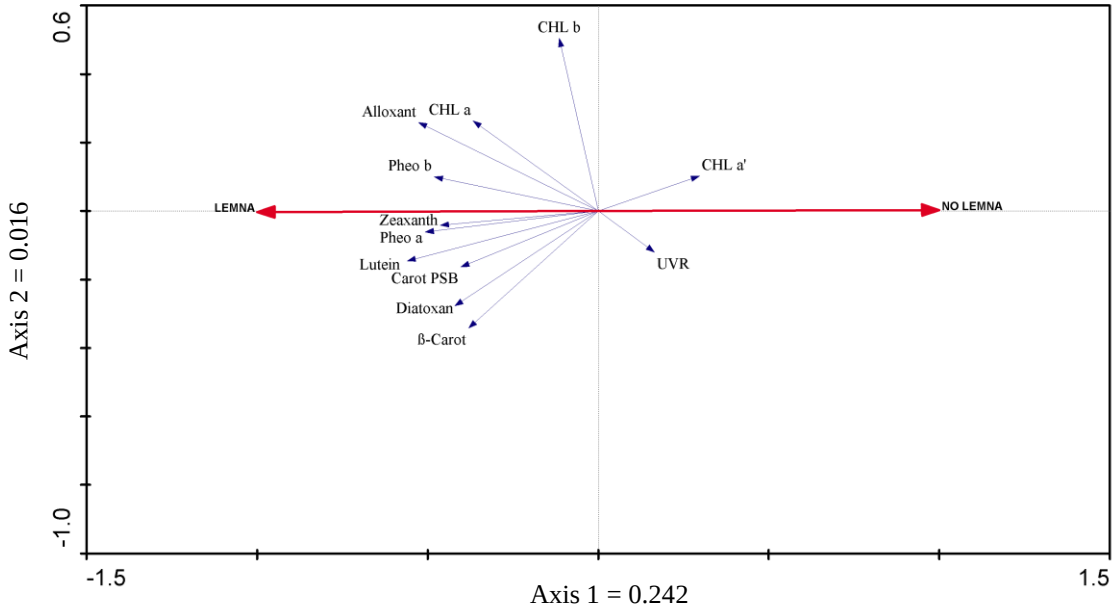

Figure 6.11. RDA plot on axes 1 and 2 of the sedimentary pigment scores for RAIL2. Chlorophyll *a* (CHL *a*), Chlorophyll *b* (CHL *b*), UVR-absorbing pigment (UVR), Zeaxanthin (Zeaxanth), Diatoxanthin (Diatoxan), Lutein, Alloxanthin (Alloxan), Carotenoid PSB (Carot PSB), Pheophytin *a* (Pheo *a*), Pheophytin *b* (Pheo *b*), Chlorophyll *a*' (CHL *a*') and β-Carotene (β-Carot). The constraining dummy environmental variables '*Lemna* and No-*Lemna*' are also shown.

The main sedimentary pigments were well correlated with *Lemna*. However, both chlorophyll *a*' and UVR absorbing pigment (both indicative of clear and oxygenated waters) were more strongly associated with 'No-*Lemna'*. In contrast to the RDA results of RAIL1, there was a statistically significant difference between the '*Lemna*' and 'No-*Lemna* dummy environmental variables when tested with a Monte Carlo permutation test (p=0.02, F-ratio=4.78, 499 permutations), suggesting a clear demarcation where *Lemna* phases were strongly influencing the main sedimentary pigment ordination, whereas pigments associated with clear water conditions (i.e. chlorophyll *a*' and UVR absorbing pigment) were strongly influenced by the absence of *Lemna* phases.

6.3.4 Plant macrofossils analysis

Twelve aquatic plant types and six riparian plant types were represented by macro-remains in core RAIL1 (Fig. 6.12). Images of some of the plant macrofossils recorded from the Rail Pit including submerged, floating and emergent aquatic forms as well as terrestrial species are presented in Figure 6.13. Cluster analysis of the aquatic plant macrofossil data revealed three major zones for RAIL1.

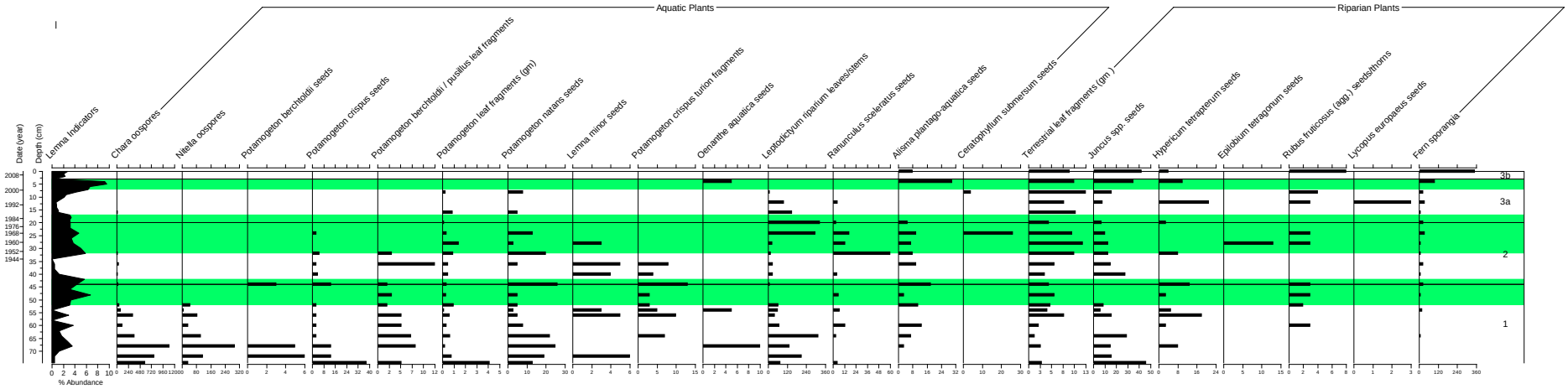

Figure 6.12. Stratigraph showing plant macrofossils for core RAIL1. The macrofossils are presented as numbers per 100 cm^3 of wet sediment and sub-divided into the groups: aquatic plants and riparian plants. The *Lemna* indicator metric is also presented as % relative abundance. The stratigraph also shows the zones derived from the aquatic plant macrofossil data. Shaded areas indicate *Lemna* dominance phases derived from the diatom data. Upper band (Phase 4) derived from recorded observations and the *Lemna* metric, lower bands (Phases 3 & 2) derived from the *Lemna* metric. Both depth (cm) and radiometric dates (year) are presented on the y axis.

Zone 1 (75-44cm)

This zone was characterised by high abundances of *Chara* (>1000 per cm^3) and *Nitella* (>100 per cm^3) oospores. This zone also sees the presence of at least three *Potamogeton* species, namely *Potamogeton berchtoldii* (and possibly *Potamogeton pusillus*), *Potamogeton crispus*, and *Potamogeton natans* with dominance by *P. crispus* and *P. natans*, although the numbers of *P. natans* seeds were drastically reduced between 62-46cm. *Oenanthe aquatica*, with the exception of a singular occurrence at 5-3cm, was only found in this zone. *Rannunculus sceleratus* and *Alisma plantago-aquatica* were also present. The aquatic moss, *Leptodictyum riparium*, was well established (>100 per cm^3) in this zone.

The *Lemna* indicator metric suggests that *Lemna* was present, albeit in low densities, in the lower part of Zone 1, increasing towards the upper part of Zone 1 indicating an early phase of *Lemna* presence but not dominance (Phase 1). This was further corroborated by the presence of *Lemna minor* seeds although the seeds were recorded just before and just after, rather than during, *Lemna* Phase 1. Characeae disappeared with the onset of the first period of *Lemna* dominance (Phase 2). Also noticeable was a decline in the *Potamogeton* species and the aquatic moss, *Leptodictyum riparium.* Riparian plants represented in this zone were *Juncus*, *Hypericum tetrapterum* and *Rubus fruticosus*. The low wet weight of terrestrial leaves, derived from the terrestrial index, suggests that riparian vegetation and trees were not well established at this time.

Zone 2 (44-20cm, c. 1900s-1980s)

P. berchtoldii/*pusillus* disappeared during this zone and *P. crispus* was found in low abundances. To date *P. berchtoldii*/*pusillus* are currently absent from the Rail Pit, but recent surveys have revealed *P. crispus* presence (2010-2012). In contrast, the numbers of *P. natans* increased again in Zone 2. A similar pattern was also seen with *R. sceleratus* and *A. plantago-aquatica*. After briefly disappearing from the macrofossil record at the 52cm level, *L. riparium* returned at 42cm and then increased in the latter period of Zone 2. *L. minor* seeds also returned between 42-38cm but then disappeared

from the macrofossil record and never reappeared. The latter period of Zone 2 saw the first appearance of *Ceratophyllum submersum* seeds which were recorded in high numbers (>25 seeds per cm^3).

The only appearance of the riparian plant *Epilobium tetrapterum* was from Zone 2, whilst Fern sporangia became more abundant in this zone. *Juncus* sp. markedly increased at 42cm but then decreased in the latter period of Zone 2, at the 20cm level. There were also increases in *R. fruticosus* seeds/thorns and also in the wet weight of terrestrial leaves suggesting increasing riparian vegetation and a concomitant increase in tree and shrub cover around the Rail Pit.

Zone 3a (20-3cm, c.1980s-2005)

P. natans persisted but then disappeared from the macrofossil record in Zone 3. The timing of the disappearance of *P. natans* seeds coincided with the disappearance of the *Potamogeton* leaf fragments, which strongly suggests that the *Potamogeton* leaf fragments were, in fact, mostly fragments of *P. natans*. Also disappearing from the macrofossil record at this time were *O. aquatica, R. sceleratus, L. riparium* and *C. submersum*. However, *A. plantago-aquatica* returned to the macrofossil record at the end of *Lemna* Phase 4. There were no *Lemna minor* seeds recorded in this zone.

Zone 3b (3-0cm, c. 2005-2010)

This zone occurred after the termination of the final *Lemna* phase (Phase 4). According to the macrofossil record, the only aquatic plant present at the Rail Pit was *A. plantago-aquatica*. However, recent surveys (2008-2010) recorded *C. submersum, P. crispus* and *Lemna trisulca* (see Table 5.2 above). The riparian plants *Juncus* sp., *R. fruticosus* and Pteridophyte (Fern) species increased in terms of numbers of seeds and sporangia respectively, and followed a similar pattern as seen with the terrestrial leaf fragments which were also more abundant at the top of the core.

Figure 6.13. Fossil plant remains from the Bodham Rail Pit. Top row (L-R): *Leptodictyum riparium* (aquatic moss) stem and leaves *Leptodictyum riparium* leaf, *Potamogeton berchtoldii*/ *pusillus* leaf sheath, *Lemna minor* seed. Second row (L-R): calcified *Chara* oospores, germinating *Potamogeton natans* seed, *Potamogeton berchtoldii* seed. Third row (L-R): *Epilobium tetragonum* seed, *Alisma plantago-aquatica* seed, *Ceratophyllum submersum* seed, *Juncus* sp. seed. Bottom row (L-R): *Schoenoplectus acutus* seed, *Oenanthe aquatica* seed, *Lycopus europaeus* seed, *Betula pendula* seed.

6.3.4.1 Chronological comparison between terrestrial and *Potamogeton* leaf indices and lithostratigraphy

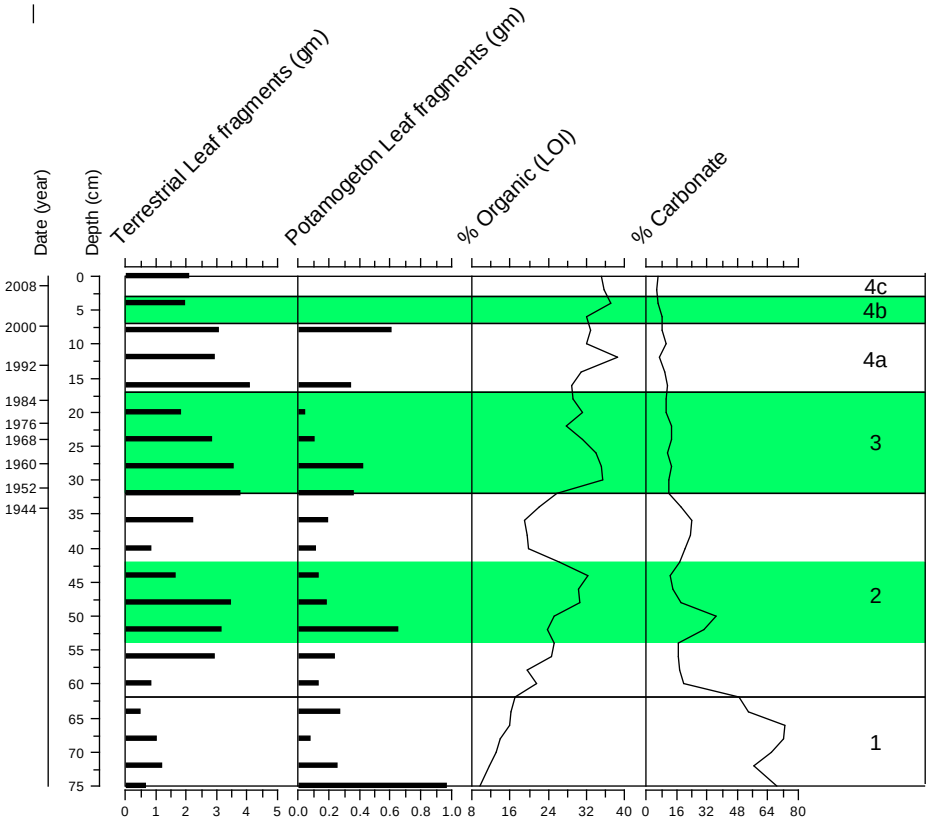

Figure 6.14. Chronologies of terrestrial and *Potamogeton* leaf indices in core RAIL1. The leaf indices are presented as wet weight (g), the lithostratigraphical data are presented as percentages. The zones derived from the diatom data are presented. The green bands denote dominant *Lemna* phases derived from recorded observations and the *Lemna* indicator metric.

Stratigraphic variation of the terrestrial and *Potamogeton* leaf fragments (g wet weight) together with the lithostratigraphic data are plotted in Figure 6.14. No clear association was evident between the *Lemna* zones and the terrestrial leaf fragments. However, it is clear that terrestrial leaves increased over time whereas the weight of *Potamogeton* leaves generally decreased. The trend of sediment organic matter (%LOI) also showed a marked increase over time, paralleling the trend of terrestrialisation.

The striking decrease in organic matter between *Lemna* Phases 2 and 3 (42-32cm) in Zone 2 coincided with the decrease in terrestrial (allochthonous) organic matter, and

equally sediment organic matter rapidly increased when the amount of terrestrial organic inputs increased. At the same time as this decrease in organic matter, there was a concomitant increase in the carbonate content of the sediment which also coincided with small increases in *Potamogeton* leaf mass. Similarly, these patterns of increasing sediment carbonate content at the same time as an increase in the *Potamogeton* leaf index were seen in Zone 1 (75-70cm) and Zone 2 (55-50cm). It is possible that the increase in the *Potamogeton* leaf index, and therefore an increase in *Potamogeton* photosynthesis, resulted in an increase in carbonate. Undoubtedly, the ending of the *Lemna* phases and the die-back of the senescent *Lemna* fronds also likely added to sediment organic matter, potentially indicated by the spike in organic matter in Zone 4a (12cm) following the ending of *Lemna* Phase 4 which coincided with an absence of *Potamogeton* leaves and a slight reduction in the terrestrial leaf index.

6.3.5 Animal macrofossils analysis

There were 16 aquatic animal types recorded from the Rail Pit sediments covering a variety of species/groups (Fig. 6.15 and 6.16). These animal types represented a wide range of animal groups including insects: *Chaoborus*, *Corixidae*, *Sialis lutaria*, and two trichopteran species: *Limnephilus flavicornis* and *Cyrnus trimaculatus*, bryozoans (*Plumatella*), bryophytes (*Leptodictyum riparium*), Crucian Carp (*Carassius carassius*) and crustaceans (ostracods) and *Cladocera* species, including small *Alona*, *Simocephalus* and *Ceriodaphnia* species, *Chydorus sphaericus*, *Daphnia magna*, *Daphnia pulex* and *Daphnia hyalina* and oribatid mites. The cladocerans recorded from the Rail Pit consisted of seven species or species aggregates covering five genera, representing different environmental and ecological conditions. The ostracods were not identified to species level, but were incorporated in the stratigraph.

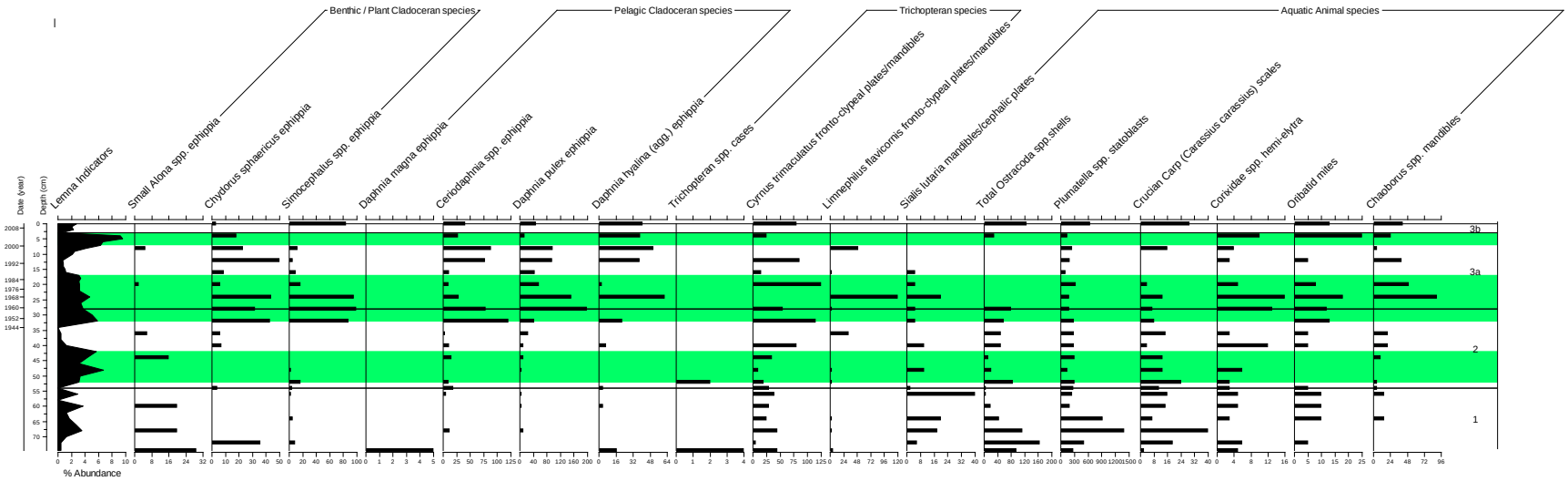

Figure 6.15. Stratigraph showing the animal macrofossils for core RAIL1. The macrofossils are presented as numbers per 100 cm^3 of wet sediment and sub-divided into the four groups: benthic/plant associated cladoceran spp., pelagic associated cladoceran spp., Trichopteran spp., and other aquatic animal spp. The *Lemna* indicator metric is also presented as % relative abundance. Shaded areas indicate periods of *Lemna* dominance phases derived from the diatom data. Upper band (Phase 4) derived from recorded observations and the *Lemna* metric, lower bands (Phases 3 & 2) derived from the *Lemna* metric. The stratigraph also shows the zones derived from the animal macrofossil data. Both depth (cm) and radiometric dates (year) are presented on the y axis.

Zone 1 (75-54cm)

Zone 1 contained macrofossils from a wide range of groups. The cladoceran assemblages were comprised of both benthic and plant-associated species such as small *Alona* species and *Chydorus sphaericus*. The large-bodied pelagic species *Daphnia magna* was recorded from this zone in the lowermost sample only and never returned to the macrofossil record after this singular occurrence.

Invertebrate macrofossils that were well represented in this zone were *Sialis lutaria* and Corixidae species. Similarly cases of the trichopteran *Cyrnus trimaculatus*, ostracods, *Plumatella* (bryozoan) and oribatid mites were also present in relatively high numbers. In contrast, the other trichopteran, *Limnephilus flavicornis*, together with *Chaoborus* sp., were present in relatively low numbers. The Crucian Carp (*C. carassius*) was the only fish species recorded from the macrofossil record and the high abundances of fish scales found suggest that it was well established in the early history of the Rail Pit.

Zone 2 (54-28cm)

There were marked changes in the cladoceran stratigraphy and the numbers of ephippia decreased sharply in this zone. However, towards the latter part of Zone 2 the benthic and plant-associated *Simocephalus* species and *C. sphaericus*, together with the more pelagic-associated *Daphnia* (*D. pulex* and *D. hyalina* agg.) and *Ceriodaphnia* species increased in abundance.

The number of fronto-clypeal plates and mandibles of the trichopteran, *C. trimaculatus* sharply decreased in the lower part of this zone but then increased towards the top of the zone. Further, fronto-clypeal plates and mandibles of the trichopteran, *L. flavicornis* suddenly increased in numbers. The numbers of mandibles and cephalic plates of *Sialis lutaria* were drastically reduced and remained at low numbers during Zone 2. A similar pattern was seen with the numbers of ostracod shells which initially sharply decreased but suddenly increased in the upper part of this zone. Again, this pattern was mirrored

with the numbers of *Plumatella* statoblasts, Corixidae species hemi-elytra and *Chaoborus* species mandibles. The number of Crucian Carp scales also decreased but did, however, remain at relatively high numbers throughout Zone 2. After suddenly disappearing from the profile, oribatid mites returned in the latter part of Zone 2.

Zone 3a (28-3cm, c. 1960-2005)

This zone was composed of *Lemna* dominance Phases 2 and 3 and the 'hiatus' between them. The numbers of *Alona* species ephippia drastically decreased but briefly returned, albeit in small numbers, towards the latter part of Zone 3a before disappearing from the macrofossil record (c. late 1990s). In contrast, numbers of ephippia of the other cladocerans (*C. sphaericus*, *Simocephalus* species, *Ceriodaphnia* species, *D. pulex* and *D. hyalina* agg.) exploded during the early part of this zone, but then they rapidly decreased during the mid interval before rapidly increasing towards the latter part of Zone 3a with the exception of *Simocephalus* species which persisted during the mid interval (18-8cm, c. mid 1980s to late 1990s) but in small numbers. *Alona*, *Simocephalus*, *C. sphaericus*, *Ceriodaphnia*, *D. pulex* and *D. hyalina* (agg.) were absent during the latter part of the zone (7-5cm, c. 1999-2005).

The numbers of fronto-clypeal plates and mandibles of the trichopteran *C. trimaculatus* continued to increase in Zone 3a. However, there was a sudden and marked appearance of fronto-clypeal plates and mandibles of the other trichopteran *L. flavicornis* seen in the early and mid intervals, and then *L. flavicornis* abruptly disappeared from the macrofossil record (c. 1999). A similar pattern to *L. flavicornis* was seen in the timing and in the numbers of *S. lutaria* mandibles and cephalic plates.

Ostracod shells also suddenly disappeared from Zone 3a, but began to reappear, albeit in small numbers, towards the end of the zone coinciding with the termination of *Lemna* Phase 4 (c. 2005). *Plumatella* statoblasts and Crucian Carp scales were consistently present throughout Zone 3a, but were sporadic and present in relatively lower numbers. The number of Corixidae hemi-elytra, oribatid mites and *Chaoborus*

sp. mandibles suddenly and dramatically increased in the early period of Zone 3a (i.e. associated with *Lemna* Phase 3) and persisted throughout this zone but at much lower abundances than previously.

Zone 3b (3-0cm, c. 2005-2010)

The benthic and plant-associated cladocerans, *C. sphaericus* and *Simocephalus* species (except *Alona* species) and the pelagic-associated cladocerans *Ceriodaphnia* species, *D. pulex* and *D. hyalina* agg, (but not *D. magna* which only occurred in the very early history of the Rail Pit) were all prevalent in Zone 3b (c. 2005).

The two trichopterans, *C. trimaculatus* and *L. flavicornis*, exhibited different responses during this zone. The numbers of fronto-clypeal plates and mandibles of *C. trimaculatus* returned to high abundances, but remains of *L. flavicornis* disappeared from the macrofossil record (c. 1999). Similarly, Corixidae hemi-elytra (c. 2005) and *S. lutaria* mandibles and cephalic plates (c. mid 1980s) also disappeared and were absent from Zone 3a. In contrast, ostracod shells, which had virtually disappeared in Zone 3a, returned in large numbers in Zone 3b (c. 2005).

Both the number of *Plumatella* statoblasts and Crucian Carp scales returned to relatively high numbers in Zone 3a (similar to those seen in earlier zones) as was the case with oribatid mites and *Chaoborus* sp. mandibles.

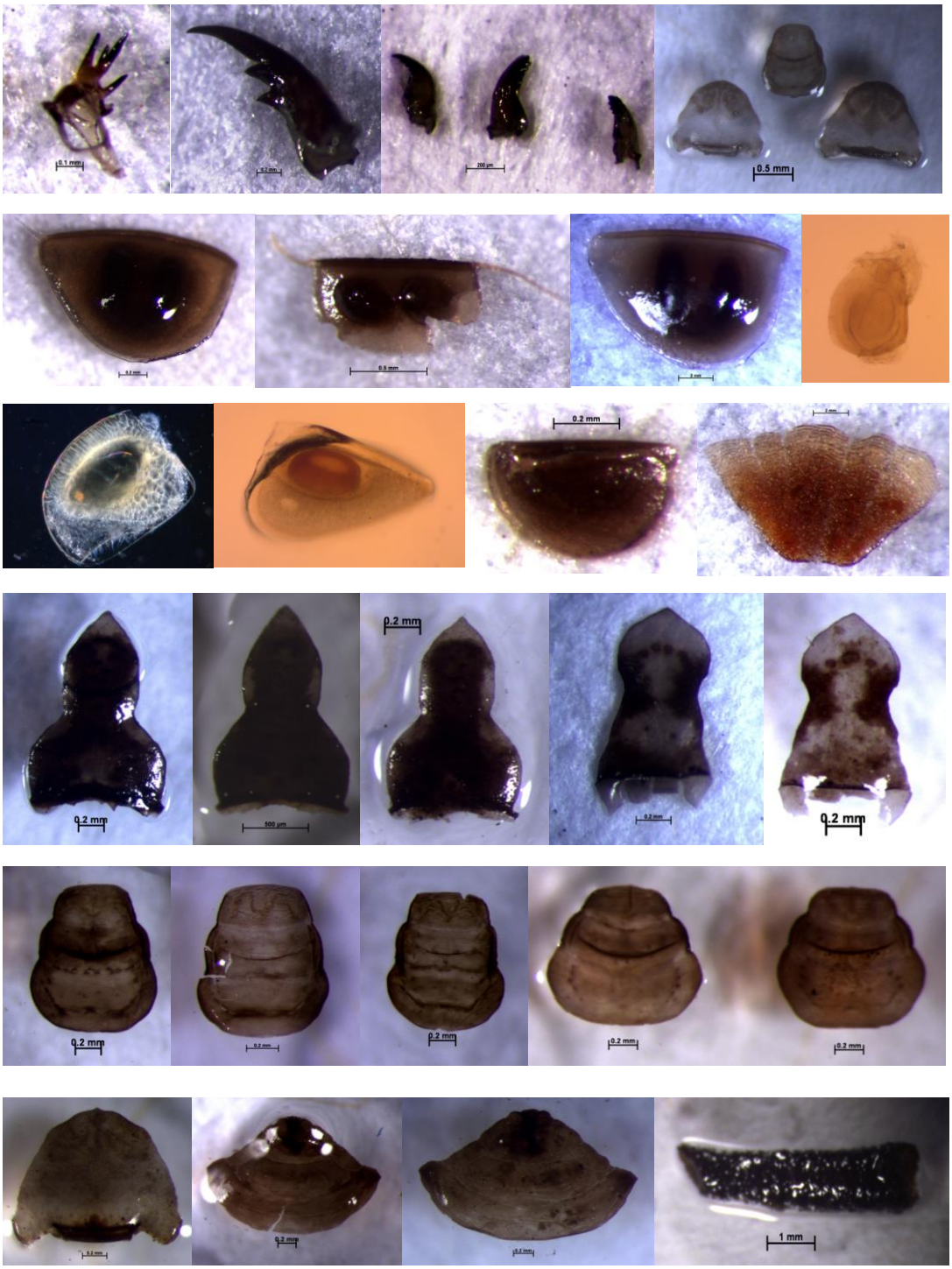

Figure 6.16. Fossil animal remains from the Bodham Rail Pit. Top row (L-R): larval mandibles of *Chaoborus* sp. (Diptera), *Sialis* (Megaloptera), caddisfly (Trichoptera) and cephalic plates of *Sialis* larvae. Second row (L-R): cladoceran ephippial egg cases of *Daphnia hyalina* agg., *Daphnia magna*, *Daphnia pulex*, *Chydorus sphaericus.* Third row (L-R): *Alona* sp, *Simocephalus* sp., *Ceriodaphnia* sp., and Crucian Carp (*Carassius carassius*) fish scale fragment. Fourth row (L-R): larval frontoclypeal apotomes of *Limnephilus flavicornis* (1-3) *Cyrnus trimaculatus* (4-5). Fifth row: unidentified larval thoracic and cephalic nota of Insect spp., Sixth row (L-R): cephalic plate of *Sialis* larva, unidentified Insect body-parts (2-3) and larval case of cased-caddis (Trichoptera) sp.

The results of the plant and animal macrofossil analyses revealed that most of the macrofossil types were often identified to genus or species level and, therefore, this increased taxonomic resolution was able to provide a more detailed reconstruction of the past animal and vegetational communities.

6.3.6 Multi-proxy data analyses

The PCA axes 1 and 2 sample scores were calculated for the fossil diatom data without inclusion of the two diatom species associated with *Lemna*, i.e. *Lemnicola hungarica* and *Sellaphora seminulum*, as the relative percentage abundances of these taxa were summed to provide the *Lemna* indicator metric. This *Lemna* indicator metric was presented alongside the PCA axes 1 and 2 scores of the other fossil groups to provide the historical timing of past *Lemna* dominance periods and to allow biological structural changes in relation to past duckweed (*Lemna*) dominance to be explored. It was found that the first and second axes explained a large amount of the variation in the various species compositional changes.

The gradient lengths of DCA axes 1 and 2 for the plant macrofossil data were 3.048 SD and 1.744 SD respectively, and both axes 3 and 4 were also similar in length to axis 2 at 1.677 and 1.084 respectively. As the axes lengths were all less than 4.0 SD units the use of linear methods was considered appropriate to explore the plant macrofossil data. The eigenvalues of the first four DCA axes explained nearly 50% of the variability in the species data, with axes 1 and 2 explaining most (i.e. >40%) of the cumulative species variation. Subsequently, PCA was employed to explore the patterns in the plant macrofossil record of RAIL1, and the first two PCA axes explained almost 99% of the variance of the species data.

The gradient lengths of DCA axis 1 and 2 for the cladocerans were 3.286 SD and 1.451 SD respectively, and both axes 3 and 4 were also similar in length to axis 2 at 1.371 SD and 1.127 SD units respectively. The gradient lengths of DCA axes 1 and 2 for the other animal taxa were 1.022 SD and 0.665 SD units, and again, both axes 3 and 4 were

similar in length to axis 2 at 0.585 SD and 0.548 SD units. As the axes lengths were all less than 4.0 SD units the use of linear methods (i.e. PCA) was considered appropriate to explore the animal macrofossil data. The eigenvalues of the first four DCA axes for the cladoceran taxa explain nearly 70% of the variability in the species data, with axis 1 and 2 explaining most (i.e. >60%) of the cumulative species variation. The eigenvalues of the first four DCA axes for the other animal taxa also explain nearly 70% of the variability in the species data, with axis 1 and 2 explaining most (i.e. nearly 60%) of the cumulative species variation. For the cladoceran data, the first two PCA axes explained over 85% of the variance of the species data, and for the other animal data, the first two PCA axes explained over 98% of the variance of the other animal group data.

In summary, PCA axes 1 and 2 scores explained: i) nearly 55% of the diatom community variation, ii) nearly 80% of the plant pigment compositional variation, iii) over 85% of the cladoceran compositional variation, iv) over 98% of the other animal (i.e. other than zooplankton) compositional variation and v) nearly 99% of aquatic plant compositional variation.

The fundamental purpose of the analyses of the various microfossil and macrofossil data was to explore potential changes in palaeoecological community structure and function and to ascertain whether the identified periods of *Lemna* dominance, derived from the diatom analyses (see Chapter 5), were driving ecological changes in the Rail Pit. To this end, the summary statistics of the PCA axes 1 and 2 sample scores for all of the main taxa included in the analyses are presented simultaneously to facilitate interpretation. The PCA axes scores summarise compositional variation in species data and provide information on the degree of concordance between the timing of major changes in the different biological groups: diatoms, pigments, plant macrofossils and animal macrofossils (Fig. 6.17).

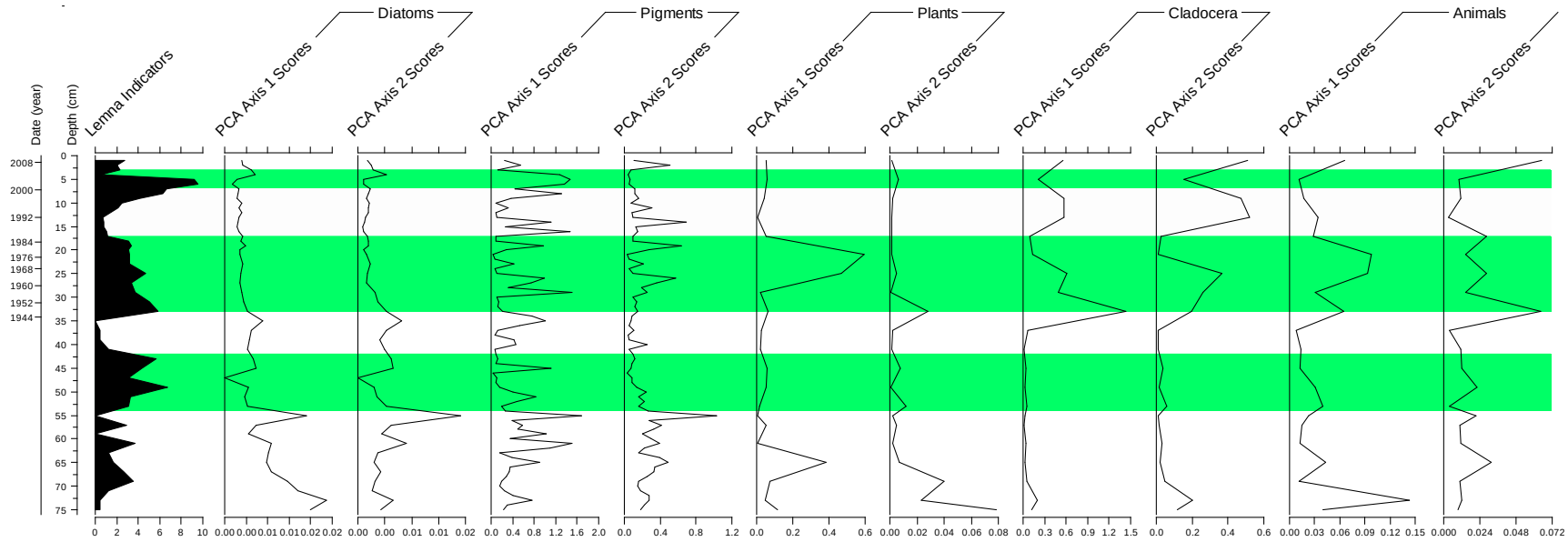

Figure 6.17. Stratigraph showing PCA axes 1 and 2 sample scores for diatoms, plant pigments, plant macro-remains, cladoceran ephippia and animal macrofossils for core RAIL1. The *Lemna* indicator metric is also presented as % relative abundance. The green bands show periods of duckweed (*Lemna*) dominance (upper band based upon recorded observations and from the *Lemna* indicator metric [Phase 4]; lower bands are based upon the *Lemna* indicator metric [Phases 3 & 2]). Both depth (cm) and radiometric dates (year) are presented on the y axis.

6.3.6.1 Synchronicity of biological change in relation to past *Lemna* dominance

- **Diatoms**

The broad pattern of diatom compositional and ecological change reflects: i) the establishment of benthic and epipelic communities in the early history of the Rail Pit (Figs. 5.15, 5.16), ii) the marked reduction in these benthic and epipelic communities coincident with the establishment of aquatic macrophytes (65-60cm; Zone 1; Figs. 5.16, 6.12), iii) an increase in epiphytic communities and the *Lemna* indicator diatoms *L. hungarica* and *S. seminulum* in particular at 65-42cm (Zone 2; Figs. 5.15, 5.16), iv) the appearance of planktonic communities, most notably between the *Lemna* dominance phases 42-32cm (late Zone 2; Figs. 5.15, 5.16) and 17-7cm (Zone 4a; Fig. 5.16), and v) the development of co-dominance of benthic, epiphytic and planktonic communities as a response to increasing eutrophication and the *Lemna* dominant Phases 2, 3 and 4 from 42cm to the top of the core (Zone 3 to Zone 4c; Figs. 5.15, 5.16). The record of planktonic diatom species suggests that there was no clear evidence of any switches between macrophyte dominance and open-water phytoplankton dominance in the past ecology of the Rail Pit (Figs. 5.16, 5.18).

- **Pigments**

As with PCA scores on axes 1 and 2 for the diatom communities, the PCA axes 1 and 2 scores of the pigments showed similarities in chronological pattern (Fig. 6.17): there were i) striking peaks (and low troughs) in pigment compositional and ecological changes, ii) the distinct peak at 62-61 cm (Zone 1) was characterised by increases in the pigments associated with algae and higher plants (including *Lemna*) with a concomitant increase in sedimentary pigment concentration (Fig. 6.4), iii) this pattern is repeated with the second distinct pigment peak (56-55cm) and the distinct trough seen immediately after this peak marks the transition between Zones 1 and 2 and coincides with the onset of *Lemna* Phase 2 (Fig. 6.4). This distinct trough in PCA axis 1 scores (Figs. 6.7a, 6.17) is mirrored with the equally distinct trough in pigment concentrations,

iv) at 41-40cm (Zone 2) there is a sudden increase in UVR absorbing pigment and also sudden increases in cryptophytes, diatoms, cyanobacteria and purple sulphur bacteria (Fig.6.4). This sudden and dramatic increase in these particular pigments coincides with the termination of *Lemna* Phase 2 (42cm), v) the peak at 29cm coincides with the establishment of *Lemna* dominance Phase 3 (Zone 3) which is associated with increases in algal and plant pigments (including *Lemna*) and also with cyanobacteria, cryptophytes and particularly diatoms and purple sulphur bacteria, and vi) the UVR absorbing pigment becomes virtually extinct at this time (25 cm, Zone 3), recovers slightly and then is again virtually extinct at the 19cm level, and then dramatically increases immediately after the termination of *Lemna* dominance Phase 3 (Figs. 6.2b, 6.4).

- **Aquatic plants**

The PCA axes 1 and 2 scores of the plant macrofossils (Fig.6.17) shows: i) distinct peaks in the early period (Zone 1) which reflects the increase in the Potamogetonaceae (*P. berchtoldii*, *P. pusillus*, *P. crispus* and *P. natans*), *Lemna minor*, the aquatic moss *L. riparium* and predominantly the dense beds of *Chara* and *Nitella* (Fig. 6.12), where this increase in Charophyta is also shown by the increase in carbonate from the sediment record (Fig. 6.14), there follows ii) a marked reduction in the PCA axes scores which coincides with the establishment of *Lemna* Phase 2 (Fig. 6.17) and a concomitant decrease in the Charophyta and both *P. berchtoldii* and *P. pusillus*, iii) the relatively small peaks in the PCA axes scores at this time probably reflects *Lemna* dominance Phase 2 and the presence of *P. natans* and *P. crispus*, whereas the small peak in PCA axis 2 located between *Lemna* Phases 2 and 3 is probably due to the sudden increase and return of *P. berchtoldii* and *P. pusillus* after the ending of *Lemna* Phase 2 and before the advent of *Lemna* dominance Phase 3 (Figs. 6.12, 6.17), this is followed by iv) a large and pronounced peak of the PCA axis 1 scores which coincides with and tracks *Lemna* dominance Phase 3, which includes the presence of *P. natans*, *R. sceleratus*, *A. plantago-aquatica*, *L. riparium* and the first and notable appearance of the submerged plant *C. submersum*, thereafter, v) the PCA axes 1 and 2 scores are

suddenly and drastically reduced and maintain at low levels after the completion of *Lemna* dominance Phase 3 as most of the aquatic plants have disappeared by this stage, then vi) there is a slight increase in the PCA axes scores which again coincides with, and reflects, the presence of *Lemna* dominance Phase 4 and also increases in riparian plants, particularly *Juncus* sp. and Ferns.

- **Cladocerans**

The cladoceran PCA axes 1 and 2 scores are remarkably similar to each other in their respective chronological profiles (Fig. 6.17). There were: i) increases in the PCA axes scores at the base of the core (75-70cm, Zone 1) which corresponds primarily to the increase in the benthic and plant-associated *Alona* species and *C. sphaericus*, and the pelagic associated *D. magna* and *D. hyalina* (agg.), then ii) there was a marked reduction in the PCA axes scores except for two relatively small peaks during *Lemna* Phase 2, which coincides with increases in *Alona*, and *Simocephalus* species (i.e. benthic and plant-associated taxa) and also *Ceriodaphnia* species (i.e. pelagic taxa), notably there were iii) sudden and large increases in PCA axes scores between *Lemna* dominance Phases 2 and 3, which saw increases in *C. sphaericus* and *Simocephalus* species (benthic and plant-associated taxa) and also increases in *Ceriodaphnia* species, *D. pulex* and *D. hyalina* agg. (pelagic taxa), however, there followed iv) a sudden and large decrease in the PCA axes scores during *Lemna* dominance Phase 3, reflecting the equally sudden and large decrease in both the benthic/plant-associated and the pelagic cladoceran communities, and v) this pattern of large increases in PCA axes scores between *Lemna* dominance Phases 2 and 3 followed by a decrease within the duration of the *Lemna* phases was repeated to a greater extent with *Lemna* Phases 3 and 4 and the 'hiatus' between them where, vi) *C. sphaericus* and the pelagic taxa in particular increased between the *Lemna* dominance phases and then underwent a very marked decrease to virtual absence during *Lemna* dominance Phase 4, and then vii) immediately after the ending of *Lemna* dominance Phase 4 all of the cladoceran taxa increased, except for *Alona* species (Fig. 6.12).

- **Other animals**

The pattern of compositional and ecological change exhibited by the other animal taxa was remarkably similar to that seen with the cladoceran PCA axes scores (Fig. 6.17) as: i) there was a large peak in PCA axis 1 scores (75-70cm, Zone 1) which corresponds primarily to the ostracod and *Plumatella* communities and Crucian Carp (*C. carassius*), then ii) smaller peaks in both PCA 1 and 2 axes scores (65-60cm, Zone 1) reflecting the insect communities of *S. lutaria*, Corixids and oribatid mites, followed by iii) a relatively small peak in PCA axis 2 immediately before the start of *Lemna* Phase 2 which signals the onset of *Chaoborus* species, and maxima of *S. lutaria*, there follows iv) small peaks in axes scores during *Lemna* Phase 2 which reflects increases in Crucian Carp and Corixids, before v) large peaks on both PCA axes 1 and 2 scores between *Lemna* dominance Phases 2 and 3 (Zone 2) which then rapidly decline with the commencement of *Lemna* dominance Phase 3, reflecting the rapid increase across most of the other animal communities, however there is vi) a rapid increase seen amongst the insect taxa, particularly *Chaoborus*, Corixids, *S. lutaria* and also oribatid mites where a large peak in PCA axes scores coincides with the greatest maxima of the trichopteran *L. flavicornis*; the changes in the PCA axes scores following the end of *Lemna* dominance Phase 3 (Zone 3) reflect vii) the fluctuations of the majority of the animal taxa, but also relates to the disappearance of *S. lutaria* (which never recovered) and ostracods, which provides evidence of the response of these particular communities to *Lemna* dominance Phase 3, thereafter viii) the PCA axes scores tail off sharply during *Lemna* dominance Phase 4 as most of the animal taxa are either absent or recorded at low abundances, and then ix) the PCA axes scores increase after the end of *Lemna* dominance Phase 4 which sees the return of *C. trimaculatus*, ostracods, *Plumatella*, Crucian Carp, oribatid mites and *Chaoborus*, but *L. flavicornis*, *S. lutaria* and the Corixids seemingly disappeared from the macrofossil record (Fig. 6.15).

6.3.6.2 Summary of the main plant and animal compositional and ecological changes

The *Lemna* phases clearly had significant effects on the submerged and floating-leaved species. Firstly, the Characeae (*Chara* and *Nitella*) disappeared with the onset of *Lemna* Phase 2 (54-42cm) but they were largely unaffected by the earlier *Lemna* presence (Phase 1) at 72-58cm (the *Lemna* indicator metric suggested a non-dominance presence of *Lemna* at this time). Secondly, the *Potamogeton* taxa *P. berchtoldii* and *P. pusillus* were drastically reduced during *Lemna* Phase 2 (54-42cm), then completely disappeared by the time of *Lemna* Phase 3 (c. late 1980s). *Lemna* Phase 3 coincided with the disappearance of *P. crispus*, whilst *P. natans* was reduced and then disappeared with the onset of *Lemna* Phase 4 (c. 1999-2005). All four *Potamogeton* taxa had disappeared from the Rail Pit by the late 1990s (8cm) and, with the exception of a small bed of *P. crispus* that returned in 2009 (Fig. 5.2), these aquatic pondweeds have, to date, never returned to the Rail Pit.

Similar effects were seen on other aquatic plants where *R. sceleratus*, *A. plantago-aquatica* and the bryophyte *L. riparium* disappeared after *Lemna* Phase 3 (although *A. plantago-aquatica* returned following *Lemna* Phase 4) whilst *O. aquatica* disappeared after *Lemna* Phase 2 but also returned following *Lemna* Phase 4. *C. submersum* first appeared, and then disappeared, during *Lemna* Phase 3 then briefly showed signs of recovery between *Lemna* Phases 3 and 4 but disappeared with the onset of *Lemna* Phase 4. However, *C. submersum* is now the dominant aquatic plant (see Table 5.2, Chapter 5) as the domination of *Lemna* phases abruptly ended in 2005.

The pattern with aquatic plant communities was also seen with the animal communities. For example, ostracods were present in high abundances in the earliest periods (i.e. pre-*Lemna* phases), then reduced with *Lemna* Phase 2, appeared to recover after this *Lemna* phase ended (54cm) but disappeared with the onset of *Lemna* Phase 3 (c. late 1980s) and only returned to former high abundances after the termination of *Lemna* Phase 4 (2005). Similarly, *Plumatella* were also very abundant in the early pre-*Lemna* period

(i.e. 1000-1500 statoblasts per 100cm^3) but drastically reduced in abundances with the timing of *Lemna* Phase 2 (i.e. <300 statoblasts per 100cm^3), and although present throughout the core profile (RAIL1) they never recovered their previous abundances. Interestingly, there were signs of recovery between *Lemna* Phases 2 and 3 (42-32cm, c. late 1980s-1999) and with the ending of *Lemna* Phase 4 (2005) *Plumatella* returned to high abundances as seen in previous pre-*Lemna* phases.

6.3.7 Summary of palaeoecological patterns using cluster analysis

The results of the numerical zonation of diatoms, pigments, cladocerans and macrofossil plants and animals (Fig. 6.18) show that there were three or four major zones indicated by CONISS for all groups with the final zone divided into one or more sub-divisions.

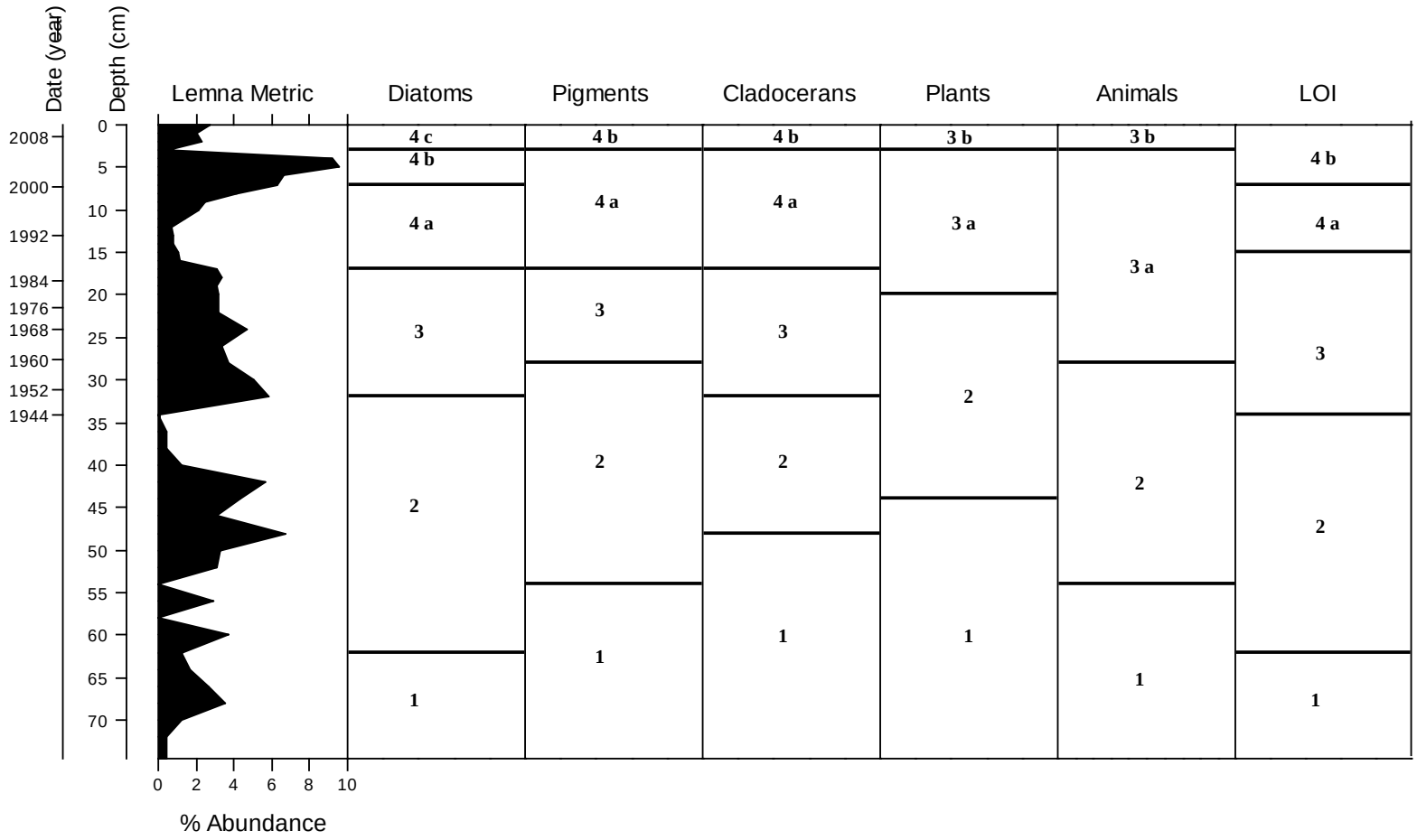

Figure 6.18. Summary diagram of the numerical zonation (CONISS) of fossil diatoms (excluding the *Lemna* indicator diatoms), fossil pigments, sub-fossil cladocerans, macrofossil plants, macrofossil animals and LOI (loss-on-ignition) data from core RAIL1. The *Lemna* indicator metric is shown as a direct comparison of the zonations. Both depth (cm) and radiometric dates (year) are presented on the y axis.

Despite considerable variation in the timing of the zone boundaries there was some concordance in responses across the various fossil groups. For example, the most recent sub-zone (i.e. post *Lemna* Phase 4, 1999-2005) was identical for all five biological groups (diatoms, pigments, cladocerans, plants and animals). The zone at 1980s to 2000 was also very similar across all biological fossil types and LOI, including identical timing for pigments and cladocerans, whilst the diatom sub-zone 4a coincided with the peak shown in the *Lemna*-indicator metric. By incorporating the *Lemna*-indicator metric with the CONISS zones it was possible to directly compare the timing of the zones of the various fossil groups and LOI with the *Lemna* phases.

The diatom and LOI zonations were remarkably similar, particularly with Zone 1 (75-62cm) and were almost identical in their chronological timing. The plant and pigment zonations were in general agreement with respect to their timing even though the pigment zones were calculated from all of the various pigment data, including alloxanthin (cryptophytes), diatoxanthin (diatoms), carotenoid PSB (purple-sulphur bacteria) and zeaxanthin (cyanobacteria) rather than just plant pigments. Zones 2 of the pigment and animal groups were identical in their timing (54-27cm), whilst for the same zone there was high concordance seen in the diatom and LOI responses (62-33cm). There were identical responses of the timing with diatom and cladoceran groups in Zone 3 (c. 1950s to 1980s).

The most striking feature is the high degree of concordance between the timing of the zones for all biological groups and LOI with that of the *Lemna* phases (Fig. 6.18). This suggests that the *Lemna* dominant phases could at least be partly responsible for some of the variation in the palaeoecological data.

6.4 Discussion

6.4.1 Palaeolimnological potential of small ponds

The literature is graced by many palaeolimnological studies that have focussed on both deep and shallow lakes but, to date, there have been very few similar studies of small ponds. This palaeolimnological study of the Rail Pit, Norfolk, is amongst the first to champion the potential of palaeolimnology in small ponds. The successful collection of both a long Big Ben core (RAIL1) and a short Glew core (RAIL2) demonstrate that palaeolimnological techniques can be applied, with confidence, to small water-bodies. Although the ^{210}Pb dating was unable to provide a complete radiometric chronology (partly owing to an apparent a sediment slump event at the 54cm level), it was nevertheless, able to successfully provide a reliable sediment chronology back to 1939 ± 11 for core RAIL1 and 1948 ± 10 for RAIL2. Moreover, it was also possible to chronologically correlate RAIL1 and RAIL2 cores from diatom and lithostratigraphical analyses, which is at least as good as is seen in the dating of shallow lakes.

There was a degree of uncertainty, at the outset of the study with regards to the integrity of the core record and whether it could be used for a multi-proxy palaeoecological analysis to reconstruct ecological change with confidence. However, the successful diatom analysis covering the entire length of core RAIL1 provided confidence in this and, furthermore, the consistently high diatom accumulation rates seen throughout the core profile (except for the anomalous 54cm level) indicate that dissolution and poor preservation were not a cause for concern. In essence, cores from small ponds preserve diatoms, pigments and a full range of macro-remains and the small size and high degree of water-level change in ponds does not preclude them from palaeoecological analyses.

Recently there have been calls for the integration of ecological and experimental investigations with palaeolimnological studies (Brodersen *et al.*, 2004, Saros 2009), and in turn the combination of contemporary ecology and palaeolimnology to better understand shallow lake ecosystem change (Sayer *et al.*, 2010a). As contemporary

ecological and monitoring studies seldom extend beyond a few decades they are unable to show how lakes (and ponds) change over longer timescales resulting from environmental stressors such as eutrophication and climate change. Palaeolimnological techniques can compliment contemporary ecological studies by placing these studies into a continuous historical context by providing understanding of biological responses and ecosystem changes over longer (decadal-centennial) timescales. The ecological snap-shots provided by contemporaneous experiments (e.g. diatom ecology) and macrophyte surveys (e.g. recent *Lemna* phases) is easier to understand when viewed within the setting of the director's cut of the full palaeolimnological movie. This study attempted to rally to this ecological 'clarion call' by combining experimental studies, contemporary biotic and abiotic surveys and palaeolimnological investigations to fully understand long-term ecosystem change in a small pond and specifically to develop and test ecological theories by modelling the effects of past *Lemna*-dominance phases.

Recent plant observations and surveys (Table 5.2, Chapter 5) in the Rail Pit are largely in agreement with the aquatic vegetation reconstruction. Firstly, the disappearance of *P. natans* from the macrofossil record in the late 1990s accords well with recent macrophyte observations which document the disappearance of *P. natans* after 1999. Secondly, *C. submersum* also declined in the macrofossil record in the late 1990s, corroborated by the macrophyte surveys which documented *C. submersum* disappearance over 1999-2008. It returned to the pond in 2008, but interestingly there were no *C. submersum* plant remains recorded from the corresponding surficial sediments. Similarly, recent surveys recorded *Cladophora* (2007-2012), *L. trisulca* (2010) and *P. crispus* (2009) none of which were recorded by macrofossils in the recent sediments. It is possible that, as *C. submersum* was only identified from the deposition of seeds (no sedimentary leaf fragments were found) insufficient time was available for seed production and deposition. Alternatively, and perhaps more plausibly, because the upper 20cm of RAIL1 was so fluid it is possible that seeds 'slipped down' through these fluid surface sediments which were largely formed by rapid breakdown of the senescent *C. submersum* vegetation. *P. crispus* was invariably rare (DAFOR=1) or occasionally (DAFOR=2) found which would account for its absence from the

macrofossil record. *L. trisulca* rarely leaves vegetative remains and seeds, and given its rarity (DAFOR=1) in 2010, it is not surprising that it was not recorded in the macrofossil data. Similarly, *Cladophora* is not known to leave remains in sediments.

An area of concern with respect to sampling in palaeolimnology is the faithfulness with which sedimentary samples represent extant biological communities, particularly given spatial heterogeneity (patchiness) across a site. A recent study of relationships between contemporary macrophytes and macrophyte fossil remains in a shallow lake found that sediment samples best represent meso-scale vegetation (20-30m) close to the coring site (Zhao *et al.*, 2006). The recent macrophyte surveys of the Rail Pit, and studies of other similar ponds in the locality (Sayer *et al.*, 2012, 2013), show that both submerged and floating-leaved plants can effectively cover the whole area of these small ponds and although several species can be present, often one species dominates. For example, over 1996-1999 the Rail Pit was dominated by *P. natans* to be replaced lately (2009-2014) by *C. submersum*. This low spatial heterogeneity of aquatic plants gave confidence in the efficacy of the core samples to accurately determine and reliably reconstruct macrophyte composition, and the core site was never far from plant beds of all macrophyte species in the pond. Moreover, diatom assemblages have also been shown to be spatially less variable in small lakes (Anderson 1986; Anderson *et al.*, 1990) and, therefore, it was assumed that a small farmland pond such as the Rail Pit would also present less spatial variability in diatom assemblages. In fact, this assumption was upheld by the remarkably similar diatom assemblages seen in the surface sediments of cores RAIL1 and RAIL2. In summary, the low spatial heterogeneity seen across these key biological indicators suggests that one core is sufficient to confidently reconstruct changes in the biological communities across whole ponds. Nevertheless, more specific studies on spatial heterogeneity are ideally needed.

6.4.2 Ecological history of the Bodham Rail Pit

The Rail Pit has seen a series of changes in its submerged and floating-leaved plants over at least two centuries and very likely since colonisation began from the original

excavation of the site as a marl pit, from at least the seventeenth-eighteenth centuries. These changes are broadly similar to those observed in studies on shallow, temperate European lakes (Brodersen *et al.*, 2001; Odgaard & Rasmussen 2001; Davidson *et al.*, 2005; Sayer *et al.*, 2010a). The evidence from the macrofossil chronologies (Figs. 6.12, 6.15), the PCA axes scores (Fig. 6.17) and the numerical zonations (Fig. 6.18) clearly show that there have been major compositional changes in both the aquatic flora and fauna over time.

- **Early ecological history**

The high UVR absorbing pigment concentrations in conjunction with: i) relatively low concentrations of sedimentary fossil '*Lemna* marker' pigments (Fig. 6.3), ii) low relative densities of the '*Lemna* marker' diatoms (*L. hungarica* and *S. seminulum*), iii) prevalence of epi-benthic diatoms and the absence of planktonic diatoms (see Fig. 5.12, Chapter 5) and v) low *Daphnia* abundances and high abundances of *Alona* cladocerans during the earliest history of the Rail Pit (Zone 1, RAIL1) suggests that the pond seemingly contained few macrophytes *per se*, and few *Lemna* in particular at this time. Furthermore, relatively low concentrations of terrestrial dissolved organic carbon (DOC) are indicated by the high UVR Index (Fig. 6.2b) and the low terrestrial leaf index (Fig. 6.14). As the pond was less shaded by riparian tree cover at that time, thereby creating a more open water-body, there would have been low allochthonous organic matter inputs. The high abundances of *Chara* (>1000 per cm^3) and *Nitella* (>100 per cm^3) oospores suggests dominance of charophyte 'lawns' (Zhao *et al.*, 2005) in the early history of the Rail Pit. All of these indicators point to a scenario of clear water conditions in the early part of the pond's history.

- **Ecological history through time**

This scenario of clear water and charophyte-dominance conditions was seemingly replaced by the establishment of other submerged macrophytes such as *Potamogetons*, namely *P. crispus* and the fine-leaved *P. berchtoldii* and *P. pusillus* (Zone 1 to early

Zone 2, RAIL1) and to a lesser extent by floating-leaved *P. natans* as indicated by high abundances of *Potamogeton* macro-remains and high concentrations macrophyte pigments. The increasing and periodic concentrations of '*Lemna* marker' pigments and *Lemna*-indicator diatoms indicated the prevalence of *Lemna* as distinct phases.

There were modest increases in cryptophytes (alloxanthin), diatoms (diatoxanthin), total cyanobacteria (zeaxanthin) and a large increase in purple sulphur bacteria (carotene PSB), but notably, however, the UVR absorbing pigment suddenly disappeared from the sediment record (Figs. 6.3, 6.2b) at this point (i.e. 60-55cm, RAIL1). There continued to be defined and sporadic increases in aquatic macrophytes, cryptophytes, cyanobacteria and diatoms. The diatom communities in the early history (i.e. Zone 1, RAIL1) were primarily epi-benthic species (e.g. *Navicula radiosa*, *Epithemia adnata*, *Staurosira elliptica*), but with the increases in aquatic macrophytes seen in Zone 2 (RAIL1) the epi-benthic diatom communities increasingly became co-dominant with epiphytic species (see Fig. 5.12, Chapter 5). This co-dominance was later replaced as epi-benthic diatoms declined and epiphytic diatoms became co-dominant with the establishment of planktonic diatom communities.

Interestingly, at 54cm level in RAIL1 there was a sudden and drastic reduction in all of the main sedimentary pigment concentrations (Figs. 6.3, 6.6a) which coincided with a paucity of diatoms, including the *Lemna* epiphytes *L. hungarica* and *S. seminulum* which indicates very low *Lemna* abundances (see Fig. 5.12, Chapter 5). Furthermore, there were marked reductions in both the plant and animal macrofossil data (Figs. 6.12, 6.15). It was also noticeable that immediately after this event witnessed the start of the first *Lemna* dominance phases (Phase 2). It is most likely that this ecological and environmental signature probably reflects a substantial drought-driven reduction in water level at the location of the RAIL1 coring site (H. Yang, pers. com.). This possible scenario was supported as a 'drying out' event covering large areas of the Rail Pit (likely including the core site) was observed during the summer of 1976 and in the early 1990s (C.D. Sayer, pers. com.). However, even though the Rail Pit experiences seasonal fluctuations in water levels and 'drying out' events, it is unlikely that the Rail

Pit experienced episodes of completely 'drying out' and it is unlikely that the Rail Pit experienced sediment slumping in the past. A succession within the *Potamogeton* taxa was also evident. From the early history of the Rail Pit (75-32cm, to c. late 1940s) several *Potamogeton* species (*P. berchtoldii, P. crispus* and *P. natans*) and possibly *P. pusillus* were present. By the c. late 1940s, however, the submerged species *P. berchtoldii, P. pusillus* and *P. crispus* disappeared from the fossil record to be succeeded by the floating-leaved canopy-forming *P. natans*. Crucian Carp were present and were seemingly present in high abundances, particularly in the earlier historical periods.

Marked changes were also observed in the animal fossil record. Ostracods were abundant from the core base, disappeared with *Lemna* dominance Phase 3 (c. 1950s-1980s) and returned after *Lemna* dominance Phase 4. The caddis *C. trimaculatus* was present throughout the core profile with highest abundances seen during periods where there were no *Lemna* dominance phases, whereas *L. flavicornis* was only present in later sequences and, interestingly, appeared to be present during the absence of *C. trimaculatus*. Other insect taxa present were the alderfly *S. lutaria* which was present in earlier sequences but disappeared with *Lemna* dominance Phase 3 whilst corixids were present throughout the entire core profile, whereas *Chaoborus* was more prevalent in the later sequences. *Plumatella* statoblasts were consistently present throughout the core profile, with highest abundances in the earliest sequences before *Lemna* dominance Phase 2; oribatid mites also were consistently present but occurred in greatest abundances during times when there were no *Lemna* dominance phases. Both benthic and plant-associated cladocerans (*Alona, C. sphaericus* and *Simocephalus*) and the cladoceran taxa (*D. pulex, D. hyalina, D. magna, Ceriodaphnia*) more associated with pelagic conditions were also present.

Crucian Carp (*C. carassius*) scales were found throughout the core profile, indicating that this fish species was always present, and, therefore, successfully reproducing. It would appear from the animal macrofossil data (Fig. 6.15) that Crucian Carp abundances fluctuated over time as suggested by the changes seen in *Chaoborus*, other

invertebrates and pelagic *Daphnia* species. As the number of Crucian Carp scales declined there were increases in *Chaoborus* and *Daphnia* remains suggesting fish kills which would, therefore, reduce the fish populations in a boom-bust manner. Indeed, it was noticeable that samples containing relatively high abundances of Crucian Carp scales invariably contained fewer remains of these invertebrates, and vice versa, with the implication that these invertebrate populations and *Chaoborus* in particular, were key prey items for Crucian Carp.

- **Recent ecological history**

The plant macrofossil data showed a general macrophyte succession from *Chara*/Nitella-*Potamogeton* (75-52cm) to *Potamogeton-Rannunculus* (52-28cm) and finally to *Potamogeton-Ceratophyllum* (28-7cm, c. 1960s-1990s). This succession has been widely observed in macrofossil studies of shallow lake macrophytes, which show a loss of *Chara* and *Nitella* followed by the subsequent establishment of canopy-forming *Potamogeton* spp. and species such as *Ceratophyllum* which effectively cover the entire water-column as a response to deteriorating light conditions (Blindow 1993; Brodersen *et al.,* 2001). *P. natans* persisted until the late 1990s (7cm) when it also disappeared. Apart from a small bed of *P. crispus* recorded in 2009, the other *Potamogeton* species never returned and the Rail Pit is now dominated by *C. submersum.*

During the course of the Rail Pit's history, and particularly during the more recent history, organic input gradually increased with terrestrialisation, namely the establishment of riparian herbage and trees. In conjunction with terrestrialisation, eutrophication occurred resulting from increasing nutrient inputs from fertilizers. A combination of shading and nutrient enrichment appeared to facilitate the rapid increase in *Lemna* productivity as *Lemna* was able to take competitive advantage over other macrophytes, culminating in periodic cycles of *Lemna* dominance. It is primarily these processes that brought about the sequential changes in macrophyte composition and abundance from charophytes to *Potamogeton* and then to *Ceratophyllum* dominance.

The prolific growth of *C. submersum* was likely attributed to the progressive increase of nutrient inputs as this prolific growth is also commonly seen in eutrophic, shallow lakes with *Ceratophyllum demersum* (Mjelde & Faafeng 1997; Kennison *et al.,* 1998). In terms of plant architecture this shift to more structurally complex *Ceratophyllum* from the more structurally less complex *Potamogeton* taxa could influence invertebrate composition and abundances (McAbendroth *et al.,* 2005). It is interesting to speculate that this could be the case at the Rail Pit which saw increases in invertebrate abundances such as the trichopterans *C. trimaculatus* and *L. flavicornis*, corixids and *Chaoborus* and also saw increases in abundances of the plant-associated cladocerans, such as *C. sphaericus* and *Simocephalus*.

Figure 6.19 shows the main ecological pathways and the direct effects on the key aquatic flora and fauna derived from the macrofossil analyses. The analyses indicate that there was a clear step-wise succession in its aquatic vegetational history: Charophyceae → Potamogetonaceae → Ceratophyllaceae. Figure 6.19a shows the negative effects upon the key aquatic flora and fauna brought about *Lemna* dominance (ecological mechanism) as a direct consequence of eutrophication (ecological driver) seen in the more recent history of the Rail Pit. In comparison, Figure 6.19b shows that in the early history of the Rail Pit, before the advent of eutrophication, *Lemna* was not a dominant floating mat but was merely present in lower abundances (indicated from the diatom based *Lemna* indicator metric, see Fig. 5.16 Chapter 5). The macrofossil analyses suggest that this early *Lemna* presence, as opposed to *Lemna* dominance seen in later years, did not negatively impact upon the key aquatic flora and fauna which saw high abundances of *Alona* cladocerans, the trichopteran *Cyrnus trimaculatus*, ostracods, *Plumatella* bryozoans and *Sialis lutaria* and also high abundances of Charophytes, Potamogetons and the bryophyte *Leptodictyum riparium* (Figs. 6.12, 6.15).

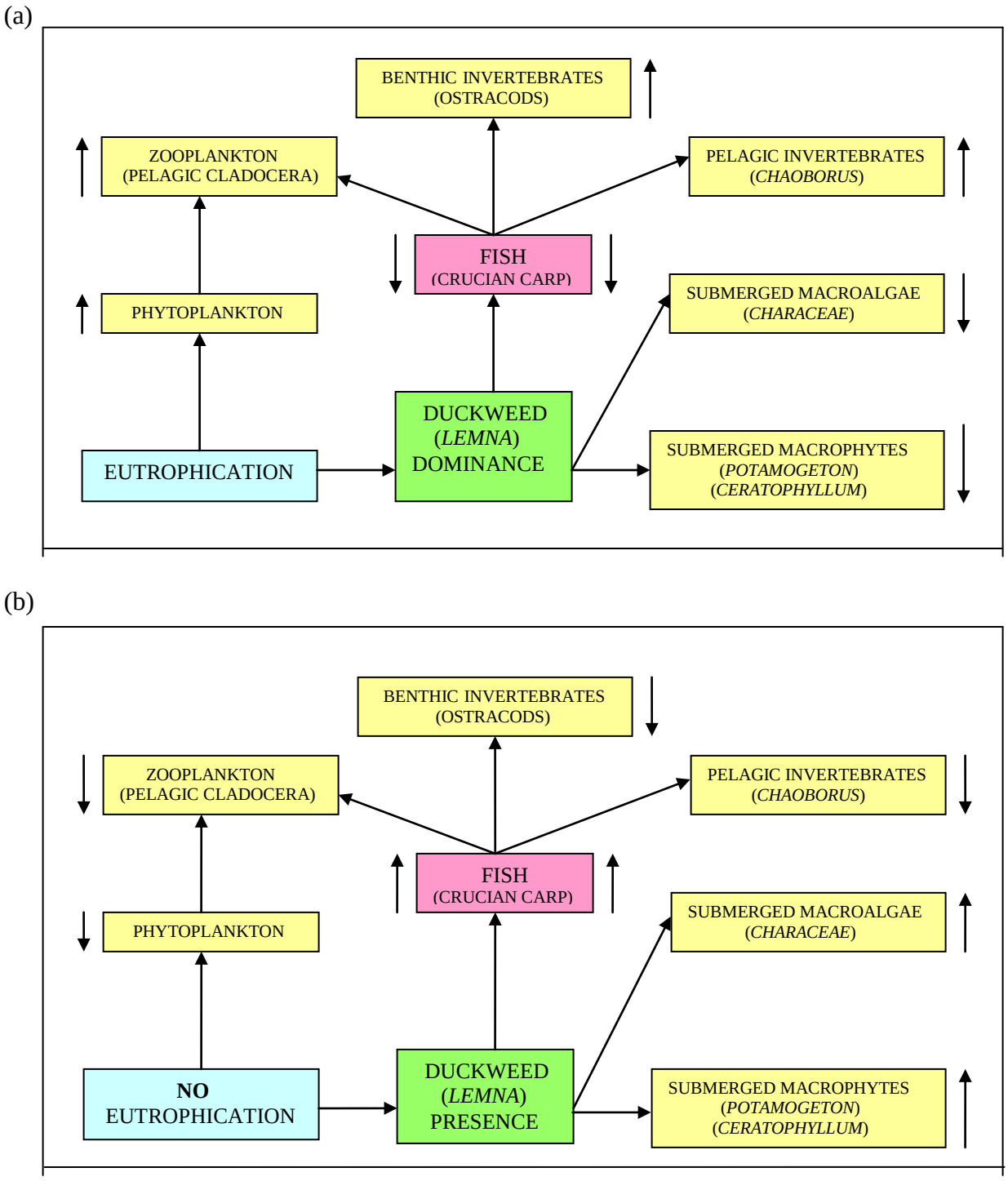

Figure 6.19. Simple schematic summary diagram showing the main ecological pathways (indicated from the macrofossil analyses) resulting from (a) eutrophication (ecological causal driver) and dominant *Lemna* cycles (ecological mechanism) and (b) before the advent of eutrophication with *Lemna* presence (i.e. non-dominance of *Lemna*). The connecting arrows signify the direction of the effect, the small arrows signify the result of the effect (i.e. arrows pointing up signify positive effects; arrows pointing down signify negative effects).

In many shallow lakes, long-term changes in biological structure are largely driven by eutrophication resulting in losses of macrophyte species diversity (Jeppesen *et al.*, 2000; Vestergaard & Sand-Jensen 2000; James *et al.*, 2005; Sayer *et al.*, 2010a, 2010b) and associated invertebrate and fish communities (Jeppesen *et al.*, 1998). In the Rail Pit, eutrophication was a likely key driver in bringing about changes in biological structure as shown by the shift from epi-benthic diatoms (e.g. *Epithemia* dominance) to planktonic diatom communities (e.g. *Cyclotella* and *Stephanodiscus* dominance) from c. 1950s onwards (Figs. 5.15 & 5.17), a pattern typically seen in many shallow lakes in response to enrichment over recent centuries (Bennion *et al.*, 2010, Sayer *et al.*, 2010a, 2010b). Research on shallow lake productivity has identified that there is a shift from benthic to planktonic pathways of production in response to nutrient enrichment (Vadeboncoeur *et al.*, 2003). This pattern was also seen in the Rail Pit where, for example, the benthic cladoceran *Alona* dominated the early periods but with increasing nutrient inputs *Alona* was replaced by planktonic cladocerans such as *D. pulex* and *D. hyalina.*

Changes in farming practice (loss of meadows, increases in arable farming and the application of fertilizers) and increasing terrestrialisation are likely to have resulted in enhanced nutrient inputs to the Rail Pit at least in the last century. The removal of hedgerows adjacent to the Rail Pit after c. 1950 (Figs. 5.1 & 5.2, Appendix 4) lends support to agricultural intensification leading to increased intensive use of agricultural fertilizers and, therefore, increased nutrient loading into the Rail Pit. Therefore, this study of a small pond also supports this diatom eutrophication signal witnessed in shallow lakes. These diatom compositional and ecological changes brought about by increased nutrient loadings and associated *Lemna* phases were also evident in both the CA and PCA axis 1 sample scores. These unstable and unpredictable 'environmental perturbations' tend to support a dynamically robust and relatively simple (diatom) community (May 1979, Connell 1979). However, a more qualitative interpretation of the macrofossil data suggests that the ecological history of the Rail Pit is more complex than simply one of progressive eutrophication. The plant macrofossil stratigraphies (Fig. 6.12) show that *Lemna* dominance likely had a major impact upon the presence,

abundance and timing of the loss of rooted and submerged macrophytes. It is likely that increased nutrient inputs were responsible for initiating periods of *Lemna* dominance and that these *Lemna* phases were a key means of bringing about changes in macrophyte composition and diversity. Thus the underlying cause of the shifts in macrophytes was increased nutrient inputs which led to dense blooms of free-floating *Lemna* mats that ultimately caused the demise of other macrophytes in the Rail Pit.

6.4.3 *Lemna* phases and *Lemna* cyclicity

There were three distinct *Lemna* dominance phases and one earlier and relatively minor phase inferred from the diatom analyses (see Chapter 5). Based upon the diatom, pigment and macrofossil data (Fig. 6.17) this earlier *Lemna* phase 72-58cm (Phase 1) was considered not to be a phase of *Lemna* dominance, but was deemed to be a phase of *Lemna* presence. The first *Lemna* dominance phase (Phase 2) occurred at 54-42cm, the second dominance phase (Phase 3) occurred at 32-17cm (c. late 1940s-late 1980s) and the third dominance phase (Phase 4) occurred at 7-3cm (c. late 1990s-mid 2000s).

The earliest record of *Lemna* (Phase 1) from the sediment profile was seen towards the bottom of the core and was likely to have first appeared shortly after the formation of the Rail Pit. The relatively low *Lemna*-indicator diatom abundances (see Fig. 5.12, Chapter 5), in conjunction with the pigment and macrofossil data (Fig. 6.17), suggest this *Lemna* phase was unlikely to be presenting in sufficient abundances to warrant a dominant *Lemna* phase and thus was not negatively impacting upon the established charophyte, *Potamogeton* (Fig. 6.12) and animal communities (Fig. 6.15). There was an accompanying spike of UVR absorbing pigment (Fig. 6.2b), indicating that sufficient light (PAR) was penetrating through the water column allowing photosynthesis to occur in the submerged phototrophic communities. Moreover, the large increase in carbonate (Fig. 6.14) strongly indicates that the charophyte communities were well established during this first non-dominant *Lemna* Phase 1.

The first *Lemna* dominance phase (Phase 2) at the 42cm level (Zone 2, RAIL1) saw a sudden and marked increase in all of the sedimentary fossil pigments reflecting increases in all types of photosynthetic taxa (Fig. 6.4), including *Lemna* (most likely *L. minor* as this time-frame precludes the arrival of *L. minuta*). Interestingly, the PCA of the sedimentary pigment data during *Lemna* dominance Phase 2 was strongly correlated with the sedimentary pigments (Figs. 6.6, 6.7). This suggests that even though *L. minor* was present as a dense floating mat (as indicated by the *Lemna*-indicator diatoms) other aquatic macrophytes, such as the floating-leaved *P. natans*, were also most likely well established at this time. There was a sudden disappearance of charophytes with the onset of *Lemna* Phase 2, and the sudden spike in sedimentary carbonate shortly afterwards suggests assimilation of the senescent and calcified charophytes into the sediment (Fig. 6.14).

The second *Lemna* dominance phase (Phase 3, Zone 3, RAIL1; Zone 1, RAIL2) predictably saw relatively high concentrations of the '*Lemna* marker' sedimentary pigments (Figs. 6.3, 6.5). However, it is reasonable to assume that this relatively lengthy and protracted *Lemna* phase was not a completely dominant and continuous dense floating mat as there were sustained cryptophyte and cyanobacterial communities present during this time. Also there were oscillating periods of UVR absorbing pigment in these zones which fluctuated between strong sedimentary presence and complete absence. This implies that there were occasions when enough PAR was penetrating through the floating *Lemna* mats and it seems likely, based on the pigment concentrations, that the *Lemna* mats could be classified as a single monocultural layer, as opposed to a thick and dense multiple layer. Although sufficient PAR was penetrating the *Lemna* mats to enable *C. submersum* to briefly make an appearance at the Rail Pit, seemingly there was insufficient PAR for the submerged fine-leaved *Potamogetons* as *Lemna* Phase 3 saw the demise of *P. berchtoldii* and *P. pusillus*.

The third *Lemna* dominance phase (Phase 4, Zone 4, RAIL1; Zone 2, RAIL2) saw substantial increases in the '*Lemna* marker' pigments but also increases in cryptophytes (alloxanthin), total cyanobacteria (zeaxanthin) and purple sulphur bacteria (carotene

PSB). However, there was some discrepancy in the UVR absorbing pigment concentrations in the corresponding zones between the two cores. In RAIL1 (Zone 4) there were substantial concentrations of UVR absorbing pigment during this *Lemna* dominance Phase 4 (Figs. 6.3, 6.2b) but in RAIL2 (Zone 2) there was a marked reduction in UVR absorbing pigment concentrations (Figs. 6.5, 6.4b). This discrepancy could be due to an error in dating due to the high sediment flocculation during core collection or alternatively it could be due to spatial heterogeneity ('patchiness') in *Lemna* abundance at the core collection sites. The latter would appear to be the most likely explanation for this discrepancy as the pigment profiles of the other sedimentary pigments were in good agreement between cores, as were the diatom profiles. This explanation is also supported by the sedimentary concentration profile of chlorophyll *a*' (oxygenic degradation of chlorophyll *a*) as there were substantial decreases seen in both cores during *Lemna* Phase 4, with complete disappearance in RAIL1. *Lemna* Phase 4 witnessed the demise and disappearance of floating-leaved *P. natans* and the submerged *C. submersum*.

The macrofossil data also revealed the presence of *Lemna* directly as *Lemna minor* seeds. Indeed, *L. minor* seeds were continuously found from the core base (74cm) to mid-core (26cm, c. 1950s). Interestingly, *L. minor* seeds were not recorded during diatom-inferred *Lemna* dominance phases, but invariably they were found just before and immediately afterwards. The *L. minor* seed maxima was recorded towards the base of the core (72cm) coinciding with the onset of *Lemna* Phase 1 but, after the early part of *Lemna* dominance Phase 3 (c. 1960), no *L. minor* seeds were found. It is interesting to speculate that perhaps the increasing presence of the invasive *L. minuta* in later years was negatively impacting on the native *L. minor* mats, as *L. minuta* is currently co-dominant in the Rail Pit. However, because of this co-dominance it was not possible to provide a direct measure of the impact of the invasive *L. minuta* upon the biological structure and the ecological function of the Rail Pit. Another hypothesis for the discrepancy between the diatom and macrofossil data is that perhaps *L. minor* propagated by sexual reproduction in these early periods (72-26cm) before the onset of the use of agricultural fertilizers and concomitant increases in nutrient load. With

agricultural intensification, especially post-1950, it is possible that *L. minor* was advantaged over other macrophytes by exploiting raised nutrient levels. In particular, it may have shifted its reproductive strategy from sexual to asexual reproduction by rapidly reproducing, and doubling, from budding of daughter fronds allowing rapid expansion across the water surface. By completely covering the pond surface *Lemna* mats prevent light (PAR) from reaching submerged plants and they also release oxygen directly to the atmosphere (Dale & Gillespie 1976; Goldsborough 1993). Further, such mats reduce gaseous exchange of oxygen and carbon dioxide resulting in a predominance of respiratory processes in the water column and consequently lower dissolved oxygen concentrations (Sayer & Emson, unpubl. data), increasing carbon dioxide levels (Janes 1998) causing physico-chemical changes in the water column (Pokornŷ & Rejmankova 1983; Goldsborough 1993; Portielje & Roijackers 1995); all of which are detrimental to the growth of other aquatic plants. Examples of the rapid decline in water column oxygen levels in small ponds resulting directly from dense *Lemna* mats are given in Appendix 5.

The uppermost part of both cores (Zone 4a, RAIL1 and Zone 3, RAIL2) included the period between the cessation of *Lemna* Phase 4 (c. 2005) and the coring date (2010). Similarly to the transitional period between *Lemna* Phase 3 and *Lemna* Phase 4 (i.e. early Zone 4, RAIL1 and Zone 3, RAIL2) there were increases in all of the sedimentary pigment concentrations in both cores in the uppermost zone. The increases of the '*Lemna* marker' pigments (and the *Lemna* epiphytes: *L. hungarica* and *S. seminulum*) were likely due primarily to the senescent *Lemna* mats sinking to the sediment. The increase in organic matter at this time may be due to high abundance of *C. submersum* which suddenly returned after the end of *Lemna* Phase 4. At the time of core collection (April 2010) *C. submersum* had become the dominant macrophyte in the Rail Pit and *Potamogeton crispus*, *Cladophora* and *Lemna trisulca* became established for the first time in recent history (see Table 5.1 & Fig. 5.2, Chapter 5).

In summary, the multi-proxy palaeoecological study of the Rail Pit shows that the occurrence of free-floating *Lemna* mats was cyclical and can therefore be described as

Lemna cycles. Increased nutrient loading is the most likely explanation for the establishment of *Lemna* mats. It is reasonable to suggest that after exploiting and exhausting nutrients from the upper water column during the spring and summer seasons, where the growth window would likely be extended to cover most of the summer months due to eutrophication, there followed rapid senescence and die-back of the *Lemna* mats presumably due to nutrient reduction in the water column which would not be replenished from sediments due to a lack of wind-induced nutrient circulation, demonstrated by the stratification processes at the Rail Pit. The extension of the *Lemna* growth season would have had a deleterious effect on the other macrophytes. These processes would explain the cyclical nature of *Lemna* which was recorded at the Rail Pit, and was particularly evident in the recent history of the site.

6.4.4 *Lemna* as a physical ecosystem engineer

The analyses sought to determine whether *Lemna* mats were ecologically engineering the structure and function of the plant and animal communities in the pond. The pigment and plant macrofossil data for the lowermost part of RAIL1 core suggest that *Lemna* was not a dominant driving force in determining, or influencing, phototrophic communities in the early history of the Rail Pit, as relatively low concentrations of the main sedimentary pigments were recorded at this time. This finding corroborates the diatom analysis (*Lemna*-indicator metric) which indicates that *Lemna*, in the early history of the Rail Pit, may not have formed dense free-floating mats and, therefore, would not be expected to significantly influence the phototrophic communities. The RDA for RAIL1 pigments, covering the entire history of the Rail Pit, showed that the main gradient of algal and macrophyte community change was not significantly associated with *Lemna* dominance, even though *Lemna* had some influence on phototrophic communities (Fig. 6.8). It appears, therefore, that the mats in *Lemna* Phase 1 were not dense and multi-layered and thus had a limited effect on the PAR transmission and thereby on the photosynthetic capabilities of the phototrophic communities. The data suggest that there were periods of dominance of macrophytes other than *Lemna* that also influenced the algal communities, notably at 62-61cm where

charophytes dominated and at 56-55cm where *Potamogeton* spp. dominated (Figs. 6.6, 6.8, 6.12).

In contrast, the results for the upper part of the RAIL1 and RAIL2 records indicate that *Lemna* dominance likely had a major influence on the structure of the phototrophic communities in more recent times. The RDA for RAIL2 pigments, which covers the most recent history of the Rail Pit, showed that the algal and macrophyte communities were significantly associated with and structured by the *Lemna* phases. This was reflected by the high concentrations of Chl *a*' and UVR absorbing pigment (pigments associated with clear water conditions) indicating periods where *Lemna* was absent (Zone 2 & 3b). The data strongly suggest that the second (Phase 3) and third *Lemna* cycles (Phase 4) ecologically engineered the community structure of the algae resulting in reductions of the cryptophytes, cyanobacteria, purple sulphur bacteria and diatoms (Figs. 6.5, 6.9, 6.11). The disappearance of *P. berchtoldii*, *P. pusillus* and *P. crispus* was very likely to be due to the negative impacts of *Lemna* Phases 2 and 3.

It is reasonable to suggest that the shift in macrophyte composition and concomitant changes in invertebrate abundances reflect increasing nutrient inputs to the Rail Pit, but *Lemna* dominance also appears to have played a role. *Ceratophyllum* abruptly appeared and briefly collapsed in the mid 1980s. It was noticeable that *Ceratophyllum* dominance collapsed during *Lemna* Phase 3, returned after this *Lemna* phase ended then disappeared with the onset of *Lemna* Phase 4 before spectacularly returning with the demise of *Lemna* Phase 4 (mid 2000s) to become the dominant macrophyte in the Rail Pit. However, this classic eutrophication-driven shift to submerged *Ceratophyllum* and, therefore, a reduction in macrophyte species richness (Jeppesen *et al.*, 2000) only occurred in the very recent history of the Rail Pit which is surprising as this shift would be expected to have occurred much earlier if it was solely due to increased nutrient inputs *per se*. This 'delayed' *Ceratophyllum* dominance appears to be due to the dominance of *Lemna* which seemingly prevented the establishment of *Ceratophyllum*. Similarly, the shift from *Chara* to *Potamogeton* was also largely influenced by *Lemna* dominance as demonstrated by the PCA axes scores (Fig. 6.17) and the zonation (Fig.

6.18). Moreover, the later shift from *Potamogeton natans* to the domination of *Ceratophyllum* was also heavily influenced by *Lemna* dominance as the third *Lemna* dominant Phase 4 (1999-2005) appeared to be solely responsible for the sudden demise of both *C. submersum* and *Potamogeton natans*. Before this *Lemna* phase, *C. submersum* and *P. natans* were established and abundant but by the second year of the *Lemna* phase both of these plants had disappeared from the Rail Pit. These data suggest that it is possible that a regime shift may have been initiated by the *Lemna* phase which brought about an alternative stable state of free-floating macrophytes, in place of submerged macrophytes (Scheffer *et al.,* 2003). After the termination of this *Lemna* phase, however, the Rail Pit eventually shifted back to *C. submersum* dominance but *P. natans* never recovered and to date remains absent. This ecological regime shift back to *C. submersum* dominance was accompanied by the appearance and co-dominance of *Cladophora* and saw the first appearance of *P. crispus* and *L. trisulca* (Table 5.2). This suggests that the more recent *Lemna*-dominance phases had a strong ecological engineering effect on the macrophyte communities. The initial 'environmental perturbation' of increased nutrients appeared to act as a precursor to bringing about a catastrophic regime shift with the sudden loss of diatom species and accompanied by the equally sudden and dramatic loss of submerged macrophytes as the ecosystem apparently switched to an alternative stable state of free-floating Lemnid dominance (Scheffer *et al.*, 2003).

With autogenic engineering the growth and extensive coverage of the *Lemna* mats became part of the new physical state by creating habitat resources (e.g. for invertebrates and diatoms) thereby engineering positive ecosystem effects. By controlling abiotic resources by forming a physical barrier to light and gas exchange thus creating dark and anoxic conditions in the water column and the benthos, the dense mats of *Lemna* created a new physical state. This allogenic engineering had profound negative effects on the ecosystem of the Rail Pit particularly on the macrophyte and fish communities.

Palm *et al*., (2011) established a relationship between *Chaoborus* remains and cyprinid fish presence whereby the past presence of Roach (*Rutilus rutilus*) was determined from fragmented *Chaoborus* mandibles recorded in lake sediments (Palm *et al.,* 2011). Although fish species such as Roach (*R. rutilus*), Perch (*Perca fluviatilis*) and Rudd (*Scardinius erythrophthalmus*) are known to be present in the Rail Pit in recent times (C.D Sayer & J. Bailey, pers. com.) no remains were found of these species in the macrofossil record. It is likely that these species were present in such low abundances that they are not recorded in the sediment profile. It is possible that the later *Lemna* dominance phases could have been responsible for their disappearance. Fish kill resulting from the effects of dense *Lemna* mats by markedly reducing light levels and creating anoxic conditions over a period of time have been reported elsewhere (Lewis & Bender 1961). The most surprising feature from the animal macrofossil analysis is that there were no egg cocoons of the fish leech *Piscicola geometra* or mollusc shell fragments found in any of the samples, as these groups are often seen in shallow lake palaeolimnological studies (e.g. Sayer et al., 2010b, Davidson et al., 2010b, Rawcliffe et al., 2010). There was no apparent preservation problems seen with the sampled diatom frustules and complete ostracod shells were also found in high abundances (>160 per 100cm^3) particularly in the earlier sediment profile. This suggests that poor taphonomic preservation is unlikely to explain the absence of mollusc shells and that their absence in the sediment profile, as with *P. geometra* egg cocoons, is possibly due to spatial heterogeneity and/or possibly low population abundances.

In contrast, the evidence from the macrofossil fish scale data revealed that Crucian Carp (*C. carassius*) was not only present from the origins of the Rail Pit (Fig. 6.15) but seemingly managed to persist throughout the history of the Rail Pit. This implies that Crucian Carp were able to sustain precarious populations, and it appears that it was the only fish species to do so. However, there was a progressive decline in Crucian Carp numbers with an apparent disappearance from the macrofossil record in the 1980s. This in turn released the predation pressure on the invertebrate communities resulting in sudden increases in their abundances, particularly *Chaoborus* (Fig. 6.15). While Crucian Carp populations were perilously low in abundance during *Lemna* Phase 3 (c.

1940s-1980s) there were signs of recovery after the ending of this *Lemna* phase, before they disappeared again from the macrofossil record with the onset of *Lemna* Phase 4. Subsequently, following *Lemna* Phase 4, Crucian Carp remains returned in very high abundances with the ending of this more recent *Lemna* phase (Fig. 6.15). This fits in with catch data as in 2012, after the final *Lemna* Phase 4, more than 500 Crucian Carp were caught (Sayer *et al.*, unpubl. data). However, it is highly likely that this cohort of Crucian Carp were produced by just a few individual adults and it appears that only one adult female ('Lucky') managed to survive the deleterious effects of *Lemna* dominance. This finding has serious implications for the future of Crucian Carp in the Rail Pit as the inequality in the sex ratio of just a few breeding individuals, which constitutes the effective population size, can potentiality of produce a genetic bottle-neck. This may enhance the opportunity for random genetic drift as the critically low effective population size contains less genetic variation. This would impede the return to a genetically healthy and viable population (Hartl 1988).

Recent studies have shown that Crucian Carp populations are seriously threatened from hybridization, habitat loss from in-filling of ponds and terrestrialisation of existing sites (Copp *et al.,* 2005, Tarkan *et al.,* 2009, Sayer *et al.,* 2011). Crucian Carp are well adapted to living and thriving in these relatively precarious water-bodies as they are able to survive for considerable periods in anoxic conditions by utilising anaerobic respiration, a facility which is highly unusual among vertebrates (Johnston & Bernard 1983). Clearly, *Lemna* dominance has played a key role in negatively impacting upon the Crucian Carp populations at the Rail Pit.

In their seminal papers on the positive and negative effects of organisms as physical ecosystem engineers, Jones *et al.,* (1997a, b) argued that ecosystem engineering has both negative and positive effects on species richness and abundances at small scales. The huge value provided by the multi-proxy analyses, together with the diatom-duckweed approach to identify past *Lemna* dominance cyclicity, has demonstrated that *Lemna* had both negative and positive effects on species richness and abundances at the small scale, namely a small farmland pond. Secondly, Jones *et al.,* (1997a, b) further

argued that models of the population dynamics of engineers suggest that the engineer/habitat equilibrium is often, but not always, locally stable and may show long-term cycles, with potential ramifications for community and ecosystem stability. Our data support this assertion as the palaeoecological analysis of the Rail Pit demonstrated that there have been distinct cycles of *Lemna* dominance which were shown to have serious ramifications for the aquatic community and ecosystem stability. Finally, the authors call for greater research on physical ecosystem engineers, their impacts, and their interface with trophic relations. This research attempted to address this call and has provided information on physical engineers and their interface with trophic relations by highlighting the negative effects on the macrophyte and fish structure, diversity and abundance and their concomitant impacts on the invertebrate and algal communities.

6.5 Conclusions

The palaeolimnological analysis revealed that the Rail Pit has experienced at least three separate *Lemna*-dominated phases, which are a classic symptom of high-nutrient loading in small water-bodies. These switches between periods of domination by free-floating *Lemna* mats and rooted, submerged macrophytes lend support to the alternative stable state paradigm whereby floating-plant dominance is seen as a self-stabilizing ecosystem state (Scheffer *et al.,* 2003). Nutrient enrichment likely reduced the resilience of this freshwater system resulting in a shift to floating *Lemna* dominance. Then, as the nutrient status of the water-column decreased (Table 5.1, Fig. 5.3, Chapter 5), due to the rapid uptake by the floating *Lemna* mats, this had the effect of precipitating a 'crash' in *Lemna* dominance and with it the implicit indication that there was a regime shift creating an alternative domain of attraction (Table 5.2, Fig. 5.4, Chapter 5). The latter resulted in a switch to domination by rooted *Potamogeton* or submerged *Ceratophyllum*. *C. submersum* is now the dominant macrophyte at the Rail Pit as this submerged plant has seemingly maintained an alternative stable state by affecting the growth of free-floating *Lemna* through a reduction of available nutrients in the water column.

The results from the multi-proxy analyses show that there have been major compositional changes in the algal, vegetation and animal communities of the Rail Pit. Despite some variation in the timing of some of the zone boundaries there was a high degree of synchronicity between all biological groups (diatoms, pigments, cladocerans, plants and animals) and the *Lemna* cycles. The implication is that *Lemna* cycles were impacting upon the algal, vegetation and animal communities. An increase in nutrient status was seen as the ecological *driver* behind the formation of the dense mats of free-floating *Lemna*. In turn, these dominant *Lemna* cycles became the ecological *mechanism* by which community structure of the plant and animals was altered, by attenuating light, reducing dissolved oxygen, water temperature and pH. It is reasonable to propose that the dense *Lemna* mats were effectively acting as autogenic and allogenic engineers of the structure, and thus the function, of the Rail Pit aquatic ecosystem.

Chapter 7. Summary, conclusions and future directions

7.1 Introduction

The primary focus of this research was to explore the palaeolimnological potential of small farmland ponds, especially the possibility for detecting impacts of *Lemna*-dominance on ecological structure and function. The different sections of this study follow a logical progression of investigation from the development of a diatom tool to identify past *Lemna* dominance cycles, and application of the resulting diatom-duckweed proxy to a sediment core sequence. With this approach, the thesis contributes to key ecological debates regarding the existence of alternative stable states in ponds and the potential for dense *Lemna* mats to operate as ecological engineers. The key findings are summarised below and future research directions are suggested with regards to the palaeolimnology of small farmland ponds.

7.2 Summary

Prologue – Before a palaeolimnological study of a small farmland pond could be undertaken it was paramount that sediment cores be collected. The successful collection of a short Glew core and a 'Big Ben' core from the Bodham Rail Pit demonstrated that it was possible to successfully collect long sediment cores from a small pond and that ponds, therefore, have considerable palaeolimnological potential. Indeed, the integrity of the sediment records allowed palaeolimnological techniques to be confidently applied to these often over-looked small water-bodies. Hence long-term dynamics and compositional changes can be determined and tracked which can then be compared with, and complimented by, contemporary ecological analyses, monitoring and surveys and ecological experiments to enhance our understanding of ecosystem structure and

function. Moreover, because of the provision of long-term data via the sediment record it is possible to develop and validate ecological theories, not only in deep and shallow lakes, but also in small farmland ponds. In the context of proposed temporal *Lemna* cyclicity this question could only be answered by palaeolimnological techniques and, as lakes are not known to present with their surface areas completely covered with dense mats of free-floating *Lemna*, it is only in small ponds, such as the Rail Pit, that the ecological effects of *Lemna* mats on aquatic ecosystems can be explored.

7.2.1 Diatom-duckweed relationships at a global scale

The exploratory global macrophyte-epiphyte study (see Chapter 3) and in particular the dissimilarity and dispersion analyses (NMDS, ADONIS, ANOSIM, HMD) suggested statistically significant differences in diatom community assemblage dispersion (β-diversity) and composition associated with the different macrophyte groups. The study also revealed that *Lemnicola hungarica* and, to a lesser degree, *Sellaphora seminulum* were significantly associated with free-floating plants *per se* including *Lemna* species, especially *L. minor* and *L. minuta*. Canonical Correspondence Analysis (CCA) indicated that *L. minor* (and other *Lemna* species) and total phosphorus (TP) were significant explanatory variables of *L. hungarica* and *S. seminulum* occurrence. Moreover, Indicator Species Analysis (INDVAL) revealed that *L. hungarica* ($p=0.001$) and *S. seminulum* ($p=0.028$) had a statistically strong association with *L. minor*, indicating that these diatom species could potentially be classified as *Lemna* indicator species.

7.2.2 Diatom-duckweed relationships at a local scale

To translate the above findings into a palaeolimnological tool it was vital that the diatom-duckweed association identified in the 'global' pilot study could be transferred to sedimentary assemblages. To this end, a space-for-time study of surface sediment diatom assemblages sampled from both *Lemna* and non-*Lemna* covered ponds was undertaken (see Chapter 3). Exploratory data analysis (especially CA) showed that both

L. hungarica and *S. seminulum* were consistently recorded from the surface sediments of *Lemna*-dominated sites. A logistic regression model indicated that *Lemna*-covered sites successfully predicted the presence of *L. hungarica* ($p=0.0001$, $r^2=0.903$) and *S. seminulum* ($p=0.002$, $r^2=0.758$). This corroborated the results from Chapter 3 (INDVAL) strongly suggesting that both *L. hungarica* and *S. seminulum* could indeed be utilised as indicator species to infer past *Lemna* presence in palaeolimnological studies.

7.2.3 Diatom-duckweed relationships: a laboratory study

In Chapter 4 an experimental approach was developed with the aim of elucidating the nature of the association between *L. hungarica* and *L. minor*. An hypothesis testing approach was taken where the null hypothesis (physical hypothesis) stated that there were no significant differences in relative abundances and growth rates between *L. minor* and inert artificial surfaces, whilst the alternative hypothesis (chemical hypothesis) stated that there were significant differences, with greater relative abundances and growth rates on the live *L. minor* in comparison with the inert artificial samples. This simple experiment, using axenic cultures of *L. minor* and *L. hungarica* placed in light and temperature controlled incubator cabinets, demonstrated that there were no significant differences between the different 'habitat' surfaces and, therefore, the chemical hypothesis was rejected and the null hypothesis accepted. Thus, it could be concluded that *L. hungarica* is not chemically interacting with *L. minor*, such as receiving nutrients from *L. minor* exudates: rather it is seemingly adapted to living in such a specialised niche at the biologically and physically stressful air/water interface.

7.2.4 *Lemna*-diatom metric and *Lemna* cyclicity: a palaeoecological approach

Chapters 3 and 4 established that both *L. hungarica* and *S. seminulum* were significantly associated with *Lemna* confirming that these diatoms could be used, with confidence, as palaeoecological proxy indicators of past *Lemna* dominance. In Chapter 5 the aim was to establish a diatom based *Lemna*-indicator model which could be

employed to identify past periods of *Lemna* at the Bodham Rail Pit, Norfolk, England where periods of *Lemna* dominance are known to have occurred. A comparison of the fossil diatom record with the historical record of *Lemna* occurrence at the site was undertaken as a means of validating the model over time. To this end, large (500 - 4700 valves per slide) absolute counts of diatoms in the Bodham Rail Pit cores were made allowing common and rare taxa to be clearly defined, and subsequently allowing the sum of the relative abundances of the two *Lemna*-indicator diatoms to be used with confidence to provide a *Lemna*-indicator metric. The *Lemna*-indicator model was based upon the significant association of the epiphytic diatom *L. hungarica* and duckweed which had, to date, been described as an anecdotal association. This study not only replaced the anecdotal evidence with a statistically significant association, but also revealed a hitherto unknown, yet significant, association between duckweed and *S. seminulum*. Although Desianti (2012) reported *L. hungarica* to be limited by high light levels and proposed a nutrient interaction between *L. hungarica* and duckweed, this study did not concur with these findings. However, this did not detract from the fact that both studies found *L. hungarica* (and *S. seminulum* in this study) being significantly associated with duckweeds.

Diatom stratigraphies from Bodham Rail Pit cores revealed four relatively distinct *Lemna* phases covering the history of the pond. *Lemna* Phase 1 occurred at 72-58cm, *Lemna* Phase 2 at 54-42cm, *Lemna* Phase 3 at 32-17cm (c. late 1940s-mid 1980s) and *Lemna* Phase 4 at 7-3cm (c. 1999-2005). It was not possible to provide dates for the first and second phases. It was concluded that *Lemna* Phase 1 was most likely recording *Lemna* presence but not dense surface coverage due to lower *L. hungarica* and *S. seminulum* counts. For *Lemna* Phases 2, 3 and 4, however, the higher relative percentage abundances of *Lemna*-diatoms suggested dense, dominant mats of duckweed. This conclusion was supported by high concordance in the abundance and timing of *L. hungarica* and *S. seminulum* from the sediment record for *Lemna* Phases 3 and 4. Moreover, temporal concordance was seen for the *Lemna*-indicator diatoms between cores RAIL1 and RAIL2.

The diatom compositional changes in core RAIL1 were largely mirrored in core RAIL2. Redundancy analysis (RDA), logistic regression analysis and Pearson's correlation coefficient analysis indicated that the diatom assemblages were significantly impacted by *Lemna* cycles. A simple comparison of the number of diatom taxa recorded from the sediment samples just before *Lemna* Phase 4 and immediately after the ending of this final *Lemna* phase saw nearly 42% and 35% of diatom species lost from the diatom assemblages of cores RAIL2 and RAIL1 respectively. These profound changes, most likely linked to the effects by *Lemna*, were found to heavily influence diatom zonation of both cores.

7.2.5 *Lemna* cyclicity and the ecological history of the Bodham Rail Pit

The influence of *Lemna* on the biological structure of the Bodham Rail Pit was investigated by examining sedimentary diatoms, pigments, and plant and animal macrofossils. Moreover, the possible engineering effects by *Lemna* on the palaeoecological communities were explored within the context of eutrophication and terrestrialisation.

The diatom, pigment, and macrofossil analysis, together with the PCA axes scores and the numerical zonations show that there have been major compositional changes in both the plant and animal communities. The timing of these changes shows a reasonable degree of concordance between the various fossil groups. These major compositional changes lend support to the possibility that the recent dominant *Lemna* cycles were directly and indirectly responsible for bringing about regime shifts in the ecosystem and that the *Lemna* cycles were producing an alternative stable state scenario to submerged plants.

The palaeoecological data indicate that in its early history the Rail Pit was less shaded by riparian tree cover than it is today, creating a more open water-body with low allochthonous organic matter inputs. However, organic input gradually increased with the establishment of riparian herbage and trees as terrestrialisation progressed.

Furthermore, eutrophication took place from the middle of the record, most likely caused by increasing nutrient inputs from fertilizers. These dual processes facilitated the rapid increase in *Lemna* productivity culminating in periodic cycles of *Lemna* dominance. It is primarily these combined processes that brought about the sequential changes in macrophyte composition and abundance from Charophytes to Potamogetons and then to *Ceratophyllum* dominance.

The palaeoecological study strongly suggests that eutrophication was an underlying ecological causal *driver* behind the changes in the ecological dynamics of the Rail Pit, leading to the formation of the dense mats of *Lemna*. In turn, these *Lemna* cycles became the ecological *mechanism* by which profound changes in both plant and animal community structure and composition occurred. It would appear that the dense *Lemna* mats were partly acting as autogenic engineers by creating a new physical state of extensive, dense free-floating mats thereby engineering positive ecosystem effects from this new habitat for epiphytic algae, and invertebrate species associated with duckweed. On the other hand, it could be argued that the dominant *Lemna* mats were also partly acting as allogenic engineers by the creation of a physical barrier to PAR and gaseous exchange resulting in dark and anoxic conditions to the underlying water column and the benthos. This negative engineering effect had detrimental impacts on the ecosystem of the Rail Pit particularly on macrophyte and fish communities. Clearly, *Lemna* dominance is a major driver of ecological change in small ponds.

7.3 Sources of uncertainty

The use of the recently developed wide-bore 'Big Ben' corer (Patmore *et al.,* 2014) in a small farmland pond presented considerable practical uncertainties. This uncertainty was two-fold: not only was there little evidence of a sediment core being collected from a small pond in the past, but also there was uncertainty in utilising a wide-bore piston corer. The collection of a 'Big Ben' sediment core was successful however, and it appeared that the sediments from the early history of the Rail Pit were fully recovered. However, the flocculation of the uppermost sediments of the core RAIL1 was a

potential problem in terms of the integrity of the upper sediment profile. This uncertainty was remedied by the successful collection of a complimentary Glew core (RAIL2) that was remarkably less flocculent. Importantly, it was possible to correlate the two cores using diatom and lithostratigraphic analyses.

Another source of potential uncertainty stems from the inherent bias associated with fossil representations of contemporary biological communities, including preservation and the degree of spatial heterogeneity of sedimentary remains. In shallow lakes macro-remains of aquatic plants have been demonstrated to accurately reflect shifts in the dominant aquatic flora of such sites (Davidson *et al.*, 2005, Zhao *et al.*, 2005). Further, a single core sample taken from a central lake position has been repeatedly used to characterise lake conditions and to infer past biological and ecological changes (Jeppesen *et al.*, 2003a). This assertion held true for the Rail Pit where at least for diatoms, the collection of two cores demonstrated that spatial heterogeneity, although present, was not a major cause for concern. Notwithstanding this finding, however, it is desirable that the spatial patchiness of sedimentary remains be further investigated in small ponds to more fully inspire confidence and to reduce uncertainty associated with the representation of sedimentary remains.

As the Rail Pit was created from marl extraction, and as marl lakes are generally known to preserve diatoms poorly (Flower 1993), the reliance on only one palaeoecological indicator such as diatoms could have been problematic. Diatom dissolution was clearly not a major issue in this study but, nevertheless, the multi-proxy approach where each indicator reflects different aspects of ecological change provided a more holistic means of exploring shifts in ecological structure and function. Further, the *Lemna*-indicator model appeared to be sensitive to such shifts.

An attempt to provide maximum confidence in the faithfulness of the fossil diatom record to accurately reflect the diatom history was addressed by the complete counting of all diatoms on the diatom slides. Although time consuming, this simple technique allowed for better representation of what would otherwise have been rare/sporadically

recorded species such that more subtle shifts in community composition were identified. This 'technique' was vindicated in the *Lemna*-indicator model being able to distinguish consistent *Lemna* presence (Phase 1) and later *Lemna*-dominance (Phases 2-4).

7.4 Conclusions and a bright future for pond palaeolimnology

7.4.1 Overall conclusions and reflections

The use of specific fossil diatom assemblages to assess temporal variation in pond ecosystems clearly has great potential. The distinct periods of *Lemna*-associated diatom species strongly indicated phases or cycles of *Lemna* dominance. Importantly, the inference model developed here was sensitive to *Lemna* despite the noise inherent to biological and palaeo-environmental data. The result is a robust model, which when applied to sedimentary data, can be compared with other palaeo-biological data to determine *Lemna*-induced changes in ecosystem structure. In this study, we assume that major changes in macrophyte community composition and alterations in fish-invertebrate relationships resulted from the strong physical ecological engineering effects of *Lemna* dominance.

The successful collection of both long and short cores from a small farmland pond demonstrates the huge potential of palaeolimnology in the over-looked "poor cousins" of lentic ecological research - namely ponds. Hopefully, this study has played a small part in putting ponds firmly on the palaeolimnological map by suggesting they are highly suitable for this kind of study. Moreover, the successful comparison between the observed *Lemna*-dominance periods and the diatom-inferred *Lemna* phases provided further support for the huge potential of palaeolimnological studies of small ponds.

The method developed here may assist in determining the causes and mechanisms leading to water quality and ecological impairment in ponds as caused by eutrophication. The major impacts on the macrophyte and fish community structure and

function at this site were likely experienced during times of agricultural intensification (post 1950s). The clear water charophyte-dominated conditions seen during the pre-industrial period may give an indication of 'baseline' or 'reference' conditions for farmland ponds in Norfolk. This fits in with the European Council's Water Framework Directive (WFD) approach (Moss *et al.,* 2003) as it allows knowledge of past pond conditions and, therefore, assessments of deviation from baseline conditions to be made, although small ponds are neglected by the WFD. Such an approach allows us to assess the quality of ponds and in turn what is needed to manage and restore ponds, such as buffering of ponds from farmland and carefully-informed management of scrub (Sayer *et al.,* 2012, 2013).

Farmland ponds are the last bastion of the Crucian Carp (*Carassius carassius*) in the UK, and this study also provides valuable information of relevance to the protection of this rare and culturally-important species. The study was able to provide evidence of the existence of Crucian Carp from the early history of the Rail Pit, suggesting it was present in the pond for some centuries, which lends support to the debate as to whether this species is native/non-native (see Maitland 1972 & Wheeler 1977, 2000). However, it was not possible to date the origins of the Rail Pit either by radiometric analysis or via dendrochronological techniques applied to an old pond-edge oak tree, thus the timing of Crucian Carp colonisation remains elusive. In the future, radiocarbon dating could be applied to the basal sediments of the Rail Pit to help with aging the pond and its Crucian Carp population. Other local studies on Crucian Carp distribution and population dynamics have corroborated the findings of this study of a negative impact by duckweed-dominance on Crucian Carp populations and recruitment (Sayer *et al.,* 2011).

This study highlights the key value of combining contemporary ecological and palaeoecological approaches to see more clearly the 'pieces' of the 'jig-saw puzzle' of pond ecosystems. In particular the 'acorn' of diatom autecological understanding derived from experiments and ecological studies developed into the 'oak tree' of a robust palaeoecological inference model capable of inferring ecological changes and

testing ecological theory from a temporal perspective. This combined ecological-palaeoecological approach, which attempts to link different timescales and methodologies, is gaining momentum in the literature (see Brodersen *et al.*, 2004, 2008; Saros 2009; Sayer *et al.*, 2010a). For example, Cuddington and Leavitt (1999) advocated that future studies in palaeolimnology should include modelling approaches. Moreover, Saros (2009) and Sayer *et al.*, (2010a) called for combined contemporary ecological and palaeolimnological research to more fully infer often complex, long-term (decades-centuries) environmental change. This study wholeheartedly echoes the authors' pleas and has gone some way to answer these calls yet there remains great scope for further research in this field.

7.4.2 Future diatom-duckweed research

Future research could test the diatom-duckweed relationship developed in this study at other sites and also within different geographical regions. Both shallow and deep lakes (as opposed to small ponds) rarely present with complete coverage of free-floating duckweeds but areas of dense, long-standing duckweed mats can occur in secluded and sheltered bays (e.g. Inner Puno Bay, Lake Titicaca, Peru - see Chapter 2) and thus the approach adapted here may be applicable to other types of water-bodies. Although the model developed in this study was not able to assess the ecological impact of the invasive *L. minuta*, due to co-dominance with the native *L. minor*, future work needs to be undertaken on such sites where *L. minuta* is solely dominant. This is particularly relevant as the established and aggressive *L. minuta* is likely to be promoted by eutrophication and possibly also by climate change. As such, the recent explosive and dominant blooms of the invasive *L. minuta* are a real and major threat to the biological structure and function of ponds. This impact could potentially manifest as increased cyclicity which will have serious ramifications on the future biodiversity management of the Rail Pit, not least because the site currently contains breeding populations of the nationally protected Great Crested Newt (*Triturus cristatus*) and the Crucian Carp (*C. carassius*) now classified as a Biodiversity Action Plan (BAP) species in Norfolk (Sayer *et al.*, 2011). These ecological changes have serious implications for the

management of the Rail Pit with respect to biodiversity, and pose questions on the sustainability of the Crucian Carp populations in the pond into the future.

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APPENDIX 1: List of diatom codes, species names (new names in parenthesis) and authorities of the 272 taxa recorded in the global pilot study. Highlighted taxa recorded in the Bodham Rail Pit RAIL1 and RAIL2 cores.

**Lemnicola hungarica* (Grunow) Round & Basson; ** Not listed in 'Diatcode' (Amphora) therefore unique codes attributed.

Code	Diatom Species and Authority
ACH0001A	*Achnanthes lanceolata* (Breb). Grun. in Cleve & Grun. (*Planothidium lanceolatum*)
ACH0001B	*Achnanthes lanceolata spp. rostrata (*Ost.) Hust.
ACH0001D	*Achnanthes lanceolata* var *dubia* Grun.in Cleve & Grun.
ACH0001E	*Achnanthes lanceolata spp. lanceolata* (Sov.) Reimer
ACH0001R	*Achnanthes lanceolata spp. frequentissima* Lange-Bertalot (*Planothidium frequentissimum*)
ACH0001T	*Achnanthes lanceolata spp. robusta* (Hust.) Lange-Bertalot
ACH0006A	*Achnanthes clevei* Grun. in Cleve & Grun. (*Karayevia clevei*)
ACH0008A	*Achnanthes exigua* var *exigua* Grun. in Cleve & Grun.
ACH0013A	*Achnanthes minutissima* Kütz. ** (*Achnanthidium minutissimum)*
ACH0016A	*Achnanthes delicatula* (Kütz.) Kütz. (*Planothidium delicatulum*)
ACH0023A	*Achnanthes conspicua* A. Mayer
ACH0032A	*Achnanthes hungarica* Grun.in Cleve & Grun.* (*Lemnicola hungarica*)
ACH0033A	*Achnanthes coarctata* (Breb) Grun.
ACH0049A	*Achnanthes ploenensis* Hust. (*Kolbesia ploenensis*)
ACH0065A	*Achnanthes exilis* Kütz.
ACH0081A	*Achnanthes kolbei* Hust. (*Kolbesia kolbei*)
ACH0083A	*Achnanthes laevis* Ostr.
ACH0085A	*Achnanthes lauenburgiana* Hust. (*Psammothidium lauenburgianum*)
ACH0134A	*Achnanthes Helvetica* (Hustedt) Lange-Bertalot. (*Psammothidium helveticum*)
ACH0136A	*Achnanthes subatomoides* (Hust.) Lange-Bertalot & Archibald
ACH0152A	*Achnanthes carissima* Lange- Bertalot
ACH0162A	*Achnanthes ingratiformis* Lange-Bertalot in Lange-Bertalot & Krammer
ACH0165A	*Achnanthes catenata* Billy & Marvan
ACH0178A	*Achnanthes straubiana* Krasske
ACH0184A	*Achnanthes ziegleri* Lange-Bertalot
ACH9999A	*Achnanthes* sp. (Sensu lato)
AML0001A	*Amphipleura pellucida* Kutz.
AMP0001A	*Amphora ovalis* Kutz.
AMP0004A	*Amphora veneta* Kutz.
AMP0005A	*Amphora normanii* Rabenh.
AMP0011A	*Amphora libyca* Ehrenb.
AMP0012A	*Amphora pediculus* (Kutz.) Grun.
AMP0013A	*Amphora inariensis* Krammer
ANO0004A	*Anomoeoneis vitrea* (Grun.) R.Ross in Patrick & Reimer (*Brachysira vitrea*)
ANO0009A	*Anomoeoneis sphaerophora* (Ehrenb.) Pfitz.
AST0001A	*Asterionella formosa* Hassall (*Asterionella ralfsii* var *Americana*)
SWA0002A	*Aulacoseira ambigua* (Grun. in Van Huerck) Simonsen
SWA0003A	*Aulacoseira granulata* (Ehrenb.) Simonsen
BAC0001A	*Bacillaria paradoxa* Gmelin in Krammer & Lange-Bertalot (*Bacillaria paxillifer*)
CAL0002A	*Caloneis bacillum* (Grun.) Cleve
CAL0004A	*Caloneis schumanniana* (Ehrenb.) Cleve
CAL0018A	*Caloneis tenuis* (Gregory) Krammer
COC0001B	*Cocconeis placentula var euglypta* (Ehrenb.) Grun.

COC0005A	*Cocconeis pediculus* Ehrenb.
CYC0001A	*Cyclostephanos dubius* (Fricke in A. Schmidt) Round
CYC0002A	*Cyclostephanos invisitatus* Theriot, Stoermer & Hakansson
CYC0003A	*Cyclostephanos tholiformis* Stoermer, Hakansson & Theriot
CYT0002A	*Cyclotella pseudostelligera* Hust.
CYT0003A	*Cyclotella meneghiniana* Kutz.
CYT0048A	*Cyclotella stelligera var woltereckii* Hust.
CYT9999A	*Cyclotella* sp.
CYP0001A	*Cymatopleura solea* (Breb & Godey) W.Sm.
CYR0003A	*Cymatosira lorenziana* Salah
CYM0003A	*Cymbella sinuata* Greg. (*Reimeria sinuata*)
CYM0004A	*Cymbella microcephala* Grun. in Van Heurck. (*Encyonopsis microcephala*)
CYM0005A	*Cymbella aspera* (Ehrenb.) H. Perag in Pell
CYM0006A	*Cymbella cistula* (Ehrenb. in Hempr. & Ehrenb.) Kirchener.
CYM0007A	*Cymbella cymbiformis* (Ag.) Ag.
CYM0015A	*Cymbella cesatii* (Rabenh.) Grun. in A. Schmidt (*Encyonopsis cesatii)*
CYM0018A	*Cymbella gracilis* (Rabenh.) Cleve.
CYM0022A	*Cymbella affinis* Kutz.
CYM0030A	*Cymbella proxima* Reimer.
CYM0031A	*Cymbella minuta* Hilse ex. Rabenh. (*Encyonema minuta*)
CYM0033A	*Cymbella hustedtii* Krasske.
CYM0041A	*Cymbella lanceolata* (Agardh) Agardh.
CYM0042A	*Cymbella tumida* (Breb. ex Kutz.) Grun. in Van Heurck.
CYM0051A	*Cymbella elginensis* Krammer.
CYM0070A	*Cymbella caespitosa* (Kutz.) Brun.
CYM0072A	*Cymbella compacta* Ostr.
CYM0086A	*Cymbella leptoceros* (Ehrenb.) Kutz.
CYM0103A	*Cymbella silesiaca* Bleisch ex. Rabenh. (*Encyonema silesiacum*)
CYM9999A	*Cymbella* sp.
CYM9999X	*Cymbella kolbei* Hust.**
CYM9999Y	*Cymbella excisa* kutzing. **
DEC0003A	*Denticula kuetzingii* Grun.
DIA0004A	*Diatoma tenuis* Ag.
DIA0010A	*Diatoma ehrenbergii* Kutz.
DIP0001A	*Diploneis ovalis* (Hilse) Cleve.
DIP0007A	*Diploneis oblongata* (Naegeli ex Kutz.) R.Ross.
EPI0001A	*Epithemia sorex* Kutz.
EPI0004A	*Epithemia turgida* (Ehrenb.) Kutz.
EPI0007A	*Epithemia adnata* (Kutz.) Rabenh.
EUN0017A	*Eunotia flexuosa* Kutz.
EUN0047A	*Eunotia incisa* W.Sm. ex Greg.
EUN0048A	*Eunotia naegelii* Migula.
EUN0070A	*Eunotia bilunaris* (Ehrenb.) F.W. Mills.
EUN0112A	*Eunotia arcubus* (Grunow) Lange-Bertalot & Norpel.
EUN0070B	*Eunotia bilunaris* var *mucophila* Lange-Bertalot & Norpel
FRA0001A	*Fragilaria pinnata* var *pinnata* Ehrenb. (*Staurosirella pinnata*)
FRA0002A	*Fragilaria construens* var *construens* (Ehrenb.) Grun.
FRA0003A	*Fragilaria bicapitata* A. Mayer.
FRA0006A	*Fragilaria brevistriata* Grun. in Van Huerck. (*Pseudostaurosira brevistriata*)
FRA0007A	*Fragilaria capucina* var *vaucheriae* (Kutz.) J.B. Petersen.
FRA0008A	*Fragilaria crotonensis* Kitton.
FRA0009A	*Fragilaria capucina* var *capucina* Desm.
FRA0006B	*Fragilaria brevistriata* var *inflata* Hust.**
FRA0009B	*Fragilaria capucina* var *mesolepta* (Rabenh.) Rabenh.
FRA0009H	*Fragilaria capucina* var *gracilis* (Oestrup) Hustedt.
FRA0009J	*Fragilaria capucina* var *perminuta* (Grun.) Lange-Bertalot.

FRA0009K	*Fragilaria capucina* var *capitellata* (Grun.) Lange-Bertalot.
FRA0010A	*Fragilaria constricta* Ehrenb.
FRA0018A	*Fragilaria elliptica* Schum. (*Staurosira elliptica*)
FRA0026A	*Fragilaria bidens* Heib.
FRA0042A	*Fragilaria nitzschoides* Grun. in Van Huerck.
FRA0057A	*Fragilaria fasciculata* (Agardh) Lange-Bertalot.
FRA0060A	*Fragilaria tenera* (W.Smith) Lange-Bertalot
FRA0068A	*Fragilaria nanoides* Lange-Bertalot.
FRA0072A	*Fragilaria similis* Krasske.
FRA0002B	*Fragilaria construens* var *binodis* (Ehrenb.) Grun.
FRA0002C	*Fragilaria construens* var *venter* (Ehrenb.) Grun. (*Staurosira construens forma venter*)
FRA9999A	*Fragilaria* sp.
FRA9999T	*Fragilaria capensis* Grunow.**
FRA9999U	*Fragilaria capucina* Desm. (sensu lato) **
FRA9999W	*Fragilaria dilatata* (Breb.) Lange-Bertalot **
FRA9999X	*Fragilaria famelica* (Kutz.) Lange-Bertalot.**
FRA9999Y	*Fragilaria nanana* Lange-Bertalot.**
FRA9999Z	*Fragilaria pulchella* (Ralfs ex Kutz) Lange-Bertalot. (*Ctenophora pulchella)* **
FRU0002A	*Frustulia rhomboides* var *rhomboides* (Ehrenb.) De Toni.
GOM0001A	*Gomphonema olivaeceum* (Hornemann) Breb.
GOM0003A	*Gomphonema angustatum* Agardh.
GOM0004A	*Gomphonema gracile* Ehrenb.
GOM0006A	*Gomphonema accuminatum* var *accuminatum* Ehrenb.
GOM0001F	*Gomphonema olivaceum* var *olivaceoides* (Hust.) Lange-Bertalot.
GOM0006F	*Gomphonema accuminatum* var *pusillum* Grun. in Van Huerck.
GOM0011A	*Gomphonema subclavatum* (Grun. in Schneider) Grun. in Van Huerck.
GOM0013A	*Gomphonema parvulum* (Kütz.) Kutz.
GOM0019A	*Gomphonema augur* Ehrenb.
GOM0020A	*Gomphonema affine* var *affine* Kutz.
GOM0023A	*Gomphonema truncatum* var *truncatum* Ehrenb.
GOM0024A	*Gomphonema clevei* Fricke in A. Schmidt.
GOM0029A	*Gomphonema clavatum* Ehrenb.
GOM0050A	*Gomphonema minutum* (Agardh) Agardh.
GOM0055A	*Gomphonema pseudaugur* Lange-Bertalot.
GOM0078A	*Gomphonema minusculum* Krasske.
GOM0080A	*Gomphonema pumilum* (Grun.) Reichardt & Lange-Bertalot.
GOM9999A	*Gomphonema* sp.
GOM9999X	*Gomphonema occultum* Reichardt & Lange-Bertalot.**
GOM9999Y	*Gomphonema pseudosphraerophorum* Kobayasi.**
GYR0001A	*Gyrosigma attenuatum* (Kutz.) Rabenh.
GYR0005A	*Gyrosigma acuminatum* (Kutz.) Rabenh.
GYR9999A	*Gyrosigma* sp.
HAT0001A	*Hantzschia amphioxys* var *amphioxys* (Ehrenb.) Grun.
MEL0015A	*Melosira varians* Agardh.
MER0001A	*Meridion circulare* var *circulare* (Grev.) Agardh.
NAV0003A	*Navicula radiosa* Kutz.
NAV0005A	*Navicula seminulum* Grun. (*Sellaphora seminulum*)
NAV0007A	*Navicula cryptocephala* var *cryptocephala* Kutz.
NAV0008A	*Navicula rhynchocephala* Kutz.
NAV0009A	*Navicula lanceolata* (Agardh) Kutz.
NAV0014A	*Navicula pupula* var *pupula* Kutz. (*Sellaphora pupula*)
NAV0021A	*Navicula cincta* (Ehrenb.) Ralfs in Pritch.
NAV0022A	*Navicula halophila* var *halophila* (Grun. ex Van Huerck) Cleve. (*Craticula halophila*)
NAV0023A	*Navicula gregaria* Donk.
NAV0027A	*Navicula viridula* var *viridula* (Kutz.) Ehrenb.
NAV0028A	*Navicula scutelloides* W.Sm. ex Greg.

NAV0030A	*Navicula menisculus* var *menisculus* Schum.
NAV0030C	*Navicula menisculus* var *upsaliensis* Grun. in Cleve & Grun.
NAV0030D	*Navicula menisculus* var *grunowii* Lange-Bertalot.
NAV0035A	*Navicula salinarum* var *salinarum* Grun. in Cleve & Grun.
NAV0042A	*Navicula minima* var *minima* Grun. in Van huerck. (*Eolimna minima*)
NAV0051A	*Navicula cari* var *cari* (Ehrenb.)
NAV0054A	*Navicula veneta* Kutz.
NAV0056A	*Navicula cuspidata* var *cuspidata* (Kutz.) Kutz. (*Craticula cuspidata*)
NAV0063A	*Navicula trivialis* Lange-Bertalot.
NAV0065A	*Navicula gastrum* (Ehrenb.) Kutz.
NAV0066A	*Navicula capitata* var *capitata* Ehrenb.
NAV0067A	*Navicula crucicula* var *crucicula* (W. Smith) Donk.
NAV0075A	*Navicula subhamulata* Grun. in Van Huerck.
NAV0084A	*Navicula atomus* (Kutz.) Grun.
NAV0095A	*Navicula tripunctata* (O.F.Muller.) Bory.
NAV0096A	*Navicula accomoda* Hust. (*Craticula accomoda*)
NAV0112A	*Navicula miniscula* var *miniscula* Grun. in Van Huerck.
NAV0114A	*Navicula subrotunda* Hust.
NAV0115A	*Navicula difficillima* Hust.
NAV0124A	*Navicula molestiformis* Hust. (*Craticula molestiformis*)
NAV0134A	*Navicula subminuscula* Manguin. (*Eolimna subminuscula*)
NAV0163A	*Navicula minusculoides* Hust.
NAV0168A	*Navicula vitabunda* Hust.
NAV0169A	*Navicula molesta* Krasske.
NAV0171A	*Navicula constans* Hust. in Lange-Bertalot.
NAV0264A	*Navicula buderi* Hust.
NAV0344A	*Navicula eidrigiana* J.R.Carter.
NAV0538A	*Navicula obdurata* Hohn & Hellermann
NAV0555A	*Navicula paramutica* Bock.
NAV0676A	*Navicula tenera* Hust. (*Fallacia tenera*)
NAV0743A	*Navicula subrhynchocephala* Hustedt.
NAV0744A	*Navicula pseudanglica* Lange-Bertalot.
NAV0745A	*Navicula capitatoradiata* Germain.
NAV0751A	*Navicula cryptotenella* Lange-Bertalot.
NAV0757A	*Navicula libonensis* Schoeman.
NAV0762A	*Navicula recens* Lange-Bertalot.
NAV0765A	*Navicula citrus* Krasske
NAV0769A	*Navicula lundii* Reichardt.
NAV0770A	*Navicula lestikowii* Lange-Bertalot.
NAV0771A	*Navicula cryptotenelloides* Lange-Bertalot.
NAV0780A	*Navicula wildii* Lange-Bertalot.
NAV9999A	*Navicula* sp.
NAV0389C	*Navicula gallica* var *laevissima* (Cleve) Lange-Bertalot.
NAV0027E	*Navicula viridula* var *linearis* Hust.
NAV9999U	*Navicula atomus* var *alcimonica* Reichardt.**
NAV9999V	*Navicula dissociata* Reichardt.**
NAV9999W	*Navicula margalithii* Lange-Bertalot.**
NAV9999X	*Navicula raederiae* Lange-Bertalot nov. spec.**
NAV9999Y	*Navicula symmetrica* Patrick in Krammer & Lange-Bertalot.**
NAV9999Z	*Navicula trophicatrix* Lange-Bertalot.**
NEI0020A	*Neidium hercynicum* A. Mayer.
NEI0036A	*Neidium ampliatum* (Ehrenb.) Krammer.
NIT0002A	*Nitzschia fonticola* Grun. in Van Huerck.
NIT0008A	*Nitzschia frustulum* (Kutz.) Grun. in Cleve & Grun.
NIT0009A	*Nitzschia palea* (Kutz.) W.Sm..
NIT0014A	*Nitzschia amphibia* Grun.

NIT0015A	*Nitzschia dissipata* (Kutz.) Grun.
NIT0017A	*Nitzschia gracilis* Hantzsch.
NIT0025A	*Nitzschia recta* Hantzsch ex Rabenh.
NIT0028A	*Nitzschia capitellata* Hust.
NIT0031A	*Nitzschia linearis* W.Sm.
NIT0033A	*Nitzschia paleacea* (Grun. in Cleve & Grun) Grun. in Van Huerck.
NIT0036A	*Nitzschia obtusa* W.Sm.
NIT0042A	*Nitzschia acicularis* (Kütz) W.Sm.
NIT0044A	*Nitzschia intermedia* Hantzsch ex Cleve & Grun.
NIT0063A	*Nitzschia agnita* Hust.
NIT0065A	*Nitzschia archibaldii* Lange-Bertalot.
NIT0083A	*Nitzschia constricta* (Kütz.) Ralfs in Pritch.
NIT0098A	*Nitzschia filiformis* (W.Sm.) Van Huerck.
NIT0139A	*Nitzschia paleaformis* Hust.
NIT0152A	*Nitzschia pusilla* Grun.
NIT0153A	*Nitzschia radicula* Hust.
NIT0157A	*Nitzschia reversa* W.Sm.
NIT0171A	*Nitzschia subacicularis* Hust.
NIT0184A	*Nitzschia umbonata* (Ehrenb.) Lange-Bertalot.
NIT0193A	*Nitzschia perminuta* (Grun.) M.Perag.
NIT0198A	*Nitzschia lacuum* Lange-Bertalot.
NIT0199A	*Nitzschia angustulata* Lange-Bertalot.
NIT0203A	*Nitzschia liebetruthii* Rabenh.
NIT0206A	*Nitzschia solita* Hust.
NIT0209A	*Nitzschia incognita* Legler & Krasske.
NIT0216A	*Nitzschia pura* Hust.
NIT9999A	*Nitzschia* sp.
NIT9999X	*Nitzschia commutatoides* Lange-Bertalot.**
NIT9999Y	*Nitzschia nana* Grun.**
NIT9999Z	*Nitzschia* [cf. *fonticola*] D.Emson (2007) **
PIN0001A	*Pinnularia gibba* (Ehrenb.) Ehrenb.
PIN0005A	*Pinnularia maior* (Kutz.) W.Sm.
PIN0014A	*Pinnularia appendiculata* (Agardh) Cleve.
PIN0019A	*Pinnularia legumen* Ehrenb.
PIN0022A	*Pinnularia subcapitata* Greg.
PIN0075A	*Pinularia brevicostata* Cleve.
RHO0002A	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot.
RHL0001A	*Rhopalodia gibba* (Ehrenb.) O.Muller.
RHL0009A	*Rhopalodia brebissonii* Krammer.
RHL0010A	*Rhopalodia acuminata* Krammer.
STR0001A	*Stauroneis anceps* Ehrenb.
STR0003A	*Stauroneis smithii* Grun.
STR0006A	*Stauroneis phoenicentron* (Nitzsch) Ehrenb.
STR0008A	*Stauroneis producta* Grun. In Van Huerck.
STR0012A	*Stauroneis kreigerii* Patrick.
STE0001A	*Stephanodiscus hantzschii* Grun. in Cleve & Grun.
STE0010A	*Stephanodiscus parvus* Stoermer & Hakansson.
SUR0001A	*Suriella angusta* Kutz.
SUR0016A	*Suriella minuta* Breb. ex Kutz.
SUR0047A	*Suriella minima* R.Ross & Abdin.
SYN0001A	*Synedra ulna* var *ulna* (Nitzsch) Ehrenb.
SYN0002A	*Synedra rumpens*. Kutz.
SYN0003A	*Synedra acus* var *acus* Kutz.
SYN0005A	*Synedra fasciculata* (Agardh) Kutz.
SYN0009A	*Synedra nana* Meister.
SYN0011A	*Synedra delicatissima* W.Sm.

SYN0019A	*Synedra capitata* Ehrenb.
SYN0002B	*Synedra rumpens* var *familiaris* (Kutz.) Hust.
SYN0003C	*Synedra acus* var *angustissima* (Grun. in Van Huerck) Van Huerck.
SYN0001H	*Synedra ulna* var *biceps* (Kutz.) Schonf.
TAL0001A	*Tabellaria flocculosa* (Roth) Kutz.

APPENDIX 2: The chemical composition of the growth media (MBL) used in the culturing of the epiphytic diatom, *Lemnicola hungarica* and (Hutner's solution) the growth media used in the culturing of *Lemna minor* for the laboratory experiment of habitat preference for the epiphytic diatom, *Lemnicola hungarica.*

MBL Stock Solution.

Chemical Constituent	Weight Used ($g\ l^{-1}$)	Element Concentration ($g\ l^{-1}$)	
$Ca\ Cl_2.2H_2O$	36.76	Ca: 10.0	
$MgSO_4.7H_2O$	36.97	Mg: 4.88	S: 7.03
$NaHCO_3$	12.60	Na: 3.45	C: 1.8
K_2HPO_4	8.71	K: 3.91	P: 1.55
$NaNO_3$	85.01	Na: 23.0	N: 14.0
$Na_2SiO_3.9H_2O$	57.05	Na: 9.24	Si: 5.63
$Na_2EDTA.2H_2O$	5.08	Na: 0.63	EDTA: 3.94
$FeCl_3.6H_2O$	3.15	Fe: 0.65	
$CuSO_4.5H_2O$	0.01	Cu: 0.003	S: 0.001
$ZnSO_4.\ 7H_2O$	0.022	Zn: 0.005	S: 0.003
$CoCl_2.6H_2O$	0.01	Co: 0.005	
$MnCl_2.4H_2O$	0.18	Mn: 0.05	
$NaMoO_4.2H_2O$	0.006	Na: 0.0006	Mo: 0.003

NB. The final experimental solution was adjusted to pH 7.2 (buffered with HCl).

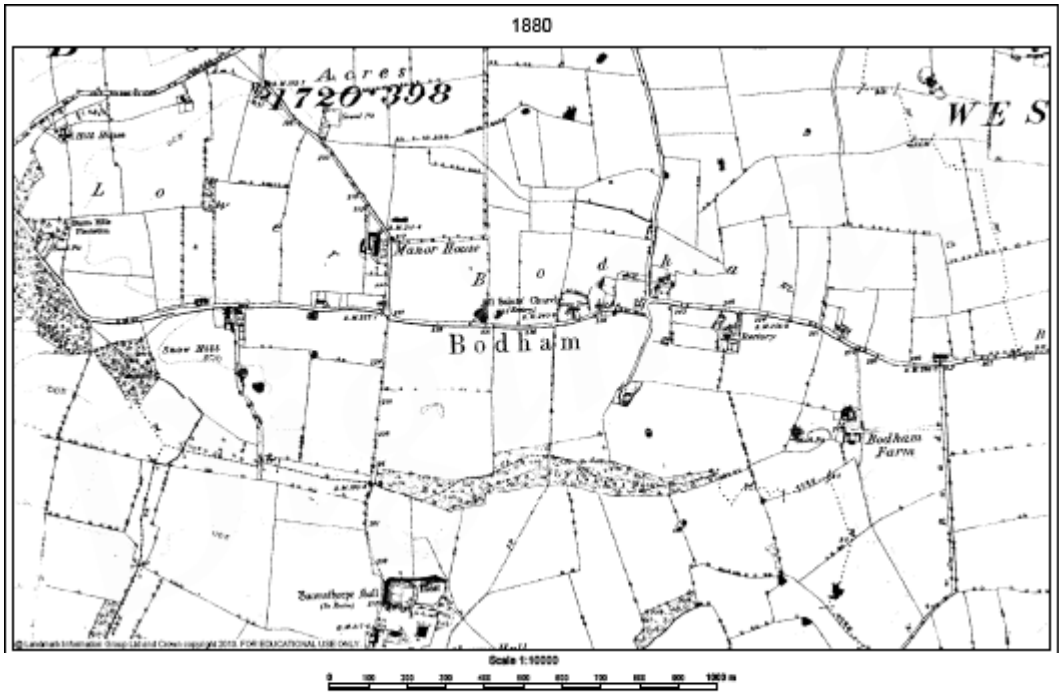
Hutner's Growth Solution

Chemical Constituent	Weight Used (mg l^{-1})	Element Concentration (mg l^{-1})	
NH_4NO_3	40	N: 14.0	
K_2HPO_4	80	K: 35.86	P: 14.25
Ca $(NO_3)_2$	40	Ca: 9.76	N: 6.83
$MgSO_4$	100	Mg: 20.0	S: 26.67
$FeSO_4$	5	Fe: 1.84	S: 1.05
$MnSO_4$	3	Mn: 1.09	S: 0.64
$ZnSO_4$	13	Zn: 5.25	S: 2.58
H_3BO_3	3	B: 0.53	
Na_2MoO_4	5	Na: 1.12	Mo: 2.33
$CuSO_4$	0.8	Cu: 0.32	S: 0.16
$CoSO_4$	0.2	Co: 0.08	S: 0.04
EDTA	100		

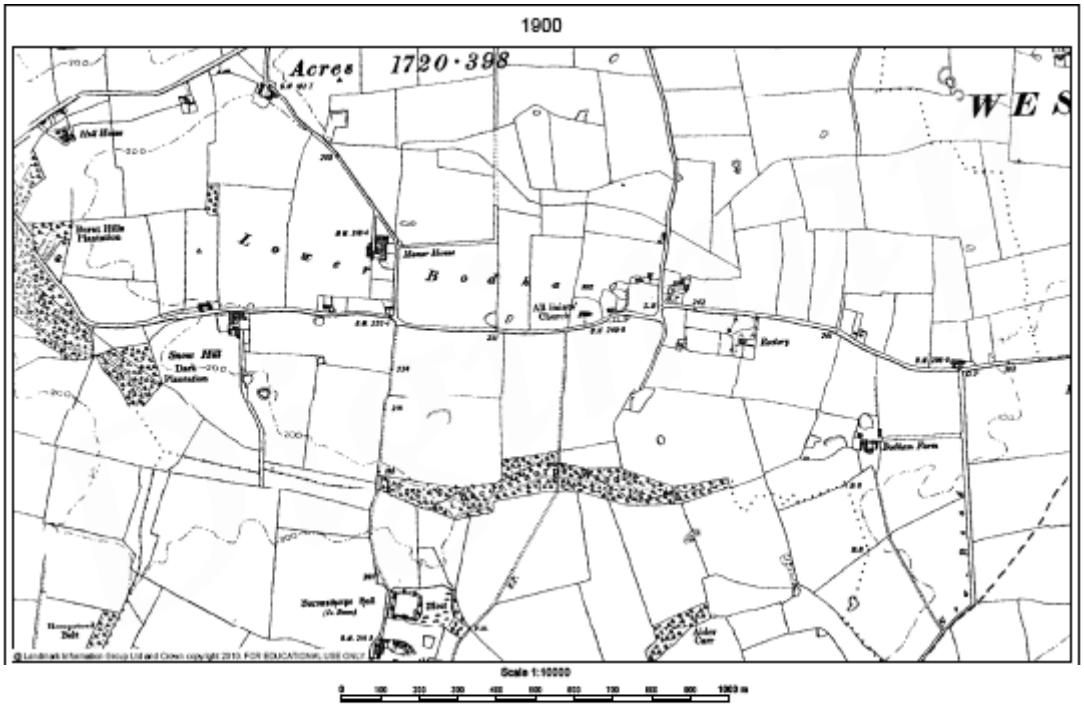
NB. The final experimental solution was adjusted to pH 7.2 (buffered with HCl).

APPENDIX 3: Historical maps of the Bodham area, North Norfolk, England showing the Bodham Rail Pit and other ponds/pits from 1880, 1900, 1920, 1950 and 1970.

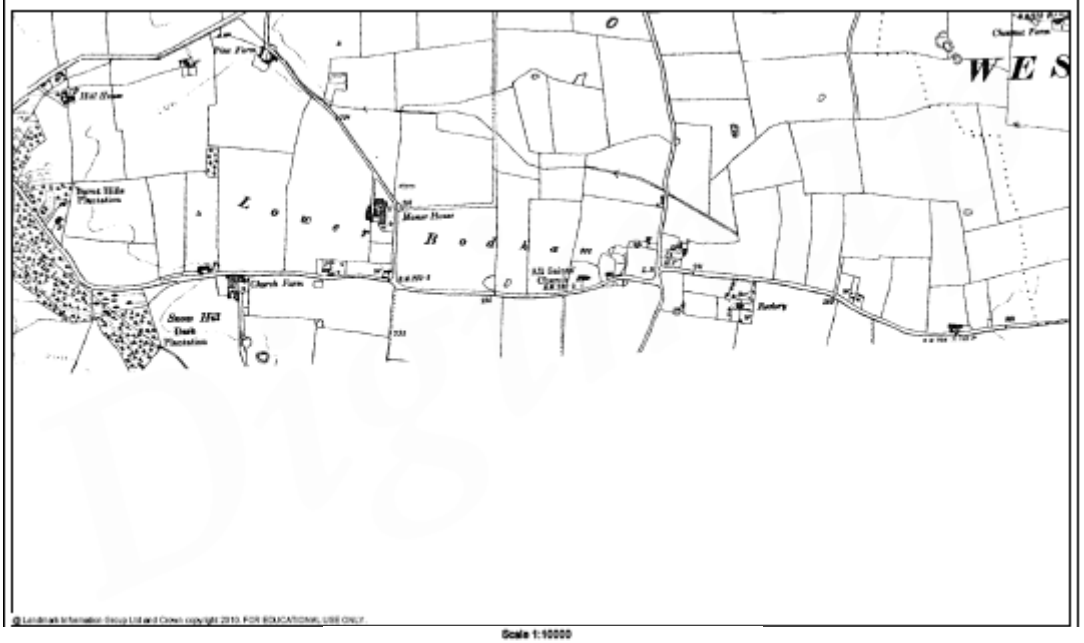
1880



1900



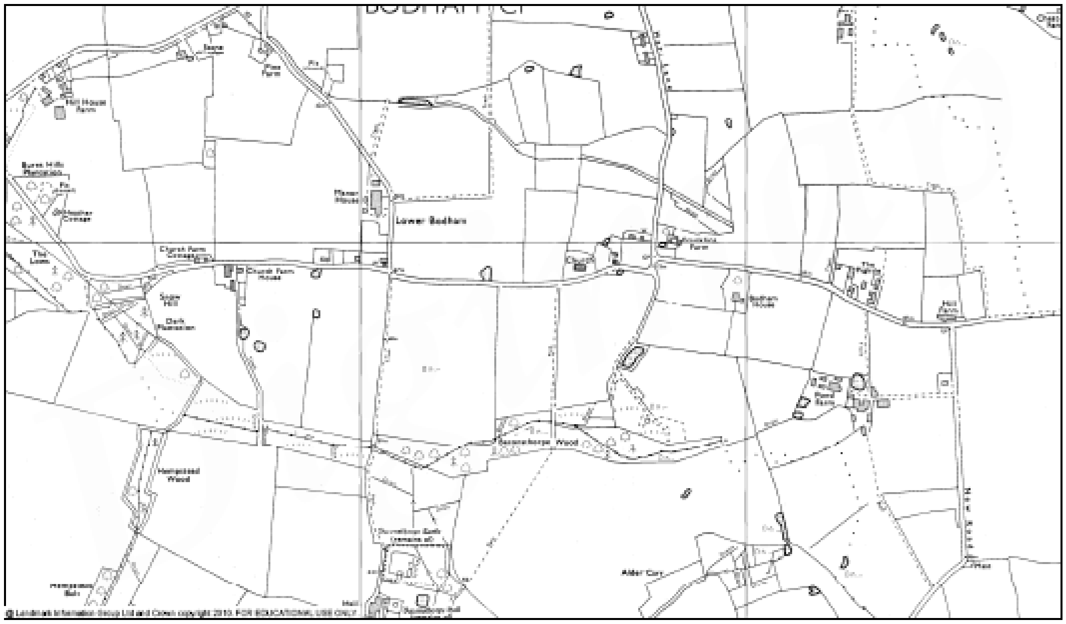
1920


Hall House
Manor House
Church Farm
Rectory
Scale 1:10000

1950

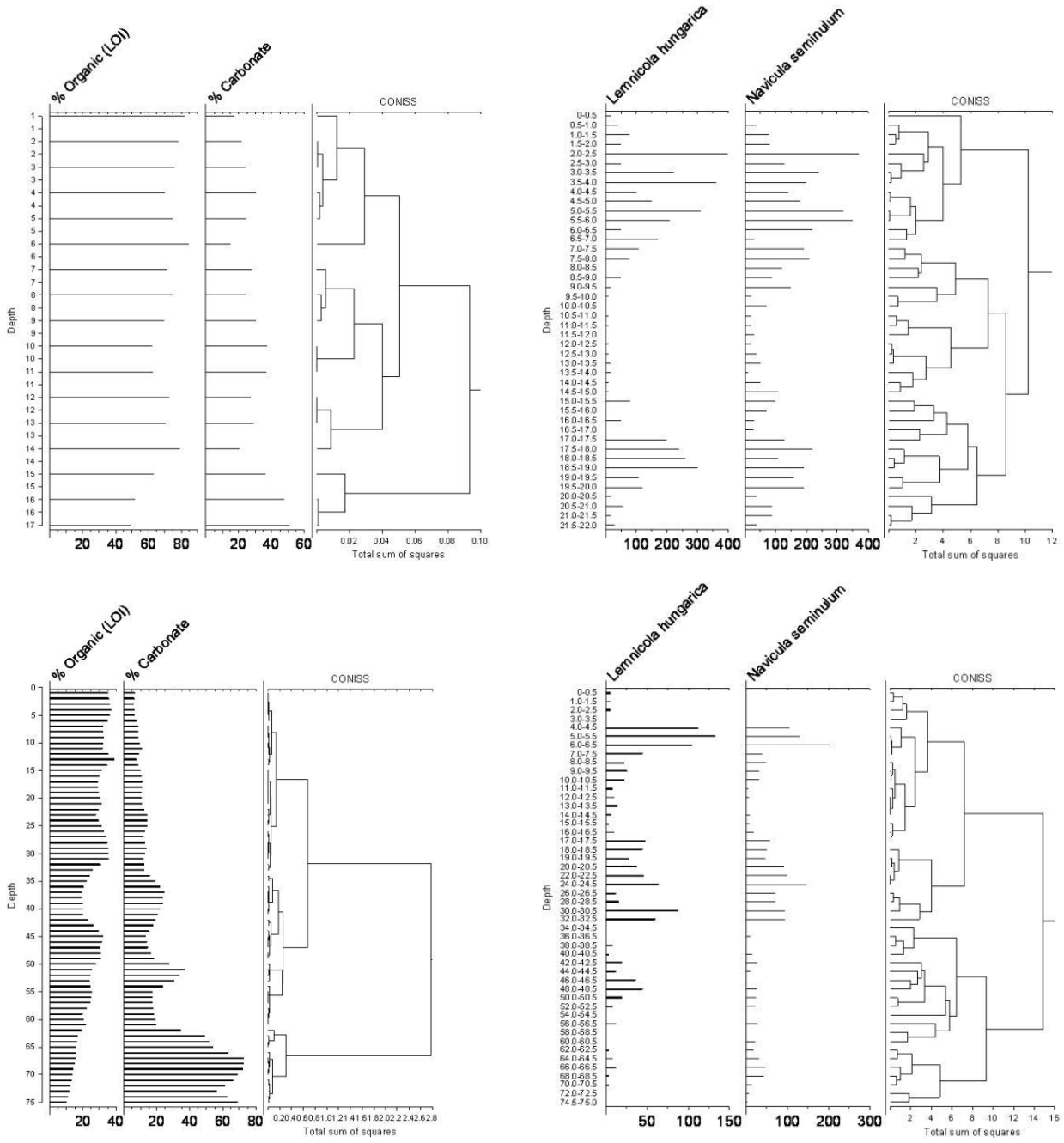

Hall House
Manor House
Church Farm
Rectory
Scale 1:10000

1970

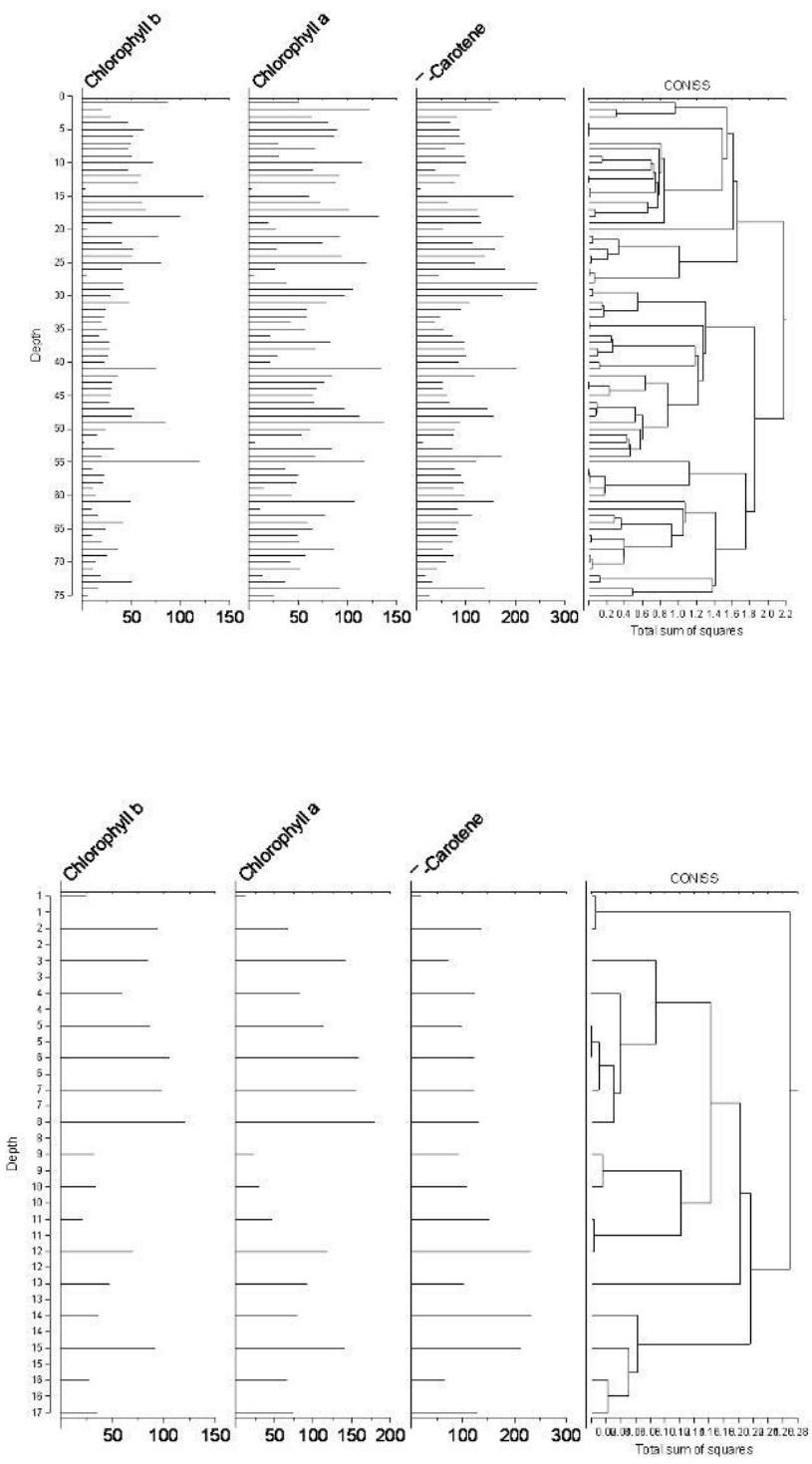


Scale 1:10000

APPENDIX 4: Results of numerical zonation of RAIL1 and RAIL2.

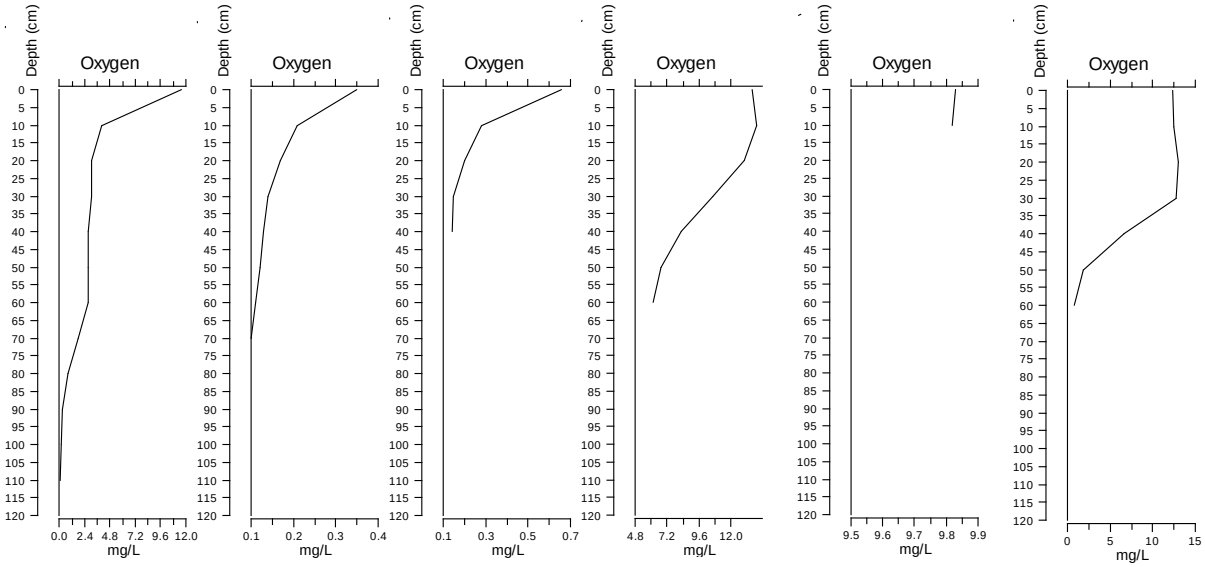


Results of numerical zonation of RAIL1 and RAIL2 using CONISS (constrained incremental sum-of-squares cluster analysis). Zonation was performed on both the diatom and the lithostratigraphic data for comparison. RAIL1 (bottom L/H = lithostratigraphic zonation; bottom R/H = diatom zonation); RAIL2 (upper L/H = lithostratigraphic zonation; upper R/H = diatom zonation). Note: diatom zonation was calculated from the total diatom assemblages recorded. Diagrams show *Lemnicola hungarica* and *Navicula (Sellaphora) seminulum*, i.e. diatoms associated with *Lemna.* The diatom zonation and the lithostratigraphic zonation are remarkably similar.

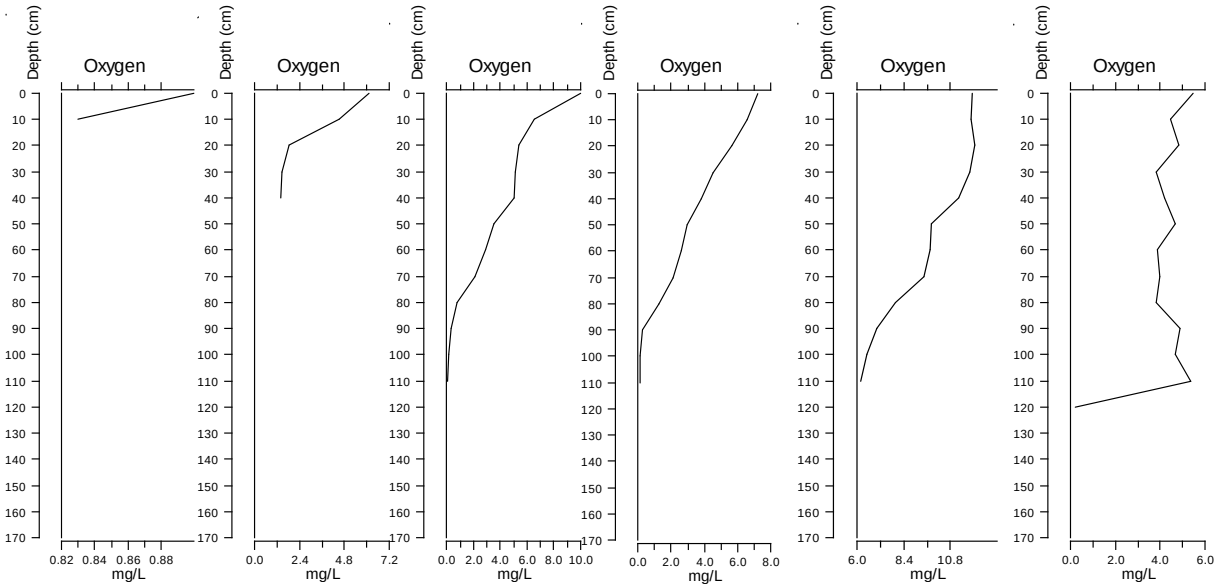


Results of numerical zonation of RAIL1 and RAIL2 using CONISS (constrained incremental sum-of-squares cluster analysis). Zonation was performed on the plant pigment data for comparison with both the diatom and lithostratigraphic zonations for RAIL1 and RAIL2. RAIL1 pigment zonation (top); RAIL2 pigment zonation (bottom). Note: pigment zonation was calculated from all of the pigment analyses recorded. Diagrams show the main chlorophyll pigments (chlorophyll a and b) and a major carotenoid pigment (β – Carotene). The pigment zonations are remarkably similar to the diatom and lithostratigraphic zonations

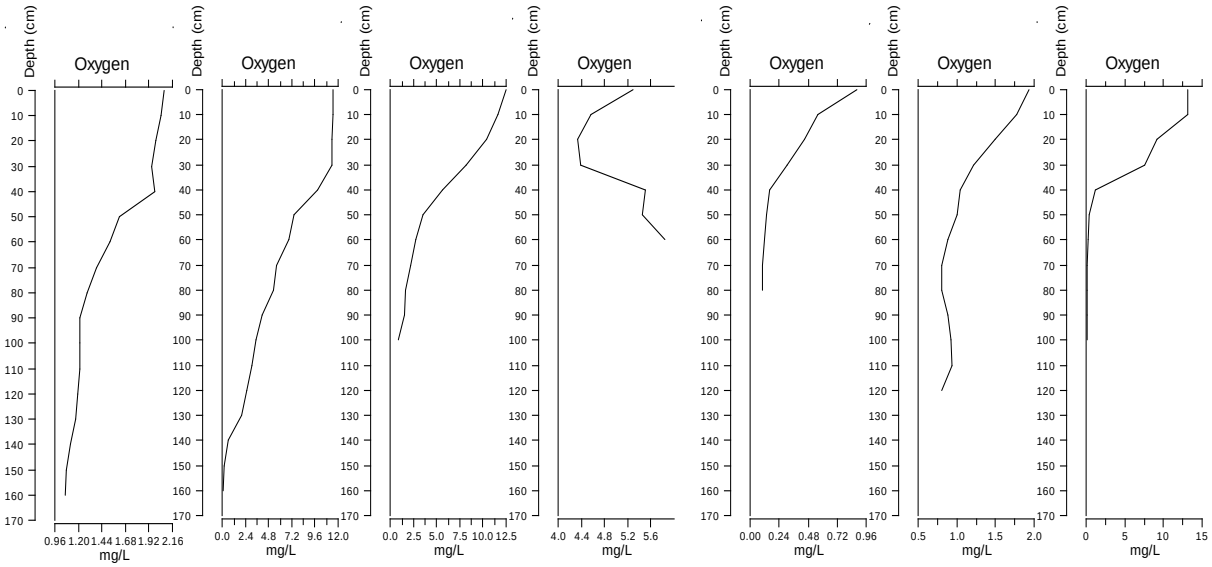
APPENDIX 5: Oxygen profiles of *Lemna* and Non-*Lemna* sites.



Oxygen profiles of the six duckweed sites used in the logistic regression and ordination analyses. The depths of the individual sites are given (cm) and the concentrations of oxygen (mg/l) are also presented. L-R: Pond Farm Pond 1 (90%), Priory Pond 1 (90%), Lower Farm Pond (80%), Church Farm Pond 1 (40%), Bullock Shed Pond 2 (5%), Ramsgate Horse Pond (25%). The percentage duckweed cover at the time of sampling is given in parentheses above. The lowest oxygen measurement for each site is the maximum water column depth.



Oxygen profiles of the non duckweed sites used in the logistic regression and ordination analyses. The depths of the individual sites are given (cm) and the concentrations of oxygen (mg/l) are also presented. L-R: Kiosk Pond, Cinders Hill Pond, Otom Pit, Bullock Shed Pond 1, Bodham Marl Pit, Pond Farm Pond 2. The lowest oxygen measurement for each site is the maximum water column depth.



Oxygen profiles of the non duckweed sites used in the logistic regression and ordination analyses. The depths of the individual sites are given (cm) and the concentrations of oxygen (mg/l) are also presented. L-R: Salle Patch Pond, Henry's Pit, Hempstead Rookery Pond, Pond Hills Pond, Sayers Black Pit, Bodham Mystery Pit, Bodham Rail Pit (post duckweed coverage).