Conduits of Contamination to Contemporary Food webs of the Norfolk Broads

Thesis submitted for the degree of the doctor of philosophy

University of London

by

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I, Jacob Laws, confirm that the work in this thesis is my own. Where information has been derived from other sources, I confirm this has been in indicated in the thesis

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Acknowledgements

First and foremost I must thank my supervisors Jonathan Grey, Kate Heppell and Dave Sheahan; without whom this would not have been possible. I know I was not always the ideal PhD student so I thank you sincerely for putting up with my incessant crises of confidence and for your guidance throughout the duration of the study. An extra thanks to Jon Grey for his mastery of stable isotopes, the chironomid sieve and for his general 'can do' approach and to Kate Heppell for enduring my chemical naivety in the early stages of the project. I must also express thanks to Gabriel Yvon- Durocher and Dan Perkins, for friendship, support and laughter throughout the past four years. Thanks to Maarten van Hardenbroek for his Dutch pancakes and Vytas Rakauskas for his unique brand of Lithuanian humour. Your help in the field was also appreciated and resulted in two unexpected friendships.

I extend my gratitude to all those who where integral to the creation and completion of the project; Carl Sayer and Dan Hoare for exposing the TBT legacy in the Broads, Charles Cator and family for access on Malthouse Broad, Henry Cator at Salhouse Broad, Jim Papworth at Wroxham Broad, Rick Southwood and Will West at Cockshoot Broad, the How Hill Trust and the Broads Authority at Cromes Broad. To all in the organotin laboratory at CEFAS Lowestoft; Thi Bolam, David James, and Boby Thomas, thank you, your guidance in TBT analysis was absolutely invaluable. Special thanks to my family; mother Jos Wickham for putting me up (and putting up with me) during field work, and Tony and Frank Laws for assistance in the field during some particularly inclement weather.

Abstract

During the 1960s-1980s antifouling applications containing the organotin tributyltin (TBT) were applied to craft on the Norfolk Broads, leaving a legacy of contaminants in sediments. Previously, no research had been undertaken to investigate the implications that this legacy may have for the ecological integrity of the Norfolk Broads aquatic ecosystem.

Eight sites in the Norfolk Broads that represented a gradient of contamination (as measured by sediment TBT concentrations) were selected. Contamination was evident in invertebrates and fish but was lower than sediments, as total organic carbon and species specific metabolic capacity for TBT controlled bioavailability

To examine TBT impacts at the community scale, novel metrics were applied to food webs defined by stable isotopes of carbon and nitrogen. Two metrics were reduced in response to increasing TBT contamination, suggesting simplification of food webs along the contaminant gradient; where loss of key food web properties such as trophic diversity and shortened food chain length could reduce resilience to further system perturbations.

I hypothesised that chironomids emerging from sites contaminated with organotins would carry with them an organotin burden, which would be reflected in terrestrial predators such as spiders via trophic transfer. A combination of spider and chironomid stable isotopes (principally δ^{13}C) and isotope mixing models indicated considerable chironomid contribution to spider biomass at all four sites (34-88%). Subsequent organotin analyses revealed consistent low level, butyltin (di-butyltin; DBT) contamination in chironomids and spider predators from the most contaminated site.

Chapter 1

Literature Review

1.1 Organotins in the Global Aquatic Environment

Organotin compounds (OTCs) were first synthesised in the mid 18th century (Van der Kerk, 1975), although they did not become commercially important until ca. 100 years later. During the 1940s it was discovered the addition of organotins (principally butyltins) to the PVC polymer had a stabilising effect that prevented weakening and discolouration of the finished product (Blunden and Evans, 1990). Around a decade later the efficacy of OTCs as biocides first became apparent (van der Kerk and Luitjen, 1954); particularly the trisubstituted butyl, phenyl and hexyl species. In light of this, they were soon used as the active toxic component in a wide range of biocidal applications; for example, as preservatives in the clothing, timber and paper milling industries, and in agriculture as fungicides, miticides, bactericides, nematocides, molluscicides and rodent repellents (Bailey et al., 1997; Hoch, 2001). However, their best known application was as the toxic component of antifouling preparations applied to boat hulls and other marine structures such as salmon pens (Gibbs, 1986; Balls, 1987).

Antifoulant treatments are designed to prevent 'biofouling' – nuisance colonisation of the hull (or other submerged structure) by barnacles, other invertebrates and algae – that results in reduced speed and economic efficiency, and increased fuel emissions (Santillo et al., 2001). For example, a small 10μm increase in hull roughness can increase fuel consumption by 0.3- 1.0% (Champ and Seligman, 1996).

Organotins were first incorporated in antifoulant preparations in the 1960s, initially as a booster biocide to complement the copper based paints popular at the time (Wade, 1988). However, it soon became evident that OTC boosters were more effective as antifoulants than copper (Evans 2000), leading to the development of a new generation of organotin-based treatments. The most common organotin added to these preparations was the trisubstituted butyltin (TBT) due to its toxicity to a wide range of fouling organisms (Hoch, 2001). Once applied to the hull, TBT leaches from the paint matrix forming a freely dissolved toxic layer that is available for immediate biological uptake. Although TBT-based antifoulants were originally designed for use on large, ocean going vessels, their effectiveness as antifoulants, coupled with successful marketing during 1960-70s, saw their popularity spread worldwide. Thus, TBT based antifoulants were soon the common treatment applied to much smaller craft in both inshore and inland waterways (Sayer et al., 2006). As organotins dissociated readily from the paint matrix, water, sediments and biotic components of the ecosystem, became highly contaminated with TBT, particularly in areas of high boating activity (Wade et al., 1991). However, the potential for widespread TBT contamination of aquatic ecosystems was initially overlooked, or simply ignored.

Coinciding with the increasing use of TBT in aquatic environments were the first English cases of undesirable impacts of TBT on non-target organisms. Blaber (1970) noted the development of male genitalia (a phenomenon later termed 'imposex'; Smith, 1980) in female dog whelks (*Nucella lapillus*) within Plymouth sound (UK). In this case, a causal link between imposex induction and TBT based antifoulants was not immediately established. However, the occurrence of the condition in the American intertidal mud snail (*Nassarius*

obseletus) was later linked directly to TBT pollution originating from marinas (Smith 1980). Further U.K-based research revealed that the incidence of imposex was widespread in Plymouth sound and other inshore areas around the UK that were subject to high boating activity (Harding et al., 1997). The full extent of the problem was manifest in severe disruptions in population ecology and in some cases, local extinctions (Gibbs, 1986).

Dog Whelks were not the only non-target organisms to be affected by early TBT use, however. Over the course of six years, (1975 – 1981) annual oyster (*Crassostrea gigas*) production in the Arcachon Bay (South - West France) fell from ~ 15,000 to 3000 tonnes (Evans, 2000). This dramatic decline in yield was attributed to declining recruitment and gross thickening of valves that rendered remaining stock unsalable (Alzieu et al., 1989). In the early 1980s, scientists ascribed the physical abnormalities and disruptions in population ecology to TBT pollution stemming from the growing number of small, recreational craft in the bay (Alzieu et al, 1986). Although evidence was to some extent circumstantial, the economic losses incurred by the virtual collapse of the industry encouraged the French government in 1982 to pass legislation prohibiting TBT-based antifoulants on all vessels < 25m in length.

Within the same time frame as the problems occurring in France, production of the introduced pacific oyster was failing on the coast of East Anglia, UK. Again, poor recruitment and shell malformations were principal causes. Key et al. (1976) cited high concentrations of water column FPOM as the cause. This hypothesis was later refuted, as experimental and survey work showed that the concentration of TBT in many U.K. coastal

waters (particularly marinas) was sufficiently high (~0.08µg l^{-1}) to reduce growth rate and induce thickening of the valves. At higher concentrations (~ 1.6 µg l^{-1}), mortalities and severe wasting of the tissues were observed (Waldock and Thain, 1983).

In light of this research, the British Government followed the path of the French, first passing legislation restricting the concentration of TBT in antifoulants in 1985, then banning their use on all craft < 25m in length in 1987. Both these case histories served to illuminate the severity of the potential ecological, and ultimately financial, implications of unrestricted TBT use. Thus, by 1989, similar legislation to that applying in France and the UK was passed in the most of Europe, North America, Australia, New Zealand and Hong Kong (Antizar-Ladislao, 2008). After these restrictions, water column concentrations of TBT fell quickly, since the compound has a short life in the water column (Gadd, 2000). However, it subsequently became apparent that degradation in the sediments could be considerably slower, (lasting for decades) particularly under anoxic conditions (Burton et al., 2004; Dowson et al., 1996). The mechanisms contributing to this considerable potential for environmental persistence are discussed further in section 1.2.

The occurrence of imposex caused by TBT has now been reported in approximately 150 wild marine gastropod species globally, representing a 50% increase in recorded incidence since 1991 (Oehlman, 1991; Vos et al., 2000). Whilst imposex is a response to toxicity restricted to certain gastropods, the number of species affected more generally indicates the widespread bioavailability of organotin compounds, and suggested a potential for uptake and accumulation by other non-target organisms. Indeed an expanding body of literature supports

this notion, documenting widespread organotin contamination in a diverse range of biota. For example, a recent review details the occurrence of butyltin compounds in a number of bivalve molluscs, gastropods and fish, from 15 countries spanning Asia, Europe and America (Antizar – Ladislao 2008). Moreover, others have documented the presence of organotins in organisms across several trophic levels, highlighting the potential for transfer and accumulation through food web interactions. For example, TBT has been detected in phytoplankton (Luan et al., 2006), bacteria (Gadd, 2000), macroalgae (Strand and Jacobsen, 2005), crustaceans (Takahashi et al., 1999; Ohji et al., 2002), in catadromous fish (eel: Harino et al., 2000; Ohji et al., 2006), anadromous fish (salmon: Ohji et al., 2006) and deep sea fish (Borghi and Porte, 2002), and in piscivorous and herbivorous birds (grebes and cormorants, Stab et al, 1996; swans, Strand and Jacobsen, 2005), otters (Kannan et al, 1999) and marine mammals (porpoises, Kannan et al., 1995; 1997; seals, Strand and Jacobsen, 2005).

The current literature documents the occurrence of organotin contamination of aquatic environments and biota on a global scale, in a variety of environmental compartments and at the levels of the individual, population (Gibbs, 1986) and community (Takahashi et al, 1999; Strand and Jacobson, 2005). Perhaps not surprisingly, there has been a strong research bias toward marine ecosystems, neglecting the fact that TBT use was not limited to the marine environment, but was also applied in freshwaters (see Stab et al., 1996; Kannan 1997; Sayer et al, 2006). The Norfolk Broads (an interconnected shallow lake system in Eastern England, UK) is one such system where TBT was used on the majority of craft from the 1960s until its ban in 1987.

A common feature of shallow lakes (by definition, those with a mean depth < 3m), such as those that characterise the Norfolk Broads, is either domination by macrophytes accompanied by clear water and a diverse faunal assemblage, or by phytoplankton with turbid water, sparse or no macrophytes and a reduced faunal diversity (Blindow et al., 1993). These two divergent ecological states can exist over a broad range of nutrient conditions and are known as 'alternative equilibria' or 'stability domains' (Scheffer et al., 1993). Shallow lakes occasionally exhibit a rapid shift between states, induced by environmental perturbations and stressor mechanisms that render the habitat open to recruits of the alternative assemblage (Scheffer, 2001; Jones and Sayer, 2003). Putative causes can be simple stochastic disturbances, such as severe storms (Mitchell, 1989), alterations in water level (Blindlow et al., 1993) and pollution from herbicides (Richards et al., 1984). However, more complex feedback mechanisms or suites of stressors may be responsible for the rapid 'flip' between ecosystem domains. For example, alterations of food web architecture, such as changes in the fish community structure, can result in trophic cascades culminating in the release of phytoplankton and periphytic algae from top down control exerted by grazing invertebrates. In turn, this may precipitate the loss of macrophytes and dominance by phytoplankton and the associated changes in the invertebrate community (see Jones and Sayer, 2003).

Throughout the period 1960-1970, such rapid shifts between ecosystem alternative states were observed in the Norfolk Broads (Moss, 1996). This was characterised by the virtually ubiquitous loss of submerged macrophytes, giving way to phytoplankton dominance of the water column. This dramatic shift occurred in parallel with an increase in pleasure craft using the system (Sayer et al., 2006). Initial hypotheses centred on boat-induced turbulence and

intensive nutrient loading from the catchment, such as waste treatment plants, as key causal factors (Moss 1996). Whilst it is possible that nutrient loading weakened ecosystem resilience, facilitating the rapid change in ecological regime, the former hypothesis invoking increased turbulence from boats has been discredited, due to the loss of macrophytes on non - navigable (but connected) Broads. Nevertheless, recent research suggests the presence of boats may have contributed in a manner more cryptic than physical disturbance alone.

Palaeolimnological surveys conducted on two lakes in the Broads system (Wroxham Broad and Hoveton Great Broad) revealed that the rapid loss of macrophytes and subsequent dominance by phytoplankton was synchronous with the first occurrence of TBT in the system (Sayer et al., 2006). In addition to reductions in macrophyte biomass and species diversity, similar simultaneous changes in the community structure of both grazing molluscs and zooplankton have been observed in the fossil record. This was typified by a shift to communities dominated by smaller species with reduced grazing efficiency (see Brooks and Dodson, 1963; Dodson, 1974). Contaminants are known to initiate indirect effects (such as trophic cascades) on recipient food webs via reductions in number of keystone or foundation species (Fleeger et al, 2003). Reductions in numbers of these species can then result in alterations of top down or bottom up food web processes that release more contaminant tolerant species from competition or grazing pressure (Fleeger et al, 2003).

Thus, in a manner similar to a trophic cascade, TBT toxicity may have significantly reduced numbers of key grazing invertebrates, thus releasing phytoplankton and periphytic algae from grazing pressure and facilitating the exclusion of macrophytes. A small number of

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experimental studies support this hypothesis. Experiments in pelagic marine enclosures demonstrated reduced numbers of large bodied zooplankton after TBT additions, and subsequent significant increases in chlorophyll ^-a and large microphytoplankton were observed (Jak et al, 1998). More direct evidence comes from the Broads. For example, in mesocosm experiments, Kerrison (1988; 1989) replicated established mollusc and zooplankton communities extant in Hoveton Great Broad. Following TBT addition (max conc. 600ng l^{-1}) overall mollusc abundance was reduced accompanied by a significant decline in zooplankton grazing. In addition, Jackson (1999) demonstrated that the number of invertebrates sensitive to TBT (especially molluscs) was reduced in navigable parts of the Broads during peak TBT use.

Indeed, water column concentrations of TBT during 1986 and 1987 in Wroxham Broad and a nearby marina on the River Bure were 898 and 1540 ng l^{-1}, respectively (Waite et al., 1989). These concentrations exceed lethal thresholds for key grazing species, such as *Daphnia magna* and *Lymnaea stagnalis*, and those for other crustaceans, various chironomids (midges) and fish. Thus, during this period, the population sizes of all of these organisms could have been reduced due to exposure to TBT.

Further research unveiled that this legacy of TBT was not only buried at depth, it was also measurable in contemporary surface sediments in the majority of navigable lakes, despite the elapse of almost 25 years since the last known use of TBT in the system (Hoare, 2007). Most likely, this stems from the application of TBT based antifoulants prior to the 1987 ban, although illegal use cannot be ruled out. The discovery of this legacy has highlighted the need

to investigate the potential implications of TBT contamination for contemporary food webs and biota of the Norfolk Broads.

Such work could have important ramifications for current science policy and aid lake management strategies at a wider scale. For example, the European Water Framework Directive (WFD) stipulates that all member states must aim to reach good chemical and ecological status in inland and coastal waters by 2015. TBT is listed as a priority substance that poses a threat to the aquatic environment under Annex 'x' in the WFD. With this date fast approaching it is imperative we have a robust understanding of the potential impacts that TBT may still have on freshwater ecosystems, and the mechanisms by which they operate.

Beyond the purely European context, shallow lake systems are globally widespread (Gratton et al., 2008) and support a variety of fundamental ecological processes such as carbon dioxide (CO_2) and methane (CH_4) efflux and carbon storage in sediments (Downing et al., 2006). In addition, they fulfil important ecosystem 'goods and services', including provision of important fisheries (Holmlund and Hammer, 1999), irrigation and drinking water (Baron et al, 2002), and encouraging tourism (Devai and Moldovan, 1983). Furthermore, they frequently have high conservation value as habitats for rare plants and animals (Davidson et al., 2008). Importantly, there is a growing recognition that once lost these functions are costly, if not impossible, to replace (Baron et al., 2002). Therefore, a clear fundamental understanding of the ecology of shallow lakes is of value in underpinning their management and conservation, if such goods and services are to be sustainable.

The recognition of this historical TBT legacy in the Norfolk Broads suggests the potential existence of similarly contaminated systems elsewhere, despite the restrictions imposed in most western countries for > 20 years. This is certainly true of the Netherlands (Stab et al., 1996), Switzerland (Becker et al., 1995), Poland (Szpunzar, 1997), New Zealand (De Mora, 1997) and Canada (Bartlett et al., 2005). Moreover, TBT use is still unrestricted in a number of countries in developing regions such as South-East Asia (e.g. India, Kannan et al., 1997; Vietnam, Hoch, 2001; China, Gui-bin et al., 2001) and the ecosystem health of freshwaters in regions could be under considerable ongoing threat. Indeed, Gui-bin et al. (2001) reported the presence of TBT and its metabolites (monobutyltin- MBT, and dibutyltin – DBT) in the water column of four Chinese lakes and four rivers at concentrations (0.8- 37 ng l^{-1}) exceeding the toxicity thresholds for many aquatic organisms, whose their persistence in these systems could therefore be compromised. Similarly, in a study from India, Kannan et al. (1997) reported TBT concentrations of up to 2000ng g^{-1} in the critically endangered Ganges river dolphin. These values were 5 - 10 times greater than those in dietary sources, highlighting the potential for bioaccumulation.

As the Norfolk Broads are connected by a series of lowland rivers they could represent an important model system in which to study the fate and occurrence of TBT contamination in both lotic and shallow lentic ecosystems. Thus, an understanding of the Broads system could facilitate more general predictions of the pattern of occurrence, fate and behaviour of organotins in fresh waters, and could underpin work aimed at the prevention of ecological

decline and the restoration of ecological integrity in those systems already affected by TBT use.

1.2 The Chemical Fate of Organotin Compounds in Aquatic Ecosystems

The persistence of a legacy of sediment-bound organotin, such as that revealed in the Norfolk Broads, is facilitated by the chemical reactions of TBT and by the effect of environmental parameters in water and sediment. Dissolved TBT (e.g. from an antifoulant paint) undergoes pH-dependent speciation on entering the water column; TBT has a pka acidity constant of 6.25; therefore, at this pH, half of the molecules occur as the cationic TBT^+ (Meador et al., 2002). An increase to pH 7 sees the prevalence of the neutrally charged species TBTOH. This is significant for two reasons: firstly, speciation influences adsorption dynamics and thus how TBT partitions between environmental phases in the aquatic environment (e.g. water, suspended material and sediments Hoch 2001); secondly, charge influences the ease with which biological membranes can be passed. Thus, the neutrally charged TBTOH can pass biomembranes more readily than TBT^+, rendering it more biologically available to aquatic biota (Looser et al., 1998).

TBT has a short half life in the water column: in natural fresh waters, estimates range from six weeks to five months (Gadd, 2000), with breakdown characterised by step-wise debutylation, first to dibutyltin (DBT), then to monobutyltin (MBT) and eventually to inorganic tin (Rudel et al., 2003). As debutylation occurs, the toxicity of compounds progressively declines (Antizar – Ladislao, 2007). Heat, light and aerobic biological activities are the principal agents of degradation, and the latter is considered the most important (Barug, 1981, in Antizar- Ladislao 2007, Dowson et al., 1993).

Thus, in the absence of an ongoing continuous source of contamination, concentration in the pelagic compartment should decline rapidly, thus limiting ecotoxicological impacts on pelagic food web components (e.g. zooplankton). Indeed, this appears to have been the case in the Norfolk Broads following the 1987 ban (Waite et al., 1989). However, TBT is a hydrophobic compound, due to a high specific gravity (~ 1.2 kg l^{-1} at 20°C; Landemeyer et al., 2004) and low solubility (< 10 mg l^{-1} at 20°C at pH 7; Fent, 1996). Octanol- water partition coefficients (log K_{ow}) from 3.7 - 4.4 have been reported in the literature, suggesting a high affinity for organic matter. Arnold et al. (1997) found that log K_{ow} values for TBT showed a positive correlation with increasing pH. This result was probably due to the increasing prevalence of the neutral species that passes biological membranes more readily (Looser et al. 1997).

Experimental work investigating equilibrium partitioning between sediment and water (measured as the partition coefficient, log K_d) has demonstrated that organic rich sediments can remove up to 100% of TBT in solution (e.g. from the Broadland River Yare: Dowson et al., 1993). More specifically, TBT has a high affinity for the organic carbon present in such sediments (Meador et al., 1997). Thus, organic carbon has frequently been demonstrated to be key parameter in controlling storage and bioavailability of TBT (Meador et al., 1997). The partitioning of TBT to organic carbon is measured as the organic carbon normalised partition coefficient (log K_{oc}- and, in the case of DOC, K_{doc}). Log K_{oc} values are partition coefficient values (K_d) that are normalised for sediment organic carbon content. This has the effect of greatly reducing variability observed in partition coefficient values between sediment types (Meador et al., 2002). This affinity for organic carbon means that TBT adsorbs readily to

suspended particulate matter, the organic fraction of sediments, and forms complexes with DOC (e.g. humic acids: Looser et al., 1997; 2002). In addition the compound may also form complexes with anions such as sulphate, nitrate and potassium (Arnold et al., 1997). TBT also associates strongly with mineral fractions, such as clays; for this reason particle size can be also be an important parameter in determining partitioning, particularly in those sediments in which organic matter is reduced (Meador et al., 1997). These factors combine to enhance the potential of sediments as contaminant sinks, a prevalent trend in systems where antifoulants containing OTCs were applied (Hoch 2001).

The parameters that influence TBT partitioning behaviour mean that a decline in water column TBT concentration may be construed as a measure of the partitioning from water to sediments rather than resulting from degradation of the contaminant in the water column (Hoare, 2007). Following partitioning to the solid phase, prevailing physical, chemical and biotic conditions within sediments contribute significantly to the persistence of TBT. This is because the sediment is frequently anaerobic (and therefore exhibits reduced biological activity), has little to no light penetration (so photolysis is reduced), and is cooler than the overlying water column.. A number of studies report slow degradation times, ranging from ~ 2 years to decades (Batley, 1996; Maguire, 1996; (in Rudel, 2003)) Dowson et al., 1996). The occurrence of degradation in sediments is manifest in the presence of the breakdown products DBT and MBT (Diez et al., 2002), the concentrations and ratios of which can be used to make inference about the rate of TBT breakdown (Diez et al., 2002). For example, in the Norfolk Broads the proportion of TBT is frequently greater than DBT, while MBT is often absent. This indicates slow degradation (Diez et al., 2002). Such measures may be useful for environmental monitoring of TBT contamination, as they can be compared over time to assess the degradation of TBT in sediments.

The partitioning and degradation behaviour of TBT both strongly influence the degree of contaminant exposure experienced by aquatic organisms. The degradation influences the length and extent of exposure of the organisms to TBT, and the partitioning indirectly controls bioavailability. Freely dissolved TBT (that which is unbound to POC, DOC, the mineral fraction and ion complexes in the water column) represents the greatest toxicological threat to aquatic organisms, as it is directly available for uptake via contact with epithelia or membranes (for example, gill filaments).

TBT dissolved in the aqueous phase is likely to have contributed significantly to the endocrine disruption observed in marine organisms throughout the 1970-80s, as continuous inputs from antifoulants maintained freely dissolved concentrations. In situations such as those that arose in the Norfolk Broads, however, the lack of a modern 'point source' (e.g. cessation of TBT use following the 1987 ban), combined with the rapid partitioning of TBT to suspended organic matter, DOC and organic rich sediments, means that water column concentrations are frequently much lower than that in the sediment (Hoch, 2001). Furthermore, TBT that is adsorbed to the organic fraction of sediments, clays and DOC and ion complexes is limited in its bioavailability, although uptake via ingestion of contaminated sediments and filter feeding on suspended organic matter represent two potential uptake routes (Rudel et al., 2003).

Despite this apparent limitation in bioavailability of sediment associated TBT, detailed studies have shown that adsorption is a fast and reversible process, and equilibrium partitioning occurs between sediments and interstitial pore waters (Berg et al., 2001). This gives rise to a fraction of freely dissolved TBT in interstitial porewater. As this fraction is unbound to any other organic or mineral compound, it represents a truly bioavailable concentration (Rudel et al., 2003). However, this concentration is known to be influenced strongly by the organic carbon in sediment and DOC (e.g. as humic acids) in interstitial waters, whereby under conditions of high OC, freely dissolved concentrations are reduced (Meador et al., 1997; 2002). Thus, the amount of OC in sediment or DOC in interstitial water controls the amount of bioavailable TBT. It follows intuitively then that the degree of bioaccumulation and toxicity to the biota of sediment-associated TBT are also controlled by the amount of organic carbon present in sediments and interstitial waters. Indeed, Meador et al. (1997) demonstrated experimentally that toxicity and bioconcentration (measured as 50% lethal concentration, LC_{50}, and bioconcentration factor, BCF, respectively) of TBT to three marine invertebrates (one polycheate, *Armandia brevis*; two amphipods, *Rheopoxinyus abronius* and *Eohaustorius washingtonianus*) was increased in those sediments with low TOC and less in those where TOC was increased. These authors concluded that the concentration of dissolved TBT in interstitial pore water was the most important uptake route to these benthic organisms. Similarly, Looser et al. (1998) found reduced bioavailability and bioaccumulation of TBT in *Daphnia* and grayling (*Thymallus thymallus*) in experimental systems with high DOC present in the form of humic acids.

While the presence of organic carbon may mitigate the amount of TBT available, the high K_{ow} values of TBT suggest a potential for accumulation in the lipids and proteins in the tissues of benthic organisms. Thus, infauna may be exposed to TBT through contact of body surfaces with interstitial pore water (bioconcentration). This is significant as it means that porewater represents a potential conduit of contamination between an otherwise 'semi dormant' reservoir of TBT and benthic, sediment-dwelling organisms. Furthermore, bioaccumulation of TBT may occur as a combination of bioconcentration from porewater and grazing/filter feeding of surficial sediments or suspended particulate matter. If either (or both) bioconcentration and bioaccumulation do occur in benthic primary consumers, this means that they may represent the second phase of a route by which TBT enters the food web. For example, in eutrophic fresh waters such as the Norfolk Broads, benthic macrofaunal assemblages inhabiting sediments (with which TBT is most frequently affiliated) are frequently dominated by abundant primary consumers such as Chironomidae (Grey et al., 2004 a). Furthermore, the ubiquitous nature of such organisms in shallow lakes means they represent an important resource for higher consumers including predatory invertebrates, fish and birds. Thus, extensive feeding on contaminated prey could potentially lead to considerable TBT burdens in many food web compartments (Traas et al., 1996). The bioaccumulation profiles and trophodynamic behaviour of organotins is discussed in section 1.3.

1.3   The Trophodynamic Behaviour of Organotin Compounds within Aquatic Food Webs.

A number of studies from diverse systems and locations document organotin contamination in a wide range of biota feeding at different heights in the food web, although the majority of these are from marine ecosystems. The study by Stab et al. (1996) is the most comprehensive freshwater-based work to date and reported the occurrence of butyl and phenyl tins in chironomids, mysid shrimps, bivalve molluscs, invertivorous and piscivorous fish and birds. This study now is over 14 years old and this reinforces the requirement for detailed research on the contamination of freshwater food webs by OTCs.

In a Danish marine web, butyl and phenyl tin contamination was recorded in 21 species ranging from primary producers, invertebrates, fish, birds, to marine mammals (Strand & Jacobsen, 2005). Similarly in a Japanese study 23 marine species including amphipods, molluscs, echinoderms, invertivorous and piscivorous fish and cetaceans (Takahashi et al, 1999) were found to be carrying butyltin burdens. In the Saguenay Fjord (Canada) butyltin contamination was widespread in fish, bivalve molluscs, echinoderms, and crustaceans (Viglino et al, 2006). In the light of the paucity of freshwater research, what further can be learned from marine studies?

Within OTC impacted food webs there are two principal accumulation pathways / uptake routes of OTCs for consumers; directly from the abiotic environment (e.g. passive accumulation / bioconcentration), and through diet. The occurrence of both (or one) of these or, uptake from diet in isolation, is known as bioaccumulation (Gray, 2002). A large proportion of the previous marine research has been conducted within systems where dissolved concentrations of OTCs in the water column were still quantifiable due to active

inputs from antifoulants. Given that this contaminant fraction is readily bioavailable it is difficult to separate the importance of uptake routes. Amongst food web consumers this is true up to the level of fish, as birds and mammals are obviously less likely to be exposed to OTCs via passive uptake. Nevertheless, it has been reported that organisms on higher trophic levels exhibit higher OTC burdens than their prey and that these concentrations are often greater than would be expected than from bioconcentration only (see Takahashi et al, 1999). Thus uptake through diet may be an important contamination pathway in higher consumers (Rouleau et al, 1999).

In contrast to many marine studies where active TBT inputs were still occurring, the water column OTC concentrations in the Broads are likely to be negligible or absent (Waite et al, 1989). Thus the principal uptake route for higher consumers (such as fish) will likely be via the benthic energy pathway and / or ingestion of contaminated sediments whilst foraging. It is also possible that passive uptake (bioconcentration) could occur to a lesser degree during feeding, via contact with interstitial porewaters. Hence, those organisms that have more contact with sediments either through life history traits or feeding ecology could be more likely to accumulate OTC compounds. There is some observational evidence for the existence of this pattern. Benthic macrofauna and fish, including chironomids, gammarids, eels, roach, tench and bream, accumulated more phenyl and butyltins than their pelagic equivalents such as mysids and smelt (Stab et al, 1996). In the same vein, flounder and eider duck were the most butyltin contaminated species in a Danish marine food web (Strand and Jacobsen, 2005). Fish such as flounder frequently conceal themselves within sediments and feed almost exclusively on benthic infauna (Carlson et al., 1997) so could accumulate OTCs

via both pathways. Alternatively diet is likely to be the principal uptake route in birds and the diet of eider duck is often subsidised heavily by benthic prey such as molluscs (Krasnov et al., 2009) that are themselves prone to OTC contamination. High butyltin concentrations in birds further highlights the potential for OTC accumulation of higher trophic levels by feeding on contaminated prey/substrate.

In contrast to other widely studied persistent organic pollutants (POPs: poly-aromatic hydrocarbons (PAHs); polychlorinated bi-phenyls (PCBs); organochlorines (DDT, DDE), organometallic compounds monomethyl mercury) butyl and phenyltin species show only moderate affinity for lipids and, as discussed previously, are relatively unstable under conditions of high biological activity and increased temperature (Sun et al, 1999). This highlights the possibility that patterns of organotin contamination and trophic transfer may not be as clearly defined as those observed with more persistent and lipophillic compounds, that are most frequently associated with lipid rich tissues (Kidd et al., 2005). Indeed, although OTCs are lipophillic, it has been reported that they have a higher affinity to proteins than lipids, and preferential accumulation in the liver is a prevalent pattern within the literature (Strand and Jacobsen, 2005).

Thus hepatic concentrations of OTCs are frequently 2-5 fold greater than tissue such as muscle in consumers such as fish (Stab et al, 1996, Takahashi et al, 1999). The same pattern has been observed in marine mammals. For example, concentrations of total butyltins ($\sum$TBT+ DBT+ MBT) in the liver (1400 ng g^{-1}) were significantly higher compared to kidneys (200 ng g^{-1}), muscle (41 ng g^{-1}), melon (190 ng g^{-1}), brain (110 ng g^{-1}), heart (50 ng

g^{-1}) and blubber (630 ng g^{-1}) in bottlenose dolphins (*Tursiops truncatus*) from the coastal waters of the United States (Kannan et al, 1997). This distribution of butytins illuminates differences in accumulation patterns between OTCs and other persistent contaminants such as organochlorines that accumulate principally in the lipid rich blubber. Moreover, this pattern singles out the liver as an important organ in the accumulation of OTCs.

Of further significance are the hepatic ratios of parent compounds and metabolites that are often higher than those in other tissues hinting at the importance of this organ in OTC degradation. For example, concentrations of TBT were accompanied by high concentrations of DBT and MBT in rainbow trout, *Onchorhynchus mykiss* (Martin et al, 1989). Conversely, concentrations of DBT in the livers of pike (*Esox lucius*) have been recorded as 15-30 times greater than those of TBT, indicating that the di- substituted compound may not be easily eliminated from the liver (Stab et al, 1996). However, in other species concentrations of the parent compound have been greater than metabolites. For example in eel (*Anguilla anguilla*) TBT concentrations followed the order TBT>DBT>MBT (Stab et al, 1996, Harino et al, 2002). These differences may be due to the existence of species specific capacity for butyltin degradation. Alternatively (or in addition) they could be the result of an imbalance in the rates of TBT uptake elimination kinetics. In other words, regular feeding on contaminated prey may result in an uptake rate of OTC that that exceeds the rate of OTC metabolism. The latter has been demonstrated to occur through species specific feeding ecology. Considerable accumulation of TBT in the Acadian redfish (*Sebastus faciatus*) stemmed from dietary specialisation on the pink shrimp (*Pandalus borealis*). The prey species had a poor metabolic capacity for TBT that indirectly lead to hepatic concentrations of up 730 ng g^{-1} in the predator

(Viglino et al, 2006). Thus species specific feeding ecology could yet prove important in determining organotin burdens.

Evidence of the existence of species specific metabolism for OTCs is supported not only by higher vertebrate consumers such as fish. Indeed, the presence of a preferential metabolic capacity for butyltins but not phenyltins has been demonstrated experimentally in primary consumers such as the chironomid *Chironomus riparius* (Looser et al, 1998). Given the importance of organisms such as chironomids as a dietary resource for higher consumers, a metabolic capacity for TBT could prove crucial in mitigating the supply of TBT to the wider food-web (Looser et al, 1998).

The apparent ease with which butyltins can be metabolised is in stark contrast to concentrations of phenyltins that are often represented by the parent compound only; suggesting TPhT is less degradable in the tissues of biota than TBT. This is a feature that is likely play a pivotal role in determining compound specific trophodynamic behaviours within impacted food webs. In a marine food web in China, Hu et al (2006) demonstrated widespread occurrence of phenyltins in primary producers, primary consumers, secondary consumers and fish. The profile of phenyltin contamination was characterised by the dominance of TPhT to the virtual exclusion of metabolites. Similar results were observed in a Japanese food web where Murai et al (2008) report the dominance of TPhT in a number of consumer species. Others have reported similar patterns; Harino et al, (2002) report the prevalence of TPhT compared to TBT in multiple tissues of eel. In the liver of pike and eel concentrations of phenyltin contamination followed the order TPhT (1000s ng^{-1}) > DPhT

(100s ng g^{-1}) > MPhT (10s ng g^{-1}), suggestive of slow degradation of TPhT. The pattern of TPhT in the tissues of many primary consumers (chironomids, gammarids, and unionid mussels (*Dressina polymorpha*) and fish (roach (*Rutilus rutilus*), perch (*Perca fluviatalis*), ruffe (*Gymnocephalus cernuus*), tench (*Tinca tinca*) and bream (*Abramis brama*)) followed the same pattern (Stab et al, 1996).

These differences in accumulation pattern appear to strongly influence the trophodynamic behaviour of the compound. For example, in the majority of studies phenyltins are reported to biomagnify (the increase of a contaminants concentration through trophic levels of aquatic food webs; Gray, 2002) whereas butyltins are not.

1.3.1   Biomagnification

Biomagnification factors (BMF) describe the ratio between contaminant concentrations in predators and prey, and by definition compounds are biomagnified when the BMF value is >1 (Strand & Jacobsen, 2005). BMF's are calculated using the following equation:

BMF= C$_{w.b}$ (predator)/ C$_{w.b}$ (prey)
(where C$_{w.b}$ is the concentration in the whole body)

Thus, using such methods Stab et al (1996) reported significant biomagnification of phenyltins only in the shallow lake food web discussed previously. In this case concentrations of phenyltins in primary consumers (such as chironomids) were lower than muscle tissue

concentrations of benthic fish such as roach, bream, tench and eel. Furthermore, phenyltin concentrations were higher in piscivorous fish than in their potential prey species. This pattern was reversed in the case of butyltins, where prey fish muscle concentrations were routinely greater than those of piscivores. Furthermore, concentrations of butyltins in primary consumers were within the same range of values as their cyprinid fish predators.

Takahashi et al. (1999) recorded the highest butyltin concentrations of all food web consumers (730 ng g^{-1}) in the liver of the Dalls porpoise (*Phocoenoides dalli*). It would perhaps be expected that high concentrations of contaminants in an apex predator such as porpoise would indicate the occurrence of biomagnification. However, there was no significant biomagnification between trophic levels. Similarly, Viglino et al (2006) reported no biomagnification of butyltins in the Saguenay Fjord food web despite ubiquitous contamination of sediments and consumers.

Nevertheless there are some apparent exceptions to this rule. Whilst previous research has found no evidence of butyltin biomagnification between trophic levels, Strand and Jacobsen (2005) concluded that when compared to putative prey species, total butyltins (∑TBT+DBT+MBT) were biomagnified in porpoise, four species of fish (cod - *Gadus morhua*; herring- *Clupea harengus*; flounder – *Platicthys flesus*; unspecified sculpin – Cottidae) and eider duck. Conversely, despite widespread contamination of consumers such as fish, no biomagnification was reported in their potential predators including harbour seal (*Phoca vitulina*), harbour porpoise (*Phocena phocena*) and cormorant (*Phalocrocorax carbo*).

However, this method of testing for biomagnification has a number of inherent problems, and these are highlighted by the work of Strand and Jacobsen (2005). Firstly, the four fish in which butyltins biomagnified were assumed to exist on the same trophic level. Herein lies the first problem: gadoid fish species such as cod, are extremely opportunistic predators and have been known to prey readily on species such as herring (Koster et al., 2000). Secondly, the opportunistic feeding behaviour of cod means they would also prey on flounder and sculpin. Such trophic interactions could be restricted to some extent by predator-prey body size relationships. Nonetheless this observation stresses a want for some empirical measure of a consumer's trophic position within its respective food web in order to prudently assess biomagnification. The third problem relates to assuming a homogenous diet (mussels, whelks, crabs) among these four species of fish. It is likely that these prey types would compose some proportion of the diets of demersal species such as flounder, sculpin and cod. However, herring are a truly pelagic species and are adapted to feed principally on zooplankton. Thus large bodied benthic invertebrates are excluded from their diet. This raises serious questions about the validity of such methods and provides one extreme example of the problems associated with simple calculation of BMFs.

Perhaps more importantly, these points highlight a lack of unification between traditional chemical techniques and ecological methods that can be used to make robust dietary investigations. For example, the application of gut contents analysis (GCA) or measures of the stable isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$ respectively) prevent the need for assigning predators discrete trophic levels and what may amount to hypothetical prey. The

latter technique (stable isotope analyses, abbreviated as SIA) is increasingly recognised as a powerful tool in contaminant studies (Jardine et al, 2006). Despite this, studies combining measures of OTCs and methods such as stable isotopes remain rare.

1.4. Stable Isotopes as Ecological Tools

1.4.1 The use of Stable Isotopes as 'Mapping' Tools

Many studies examining the occurrence of biomagnification have done so through food webs recreated from gut contents analysis (GCA) or from assumed predator prey interactions (see last section and references therein). Using these traditional methods to identify biomagnification can be difficult because the diet of an organism can: (1) comprise prey from several different trophic levels; (2) be strongly influenced by seasonal variation in prey availability; and (3) feature prey that are very small and therefore difficult or impossible to identify (Grey, 2006). However, relatively modern advances in the field of stable isotope ecology have gone some way to alleviating these confounding factors.

The heavier isotope of nitrogen (^{15}N) is conserved through trophic interactions due to preferential excretion of the lighter isotope (^{14}N) during metabolic processes (Gaebler et al, 1996). The ratio of the two ($^{15}N/^{14}N$) is reported as $\delta^{15}N$, calculated as

$$\delta = [(X_{sample} /X_{reference}) - 1] \times 1000$$

expressed in units of per mille (‰) where $X = {}^{15}N/^{14}N$. This ratio has been demonstrated to increase in a relatively predictable manner from prey to predator. Typically, these increases are between 3-5 parts per mille (‰) (DeNiro and Epstein, 1981; Minagawa & Wada, 1984).

Thus, measures of $\delta^{15}N$ can be used to provide a continuous measure of trophic position. Furthermore, nitrogen isotope ratios represent a time integrated indication of food consumed over the period of tissue turnover (Grey, 2006). If augmented by representative sampling, these features combine to offer a high degree of resolution when determining the relative trophic position of the consumers within food webs, and when combined with measures of organism contaminant concentrations, $\delta^{15}N$ provides a powerful tool for testing for the occurrence of biomagnification.

1.4.2   Stable Isotopes and Contaminants

The utility of applying stable isotope characterisation of food webs to contaminant studies was first demonstrated in 24 Canadian Shield lakes. An earlier study classified these lakes on the basis of the presence / absence of *Mysis relicta* and or pelagic forage fish (alewife, sculpin, smelt) and assigning organisms to discrete trophic levels (relative to zooplankton at trophic level 2), and used this to predict mercury (Hg) concentrations in a common top predator (lake trout, *Salvelinus namaycush*). Further, it was found that the food chain model could be used to predict mercury and PCB concentrations in the common top predator among lake class. Those lakes that had longer food chains due to presence of mysids and / or forage fish contained lake trout with higher Hg concentrations, than lakes with shorter food chains lacking the intermediate trophic level where lake trout fed principally on zooplankton (Rasmussen et al., 1990). However, with the application of stable isotope measurements comparing lake trout $\delta^{15}N$ within lake class, a later study (Cabana et al., 1994) demonstrated considerable in variability in food chain length. Thus in lakes where mysids and forage fish where absent then lake trout $\delta^{15}N$ was 9.9‰, increasing to 13.7‰ in lakes with the mysids

and forage fish were present. This suggested variable degrees of omnivory exhibited by lake trout between lake class, and trout δ^{15}N correlated positively with mercury concentrations. This study highlighted δ^{15}N as better predictor of food web biomagnification than discrete food chain or discrete trophic level classification (Cabana and Rasmussen, 1994).

A similar Canadian study examined the relationship between concentrations of hexachlorocyclohexane ($\sum$HCH), $\sum$DDT, and chlorinated bornanes (toxaphene, $\sum$CHB) and δ^{15}N of fish and invertebrates of Lake Laberge (Yukon Territory; Kidd et al., 1995). Each of these contaminants was found to increase through the food web in response to trophic position (as measured by δ^{15}N). However, comparing the slopes of these relationships allowed for inference about the respective biomagnification potential of each contaminant. Thus a significantly lower slope of the less lipophillic $\sum$HCH suggested reduced biomagnification potential relative to the other more lipophillic compounds analysed (Kidd et al., 1995).

Despite a number of studies that investigate the trophodynamic behaviour of OTCs within aquatic food webs (see previous section), only two studies compare measures of δ^{15}N with concentrations of organotins and both have been conducted in marine systems. Thus, similar to Kidd et al. (1995), Hu et al. (2006) were able to conclusively demonstrate differences between the biomagnification potential of butyl and phenyltins in a Chinese marine food web. In their study, triphenyltin (TPhT) was found to have a significant positive correlation with δ^{15}N; biomagnifying through the food web by a trophic magnification factor (TMF, see below) of 3.7. However, a negative relationship was evident between δ^{15}N and tributyltin

burden, and no significant biomagnification occurred between consumers (TMF < 1). The same compound-specific relationship was detected within a marine food web from Japan (Murai et al, 2008). Again, significant correlations were observed between increasing trophic position (as represented by δ^{15}N) and respective TPhT concentrations of consumers, but not in the case of TBT. The application of stable nitrogen isotope measures allowed these researchers to quantitatively demonstrate for the first time expected differences between the accumulation patterns and biomagnification potential of OTCs in aquatic food webs.

There are some factors that must be taken into consideration when δ^{15}N is applied in such studies. For example, the slopes of contaminant - δ^{15}N derived regressions quantify the increase in contaminant with each per mille increment (see Broman et al, 1992; Kidd et al, 1995). The equation is described below:

$$[\text{contaminant}] = e^{(b + m \times \delta15N)} \tag{1}$$

This can be transformed to:

$$\ln[\text{contaminant}] = b + m \times \delta^{15}N \tag{2}$$

Here the slope (m) represents a measure of contaminant biomagnification through the food web, and the intercept (b) can be seen as the concentration at the base of the food web. Given the manner in which δ^{15}N enriches between predator and prey (e.g. typically 3-5‰), measuring the contaminant against single unit increments of δ^{15}N is somewhat alien to this type of application (Jardine et al, 2006). To improve the application, δ^{15}N values used in

equation 2 can be replaced by an index of trophic position (referred to as TL; Hobson and Welch, 1992) and is calculated thus:

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{primary\ producer}) / \Delta^{15}N + 1 \tag{3}$$

In this case, $\delta^{15}N_{primary\ producer}$ is presumed to occupy TL 1 and $\Delta^{15}N$ is the enrichment factor from one trophic level to the next. The result of this equation (TL) can then be substituted in to in to equation 2:

$$\ln [contaminant] = b + m \times TL \tag{4}$$

a trophic magnification factor (TMF) can then be calculated:

$$TMF = e^m \ (or \ 10^m) \tag{5}$$

This method was used by Hu et al (2006) to elucidate the TMF of TPhT compared to TBT. The advantage of such a method is that the TMF represents the increase in contaminant from one TL to the next averaged over the whole food web, so is analogous to a food web-wide biomagnification factor (such as those detailed in the previous section). Furthermore, it is corrected for the variation in isotopic baselines often observed between systems (Jardine et al., 2006). However, the use of primary consumers as a baseline can present problems due to the wide variability of the isotopic signatures of primary producers (see Heckey and Hesslein, 1995).

Thus, $\delta^{15}N_{primary\ producer}$ may be too variable to make valid comparisons across and between systems. However, this can be alleviated by the careful selection of long-lived primary consumers that better integrate the isotopic signatures of other long-lived secondary consumers such as fish (see Post, 2002). Long-lived unionid mussels are a useful baseline 'anchor', and can be used to standardise isotopic signatures between habitats and across ecosystems (Post, 2002; Vuorio et al., 2007). Thus equation 3 is modified:

$$TL_{consumer} = (\delta^{15}N_{consumer} - \delta^{15}N_{baseline}) / \Delta 15N + 2 \qquad (6)$$

A further advantage of the Trophic Level approach is that individual enrichment factors for specific groups of organisms can be incorporated into TMFs. For example, to utilise different enrichment factors for invertebrates, fish, birds, and marine and terrestrial mammals (Fisk et al., 2001; Hobson et al., 2002). In this manner, both adjusted and unadjusted contaminant TL relationships can be used in examining biomagnification potential.

Measures of $\delta^{15}N$ in isolation do not make full use of the potential of the stable isotope techniques, and ignores the ability of isotopic ratios to capture contamination patterns and processes across entire food webs. For example, in contrast to nitrogen, carbon isotope ratios ($^{13}C/^{12}C$) are relatively unaffected by trophic transfer and are typically reported as enriched by 1‰ relative to dietary source (DeNiro & Epstein, 1978). This somewhat of an oversimplification as detailed feeding experiments have revealed trophic fractionation varies between -1 and +2.5‰ (for example, see the meta-analyses of McCutchan et al., 2003) but serves as a useful 'rule-of-thumb'. Thus, the conservative nature of the heavier carbon

isotope means that it can be used to trace energy flow with aquatic food webs provided that basal resources exhibit distinct carbon signatures. For example, detritus and periphyton of the littoral / benthic zones are typically more ^{13}C -enriched than those primary producers (e.g. phytoplankton) that inhabit the pelagic zone of lentic waters because of a combination of differences in the origin of CO_2 fixed during photosynthesis and differences in liquid / solid boundary-layer kinetics. For example, high rates of CO_2 from respiration and microbial decomposition can produce a photosynthate that is isotopically light. In contrast, high rates of photosynthesis can deplete dissolved CO_2, and the resulting photosynthate is isotopically heavier (Hecky and Hesslein, 1995). In addition, the thickness of the stagnant boundary layer that surrounds aquatic primary producers controls the rate of CO_2 diffusion and therefore represents a limiting step in photosynthesis. The boundary layer associated with epiphyton, periphyton and benthic algae can be thick (>1mm), reducing the supply of CO_2, leading to an accumulation of ^{13}C that would normally be expelled. This increases the ^{13}C/^{12}C ratio and plant tissues become accordingly δ^{13}C enriched. In contrast the more turbulent pelagic environments of lakes results in thinner boundary layers which increases the supply rates of CO_2 to pelagic primary producers. Thus, discrimination of the heavier ^{13}C isotope is increased, and is reflected by typically lower δ^{13}C values of pelagic primary producers.

These patterns are applicable in lentic ecosystems on a wide scale. For example, in a meta-analysis of divergent algal sample types, France (1995) demonstrated that among the lentic littoral algae, more than 80% displayed values between -29 to -20‰. In the case of planktonic species, the majority of values lay within the range -39 to -32‰. A combination of these distinct isotopic signatures and the minimal fractionation between source and consumer

means that it is possible to trace the origin of consumer carbon source across multiple trophic levels. Thus, it is possible to create 'maps' that depict energy pathways, allowing for the differentiation of organisms relative reliance carbon of benthic or pelagic origin, and describing the trophic height of consumers within the food web by simultaneously measuring the stable isotope ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ of primary producers and consumers within food webs (Grey, 2006). Such 'maps' can be used to examine both 'vertical' food web features that may influence patterns of contamination accumulation through trophic levels, as well as 'horizontal' features that may influence contamination accumulation in other ways. For example, habitat use, dietary shifts, and / or benthic-pelagic energy pathway dependencies may all influence the distribution of contaminants in food webs (Power et al., 2002). Power et al. (2002) were able to demonstrate that mercury contamination increased as a function of trophic position (as measured by $\delta^{15}N$) in the fish community of Stewart Lake (Canada) but was inversely related to the $\delta^{13}C$ signature of fish consumers. Those fish feeding in the pelagic had higher Hg burdens than those feeding from the benthos. Furthermore, larger lake trout had lower $\delta^{13}C$ values and correspondingly higher Hg concentrations as individuals underwent size-based ontogenetic dietary shifts to reliance on pelagic prey fish.

1.4.3   Stable Isotopes and Quantification of trophic resources

In a similar manner to Power et al. (2002), Kidd et al. (2001) combined measures of $\delta^{13}C$ and $\delta^{15}N$ to assess differences in the biomagnification of DDT through benthic and pelagic food webs of Lake Malawi (Africa). Comparison of the slopes of $\delta^{15}N$ versus DDT concentrations of respective food webs revealed that the contaminant biomagnified at a significantly greater

rate per trophic level in the pelagic food web than through the benthic food web. In this case, $\delta^{13}C$ values at the base of the food web were used to determine the relative importance of benthic and pelagic autochthonous carbon in the diets of consumers. In order to quantify the relative importance of two distinct carbon sources to invertebrates and fish, these authors used mean epilithic algae $\delta^{13}C$ and mean zooplankton $\delta^{13}C$ as endpoints in a two-source isotopic mixing model. Zooplankton was used in place of POM as the pelagic endpoint as it was less variable in $\delta^{13}C$ over time. The equation used is given below:

$$P_{pelagic} C = (\delta^{13}C_{organism} - \delta^{13}C_{benthic\ algae})/ (\delta^{13}C_{zooplankton} - \delta^{13}C_{benthic\ algae}) \tag{7}$$

where P is the proportion of pelagic carbon in fish and invertebrates.

In the model described above, no fractionation of $\delta^{13}C$ is assumed through the food web, however this can be accounted for if knowledge of the likely fractionation between source and consumer is known. The meta-analysis of McCutchan et al. (2003) provides a useful database for information on isotopic fractionation. One limitation of the model described (7) is a failure to account for the error that is likely to exist around mean estimates of the sources and mixture. However, recent developments in this field have led to the creation of software that covers a variety of situations in which these models are useful. For example, in the program 'Isoerror' (Phillips and Gregg, 2001), it is possible to input source and mixture data including mean and standard deviation for both standard one-isotope, two-source models, and two-isotope (e.g. $\delta^{13}C$ and $\delta^{15}N$) three-source models. The addition of variability into these models greatly improves their accuracy and reliability.

1.4.4 Stable Isotopes and Subsidies / Contaminant Transfer

The ability to qualify and quantify energy sources or trophic linkages using stable isotopes is not necessarily limited to within the aquatic or the terrestrial realm. Indeed, the application of stable isotope measures has been instrumental in demonstrating the strength of linkage between ecosystems such as the subsidy of terrestrial food webs by aquatic-derived resources (for a review see Baxter et al., 2005). This is because aquatic and terrestrially-derived carbon sources typically exhibit markedly different $\delta^{13}C$ values due to differences in the source CO_2 pools and subsequent fractionation by primary producers (Peterson & Fry, 1987). For example, carbon uptake by terrestrial C_3 plants typically involves a net fractionation of ~ 21‰ between the atmospheric CO_2 pool (-7 ‰) and plant biomass (-28‰). As discussed previously, aquatic-derived biomass is usually more ^{13}C-depleted than this, particularly if from pelagic sources, because the CO_2 could have originated from respiration or dissolution as well as exchange across the air-water interface. Thus, if material such as terrestrial leaf litter is transferred across ecosystem boundaries, then the distinguishable $\delta^{13}C$ from which it is composed should allow that material to be traced with confidence (e.g. Grey et al., 2001). Thus, a number of studies have been able to quantify the importance of aquatic-derived prey to terrestrial consumers by combining mixing models and isotope tracer techniques (see Collier et al., 2002; Kato et al., 2003; Paetzold et al., 2005). A reoccurring feature of these studies is the importance of emergent aquatic invertebrates (particularly the Chironomidae) as a subsidy to terrestrial food webs.

If larvae live for considerable periods in contaminated sediments, then the emergent imagos may carry with them a contaminant burden that could be transferred to terrestrial consumers.

The role of emergent invertebrates as contaminant vectors is still poorly understood and studies detailing the occurrence of cross-ecosystem contaminant transfer are rare. However, two recent articles document the occurrence in freshwater ecosystems. In a PCB contaminated stream in the U.S.A, Walters et al. (2008) were able to demonstrate that increasing aquatic prey utilisation (indicated by markedly low $\delta^{13}C$) among riparian terrestrial invertebrate predators such as spiders led to significant increases in PCB concentrations within spider tissues. Similarly, in a comparison of upland and riparian spiders (Lake Hartwell, USA) a combination of SIA and mixing models indicated that riparian spiders were primarily subsidised by aquatic insects whereas spiders from upland habitats comprised carbon from predominantly terrestrial sources. Aquatic prey such as chironomids were found to have PCB concentrations up to two orders of magnitude higher (1240 ng g^{-1}) than terrestrial counterparts (15 ng g^{-1}). These differences were mirrored in their respective predators where riparian spiders had significantly greater PCB burdens (820-2012 ng g) than those feeding on terrestrial prey (30ng g^{-1}).

The potential impacts of cross–ecosystem transfer of contaminants are still relatively unknown. However, many invertivorous birds prey upon chironomid imagos and spiders and target their breeding to coincide with mass emergence of insects to better provision for their young. It is not unreasonable to speculate that chicks and juveniles are more vulnerable to contaminant exposure due to their small body size and high ingestion rate (Walters et al., 2009). Further, if contaminant transfer resulted in alterations in abundance of terrestrial invertebrate predators (e.g. bioaccumulation to levels inducing reproductive failure or

mortality) this could result in trophic cascades, similar to those initiated by contaminants in aquatic ecosystems (e.g. Knight et al., 2005).

1.4.5 Stable Isotope Metrics

As the costs of stable isotope analysis have decreased (a function of supply and demand as more people use the 'tools'), many ecologists are turning to analysis of intrapopulation and intraspecific isotopic variance to provide valuable insight into niche dynamics. Such studies range from elucidating patterns in trophic niche shifts throughout ontogeny in fish (Grey, 2001; Post, 2003), demonstrating intraspecific and intrapopulation feeding specialisation in primary consumers (Grey et al., 2004), and developing novel methods by which to test classical niche theory (Bearhop et al., 2004).

A recent novel application for carbon and nitrogen stable isotope ratios is the development of a set of quantitative metrics that can be used to infer important 'community wide' aspects of trophic structure (Layman et al., 2007). The approach builds on techniques commonly used within the field of ecomorphology, where metrics are calculated based on two-dimensional representations of species morphological characteristics and used to assess trophic roles (e.g. niche differentiation) between specific taxonomic groups with similar morphologies. The approach of Layman et al. (2007) differs in that it extends beyond specific taxonomic groups, using the realised trophic niches of species (as represented by isotopic ratios). Layman et al. (2007) suggest that as the isotopic ratios of an organism are the culmination of all trophic pathways leading to it, they provide one representation of its trophic niche. Thus, the traditional $\delta^{13}C$ / $\delta^{15}N$ bi-plot space becomes 'niche space' and metrics are calculated by measures of the relative distribution and distances between species within it.

These metrics may be of use in systems that have been subject to environmental perturbations such as habitat fragmentation or contamination by anthropogenic contaminants. For example, total niche space (TA) can be used as a quantitative method of measuring a population's total niche width (Layman et al. 2007a) and could provide valuable information on community response to gradients of perturbations. In order to test this idea, Layman et al. (2007a) plotted the $\delta^{13}C$ and $\delta^{15}N$ of a top predator (grey snapper, *Lutjanus greisus*) in bi-plot space along a gradient of habitat fragmentation of tidal creeks in the Bahamas. It was hypothesised that the trophic niche width of the snapper would contract (e.g. a reduction in the range of both $\delta^{13}C$ and $\delta^{15}N$), reducing TA values in response to increasing fragmentation. Indeed, TA was reduced by 93% in the most fragmented habitats compared to that of snapper in un-fragmented systems. Such an approach could prove useful in contaminant impacted systems. For example, in the Norfolk Broads TBT loading of sediments is characterised by a gradient contamination in response to the degree of boating activity and connectivity. For this reason, TBT is absent in those lakes isolated from navigation, but increases in connected lakes depending largely on their popularity as a part of a navigable route along the river system. The most contaminated sites have been demonstrated as boat yards where holiday craft are stored and maintained (Hoare, 2007). Hence, facilitated by the existence of isolated reference sites, it could be possible to test metrics such as TA (of entire communities), in response to the gradient of TBT contamination.

1.5   Project Aims

My review to date has identified that OTC studies are generally under-represented in freshwater ecosystems, that a contamination legacy of OTCs occurs in the Norfolk Broads

and that the Norfolk Broads are not unique in such contamination. There has been no contemporary study of a food web still containing OTCs from a sediment legacy. Furthermore, there appears to be no study of the potential transfer of OTCs from aquatic to terrestrial ecosystems. Stable isotope analysis represents a useful tool in the study of such contaminants. Thus, I have identified several gaps in our knowledge which I will address in this thesis:

1. I aim to confirm the continued presence of the contamination gradient unveiled by Hoare (2007), and determine any changes in the degree of TBT degradation between the two study periods. This will provide important information regarding the likely persistence times of such contaminants. Hypothesis: *There has been a decline in the concentration of OTCs in the sediments of the broads congruent with the ban of their application to pleasure craft.*

2. I will examine the role of abiotic factors such as pH, and organic carbon content (TOC) in the control of speciation and bioavailability of OTCs to food web consumers such as benthic primary consumers. Hypothesis: *Sediment TOC and pH will represent the dominant abiotic factors controlling supply of OTCs to the wider food web*

3. I will assess the role of primary consumers in the supply of OTCs to the food web using stable isotope analyses in combination with measures of TBT to examine the importance of uptake routes, and food web energy-pathway dependence (via $\delta^{13}C$) on determining OTC burden of higher consumers such as fish. Hypothesis: *Those species more reliant upon benthic basal resources routed via organisms such as the*

Chironomidae will contain a higher OTC burden reflecting the source of contamination in the sediments.

4. Using the $\delta^{15}N$ values of components of the food web primary consumers to top predator, I will test for the occurrence and potential for food web biomagnification of OTCs. Hypothesis: *There will be a positive relationship between trophic position as measured by $\delta^{15}N$ and OTC burden.*

5. I aim to test the potential for the recently developed community metrics of Layman et al. (2007) to reveal changes in community structure (or niche dynamics) in food webs along a gradient of TBT contamination in the Norfolk Broads. Hypothesis: *Those ecosystems that are more heavily contaminated with OTCs will have a simpler architecture and reductions in both trophic width and height (leading to a reduced total area in isotope-space).*

6. Lastly, using a combination of SIA and measures of TBT I will examine the potential for emergent aquatic invertebrates such as chironomids to act as vectors of OTCs across the aquatic-terrestrial interface. Hypothesis: *There will be a positive correlation between the OTC burden found in terrestrial insectivorous predators collected from the immediate riparian and the degree to which they are subsidised by aquatic imagos*

The site descriptions and both laboratory and field methods that are used widely throughout the thesis are presented in Chapter 2. My first four aims are combined within the structure of

Chapter 3, while my fifth and sixth aims are assigned to Chapters 4 and 5, respectively. I summarise my findings in Chapter 6.

The findings of this study of OTCs in a freshwater shallow lake system, should improve the understanding of the likely implications of OTC legacies, and the behaviour of these contaminants in the food webs upon which they impact. Thus the Norfolk Broads may serve as model system from which it is possible to extrapolate findings to other similar systems and may aid lake management strategies on a wider scale.

Chapter 2

Study Sites and General Methods

2.1   Study sites

Based on the work of Hoare (2007) six sites were selected within the Norfolk Broads that had previously been demonstrated to represent a sliding scale of sediment TBT contamination. Sediment TBT concentrations followed the order Horning Ferry Marina > Loyne's > Malthouse > Ranworth > Salhouse > Wroxham. A further two isolated control sites where TBT had been previously undetected were added to represent control sites. The location of all study sites is shown in figure 2.1.
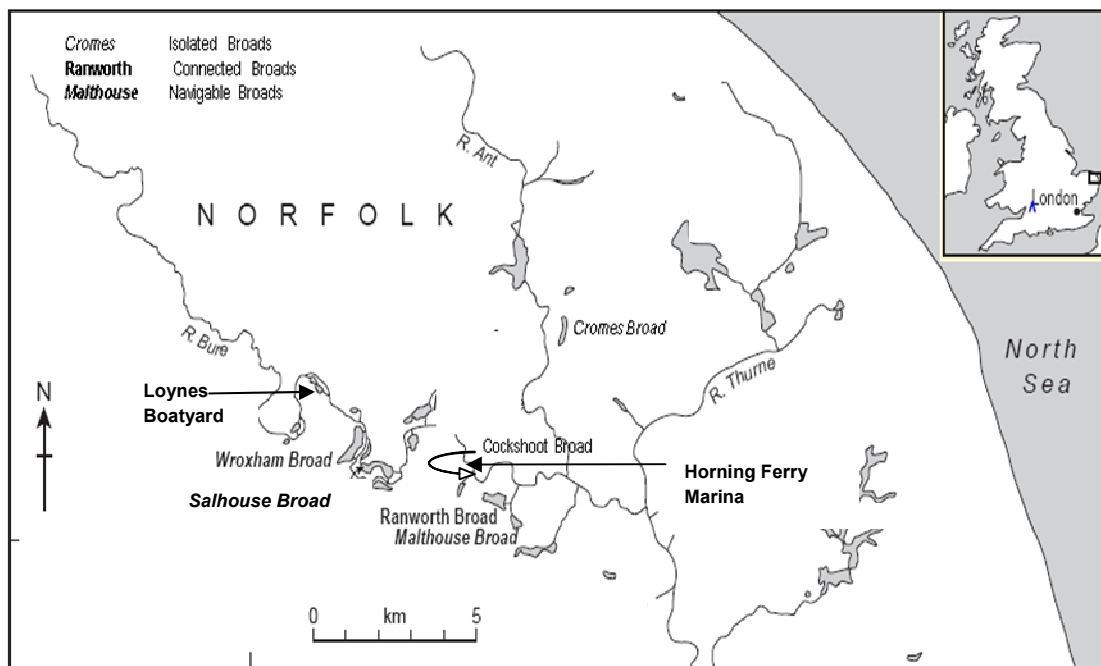
Figure 2.1 Map of the Norfolk Broads, showing the large broads and the positions of the eight study sites. Cockshoot Broad (River Bure) and Cromes Broad (River Ant) were control sites; Wroxham and Salhouse (low TBT); Ranworth and Malthouse Broads (intermediate and high TBT, adjacent on the River Bure); and Loyne's boatyard and Horning Ferry (high TBT). Positions of the smaller boatyards are indicated by arrows.

Site	SA (ha)	Z_{mean} (m)	Mean TP (ug l^{-1})	Mean NO$_3$-N (mg l^{-1})	Secchi Depth (m)	Plant richness
Cromes Broad	2.3	0.58	59	0.2	0.58	5
Cockshoot Broad	5.1	1.0	48	0.01	0.92	4
Wroxham Broad	34. 3	1.5	91	0.91	1.0	1
Salhouse Broad	8.5	1.2	*	*	*	*
Ranworth Broad	29.2	1.3	72	0.7	0.8	3
Malthouse Broad	10	1.2	*	*	*	2
Loynes Boatyard	< 1	1.7	*	*	*	0
Horning Ferry	< 1	1.8	*	*	*	0

Table 2.1 Limnological characteristics of the four study sites in the Norfolk Broads: surface area (SA) and mean annual values ($n = 22$ each site) for total phosphorous (TP), total nitrate, Secchi depth and plant richness. * indicates data not available. Physiochemical data for Wroxham, Ranworth and Cockshoot Broads provided by the Environment Agency, and from Sayer et al, 2009 for Cromes Broad. Plant species richness refers to number of species of submerged and floating-leaved macrophytes found (Cromes, and Cockshoot data from Sayer et al 2009, remainder from current study).

The main physical and chemical parameters of the eight sites are summarised in Table 1. Although chemical data is lacking for the boatyards, these are likely to be similar to the broads connected to the river systems. All sites apart from Cromes Broad are located on the River Bure, although connectivity varies between sites. Cromes Broad is situated on the

River Ant and connected by a fragmented network of small dykes. The lake has been non-navigable throughout its history. Cockshoot Broad is isolated from the River Bure by a damned dyke and has been non-navigable for >20 years. Wroxham and Salhouse broads are connected to the Bure and are both fully navigable. Malthouse and Ranworth broads are situated adjacent to one another and are connected via a gated dyke which currently renders Ranworth Broad non-navigable; both broads are connected to the tidal River Bure via Ranworth Cut. Ranworth Broad is a designated nature reserve with Site of Special Scientific Interest status.

2.2   Sampling of sediments and food web components

2.2.1   Sampling frequency

To account for potential temporal variation in the components comprising the food web, and the isotope values for the different components, sampling was conducted (where possible) on a quarterly basis. Thus, with the exception of January 2008, the food webs of Wroxham, Ranworth, Cromes, and Cockshoot broads were sampled throughout March 2007, June-July 2007, September-October, 2007 and March 2008. Malthouse and Salhouse broads were sampled during March 2007, June/July 2007, and March 2008. Additional sampling of chironomids for organotin analyses was conducted in Malthouse and Ranworth broads in July 2008. Access to the boatyards was extremely limited by the owners due to boat traffic so these sites were sampled in late Autumn 2007 and early Spring 2008 when boat activity was at a minimum.

2.2.2 Sampling methods – sediments

Surficial sediments (0-1cm and 1-5cm depth) for organotin analyses were collected using a 7cm I.D. Glew gravity corer (Glew, 1991). A minimum of three samples were collected from around the boat in each location. Prior to extrusion, excess water was pippetted from the surface of the core and the sediment sample placed in decon-90 washed, hexane rinsed 120ml glass jars, pH was measured using an electronic probe and jars capped with hexane rinsed foil lids to eliminate any potential cross contamination. The remainder of the core was then extruded into hexane rinsed foil lined sealable plastic bags. Once at the laboratory sediments were stored at -80˚C until analysis.

2.2.3 Sampling methods- lake water

Lake water was collected from around the boat in 2.5l hexane rinsed Winchester bottles. Once at the laboratory samples were frozen at -80˚C until analysis.

2.2.4 Sampling methods – invertebrates and fish

Due to the inaccessible nature of study sites all samples were collected from boats. Chironomids were collected from benthic sediments using an Ekman grab. Due to the numbers required for organotin analyses (typically 70-100), a large (500mm diameter, 1mm mesh size) was used to sieve sediments *in situ*. Any chironomids present were then removed using forceps and as they were required for organotin analysis, immediately placed in decon-90 washed, hexane rinsed 120ml glass jars filled with clean water. Unionid mussels (*Anodonta* spp.) were collected from benthic sediments by dragging a large hand-held D-shaped net through sediments at a depth of 15-20 cm, or located visually where transparency permitted and removed with a large hand-held net. Other macroinvertebrates were collected

from macrophyte stands, reed cover and littoral margins using kick nets and were immediately sorted into separate water-filled 500ml plastic screw-top pots according to taxa to prevent predation. Zooplankton samples were collected by slow towing of zooplankton nets (mesh size 250 μm) behind a boat. All invertebrates were left for a minimum of 12 to a maximum of 24 hrs for gut clearance prior to sacrifice (Feuchtmayr and Grey, 2003). Water samples (for fine particulate organic matter, FPOM) were taken from the pelagic zone at all sites (25-50 litres, depending on turbidity). Zooplankton and FPOM samples were filtered either directly onto pre-ashed (500 ^{o}C for 48 h) Whatman 47mm diameter GF/F discs or pre-concentrated in the case of FPOM via an ultra-filtration system (Minitan, effective filter area 250 cm^2). All samples were then stored at -80˚C.

Fish were captured using large fyke nets, electrofishing, and by rod and line. In order to allow the capture of fish with maximum dorsal-ventral measurements greater than 90mm (standard measurement of legally required otter guards as specified by the UK Environment Agency), the otter guards were omitted and instead, the cod end of the fyke net opened in to a large open topped steel cage (H 1.6m, 0. 8m, L, 1.4m; Figure 2.2) from which any captured otter could escape. Fish were sacrificed in the field and placed on ice until return to the laboratory where they were stored at -80 ^{o}C until further preparation.

Figure 2.2 Otter escape cage attached to the cod end of a fyke net (arrow A). The top of the cage is proud of the water surface (arrow B) surface allowing any otters to escape but keeping fish entrapped.

2.3   Laboratory methods

2.3.1   Sediment pore water chemistry

Porewater was extracted from sediments by centrifugation of field sediments at 3000rpm for 20 minutes. Following centrifugation, 5 ml of porewater was used for anion analysis (NO^-_3, Cl^-, SO_4, and PO^{3-}_4) by ion exchange chromatography (ICS-2500, Dionex Corp., Sunnyvale, California, U.S.A.). Anions were separated on a 2 x 250 mm RFICTM IonPac® AS18 analytical column with an IonPac® AS18 guard column. The eluent used was 100 mM KOH pumped by a GP50 gradient pump at a flow rate of 0.25 mL min-1 in gradient mode. Conductivity was measured by an ED50 detector and was linear between 0 and 45 µS. The system had an AS50 autosampler for automated sample injection (25 µL

injection volume) and Chromeleon® 6.5 software for system control, peak integration and data processing.

2.3.2 Total organic carbon content of sediments (%TOC)

Preparation for the removal of calcareous deposits in sediments followed the method of Hedges & Stern (1984).

Sub samples of 50-100mg were taken from homogenized field sediments and dried overnight at 50 °C in pre-weighed, 20ml glass scintillation vials and then weighed to obtain initial dried weight (W_o). To determine organic carbon content, the sample was treated with ≥ 2ml of organic- free 1M HCL. The slurry was then agitated for 5 minutes and then dried overnight at 50 °C. Following drying, samples were rested for 24h to allow for equilibrium of hydroscopic salts, and sample vials re weighed to determine W_f and 1-10mg subsamples weighed in to ultraclean tin sample boats for determination of carbon content by isotope mass spectrometry (see section 2.3.4). The percent organic carbon of samples could then be calculated thus;

$$\%TOC = [(C_{sample}/1000)/ W_{sample}] * 100$$

Where C_{sample} is the amount of carbon (µg) in the sample as measured by mass spectrometry (see section 2.3.5) and W_{sample}, the weight of the sediment sample analysed (mg).

2.3.3   Sediment particle size analyses

Preparation of sediment samples were conducted following the method of Rowell (1994).

Approximately 5 g of air-dried sediment was disaggregated and then sieved through a 2 mm mesh. Around 1 g of fines were weighed to 3 d.p. and placed in a 50 ml glass conical flask with 5 ml of hydrogen peroxide to remove organic matter. A further 10 ml of hydrogen peroxide was then added and left overnight. The mixture was gently heated on a hotplate with repeated addition of hydrogen peroxide until effervescent reaction had ceased. Liquids were then boiled off until completely dry. The cooled flask and contents were then reweighed and the remaining inorganic sediment fraction calculated. To disaggregate and disperse the dried inorganic fraction in a solution, 30 ml of ~2% "Calgon" solution was added and left overnight on a wrist-action shaker.

Analysis of the disaggregated sediment was performed on a Beckman Coulter LS™ 13 320 Laser Diffraction Particle Size Analyser with an integrated autosampler. Prior to analysis the samples were sonicated for 10 seconds. The particle size range measured was between 0.4 – 2000 μm. Percentages of clay (0.4 – 2 μm), silt (2 – 64 μm), fine sand (64 – 200 μm) and coarse sand (200 – 2000 μm) fractions were determined for each sample.

2.3.4 Organotin analyses

To provide sufficient wet weight of material (~3 g) for organotin analyses, 70-100 individual chironomids (*Chironomus plumosus*) were amalgamated to form composite samples. Due to the larger body size of *Anodonta* and fish, individuals or tissues from those individuals could be analysed in most cases.

Analyses of the organotin compounds tributyltin (TBT) and triphenyltin (TPhT), and the di- and mono-substituted daughter compounds (DBT, MBT, DPhT, and MPhT, respectively), were carried out at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) laboratory facility at Burnham on Crouch, Essex and Lowestoft, Suffolk. Standard operating procedures (SOPs) for the determination of sediments and biota used by the organotin laboratory were used. The method was originally developed by Waldock et al (1989).

Sediment, fine particulate organic matter (FPOM) and biota samples were extracted wet, and in the case of sediments and FPOM a separate solid determination performed in order to express sediment concentrations on a dry weight basis. Following homogenisation of each sample, a tripropyltin standard was added to each sample to act as a surrogate compound by which to correct for variability in the extraction efficiency of each sample. Organotin compounds were then extracted from the sample matrix (either tissues or sediments) by addition of sodium borohydride and methanol, converted to hydrides, and partitioned into hexane. Derivatives were then analysed by gas chromatography with flame photometric detection (GC-FPD). Quality control in each sample batch included an analytical blank, a certified reference material (CRM 646, for sediments; CRM 477 for biota) spiked with target compounds, and a Response Factor sample (containing known concentrations of all organotin compounds and a TPT internal standard) analysed prior to every three environmental samples. Quantification of sample contamination was calculated by the peak height of each organotin compound as a percentage of the peak height of the relevant standard in the previous Response Factor sample. No contamination was detected in any analytical blank. Limits of detection for the method were approximately 2ng g^{-1} for butyltin species and 14ng g^{-1} for phenyltins. Relative Standard Deviations (RSDs) of the Response Factor sample

heights were <15 % for butyltins for all sample runs. Organotin concentrations are expressed in terms of the cation (TBT^+, DBT^+ and MBT^+) as ng g^{-1} dry weight of sediment (dw), and wet weight (ww) in biota.

Organotin analysis of water samples was conducted the standard operating procedure (SOP) used by the organotin laboratory. Following homogenisation of each sample, a tripropyltin standard was added to each 2L sample to act as a surrogate compound by which to correct for variability in the extraction efficiency of each sample. Organotin compounds were then extracted from the water sample using a liquid – liquid extraction. Following addition of sodium borohydride and dichloromethane organotins were converted to hydrides, and partitioned into dichloromethane. After pre –concentration of samples to 300µl derivatives were then analysed by gas chromatography with flame photometric detection (GC-FPD). Quality control in each sample batch included an analytical blank and a Response Factor sample (containing known concentrations of all organotin compounds and a TPT internal standard) analysed prior to every three environmental samples. Quantification of sample contamination was calculated by the peak height of each organotin compound as a percentage of the peak height of the relevant standard in the previous Response Factor sample. No contamination was detected in any analytical blank. Limits of detection for the method were approximately 5ng l^{-1} for butyltin species and 14ng l^{-1} for phenyltins. Relative Standard Deviations (RSDs) of the Response Factor sample heights were <15 % for butyltins for all sample runs. Organotin concentrations are expressed in terms of the cation (TBT^+, DBT^+ and MBT^+) as ng l^{-1} of water.

2.3.5   Experimental determination of the sediment – water partition co-efficient K_d

Sorption experiments were carried out to determine the sediment- water partition coefficient of TBT for site specific sediments from Cockshoot, Wroxham, Salhouse, Ranworth, and Malthouse Broads. All sorption work was based on OECD (2000) guidelines.

Air dried field sediments were pre- equilibrated on a wrist action shaker at 120 oscillations per minute for 12h with 5ml of synthetic pore water solution consisting of concentrations of potassium salts of KNO_3, K_2SO_4, KCl. Concentrations were replicated from measures of relevant anions in pore water from field sediments. Following initial equilibration a further 15ml of synthetic pore water was added to experimental systems, pH recorded and TBT spikes of 53, 107, and 160 µg l^{-1} added to respective treatments to achieve initial TBT concentrations of 40, 80 and 120 µg l^{-1}. Samples were then shaken for a further 24h to allow for contaminant equilibration between aqueous and solid phases. Experiments were run in triplicate for each concentration treatment and site. For each site, duplicate control samples were run with pore water solution containing no TBT. Following termination of the experiment pH was re-recorded and samples were centrifuged at 3000 rpm for 20 minutes to separate solid and aqueous phases. Sediments were then analysed for TBT following the GC-FPD method outlined in section 2.3.3. Results were corrected for existing contamination by subtracting TBT concentrations measured in control samples from those measured in experimental sediments. Sorption isotherms were plotted using aqueous and solid phase concentrations, and K_d values calculated. The K_d value for each concentration was calculated using the following equation.

$K_d = C_s/C_{aq}$

Where C_s is the TBT concentration quantified in sediment (corrected by control samples) and C_{aq}, the TBT concentration in solution (calculated as total TBT inputted – TBT quantified in sediment).

To ensure experimental results were not an artefact of TBT sorption to centrifuge tubes a separate experiment was carried out by spiking pore water only with120 µg l^{-1} of TBT. Tubes were shaken for 24h and porewater analysed for TBT using the methods outlined in section 2.3.3. Recovery was equal to or exceeded 100% in all cases.

2.3.5   Stable isotope analyses

For those taxa with body size insufficient for individual analysis, three replicate samples comprising a minimum of five pooled individual macroinvertebrates (but in the majority of cases when and where each taxa was abundant then up to 20 individuals were used) were analysed for each quarterly sampling event (i.e. n = 9 or n = 12, depending on site). Where replication was not possible due to low taxa abundance a sample comprising a minimum of 5 bulked individuals was used for isotopic analysis. For acaridae and large bodied predators such as the zygopteran larvae (*Sympetrum* spp. and *Aeshna* spp.) sampling was conducted until a minimum of three individuals (in some cases five) were captured to provide sufficient sample replication (i.e. n = 15 or 20). For *Anodonta* spp., five individuals were analysed separately for each sampling event (n = 15 or 20, depending on site). For the construction of each food web, fish samples were represented by a minimum of three individuals for less abundant species such as pike (*Esox lucius*) and tench (*Tinca tinca*) and a maximum of 15

individuals for species such as eel (*Anguilla anguilla*), bream (*Abramis brama*), perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), and rudd (*Scardinius erythrophthalmus*).

After thawing, samples were oven-dried at 65°C for a minimum of 48 h before being homogenised using an agate pestle and mortar. Shells were removed from molluscs prior to drying due to the incorporation of DIC in $CaCo_3$ that is typically ^{13}C enriched relative to diet. Small bodied invertebrates were analysed whole. For individual *Anodonta* spp. a small portion of the foot muscle was excised and used for analyses. In larger predatory invertebrates such as dragonfly larvae the softer abdomen was used. For fish a small piece of muscle tissue was removed from above the lateral line, below the region of the dorsal fin. Samples were then weighed (0.6 ± 0.05 mg) into tin capsules and were then analysed for stable carbon and nitrogen isotope composition using an elemental analyser coupled to a continuous flow isotope ratio mass spectrometer (Thermo-Finnigan, Delta Matt Plus) in house at Queen Mary, University of London. Results are given using the standard δ notation:

$$\delta = [(X_{sample} / X_{reference}) - 1] \times 1000$$

expressed in units of per mille (‰) where $X = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$. Reference materials were internal standards of ammonium for nitrogen, and sucrose for carbon. A secondary internal standard of a known relation to international standards was run every ten samples to provide a measure of instrumental precision (± 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$).

2.4   Statistical methods and Data analyses

Minitab 15 (Minitab Inc., 2007) was used for statistical testing, with a significance level of $p \leq 0.05$. Normality and equality of variances were ascertained for all data by performing Anderson-Darling and Levene's tests, respectively, with data accepted as normal if $p > 0.01$. If data were below acceptable normality criteria they were log transformed. If transformation was not possible then the appropriate non-parametric test was used.

2.4.1   Sediments

Regression analyses were used to examine relationships between butyltin concentrations and environmental variable data, and ANCOVA was used to compare the slopes of regression relationships for sediment DBT and TBT concentrations between years 2004-2008. To compare experimentally derived log K_{oc} values between sites, one-way ANOVA (with *post-hoc* Tukey's tests) were used. Students T-tests were used to test for statistical differences in the butyltin degradation index between years 2004-2008. For non-normal sediment data a Kruskall-Wallis test was used to test for differences between site specific sediment concentrations.

2.4.2   Biota

To compare sediment concentrations to those of biota such as *Anodonta* spp. and to examine the relationship between $\delta^{15}N$ values of consumers and butyltin burdens (where necessary) TBT sediment data were log transformed. To statistically compare TBT concentrations in *Anodonta* spp. between site, and isotopic distinction between fish species, one-way ANOVA and general linear models were used. For non-normal data, Kruskall-Wallis tests were used in

place of ANOVA. To compare groups of samples such as butyltin concentration in specific fish body parts between sites a General Linear Model (with *post-hoc* Tukey's tests) was used with tissue type and site as fixed factors.

2.4.3 Stable Isotope metrics and Standard Ellipse Area (SEA)

The calculation of isotopic metrics, and standard ellipse area (SEA) were conducted using the 'R' statistical package (R Development Core Team, 2007). The metrics and what they represent ecologically are described in detail in chapter 4, section 4.2.3.   The methods used to create the 'R' codes are presented in detail in Jackson et al (2010) and are available to researchers via the package SIAR (Stable Isotope Analysis in R - Parnell et al., 2008, Parnell et al., 2010) available to download from the packages section of the Comprehensive R Archive Network site (CRAN) - http://cran.r-project.org/.

The community metric total area (TA: see Chapter 4; section 4.2.3) can be converted to a measure of population niche area (Layman et al. 2007b). However, calculation of TA from a convex hull drawn around the most extreme data points on an isotope bi-plot results in an incomparable measure of niche area when applied to samples of different sizes. This is because the convex hull area increases in response to sample size even if the underlying population remains the same. To avoid this error the standard ellipse area (SEA) was employed to compare the isotopic niches of bream and rudd in Malthouse and Ranworth Broads (Chapter 3, section, 3.5.1.). Briefly, the standard ellipse area is a measure of mean population isotopic niche area which is robust to variation in sample size and is to bivariate data as standard deviation is to univariate data. The standard ellipse of a set of bivariate data is calculated from the variance and covariance of the x and y data and contains approximately

40% of the data (Batschelet 1981, Ricklefs and Nealen 1998) and is expected to be insensitive to sample size. However, the use of a (n-2) correction on the denominator in place of the standard (n-1) when calculating variances is employed due to the loss of an extra degree of freedom involved when dealing with bivariate data. A sample size corrected version of the standard ellipse area, referred to as SEAc is employed to circumvent the bias that arises when sample sizes are small. Explicitly:

SEAc = SEA * (n-1) / (n-2)

This correction has the property of increasing SEAc at small sample sizes in order to correct bias towards underestimation but asymptotes to 1 at infinity. The calculation of SEAc allows the degree of isotopic niche overlap to be calculated which can be then used as a quantitative measure of dietary similarity among populations. This method was used to calculate and compare the degree of isotopic niche overlap between rudd and bream samples from Malthouse and Ranworth Broads (Chapter 3, section, 3.5.1)

Chapter 3

Organotin Contamination in the Norfolk Broads: from a Sediment Legacy to Contemporary Food Webs

3.1   Introduction

Despite nearly 25 years since its last legal, known use in the Norfolk Broads system recent research has unveiled a historical legacy of TBT contamination in the sediments of many connected lakes (Hoare, 2007). Specifically a gradient of contamination was revealed that increased in response to boating 'activity'. Thus, in those Broads that were non-navigable, TBT was undetected, as boat traffic increased concomitant increases in TBT were observed in sediments, whereby those sites with the most heavily contaminated sediments were boat yards, where holiday craft are moored and maintained.

In this chapter I will attempt to establish whether this gradient of contamination still exists and whether significant degradation of the contaminant has occurred between studies. I will also asses the role of abiotic factors such as pH, anion concentration, sediment type and TOC in the speciation and bioavailability of OTCs. A further key aim is to establish whether this historical sediment OTC reservoir can act as source of contamination to contemporary food webs of the Norfolk Broads. I hypothesised that sediment dwelling primary consumers such as chironomids and unionid mussels could potentially accumulate OTCs due to benthic life histories. For example, mussels are long lived and therefore have the potential to accumulate considerable OT burdens. In contrast chironomids are comparatively short lived but can be numerically abundant and therefore often represent an important prey for a range of food web consumers such as benthivorous fish. For this reason primary consumers such as chironomids could represent a conduit via which OTCs could enter the wider food web. Fish represent an ideal group of consumers in which to test for the presence of OTCs in food

webs, as different feeding ecologies and habitat utilisation may influence their exposure to contaminants. For example, benthivorous fish such as bream (*Abramis brama*) are likely to feed almost exclusively in the benthic compartment of food webs so may come be exposed to organotins both through contact with contaminated sediments and via feeding on contaminated prey. In contrast species such as rudd (*Scardinius erythrothalamus*) are adapted as surface or mid – water feeders so their OTC exposure risk may be reduced. Similarly piscivores such as pike (*Esox lucius*) should in theory only be exposed to OTCs through trophic interactions. Due to the factors outlined above, I hypothesised that in contaminated broads the principle route of TBT transfer to the wider food web will stem from profundal sediments. Specifically, in order to test the idea that TBT will enter food webs via the benthic route of energy transfer, fish species with divergent feeding ecologies will be analysed for the presence of TBT. This can be developed further as others have used measured concentrations of OTCs in specific body parts and used them as an indication of the importance of uptake route (Morcillo et al., 1997; Viglino et al., 2006). Hence, I will ascertain the importance of uptake routes by comparison of specific body parts/tissues. Furthermore, by combining measures of food web consumer $\delta^{15}N$ with corresponding measures of OTC burden, it should be possible to assess the biomagnification potential of OTCs in representative broadland food webs in a similar manner to Kidd et al. (1995). Few studies have used the stable isotope approach in studies concerning OTC transfer (but see Hu et al, 2006; Murai et al, 2008), and to the best of my knowledge my work is the first to use such methods in freshwater ecosystems. Thus, results could provide important future reference for similarly contaminated shallow lake systems.

The generic field and laboratory methods outlined in Chapter 2 are used in many of the following sections. Rather than detail specific methods here, I have integrated any specific methods / protocols in the respective sections that follow a logical progression from investigations of the organotins in the sediment, the occurrence of organotins in the primary consumers, and finally, the potential for transfer of those organotins upwards through the food web.

3.2   Organotin Contamination in Water and Surficial Sediments

Quantifiable concentrations of TBT were detected at six sites (Fig. 3.1) and ranged from 87 to 4625 ng g^{-1}, with a mean (±SE) value of 1150 ± 230 ng g^{-1}. DBT was also present in quantifiable concentrations at all connected sites, although concentrations were lower overall, ranging from 165 to 850 ng g^{-1} (mean 286 ± 46 ng g^{-1}). MBT was quantifiable from boatyards only and ranged from 267 to 514 ng g^{-1} (mean 268 ± 37 ng g^{-1}). Results confirmed the presence of the expected gradient of increasing sediment contamination in the order of Wroxham < Salhouse < Ranworth < Malthouse < Loyne's Boatyard < Horning Ferry Marina (Fig. 3.1). Total butyltin (∑MBT+DBT+TBT) concentrations from boatyard sites were up to an order of magnitude greater than those from three of the four connected broads (Fig. 3.1). Samples from Malthouse Broad yielded concentrations of DBT (266 ± 11 ng g^{-1}) and TBT (1368 ± 68 ng g^{-1}) similar to those observed in boatyard sediments. Total butyltin concentrations were significantly greater in sediment samples from Malthouse Broad and the two boatyard sites (Kruskall-Wallis χ^2 =14.8, DF = 5, p< 0.05) compared to those from Wroxham, Salhouse and Ranworth. No organotins were detected in any water sample. Phenyltins were not detected in any sediment samples. Organotin analyses from the two

isolated control sites (Cromes and Cockshoot Broads) yielded no quantifiable butyltin species.
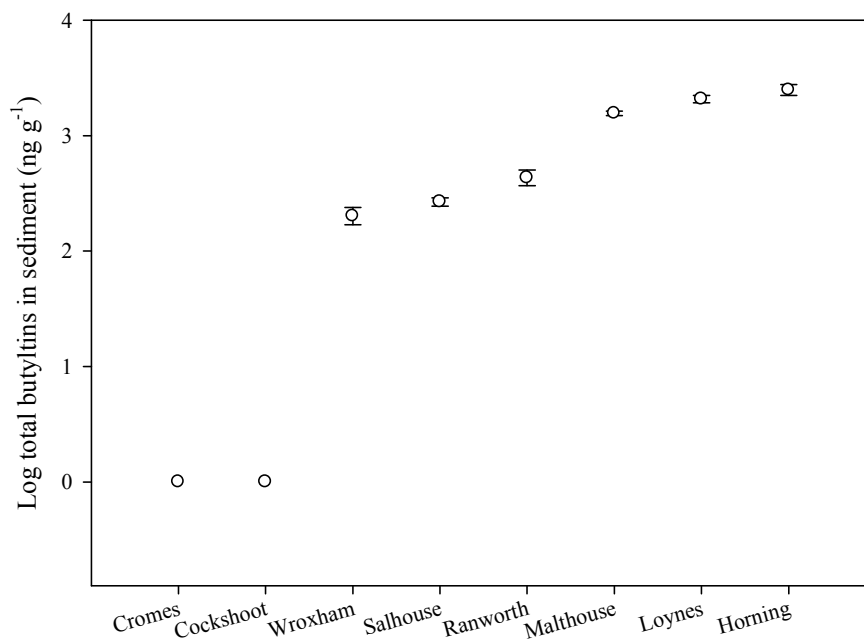
Figure 3.1. Mean log total butyltin ($\sum$ MBT + DBT +TBT) concentrations in surficial sediments from sampled sites. Error bars represent ± SE. For all sites mean values derived from pooled data from 2007 and 2008 (n=6), except Malthouse and Salhouse Broads where data available from 2008 only (n=3)

3.2.1 TBT degradation in sediments

If degradation of TBT is occurring in sediments then I expected that there would be a relationship between concentrations of the parent compound and metabolites. In the previous

section it was demonstrated that TBT was the dominant metabolite in sediments. Regression analyses of data from sampling in 2006 and 2008 revealed a significant relationship ($y= 0.817 x - 0.043$. $r^2= 0.98$, $p < 0.05$; Fig. 3.2) between TBT and DBT throughout boatyards and broads alike.
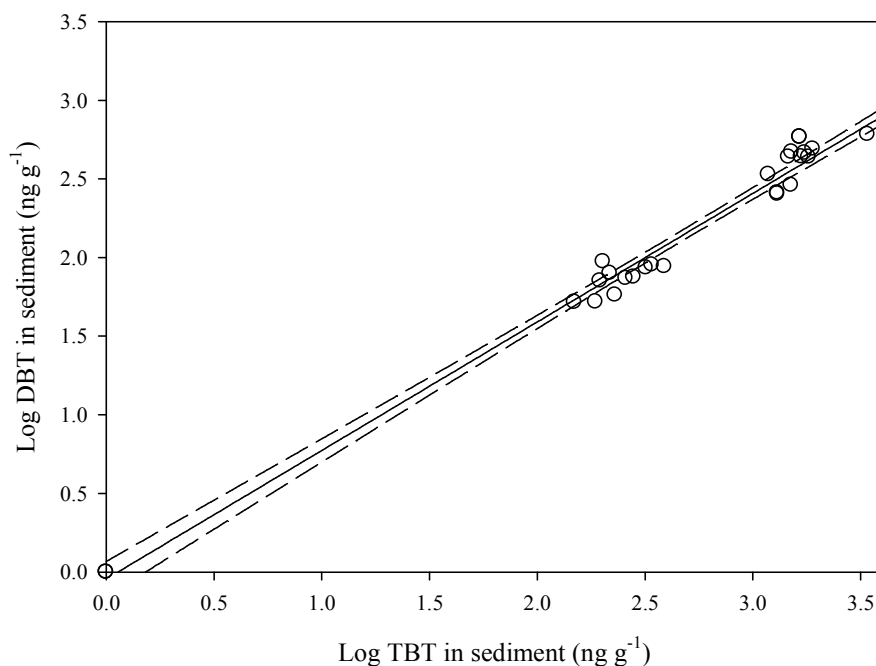
Figure 3.2. Relationship between TBT and DBT at all sampled sites from the current study. Data from 2007 and 2008 (n = 6), except Salhouse and Malthouse Broads where data available from 2008 only (n=3). The solid regression line was fitted using the equation (± 95% CI) $y= 0.817 x - 0.043$. $r^2= 0.98$, $p < 0.05$.

A butyltin degradation index (BDI) was calculated for TBT data from all of the contaminated sites in the current study (Table 3.1) and with comparable data obtained from a previous

study in 2003/2004 (Hoare 2007). Where MBT was below the LOD, concentrations of DBT only were used to calculate the BDI, in accordance with the methods proposed by Diez et al (2001). Calculated BDI values were < 1 for all sites and years, suggesting slow de-butylation of TBT to DBT and MBT (Table 3.1).

	WR	SA	RA	MA	LB	HF
BDI 2003/2004	0.36	0.22	0.34	0.26	0.66	0.29
BDI 2006/2008	0.17	0.28	0.26	0.19	0.41	0.37

Table 3.1. Comparative butyltin degradation index (BDI) for 2003/2004 and 2007/ 2008 from all contaminated sites. Data from 2003/2004 are mean values derived from n = 2 samples, excluding Malthouse Broad (n = 1). Data from 2006/2008 are mean values from n = 6 samples, excluding Malthouse and Salhouse Broads where n = 3. WR, Wroxham Broad; SA , Salhouse Broad; RA , Ranworth Broad; MA, Malthouse Broad; LB, Loynes Boatyard; HF, Horning Ferry Marina. At Boatyard sites BDI calculated using MBT & DBT. Due to non detection of MBT from all other sites, BDI calculated using DBT only.

To examine whether the TBT:DBT ratio had altered in the four years since the study of Hoare (2007) ANCOVA was used to compare the slopes of the two data sets. Results were not significant ($p > 0.05$), indicating little temporal change in TBT degradation (Fig. 3.3).
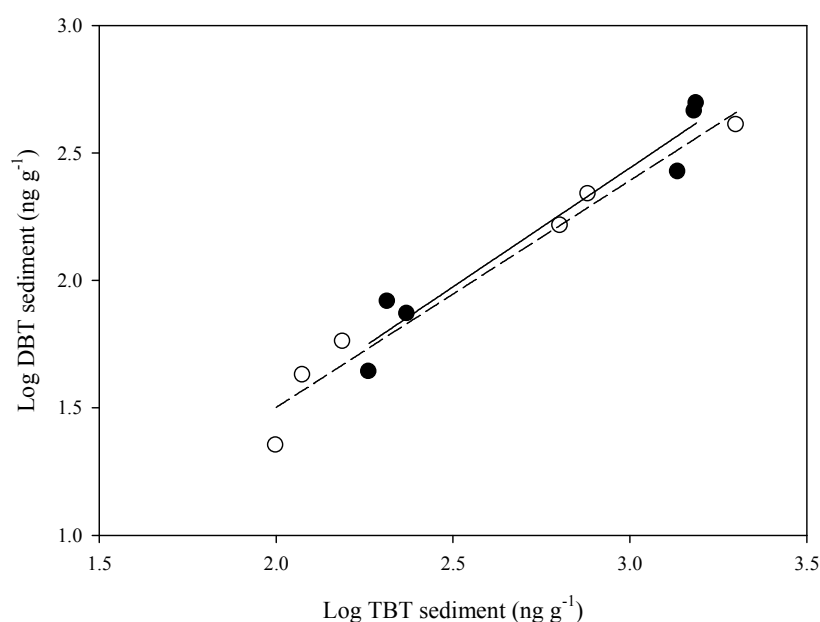
Figure 3.3 Relationship between TBT and DBT in surficial sediments from contaminated sites. Open circles represent single samples from 2004 (Hoare 2007). The dashed regression line was fitted using the equation; y= 0890 x-0.279, r^2= 0.96, p <0.05. Closed circles represent mean of three samples from 2008. The solid regression line was fitted using the equation y= 0.934 x- 0.360, r^2= 0.95, p < 0.05).

3.3   Parameters Controlling Speciation and Partitioning of TBT in Sediments

Sediment characteristics and chemical parameters are known to influence the partitioning behaviour and speciation of OTCs. In turn, partitioning behaviour and speciation may control the fraction of the contaminant that is biologically available to aquatic biota (see Chapter 1). Thus the aim of this section was to determine the dominant TBT species in the study sites and whether other parameters would control the degree of contamination in sediments, and the implications this could have for the supply of TBT to the wider food web.

3.3.1   Particle size distribution of surficial sediments

With the exception of Salhouse Broad, sediments were comprised primarily of clays that frequently represented > 85 % of the total particle size distribution (Table 3.2). At these sites silts made up the remaining size fraction but were usually < 15% of the total. In contrast to other sites, Salhouse sediments were comprised principally of silts, with clays and fine sands of less importance in sediment composition (Table 4.2). Coarse sands were absent from the surficial sediments of all eight sites (Table 4.2).

Site	% Clay	% Silt	% Fine sand	% Coarse sand
Cromes	92.7 ± 0.9	7.3± 0.9	-	-
Cockshoot	87.8 ± 1.0	12.2 ± 2.1	-	-
Wroxham	92.0 ± 2.0	8.0 ± 2.6	-	-
Salhouse	21.9 ± 0.5	64.2 ± 0.9	14.1± 2.2	-
Ranworth	91.6 ± 0.6	8.4 ± 0.6	-	-
Malthouse	54.6 ± 32.1	48.5 ± 22.9	-	-
Loyne's	89.7 ± 0.1	10.3 ± 0.1	-	-
Horning	90.1 ± 0.1	9.7 ± 0.2	-	-

Table 3.2. Mean (± SE) % particle size distribution of the eight sample sites, dash indicates absence of respective fraction from samples.

3.3.2 Sediment pore water chemistry

The pH of interstitial pore water was measured in fresh field sediments (1-4 cm depth). Values were similar across all sites and ranged from 7.3 to 7.6 the mean (±SE) value being 7.5 ± 0.1 (Figure 3.4).
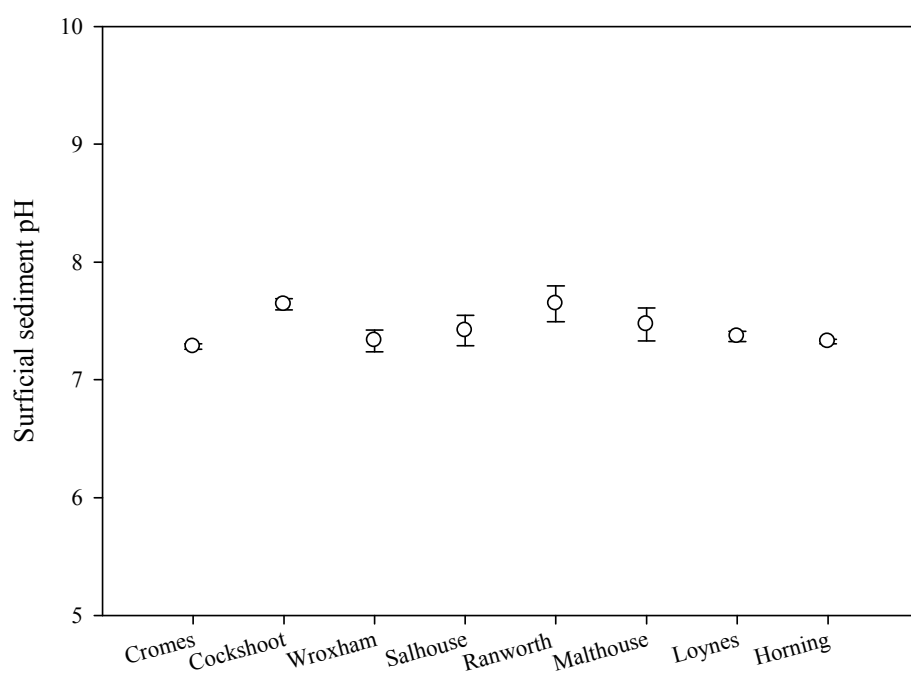
Figure 3.4 Mean pH of interstitial pore-waters of surficial sediments (1-4cm) from all sample sites. Error bars represent ± SE. At each site mean pH was derived from six separate measurements in April 2008.

The anion concentrations of pore water extracted from surficial sediments (1-5 cm depth), in contaminated broads are summarised in Table 3.3. Concentrations of all pore water anions were greatest in samples from Malthouse Broad. This was particularly marked in terms of chloride where concentrations were an order of magnitude greater than those from other contaminated broads (Table 3.3). The anion concentrations in the sediment pore water of the other three contaminated broad sites were similar, with the exception of sulphate which was considerably lower in pore water extracted from Salhouse (Table 3.3).

Site	Cl^- (mg l^{-1})	SO_4 (mg l^{-1})	NO_3^- (mg l^{-1})	$PO^3_4^-$ (mg l^{-1})
Wroxham	129.8	53.6	0.70	4.28
Salhouse	89.9	9.4	1.5	*
Ranworth	139.3	59.9	1.9	*
Malthouse	1201.6	230.5	34.5	*

Table 3.3 Concentrations of chloride, sulphate, nitrate and phosphate in sediment (1-5cm depth) pore-water from contaminated broads. * Indicates anion not detected.

3.3.3 Total organic carbon content (%TOC) of sediments

Total organic carbon (%TOC) in surficial sediments was quantified for all sites (Fig. 3.5) and ranged from 6 to 42 %, the mean (±SE) value for all sites being 17 % ± 4.0. Sediments from the control sites, Cromes and Cockshoot Broads, yielded the highest %TOC (Fig. 3.5). Excluding control sites, TOC in sediment followed a similar pattern to that of butyltin contamination. For example, sediments from Wroxham and Salhouse Broads contained the lowest organic carbon of all broads (7 % ± 0.4 and 9 % ± 0.4 respectively). TOC content measured from Ranworth (11% ±0.5), Malthouse Broad (12 % ± 1), and Horning Ferry (12% ± 0.2) were similar to samples from Malthouse. Sediments from Loyne's boatyard displayed the greatest TOC content of all contaminated sites (20% ± 1.4). Thus, TBT concentrations in the sediment were significantly positively correlated with total organic carbon content ($Y = 2.091, x - 0.068; r^2 = 0.46, p < 0.05$; Fig. 3.6), but not with pH or particle size. Accordingly, total butyltin concentrations ($\sum$MBT+DBT+TBT) were normalised using organic carbon content to account for the effect of organic carbon content on TBT concentration. Normalisation of total butyltin concentration for sediment TOC revealed little change in the pattern of increasing sediment TBT with boating activity (Fig. 3.7).
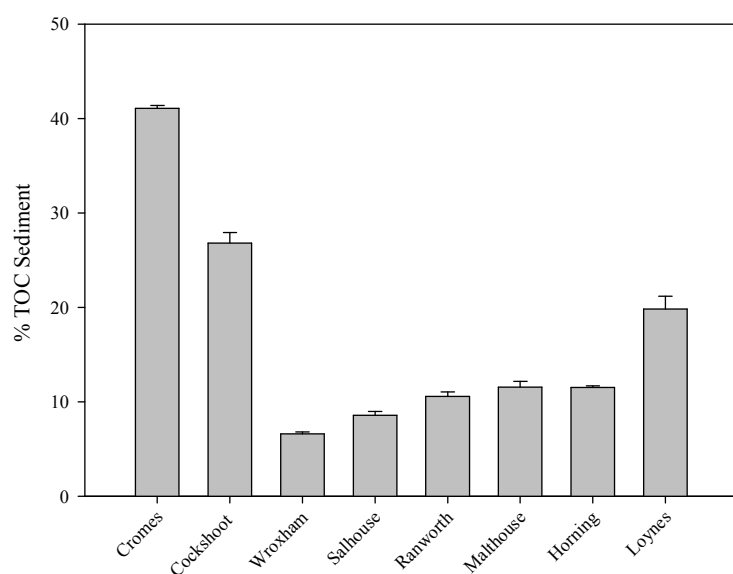
Figure 3.5 Mean %TOC in surficial sediments from all sample sites. N = 3; error bars ± SE.
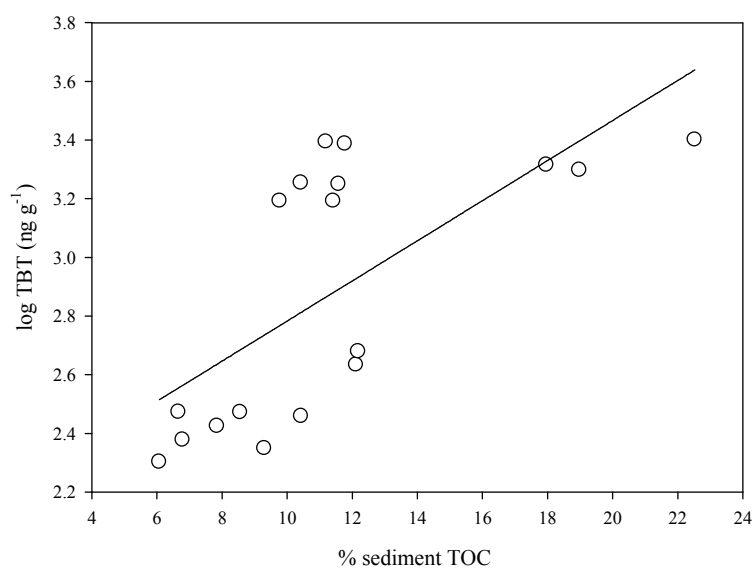
Figure 3.6 Relationship between % TOC and log transformed TBT concentration from all

sampled sites (n=3 each site). The regression line was fitted using the equation Y= 2.091, x-

0.068; r^2= 0.46 p< 0.05. Data from 2008 sampling only.
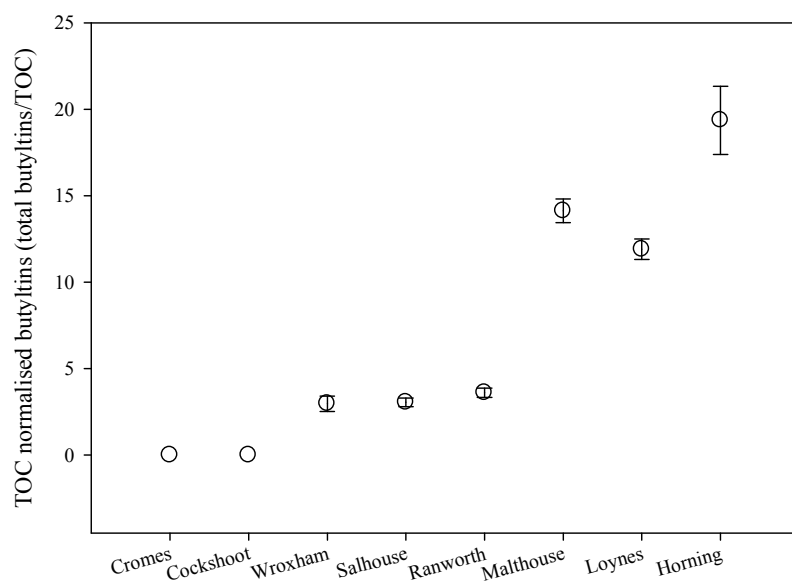
Fig 3.7 Mean TOC normalised total butyltin ($\square$ MBT + DBT + TBT) concentrations in surficial sediments. Error bars represent ± SE. For all sites mean values derived from pooled data from 2006/2008 (n=6) except Malthouse and Salhouse Broads where data available from 2008 only (n=3)

3.3.4 Experimental determination of sediment-water partition coefficients *(KD)*

Sorption isotherms for TBT were linear (Fig. 3.8) and of the form *S=KdCe* where *S* is the amount of TBT sorbed (μg kg^{-1}), K_d is the partition coefficient (L kg^{-1}) and *Ce* is the concentration in solution at equilibrium (μg l^{-1}). Linearity of isotherms suggested no exhaustion of TBT binding sites throughout the 24hr equilibration. Mean (± SE) calculated K_d values were lowest in sediments from Ranworth Broad (2.6 ± 0.1), K_d values were similar for Wroxham and Salhouse sediments (3.8 ± 0.2 and 3.6 ± 0.3, respectively) and highest in sediments from Malthouse Broad (4.3 ± 0.9). There was no significant relationship between site specific log total ionic strength ($\sum Cl^{-1} + SO_4 + NO_3^- + PO^3_4^-$) of pore-water and mean log K$_d$ values (p > 0.05) suggesting little influence of total anion concentrations on K$_d$ values.
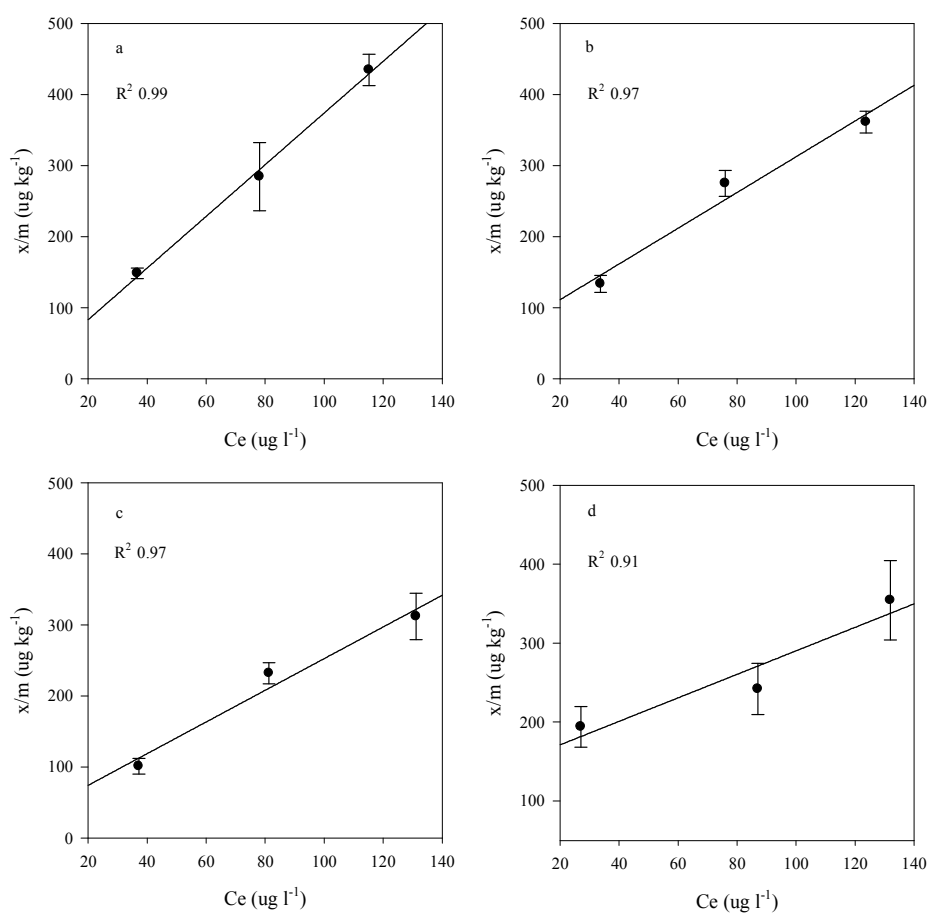
Figure 3.8 Sorption isotherms for TBT in replicated sediment pore-water systems for a, Wroxham Broad; b, Salhouse Broad; c, Ranworth Broad and d, Malthouse Broad. Where Ce is the concentration of TBT in solution at equilibrium (mg l^{-1}); x/m is amount of TBT sorbed to solid at equilibrium (μg kg^{-1}). Each data point represents mean ($\pm$ SE) of 3 samples.

The results of control experiments, which involved shaking TBT standards in the centrifuge tubes for 24 hours without sediment, indicated that there was no sorption of TBT to centrifuge tube walls. Recovery concentrations ranged from 108 to 144%, with a mean ($\pm$SE) value of 121 ± 9.0 ng l^{-1}. pH was measured at t_0 and t_{24} for each experimental pore-water system and showed no significant change throughout the experiment.

As TBT concentrations in sediment were strongly correlated with organic carbon content, organic carbon partition coefficients were calculated for the sediments. There were significant differences in log K_{oc} values between sites (ANOVA, $F_{3,\,35}$ 10.14, $p < 0.05$ with post hoc Tukey's tests); specifically the mean K_{oc} value from Wroxham sediments was significantly different to those from Ranworth and Malthouse and the mean K_{oc} from Salhouse sediments significantly different to those from Malthouse only (Figure 3.9). The full ranges of K_{oc} values for each experimental treatment are recorded in appendix 2.
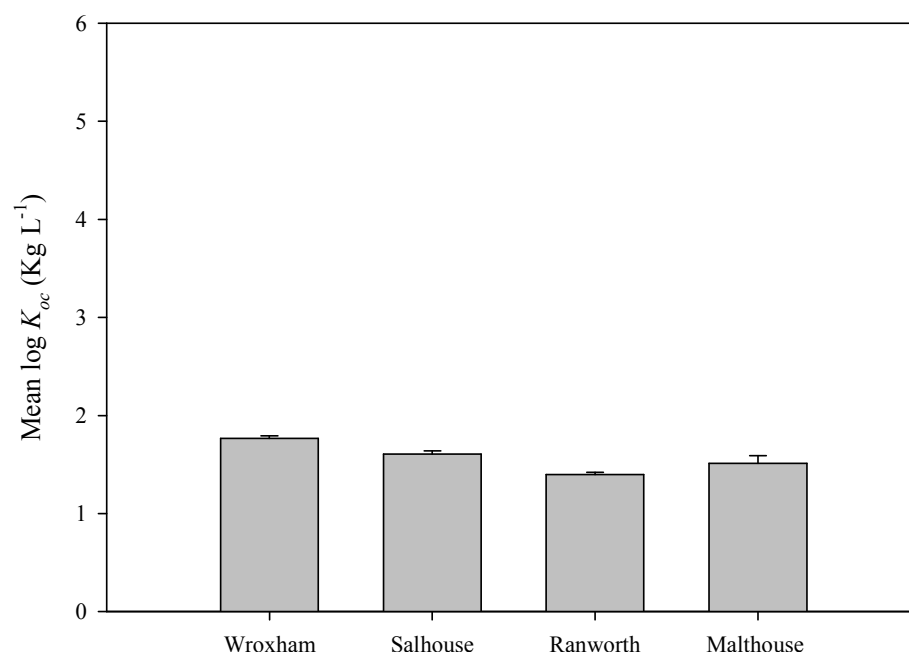
Figure 3.9 Mean ($\pm$ SE) Log K_{oc} values for TBT in replicated sediment – pore-water systems for Wroxham, Salhouse, Ranworth, and Malthouse Broads. Values derived from 3 replicates of 3 different TBT concentration treatments (n=9, each site)

3.3.5   Comparison of K_{oc} values between contaminated and non-contaminated sites

To ensure that the low K_{oc} values obtained in the first experiment were not an artefact of reduced availability of binding sites due to existing TBT contamination of experimental sediments (Burton et al., 2004), a second experiment was conducted using sediments from Cockshoot Broad (in which TBT concentration were lower than detection limits), in tandem with sediments from the most contaminated site (Malthouse Broad). The mean log K_{oc} from Malthouse Broad sediments (1.4 ± 0.6 Kg l^{-1}) was approximately double that of Cockshoot and ~ equal to the value obtained in the first experiment (log K_{oc}; 1.3 Kg l^{-1}). Thus, results suggested sorption was not inhibited by previous contamination of sediments.

3.4   Organotin Contamination of Consumers

Following the establishment of the TBT gradient in sediments, I aimed to determine whether sediments could act as source of contamination to consumers. Further, to determine whether benthic primary consumers (*Anodonta* spp. and *Chironomus plumosus*) would display quantifiable TBT burdens and whether there were relationships between variables such as consumer TBT concentrations in response to the gradient of contamination, and TBT concentrations and body size. I also attempted to assess the importance of uptake routes using TBT analysis of FPOM, biota sediment accumulation factors (BSAFs), and isotope mixing models.

3.4.1   Organotin Contamination in Benthic Primary Consumers: *Anodonta* spp.

Thirty unionid mussels (*Anodonta* spp.) from broad sites (n = 5 each site) were analysed for organotin contamination. In order to avoid the possibility of an effect of size on TBT burden (see next section), individuals of a similar size were selected; the mean ($\pm$ SE) measured shell length was 87.4 ± 5.6 mm.   No phenyltins were detected in any sample, and butyltins were not detected in mussels from control sites (Cromes & Cockshoot Broads).

At contaminated Broads, all mussel samples showed butyltin contamination although concentrations were typically an order of magnitude less than those of surrounding sediments (Figure 3.10). TBT was the dominant organotin species in all samples and ranged from 23.4 to 106.9 ng g^{-1} (mean $\pm$ SE: 43.8 ± 4.0 ng g^{-1}). MBT was not detected and DBT was detected in two individuals from Malthouse Broad only (13 and 8ng g^{-1}). There was a significant difference in mussel TBT contamination between sites (ANOVA, $F_{3,19}$, 21.26, p <0.05 with post hoc Tukey's tests); specifically concentrations of TBT were significantly greater in mussels from Malthouse Broad, compared to other sites and thus, a positive relationship existed between mean sediment TBT and mussel TBT burden (y = 12.34x + 0.050 r^2=0.78, p < 0.05)
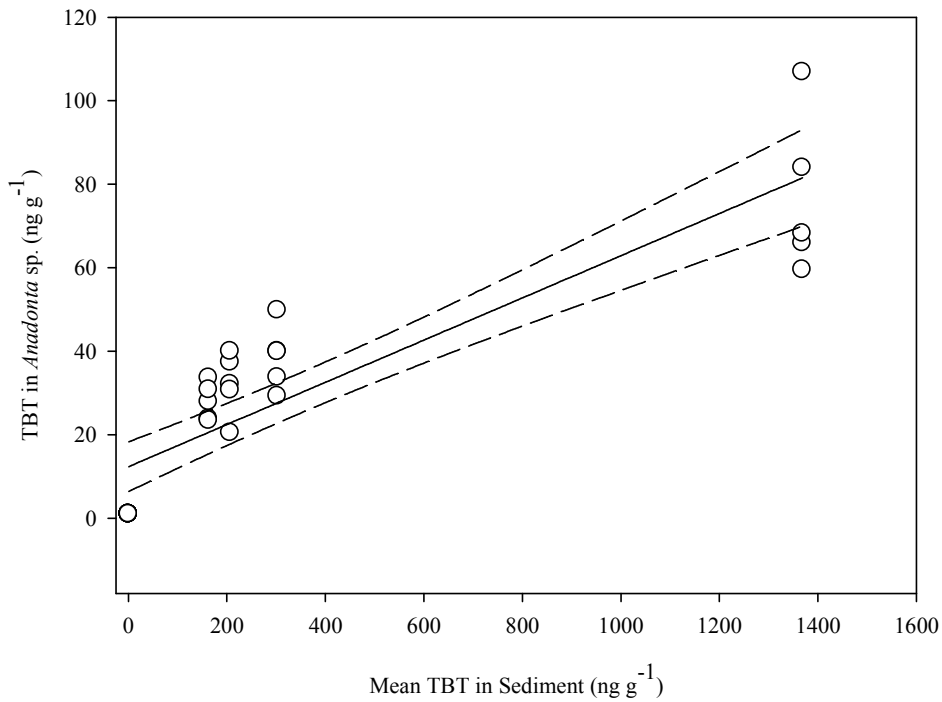
Fig 3.10 Relationship between site specific mean TBT in sediments and TBT in individual *Anodonta* spp. from six broad sites. The regression line (± 95% CI) was fitted using the equation y = 12.34x + 0.050 r^2=0.78, p < 0.05

3.5.2   Body size:TBT burden relationships

Unionid mussels may reside in sediments for decades and thus one might predict a positive relationship between age (inferred from body size) and organotin burden. To test for such a relationship, three different size classes of mussels collected from Malthouse Broad were analysed for butyltin contamination. Mean shell length, wet weight, and TBT concentration for each size class are given in Table 3.4. On average, the smallest sized individuals had the

highest TBT concentrations, ranging from 51 to 79 ng g^{-1}. In intermediate sized individuals, concentrations were lower on average ranging from 37 to77 ng g^{-1}. The largest mussels showed the lowest concentrations of TBT, and ranged from 19 to 48 ng g^{-1} (Table 3.4). There was a significant negative relationship between *Anodonta* spp. body mass and TBT concentrations in body tissue (Figure 3.11 (a); $y = 2.439x - 0.543$, $r^2 = 0.58$, $p < 0.05$). Thus, smaller sized individuals exhibited greater concentrations of TBT per unit mass. However, when these concentrations were converted to total body burden (calculated as individual TBT (ng g^{-1}) x individual whole body weight (g)), then there was a significant relationship between body mass and total TBT burden (Fig 3.11 (b) $y = 2.439x + 0.543$, $r^2 = 0.66$, $p < 0.05$).

Size class	n	Shell length (mm)	Body Weight (g)	TBT (ng g^{-1})	Whole Body TBT (ng)
Small	5	67 ±0.8	20.4 ±1.9	72.4 ±7.8	1443.2 ± 128
Medium	5	105.6 ± 2.4	44.1 ±4.3	51.0 ±9.3	2212.4 ± 450
Large	5	142.1± 3.1	132.8 ± 4.8	32.4±5.9	4267.0 ± 767

Table 3.4. Mean shell length, wet weight and TBT concentrations in *Anodonta* spp. from Malthouse Broad used for size based analyses.
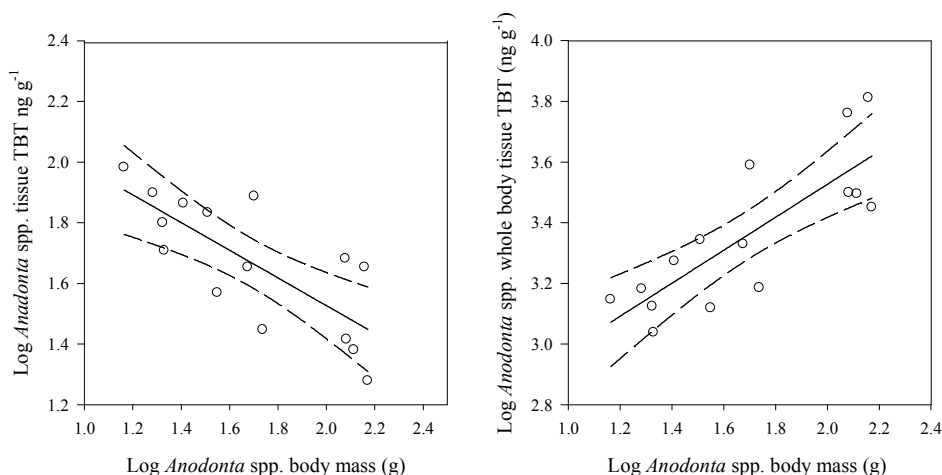
Figure 3.11 (a) Relationship between body mass and tissue TBT concentration in *Anodonta* spp. from Malthouse broad. The regression line (± 95% CI) was fitted by the equation y = 2.439x - 0.543, r^2 = 0.58, p <0.05. (b) Relationship between individual body mass and total body TBT burden. The regression line (± 95% CI) was fitted with the equation y = 2.439x + 0.543, r^2 = 0.66, p <0.05.

3.4.3 Organotin Contamination in Benthic Primary Consumers: *Chironomus plumosus*

Chironomids are relatively short lived organisms but *C. plumosus* is tubicolous in the soft sediments and an important prey species for fish and therefore may represent an important link from the sediments to the higher food web.

Chironomids were absent from boatyards during all sampling events. No phenyltins were detected in any chironomid sample, and analyses of chironomids from isolated control sites (Cromes and Cockshoot Broads) yielded no quantifiable butyltin species. TBT was < LOQ in

chironomids from the remaining four butyltin contaminated sites. Where butyltins were detected in chironomids, it was in the form of DBT and <10 ng g^{-1}, meaning organismal concentrations were at least one order of magnitude less than respective sediment concentrations (Fig. 3.12). Only one replicate from Ranworth Broad showed a quantifiable DBT concentration of 3 ng g^{-1} whereas all samples (n = 4) from the most contaminated site (Malthouse Broad) showed consistent low level DBT contamination ranging from 6 to 9 ng g^{-1} (Figure 3.12). Thus it was not possible to determine a relationship between the TBT sediment gradient and chironomids.
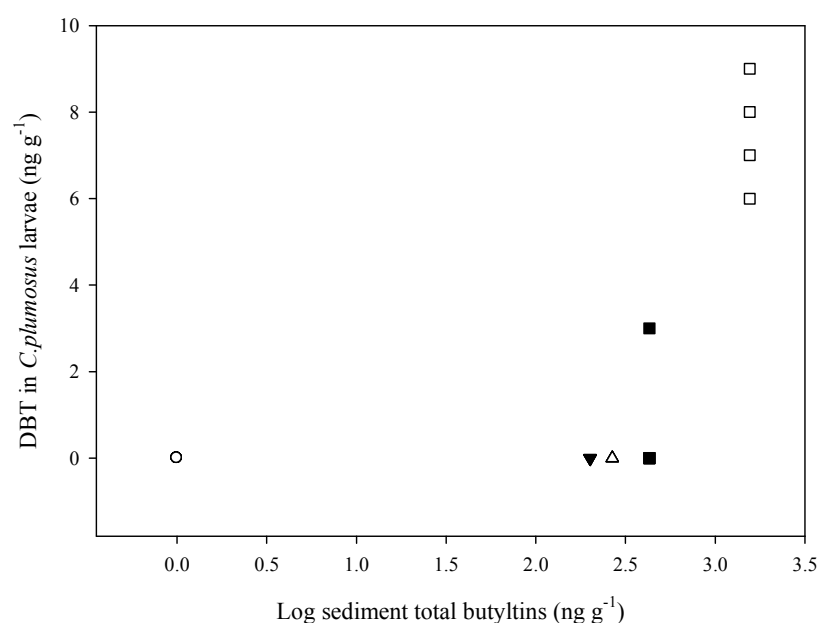
Figure 3.12. DBT concentrations in *C.plumosus* larvae as a function of total butyltins in sediments. Open circles: control sites; down triangles: Wroxham; up triangles: Salhouse; solid squares: Ranworth; open squares: Malthouse.

3.4.4 Bioaccumulation Factors (BAFs) and Biota Sediment Accumulation Factors (BSAFs) in *Anodonta* spp.

Calculations of BAFs were not possible as organotins were not detected in FPOM samples, suggesting uptake from this potential dietary route was of little importance to *Anodonta* spp. TBT body burden. The biota sediment accumulation factor was calculated for *Anodonta* spp. from contaminated broad sites. Table 3.5 shows the mean exposure organotin concentration (e.g. sediment concentration), concentration in mussels and the resulting BSAF. The BSAFs were low in comparison to sediment concentrations at all sites, suggesting little bioaccumulation from sediment. Unlike TBT in mussel tissue that increased in response to the sediment TBT gradient; BSAFs were lowest in mussels from Malthouse Broad and highest in those from Wroxham and Salhouse (e.g. BSAF Wroxham = Salhouse > Ranworth > Malthouse). There was a significant negative relationship between TBT in sediments and BSAF's of individual *Anodonta* spp. (Figure 3.13 y = 0.436x - 0.217; r^2 = 0.76, p < 0.05). As concentrations in chironomids were low compared to sediments BSAFs are not presented for this species.

Site	Exp. route	Exp. (ng g^{-1})	Organism (ng g^{-1})	BSAF
Wroxham Broad	Sediment	163.4 ± 18.2	27.9 ± 1.9	0.2 ± 0.01
Salhouse Broad	Sediment	206.8 ± 8.4	32.1 ± 3.4	0.2 ± 0.04
Ranworth Broad	Sediment	302.7 ± 23.9	38.5 ± 3.5	0.1 ± 0.01
Malthouse Broad	Sediment	1368.8 ± 68.0	78 ± 8.5	0.05 ± 0.001

Table 3.5. (Previous page) Mean (±SE) exposure concentration (sediment), organismal concentration, total body burden and resulting BSAF (mean ± SE of n = 5 samples) in *Anodonta* spp. from contaminated broads.
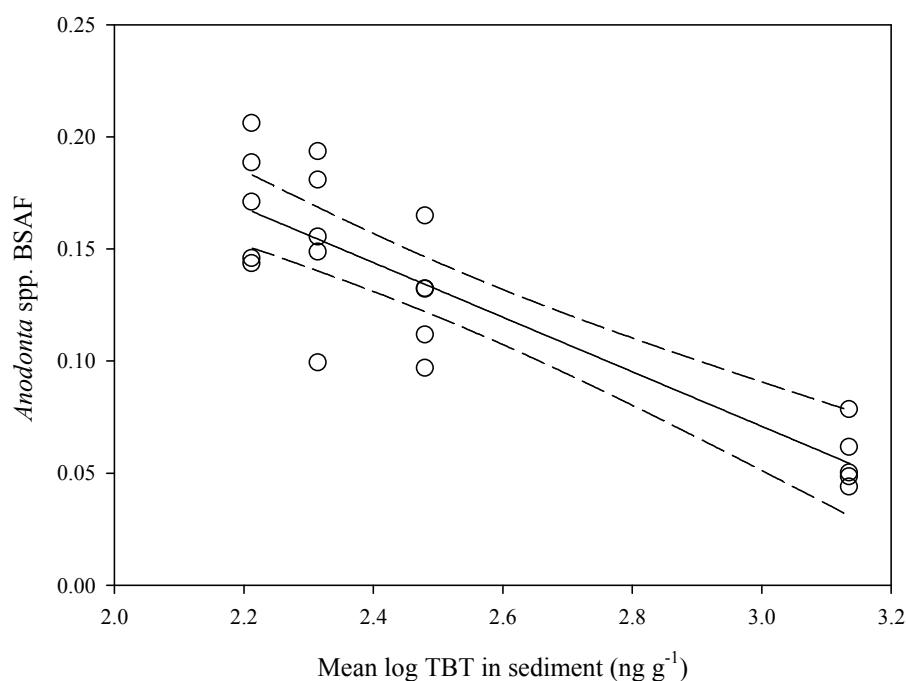
Figure 3.13 Relationship between mean log transformed sediment TBT concentrations (ng g^{-1}) and *Anodonta* spp. BSAF from contaminated broads. The regression line (± 95% CI) was fitted using the equation y = 0.436x - 0.217; r^2 = 0.76, p < 0.05

3.4.5   Isotopic mixing models

In order to assess the potential importance of diet as a contaminant uptake route in *Anodonta* spp. and *C.plumosus* isotope mixing models (Isoerror04; Phillips & Gregg 2001) were

applied to assess the relative importance of FPOM and surficial sediment as dietary components in mussels and chironomids.

In Malthouse Broad the contribution of the FPOM end member to *Anodonta* spp. δ^{13}C was 100%. The contribution of the sediment end member for chironomids was 94% and FPOM 6%. At all other sites contribution of one end member source exceeded the limits of the model. In *Anodonta* spp., this was always the contribution from the FPOM δ^{13}C end member. In contrast, sediment δ^{13}C end member frequently exceeded the limits of the model in chironomids. Data from Malthouse Broad are given in Table. 3.6.

	Mean δ^{13}C cons.	Mean δ^{13}C FPOM	Mean δ^{13}C sed.	% FPOM	% Sed
Anodonta spp.	-32.6 ‰ ± 0.3	-32.6 ‰ ± 3.1	-34.5 ‰ ± 0.9	100	0
C. plumosus	-34.4‰ ± 0.3	- 32.6 ‰ ± 3.1	- 34.5 ‰ ± 0.9	6	94

Table 3.6 Annual mean δ^{13}C of consumers, FPOM, and sediments from Malthouse Broad used in mixing models to calculate source contributions to consumers. % denotes the calculated model contribution of the relevant end member to the consumer. A trophic fractionation factor of 0.4‰ was added to end members (McCutchan et al., 2003).

3.5   Organotin contamination in fish

3.5.1   Tracing the route of contamination: Do fish with divergent feeding ecology have different butyltin burdens?

So far, I have been working on the assumption that the sediment legacy of organotins is the principal route for contamination of the food web. If that is correct, then components of the food web which have little direct (i.e. contact via residence in the sediment) or indirect (i.e. trophic transfer) connection to the sediment should have comparatively low organotin body burdens compared to those organisms are reliant upon the sediments. I sought to test this hypothesis by comparing bream (a classic benthic invertivore) with rudd (more typically associated with surface / mid-water feeding) from Malthouse and Ranworth. To assess the isotopic niche of each, I could calculate a total area (the metric TA I use in Chapter 4 for communities rather than populations) from a convex hull drawn around the most extreme data points on an isotope bi-plot. However, for a small sample size and limited temporal sampling, the convex hull area is heavily influenced by sample size (Jackson et al. 2010a). Consequently, I used a new metric developed in house at QMUL, standard ellipse areas (SEAs), as a measure of population isotopic niche area which is robust to variation in sample size (Jackson et al. 2010b).

The fish lengths, weights and isotopic compositions are presented in Table 3.7. At Malthouse Broad, rudd fork length ranged from 103 to 188 mm and weight ranged from 16.5 to 135.7 g while the bream analysed were longer (400 to 495 mm) and heavier (1195.1 to 1893.3 g). Rudd muscle $\delta^{13}C$ from Malthouse spanned 3‰ (-30.7 to -27.7‰) while those of bream spanned <1‰ (-30.1 to -29.2‰) and thus, isotopically distinct (ANOVA, $F_{1, 22} = 6.84$, $P <$ 0.05). In contrast, muscle $\delta^{15}N$ values were isotopically indistinct between species with rudd $\delta^{15}N$ spanning ~4‰ (15.1 to 19.4‰) compared to <2‰ in bream (Table 3.7). Calculation of Standard Ellipse Areas (SEAs) revealed no overlap in the trophic niche of bream and rudd

from Malthouse (Fig 3.14). In terms of SEA size, rudd (4.33) occupied a greater area than bream (0.262) suggesting a wider trophic niche.

The $\delta^{13}C$ values of rudd and bream from Ranworth Broad were similar, both spanning ~2‰ (Table 3.7; rudd, -29.6 to -27.7‰; bream, -30.1 to -27.7‰). However, muscle $\delta^{15}N$ of rudd was lower than that of bream (Table 3.7) and with a greater span (~2‰; 16.5 to 18.6‰). Bream were remarkably consistent in terms of muscle $\delta^{15}N$ spanning only 0.4‰ (18.3 to 18.7‰). There was significant isotopic distinction in terms of muscle $\delta^{15}N$ values between bream and rudd from Ranworth (Kruskall-Wallis χ^2 =4.81, $p < 0.05$). Calculation of SEAs for rudd and bream from Ranworth resulted in some overlap in trophic niches. Again, as in Malthouse, the SEA of bream (0.548) was smaller than that of rudd (1.63).

Site	n	$\delta^{13}C$	$\delta^{15}N$	Length (mm)	Weight (g)
Malthouse Broad					
Rudd	15	-29 ± 0.2*	16.9 ± 0.4	138 ± 1.8	58 ± 3.8
Bream	8	-30 ± 0.2*	17.9 ± 0.2	445 ± 14.6	1624 ± 121.3
Ranworth Broad					
Rudd	5	-28.4± 0.2	17.6 ± 0.4*	139 ± 8	53.4 ± 10
Bream	5	-29.1± 0.4	18.6 ± 0.1*	415 ± 14.6	1232 ± 135

Table 3.7 Mean (±SE) $\delta^{13}C$, $\delta^{15}N$, length, and weight of rudd and bream, from Malthouse and Ranworth Broads. *indicates significant inter-species difference between relevant variable at same site.
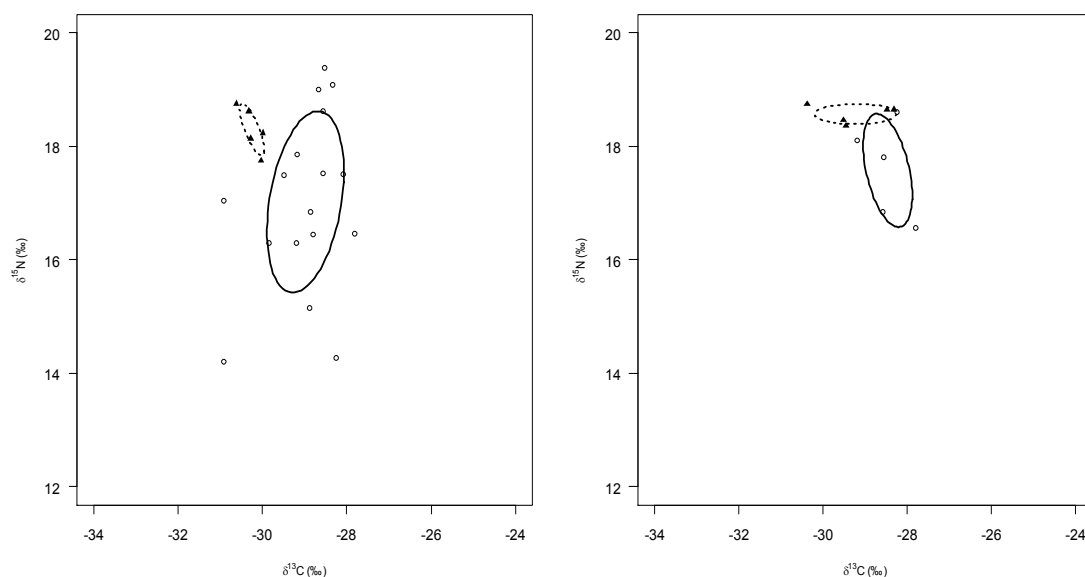
Figure 3.14 Standard ellipse areas for bream (filled triangles) and rudd (open circles) from Malthouse (left panel) and Ranworth Broad (right panel).

In order to check for no effect of size on hepatic butyltin concentrations in rudd, 15 individuals captured from Malthouse Broad were broken down into three size groups and respective liver samples analysed separately. In total five samples were analysed; 3 single livers from 3 individuals (mean FL, 180.7mm ± 4.0; mean ww, 120.6 g ± 8.3); 1 composite sample from four medium rudd (mean FL, 126 mm ± 3.7; mean ww, 35 g ± 3.8), and another composite sample from the eight smallest individuals (mean FL: 106.7mm ± 1.4; mean ww, 18.4 ± 1.4). No Phenyltins were detected in any sample, and only a single liver from large rudd showed a quantifiable concentration of TBT (13ng g^{-1}). Regression analyses revealed no significant relationship between rudd weight and hepatic butyltin concentrations ($p > 0.05$).

In contrast to rudd, all five bream livers analysed for butyltins from Malthouse broad yielded quantifiable concentrations of MBT, DBT, and TBT (Figure 3.15 a). Concentrations of TBT were consistently an order of magnitude greater than those of both MBT and DBT, and ranged from 125 to 366 ng g^{-1} with a mean value of 122.2 ± 44.5 (Figure 3.15 a). Hepatic concentrations of DBT were marginally greater than MBT 54.4 ng g^{-1} compared to 38.8 ng g^{-1} (Figure 3.15 a). Variability was reduced in MBT and values ranged from 25 to 51 ng g^{-1} compared to 29 to 78ng g^{-1} in DBT. Concentrations of TBT were significantly greater than those of metabolites (ANOVA, $F_{2,14}$= 23.02, p <0.05). Further statistical analysis of rudd and bream TBT values showed a significant difference between inter-specific hepatic concentrations (Kruskall –Wallis χ^2 =7.26, DF=1, p < 0.05).

As with rudd from Malthouse, no phenyltins were detected in any Ranworth sample and only a single liver showed quantifiable concentrations of TBT (23 ng g^{-1}). This was in contrast to bream livers that, although concentrations were generally lower, showed a similar pattern of contamination to those observed in bream from Malthouse; following the pattern TBT > DBT > MBT (Figure 3.15 b). TBT contamination was apparent in all samples, though detection of metabolites was less consistent than Malthouse; DBT was < LOD in one liver sample, and MBT < LOD in a further two. Concentrations of TBT ranged from 69-138 ng g^{-1} (mean; 105.8 ± 12 ng g^{-1}); DBT, 31-45 ng g^{-1} (mean; 27.2 ± 8.2 ng g^{-1}) and MBT, 29-43 ng g^{-1} (mean; 22.6 ± 9.3 ng g^{-1}). As with samples from Malthouse log transformed TBT concentrations were significantly greater than metabolites (ANOVA, $F_{2, 11}$ = 18.25, p <0.05) and there was a significant difference in hepatic concentrations of TBT between rudd and bream (Kruskall – Wallis, χ^2 =7.26, DF=1, p< 0.05). Regression analyses of concentrations of

TBT and DBT in bream liver revealed no significant relationship in samples from either Malthouse or Ranworth broads ($p > 0.05$).
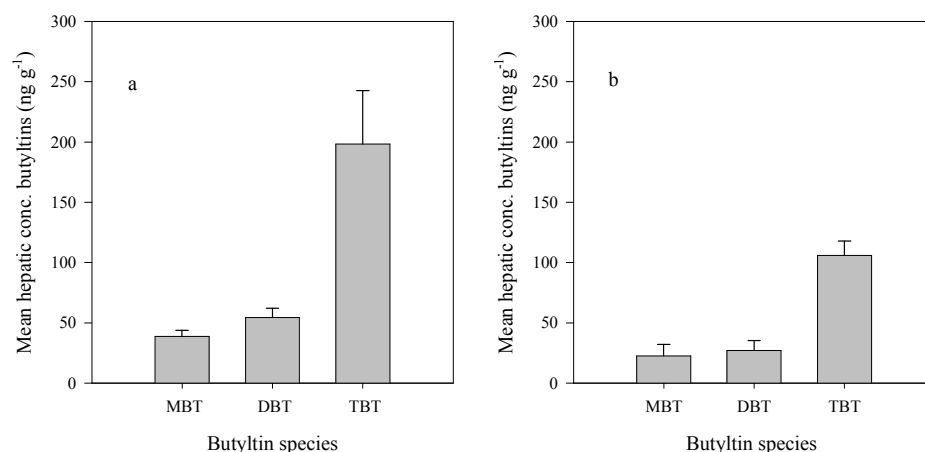
Figure 3.15 Mean hepatic concentrations of butyltin species in bream captured from a, Malthouse Broad, and b, Ranworth Broad. Error bars are ± SE.

3.6.2   Can analyses of key body parts give an indication of contaminant uptake route?

Hepatic organotin concentrations can be viewed as an amalgam of the contaminant loading to the body irrespective of uptake route as the liver is the metabolic processing centre of the body. This was demonstrated in the previous section where high concentrations of TBT were detected in the livers of bream. I hypothesised that separate analysis of gill filaments and intestinal tract wall might provide further evidence of uptake route from the water-column (via respiration) and from the diet, respectively.

In addition to the 10 bream livers analysed in the previous section (5 from Malthouse, 5 from Ranworth) a further 5 livers from bream captured in Wroxham Broad (mean FL: 361 ± 26.1 mm; mean weight: 849.8 ± 197.7 g), plus the gill filaments and intestinal tracts from all 15 individuals were analysed for butyltin contamination (Table 3.8). Organotin analysis of muscle samples from Wroxham bream was not possible due to cost restrictions, and gill, intestine and livers were analysed for DBT and TBT only.

Overall the highest concentrations of butyltins were represented by TBT in all body parts (Table 3.8) and ranged from 17 to 684 ng g^{-1}; where present, the concentrations of metabolites were always lower than those of the parent compound. In all bream, concentrations of butyltins were greatest in the liver, and hepatic TBT concentrations were typically ~4-5 greater than in muscle, gills, and intestine (Table 3.8) Hepatic concentrations of TBT and DBT in Wroxham bream were present in similar proportions to Ranworth and Malthouse bream, where concentrations of DBT were ~60-70% < than those of TBT (Table 3.8). Despite this, there was no significant difference between respective concentrations. Concentrations of TBT were similar in gills and intestines at all three sites and consistently an order of magnitude lower than those found in the liver (Table 3.8). No DBT was detected in the gills or intestine from Wroxham bream and was not detected in muscle tissue in bream from Wroxham and Ranworth. Low concentrations of DBT were detected in the gills of Malthouse bream only (Table 3.8).

No DBT was detected in muscle tissue of bream from either Ranworth or Malthouse Broads. Low concentrations of MBT were quantified in muscle tissue of bream from both sites (Table 3.8) and were marginally greater in samples from Ranworth. TBT contamination of muscle

tissue was apparent at both sites although concentrations were ~ 50 % higher in Malthouse bream.

TBT concentrations in gill filament were lowest in samples from Ranworth Broad, where only two samples showed quantifiable concentrations of the parent compound. Mean concentrations of TBT were similar in gills of bream from Wroxham and Malthouse Broads, despite only two Wroxham samples containing quantifiable concentrations. Conversely, all gill samples from Malthouse contained TBT, and ranged from 20 to 35 ng g^{-1} (Table 3.8). DBT was detected at low concentrations (< 5ng g^{-1}) in gills from two Malthouse bream only. Low concentrations of MBT were detected in bream gill from Ranworth and Malthouse bream. The contamination profile in intestinal tract was similar that of gill, thus TBT concentrations decreased in the order Wroxham > Malthouse > Ranworth (Table 3.8). No DBT was detected in any sample and concentrations of MBT in Malthouse and Ranworth samples were similar to those in gill.

Concentrations of TBT and metabolites were greatest in the liver in all bream from all sites. As with gill and intestines, hepatic concentrations of TBT in bream from Wroxham were greater than those from Ranworth and Malthouse, ranging from 94 to 684 ng g^{-1} (Table 3.8). I used total butyltin ($\sum$MBT, DBT, TBT) concentration in each body part to assess the potential importance of different uptake routes. Concentrations of total butyltins in muscle tissue (Ranworth and Malthouse only), gill filament and intestines were statistically indistinct; both within and between sites. This was in contrast to hepatic concentrations of total butyltins that were significantly greater compared to gills and intestines (Kruskall-

Wallis $\chi^2 = 28.7$, DF=2 p <0.05). Additional analyses of hepatic TBT concentrations revealed no statistical difference between sites (p> 0.05).

Site	□	Muscle MBT	DBT	TBT	Gill MBT	DBT	TBT
Wroxham		n/a	n/a	n/a	n/a	nd	34.4±25.4
Ranworth		6.2±1.6	nd	12.8±3.3	12.2±5.0	nd	14.4±9.0
Malthouse		3.4±2.0	nd	19.0±5.8	5.1±2.0	2.5±1.5	30.2±2.7
		Gut MBT	DBT	TBT	Liver MBT	DBT	TBT
Wroxham		n/a	nd	42.8±24.1	n/a	83±30.2	276±105.2
Ranworth		12±5.0	nd	12.4±9.0	22.6±9.5	27.2±8.2	105.8±11.9
Malthouse		4.6±4.6	nd	38.8±16.0	38.8±5.0	54.4±7.8	198.2±44

Table 3.8 Mean ± SE (ng g^{-1}) values of butyltin species in gill, intestine, and liver of bream, from Wroxham, Ranworth, and Malthouse Broads. n/a- metabolite not analysed for; nd − metabolite not detected in any sample.

3.6 Consumer butyltin burden with respect to trophic position as measured by δ^{15}N

As I outlined in my review in Chapter 1, there is still uncertainty concerning the trophodynamic behaviour of butyltin compounds, with different studies presenting conflicting results on their biomagnification potential. However, much of this uncertainty stems from the methods that are used to describe biomagnification of butyltin compounds such as the

delegation of discrete trophic levels from assumed feeding links, and/or over/under-representation of certain tissues or body parts (see Chapter 1). In this section I will use estimated body burdens of consumers derived from representative body parts and test for a relationship with respective measures of $\delta^{15}N$. Muscle tissue, intestinal tract, liver, and gill filament were analysed separately for butyltins as previously. The resulting concentrations were then multiplied by the fraction that each respective organ or tissue represented of the total wet weight of the consumer, these values were then summed to give a weighted total body butyltin burden concentration. In order to represent a conservative an estimate as possible it was assumed that these tissues would be the most important in terms of butyltin storage. In consumers (or body parts thereof) where butyltins were below the limit of detection 50 % of the LOD was assumed for statistical purposes (Murai et al., 2008).

Measurements of $\delta^{15}N$ allowed for the determination of the relative trophic positions of consumers in the four studied food webs (Fig. 3.17 a-d), and these were relatively consistent among consumers between lakes. Primary consumers were consistently the most $\delta^{15}N$ depleted food web members. In Wroxham and Salhouse pike were the top predator although this was not reflected in their butyltin body burdens (Fig 3.17 a-b). In Malthouse and Ranworth pike were substituted by perch as the most $\delta^{15}N$ enriched consumer (Fig 3.17 c-d). Either roach or *Anodonta* spp. represented the consumer species with the highest butyltin burden in all food webs. In general there was negative trend of total butyltin burden with increasing consumer $\delta^{15}N$, and regression analyses revealed no significant relationship between these data.
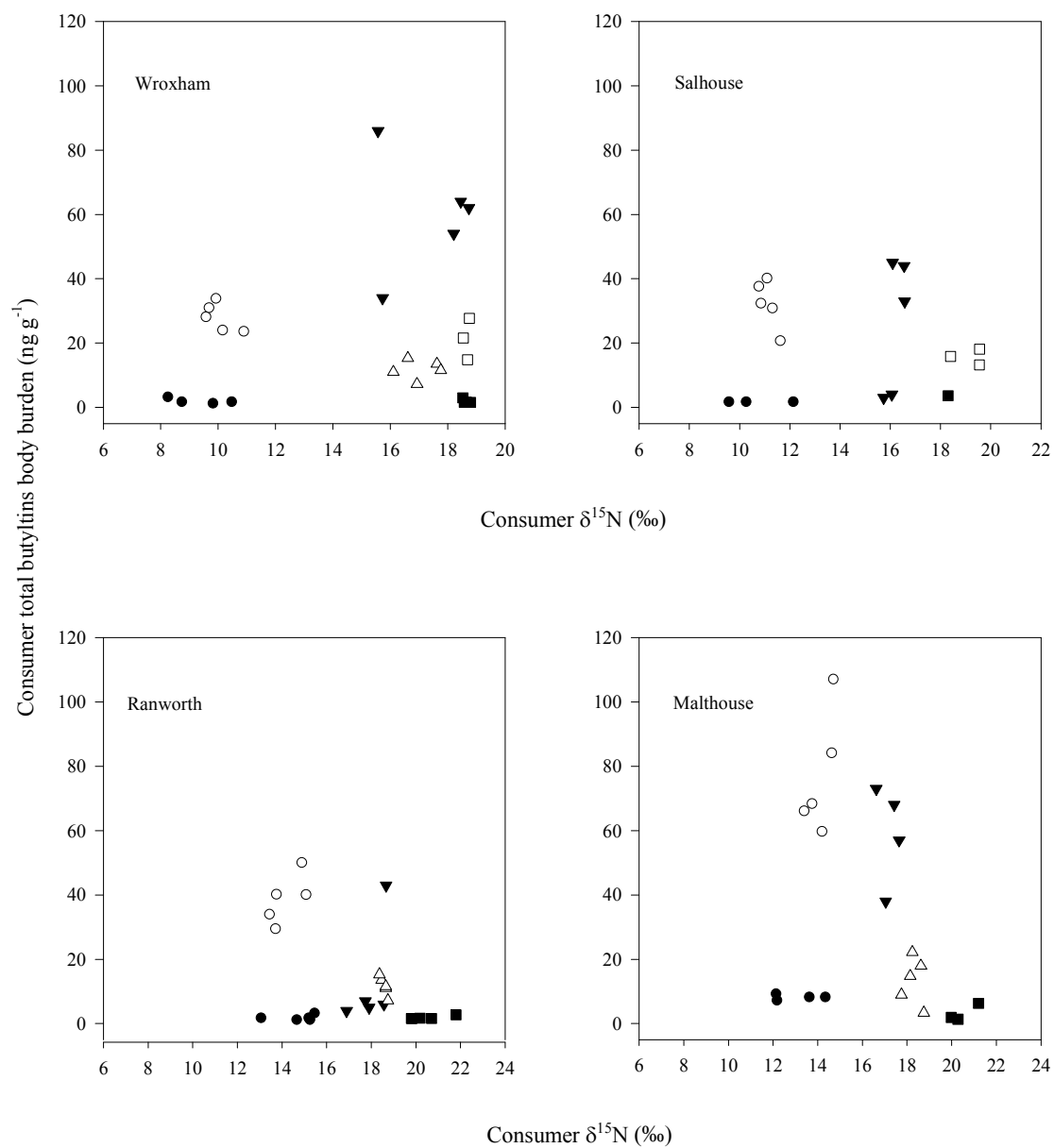
Figure 3.16 a-d. Mean consumer organotins burden as a function of trophic position measured as δ^{15}N. Open circles: *Anodonta*; solid circles: chironomids; up triangles: bream; down triangles: roach; solid squares: perch; open squares: pike

3.7   Discussion

The existence of the TBT gradient with increasing boating activity, as documented by Hoare (2007) was confirmed by the current results. However, results in this study suggested that sediment TOC may also explain some of the variability between sites and it appears TBT concentration in sediments of connected broads are controlled by a combination of organic carbon and boating activity. Regarding the longevity of the contaminant legacy my results suggest degradation times may be slow. Certainly the quantification of DBT in addition to TBT in sediments of the broads indicates some degradation of the contaminant has occurred. However, the lack of change between the ratio of TBT:DBT between this and the other comparable data set (Hoare, 2007) indicates degradation is negligible over a relatively long time span. This finding supports the notion of persistence of TBT in anoxic sediments (Dowson et al. 2006). The similarity of ratios between studies could also be seen as evidence that fresh inputs of TBT are no longer entering the system (i.e. illegal use). However, further investigation is required as TBT:DBT ratios were higher than might be expected in some boatyard samples.

The pH and anion concentration of the sediments in the current study suggest that the neutral species TBTOH should be dominant in the broads (e.g. Burton 2004., Arnold et al., 1997), and this may have important implications for the wider food web as TBTOH is more bio-available than the cationic TBT^+ species (Looser et al, 1998). The dominance of the neutral species combined with the high organic carbon content of the sediment suggests that in the broads hydrophobic partitioning to organic matter in the sediments may be the dominant sorption mechanism.

Attempts at direct measures of field pore water concentrations revealed concentrations < LOD and so direct measurement of pore water concentration was not possible. Instead, the intention was to calculate theoretical pore water concentrations of TBT using experimentally determined K_d values. However, the results of this experiment were not as expected as values were far lower than those reported in the literature. Although TBT did not sorb to container walls, a mass balance was not possible as TBT recovered from experimental pore water systems was below the limit of detection. This suggests the influence of other factors. For example, Arnold et al., (1998) demonstrated that up to 70% of TBT was sorbed to DOC (in the form of humic acids) under similar anion and pH conditions to those found in the broads. Similarly, Looser et al. (2000), demonstrated that the presence of DOC (in the form of humic acids) significantly reduced the bioavailability of TBTOH to chironomids (*Chironomus riparius*). Given the organic rich character of broadland sediments then binding of TBTOH to DOC in pore water could represent an important sorption mechanism in addition to that which occurs to TOC in sediments. This could go some way to explaining why the organic carbon partition coefficients generated from experiments were around 50% less than the lower limit of those reported within the literature (e.g. log K_{oc} TBT of $\sim$ 4- 5.0; Meador et al., 2002).

These observations are likely to be important in the context of TBT supply to the wider food web. For example, low TBT concentrations observed in benthic invertebrates (chironomids and swan mussel) compared to sediments are most likely to be related to the influence of organic carbon. Thus, although the more bioavailable TBTOH is the dominant species in the broads (*sensu* Arnold et al., 2007), if it is strongly sorped to TOC in sediment, and/or DIC in

pore water then bioavailability will be reduced. The low BSAFs calculated for mussels in this study support this notion and suggest that bioaccumulation does not occur from contact with (or ingestion of) contaminated sediments (e.g. BSAF<1).

If sediments are not a direct source of contamination then the remaining uptake routes for benthic consumers are diet and passive uptake via interstitial pore water. It seems improbable that diet is the principal uptake route. For example, in Malthouse Broad, my isotopic mixing model outputs suggested dietary reliance on FPOM in mussels and sediments in chironomids (e.g. deposit feeding), and although outputs exceeded the limits of the model for both organisms at other sites they were directionally indicative of similar feeding behaviour. Organotin analyses of FPOM revealed no evidence of contamination in this food web component. Therefore uptake through diet can effectively be ruled out in mussels. If neither sediment contact nor diet contribute significantly to TBT burden then passive uptake via freely dissolved TBT pore water is the most probable uptake mechanism. However, as both field and experimental measures of pore water concentrations were below the organotin method limit of detection, it was not possible to calculate a bioconcentration factor (BCF) to assess the contribution of this uptake route; and this notion remains speculative. Despite this it is possible for the purpose of discussion to calculate a theoretical BCF using 50% of the LOD for the organotin water extraction method (5ng L^{-1}) and the mean TBT concentration of mussels from Malthouse Broad. The bioconcentration factor is calculated in the same manner as the BSAF and where the BCF is > 1 the compound is bioconcentrated. The BCF is given by the following equation:

BCF = C_i / C_{aq}

Where C_i is the concentration in the organism and C_{aq} the concentration in the aqueous medium . Inserting the mean TBT concentration in *Anodonta* spp. from Malthouse (76.9 ng g^{-1}) and 50% of the LOD (2.5 ng g^{-1}) of the organotin in water extraction method gives:

$$76.9/2.5 = 30.76$$

In this hypothetical case, TBT is bioconcentrated in *Anodonta* spp. Given that the real concentration in pore water is likely to be less than, or at most equal to, this assumed value then bioconcentration of TBT could occur in mussels in the Norfolk Broads. Despite consistent TBT contamination in all 55 mussel samples analysed, the metabolites were almost entirely absent suggesting a poor metabolic capacity for TBT in this species. Unionid mussels such as *Anodonta* are long lived (Chojnacki et al., 2007) and so these results could be a reflection of a reduced metabolic capacity for TBT due to slow growth / tissue turnover times.

Excepting juveniles, the large body size of *Anodonta* spp. may limit their potential to act as a source of contamination to higher trophic levels within aquatic food webs. Individuals in this study had large shell sizes (up to 140mm), and for this reason would be unavailable to gape-limited predators such as benthivorous fish. Although not available to fish, coots (*Fulica atra*) were frequently seen taking large mussels to the shore, breaking the shells and consuming the soft body tissues (pers. obs.). Opportunistic predators such as otter (*Lutra lutra*) may also feed on mussels where available. Although further research is required to confirm such trophic linkages, TBT may cross ecosystem boundaries via such routes (see Chapter 5).

Assuming deposit feeding as the dominant feeding mode in chironomids from the stable isotope data, then it could be expected that body burdens would be high as a result of ingestion of contaminated sediments. However, compared to mussels chironomid butyltin burdens were low in Malthouse broad and absent in chironomid samples from other broads (excepting Ranworth n=1). Furthermore, the consistent detection of DBT as opposed to TBT in Malthouse suggests some metabolic capacity for degradation of the parent compound. Other research supports this notion. For example, Looser et al. (1998) reported short half lives (16-22h) of TBT in *Chironomus riparius* tissues in experimental systems, indicating the presence of a rapid metabolism for TBT in this species. The absence of butyltins in chironomids sampled from other broads suggests a threshold exposure concentration beyond which the rate of uptake exceeds or is equal to that of elimination (see Looser et al., 2000). Although sediment TBT concentrations were high in broads such as Malthouse it should be considered that the fraction of this concentration ingested with sediments (by chironomids) would be small. Furthermore, the soft bodied, tubiculous nature of chironomids means passive uptake is sure to occur. Thus, a relatively low exposure concentration coupled with an efficient TBT metabolism (assuming a similar metabolism in *C. plumosus*) could result in the maintenance of a low TBT body burden. In turn this may limit the potential of chironomids to act as source of TBT to the contamination to the higher food web.

I hypothesised that fish with divergent feeding ecologies (as indicated by stable isotopes) would exhibit different butyltin burdens, as a result of utilisation of different feeding modes/habitat utilisation. Firstly, it was possible demonstrate that rudd and bream were

isotopically distinct. The increased standard ellipse area of rudd from both Malthouse and Ranworth broads indicated occupation of a greater trophic niche than bream. This is supported by other studies that report a wide range of prey items in the gut contents of rudd. For example, Jones and Waldron (2003) recorded the occurrence of 29 different prey species in the gut of a single rudd in another shallow Norfolk lake, and Rawcliffe et al (2010) reported the occurrence of both terrestrial and aquatic prey types in the stomach of rudd from Felbrigg Hall Lake (Norfolk). In the current study (see Chapter 4) rudd from Cockshoot Broad were extremely ^{13}C-enriched compared to other fish species, which could be seen as evidence of a diet partially subsidised by aquatic prey. The occurrence of terrestrial prey in the diet of rudd is not surprising as this species has specialised mouthparts that facilitate the capture of prey trapped on the water surface. Accordingly, I recorded only two occurrences of TBT in the livers of rudd. Almost certainly rudd feed in the benthos of the broads at times, although it is likely that this is more typically in the littoral zone where prey diversity is higher. Thus the more generalist feeding strategy of rudd appears to mitigate their exposure to TBT.

In contrast to rudd, adult bream can be regarded as a classic benthic invertivore, and are physically adapted to foraging for buried prey deep in sediments. This method of feeding necessitates sorting of small bodied prey from sediments in the mouth. Indeed, bream have been reported to repeatedly sample sediments in order to locate small bodied prey such as chironomids (Lammens and Hoogenboezem, 1991). Furthermore, larger bream are more efficient at this type of feeding than smaller conspecifics (Lammens and Hoogenboezem, 1991; Harrod & Grey 2006). The more specialised approach to feeding of bream compared to

rudd is almost certainly the cause of the consistently higher TBT contamination in bream livers compared to rudd observed in this study. For example ingestion of sediments would certainly occur when bream are foraging, and thus TBT burden may result from a combination of consumption of contaminated prey and feeding mode.

The benthic feeding of bream may also influence the pattern of TBT and metabolites. A number of studies have highlighted the importance of the liver in the metabolism of TBT to the less toxic di- and mono- substituted daughter compounds (Martin et al., 1989; Bailey et al., 1997; Morcillo et al., 1997; Viglino et al., 2006). In a general sense this pattern was confirmed by the presence of high concentrations of both TBT and metabolites in the liver (Figure 3.16). However, butyltin profiles are commonly characterised by increasing concentrations of the metabolites as debutylation of the parent compounds occurs (Morcillo et al, 1997). This was not the case in bream from either Ranworth or Malthouse where TBT concentrations were significantly greater than those of both DBT and MBT. This suggests that rate of uptake may exceed that of elimination and this could expose bream to an increased risk of deleterious health effects when compared to other fish consumers (Stab et al. 1996).

In order to confirm that diet was the principal uptake in bream TBT analysis was conducted on gill filament and intestinal tract, with the addition of bream from Wroxham broad. This was based on the premise that if diet was the dominant uptake route then concentrations in the gut tract would be higher than those of the gill, the latter representing passive uptake from water. A similar method was used by Morcillo et al. (1997) who found that concentrations

were consistently greater in the gills than both digestive tract and liver in two species of marine teleosts (grey mullet, *Liza aurata* and red mullet *Mullus barbatus*) as a result of uptake of TBT via the water column. Similarly, Viglino et al. (2006) suggested the occurrence of high butyltin burdens in the intestinal tracts and livers of eel pout (*Licodes vahlii*), Acadian redfish (*Sebastus faciatus*), and shorthorn sculpin (*Myoxocephalus scorpius*) was indicative of the importance of diet over other assimilation routes. In contrast to these studies and despite contamination in both organs in bream from the broads I found no significant pattern of butyltin distribution between these two organs, suggesting the method may be ineffective in determining the degree of uptake from discrete sources in some circumstances. However, hepatic concentrations were consistently and significantly greater in the liver than all other all organs, further confirming the importance of this organ as a site of butyltin degradation. The tendency of bream to bury the head in sediments when feeding (Lammens and Hoogenboezem, 1991) may have complicated this analysis to some degree as concentrations in the gill could be accumulated through contact with contaminated pore-water or sediments rather than passive uptake via the water column.

A key topic in organotin research is compound specific trophodynamic behaviour within food webs. Specifically, there is some debate as to whether the butyltin species (TBT, DBT, MBT) have the ability to biomagnify in food webs (Strand and Jacobsen, 2005; Murai et al., 2008). Using the range of consumers that were sampled within the current study allowed me to examine the potential for biomagnification across the food webs of four contaminated broads. I hypothesised that there would be a positive relationship between consumer $\delta^{15}N$ and respective total butyltin burden, and if significant this could indicate the occurrence of

biomagnification (*sensu* Kidd et al., 1995). This notion was not supported by my results. In fact generally total butyltin burden decreased in response to increasing consumer $\delta^{15}N$, and regression analyses of these data revealed no significant relationship in any of the four studied food webs. Such a pattern would appear to suggest there is no clear trend of TBT accumulation with relation to the trophic position of consumers (Hu et al, 2006). With reference to my results some interesting features were apparent. For example, bream were consistently contaminated within the three food webs in which they were analysed. Although as top predator butyltins were not biomagnified in pike they were nonetheless consistently butyltin contaminated. Given that these two species have specialised trophic niches, contamination could be a result of this specialisation. I previously suggested that butyltin contamination in bream could stem from their benthic feeding habits. In pike contamination could come from feeding on contaminated prey such as roach, although feeding on potentially TBT contaminated predatory invertebrates cannot be ruled out (see Beaudoin et al., 1999). Roach often displayed relatively high butyltin burdens within these food webs, although this was less consistent than for pike and bream which may be a reflection of their more generalist feeding strategy (Syvaranta, et al., 2010).

My observations are in good agreement with the few studies that have attempted to track patterns of biomagnification using stable isotope techniques. Thus, Hu et al., (2006) observed a generally decreasing (but non-significant) relationship in the $\delta^{15}N$ of food web components and their corresponding TBT burden in a marine food web in China. Similar to the food webs of the Norfolk Broads there was no evidence of TBT biomagnification between trophic levels. A similar Japanese study (Murai et al., 2008) also detected no biomagnification of

TBT in response to consumer $\delta^{15}N$. In contrast to TBT, both these studies found significant biomagnification of the phenyltin species TPhT. However, this compound was not detected in the current study which suggests it is of little concern in the context of broadland ecosystem health.

A number of other studies that do not use isotope techniques also document a pattern of TBT burden in food web consumers that is similar to those observed currently in TBT contaminated Broadland food webs. In another Japanese food web, for example, butyltin contamination was evident on multiple trophic levels, yet no significant differences were recorded between them (Takahashi et al., 1999). Stab et al. (1996) recorded TBT concentrations in species such as roach and bream in a shallow freshwater lake in the Netherlands, revealing that these were in excess of putative predators such as pike and perch. Where included in analyses (all except Salhouse, where organotin analyses were insufficient to calculate total body burden) this trend was mirrored in the same suite of species in the broads. Again, in this Dutch study no biomagnification was reported, further confirming that biomagnification of TBT is unlikely to occur in most shallow lake food web scenarios.

In contrast to results of the current study, and those previously detailed in Asia and the Netherlands, a study in Denmark reported the apparent biomagnification of TBT in a marine food web (Strand and Jacobsen, 2005). In order to assess the biomagnification of butyltins in this food webs Strand and Jacobsen, (2005) used biomagnification factors (BMFs) that can be calculated thus:

BMF= $C_{w.b}$ (predator)/ $C_{w.b}$ (prey)

Where $C_{w.b}$ is the concentration in the whole body

The majority of concentration data in this Danish study was derived from analysis of consumer liver. As discussed previously, hepatic concentrations of butyltins are frequently greater than other tissues as it is an important site for the metabolic degradation of these compounds (this study, Martin et al., 1989; Bailey et al., 1997; Morcillo et al., 1997; Viglino et al., 2006). Therefore, if direct comparisons are made between concentrations in the liver and those of the whole body in prey then this will create a bias that will likely lead to the calculation of an erroneous BMF.

In order to attempt to overcome this potential bias Strand & Jacobsen (2005) suggested the use of two parameters *L* and *P* to estimate whole body concentrations in larger predators. *L* being the ratio between hepatic and total weights as measured during dissection. *P* is the ratio between the liver and total body residues of butyltins based on related species assuming the same distribution between liver and total body burden. There are a number of problems with this approach. For example the *P* value used by Strand & Jacobsen (2005) was derived experimentally through diet only. The validity of comparisons between field and experimental concentration measures is questionable. For example in the 'wild' organism the hepatic concentration will potentially represent an amalgam of all assimilation pathways. Contrastingly, hepatic concentrations of an organism raised on a contaminated diet represent the uptake of the contaminant from this pathway only.

Another caveat of this method is the assumption that the distribution of butyltins is the same in the rest of the body as the liver. As previously discussed, liver concentrations are consistently greater than other organs (e.g. Stab et al, 1996, Bailey et al, 1997, Morcillo et al 1997, Takahashi et al 1999, Harino et al, 2002, Strand & Jacobsen, 2005, Viglino et al, 2006). Thus, inappropriate use of hepatic concentrations in the calculation of BMFs could misrepresent the occurrence of biomagnification. Furthermore, in most cases the 'trophic level' of consumers is assumed rather than measured empirically which introduces another potential margin of error (see Chapter 1, section 1.3.1).

In this study I sought to overcome the caveats of methods such BMFs by separate organotin analyses of body parts that when combined, would represent a more realistic overview of body burden (see section 3.6). In addition, combining these measures with empirical measures of $\delta^{15}N$ of consumers it was possible to plot the time integrated trophic position of food web consumers without the need for assuming discrete trophic levels, as is required with traditional calculations of BMFs.

Chapter 4

Changes in the Stable Isotopic

Architecture of Food Webs across an

Organotin Contaminant Gradient

4.1   Introduction

The application of stable isotope ratios (most commonly those of $\delta^{13}C$ and $\delta^{15}N$) has enabled ecologists to investigate various aspects of food web structure, nutrient flux, and biogeochemical processes within aquatic ecosystems (see detail in Chapter 1). To briefly recap for aquatic ecosystems, primary producers that form the basal resource for food webs exhibit differing $\delta^{13}C$ values; typically, pelagic primary producers are more ^{13}C-depleted than periphyton and detritus of the littoral / benthic zones because of the original source/s of CO_2 and boundary layer kinetics. The tissue $\delta^{13}C$ values of consumers become heavier (i.e. ^{13}C-enriched) by approximately 1‰ relative to the dietary source (DeNiro & Epstein 1978) but the relatively conservative nature of the trophic transfer means that $\delta^{13}C$ can be a good indicator of the primary carbon source and allow for confident tracing of energy flow within food webs. Thus, with a knowledge of the $\delta^{13}C$ values typical for specific habitats and biogenic processes, it has been possible to, for example, quantify (using mixing models) the relative importance of pelagic and littoral production to consumers (Vander Zanden & Vadeboncoeur 2002); identify and assess the roles of autochthonous production and allochthonous subsidies in lake productivity (Grey et al 2001); illuminate novel energy transfer pathways within food webs (Jones et al 1999; Trimmer et al 2009); and trace energy flux across ecosystem boundaries (Kato et al., 2003; Paetzold et al., 2005).

Furthermore, predictable enrichment of ^{15}N in consumers relative to their diet (e.g. 3-5‰; see Minagawa & Wada 1984; Jardine et al, 2001; McCutchan et al 2003) allows for delineation of important 'vertical' features of food web structure, such as the trophic position of consumers (Vander Zanden & Rasmussen 1999; Post 2002) and food chain length (Vander

Zanden et al 1999), provided that there has been establishment of adequate trophic baselines as an 'anchor' (see Post, 2002) These baselines also allow for standardisation when comparing isotope values of consumers from different habitats or ecosystems (Cabana & Rasmussen 1996, Post 2002). Values of $\delta^{15}N$ can also be substituted into mixing models to estimate relative prey contribution to consumer production and in conjunction with stable carbon and sulphur or hydrogen isotopes, can increase the resolution of contributions from different sources (Phillips & Gregg, 2002).

Characterisation of energy sources and trophic transfer at scales from the individual to the ecosystem using stable isotope approaches has proven a powerful tool for ecotoxocologists. A review of the literature reveals a wealth of contaminant studies using SIA in both terrestrial and aquatic systems (Jardine et al. 2006; see Chapter 1 for further details and references therein). The majority typically use either $\delta^{13}C$ or $\delta^{15}N$ data in isolation to assess source and trophic transfer, respectively but much important and potentially very informative data is wasted in this manner. A recent development to capture and incorporate such information has been proposed by Layman et al. (2007 a, b). They set out a series of quantitative metrics (see Methods Section 4.2.3 for the full details of how the metrics are calculated and what each represents ecologically) that can be used to infer important 'community wide' aspects of trophic structure. The approach builds on techniques commonly used within the field of ecomorphology, where metrics are calculated based on two-dimensional representations of species morphological characteristics and used to assess trophic roles (e.g. niche differentiation) between specific taxonomic groups with similar morphologies. The approach of Layman et al (2007a) differs in that it extends beyond specific taxonomic groups as the

isotopic ratios of an organism are the culmination of all trophic pathways leading to it and, as such, provide one representation of its trophic niche. Thus, the traditional $\delta^{13}C$ - $\delta^{15}N$ bi–plot space becomes 'niche space' and metrics are calculated by measures of the relative distribution and distances between species within it.

The community metrics of Layman et al. (2007a) were criticised initially as being too simplistic by Hoeinghaus and Zueg (2007) but as Layman et al. responded, such 'tools' should not be treated as a panacea and like with any other tool, care should be taken in their application. A major criticism was that the $\delta^{13}C$ of a particular basal resource or consumer can vary substantially over time. Thus, if considerable temporal variation is evident in a given source (e.g. $\delta^{13}C$ ~ 8‰ in cyanobacteria and ~ 4‰ in microphytoplankton; Vuorio et al., 2006) then to some degree this will be reflected in the isotopic composition of consumer tissues. This is particularly likely in primary consumers with rapid tissue turnover that feed on an isotopically variable food source (e.g. selective phytoplankton feeding by zooplankton; Post, 2002). Thus, the isotopic position of a given consumer in a food web sampled only once in a season may not be representative of its actual time integrated position, and could exert a disproportionate influence on overall community metric calculations (Hoeinghaus and Zueg, 2007). Similarly, dietary switches or changes in diet throughout ontogeny could result in a similar skew in calculated metrics (e.g. zooplankton and chironomids changing in $\delta^{13}C$ by >20 ‰ over an annual cycle; Grey et al., 2004, Harrod & Grey 2006). The same applies of course to $\delta^{15}N$ of sources and consumers that may vary considerably with time (e.g. $\delta^{15}N$ 6‰ in microalgae; Vuorio et al, 2006) If such variability is unaccounted for then the resultant metrics (such as TA) could lead to erroneous inference regarding the trophic structure of a

given food web. However, these shortcomings could potentially be overcome by a sampling regime that is designed to take in to account temporal variability and therefore constrain any exaggerated influence on the calculated metrics.

The potential for these metrics to reveal changes in community structure (or niche dynamics) with respect to environmental gradients or stressors is of considerable interest in this study of organotins in shallow lake ecosystems. Of course, having sampled in the field only, I will only generate correlational data from which I can infer a relationship between community architecture and organotin as the stressor. The effect of organotins may well be confounded by boating 'activity' and other types of habitat alteration / destruction associated with boating. However, contaminants are known to initiate indirect effects (such as trophic cascades) on recipient food webs via reductions in the number of keystone or foundation species. Contaminant induced alterations in the abundance of such species can result in alterations of top down or bottom up food web processes that release more contaminant tolerant species from competition or grazing pressure (Fleeger et al, 2003).

There is some evidence to suggest that TBT may have initiated this type of indirect effect in the Norfolk Broads. Throughout the 1960-1970s, a rapid decline in ecosystem health was observed characterised by the virtually ubiquitous loss of submerged macrophytes, that gave way to phytoplankton dominance of the water column (e.g. a shift between alternative stable states, see Scheffer et al., 1993, 2001; Jones and Sayer, 2003). Palaeolimnological surveys conducted on two lakes in the Broads system (Wroxham and Hickling Broads) revealed that the rapid loss of macrophytes and subsequent dominance by phytoplankton was synchronous

with the first occurrence of TBT in the system (Sayer et al., 2006). In addition to reductions in macrophyte biomass and species diversity, similar simultaneous changes in the community structure of both grazing molluscs and zooplankton were observed in the palaeo-record. This was typified by a shift to communities dominated by smaller species with reduced grazing efficiency (see Brooks and Dodson, 1963; Dodson, 1974); which could have further exaggerated conditions of phytoplankton dominance.

Thus, TBT toxicity may have initiated indirect effects similar to a trophic cascade, significantly reducing numbers of key grazing invertebrates, thus releasing phytoplankton and periphytic algae from grazing pressure and ultimately facilitating the exclusion of macrophytes. A small number of experimental studies support this hypothesis. In pelagic marine enclosures experiments have demonstrated reduced numbers of large bodied zooplankton after TBT additions, and subsequent significant increases in chlorophyll a and large microphytoplankton were observed (Jak et al, 1998). Further direct evidence comes from the Broads: in mesocosm experiments, Kerrison (1988, 1989) replicated established mollusc and zooplankton communities extant in Hoveton Great Broad (TBT undetected). Following an addition of TBT (max conc. 600ng l^{-1}), the overall mollusc abundance was reduced accompanied by a significant decline in zooplankton grazing. In addition, Jackson (1999) demonstrated that the number of invertebrates sensitive to TBT (especially molluscs) was reduced in navigable parts of the Broads during peak TBT use. Although it may not be possible to rule out other stressors such as habitat degradation through eutrophication and mechanical disturbance the application of community wide metrics to isotopically defined food webs could provide insight into whether differences in food web architecture exist along

a gradient of TBT contamination. This has been previously highlighted in the collapse of the trophic niche width of a top predator along a gradient of habitat fragmentation (e.g. Layman et al., 2007a, see Chapter 1).

4.1.1   Specific aims and hypotheses

I aimed to isotopically characterise the food webs of a series of broads and boatyards in Norfolk representing an organotin gradient as measured by sediment concentration (see Chapter 3) to test the following hypotheses:

1. Since these broads are all relatively shallow (<2m) and productive, then I expected that the highly mobile, top secondary consumers (fish) would access both pelagic and littoro-benthic resources, and thus, for the food web architecture to be essentially in the form of a square or an isosceles triangle (i.e. the apex predator sitting almost equidistant between the two habitat-influenced basal resources with well developed food chains stemming from each (Figure 4.1). However, with respect to transfer pathways of organotins, then I have previously shown that sediments and not the water column are the most likely reservoirs (Chapter 3) and thus I would expect altered population structure in the benthic components which ultimately would be reflected in the community architecture as a shift toward reliance upon pelagic resources (i.e. a scalene triangular form).
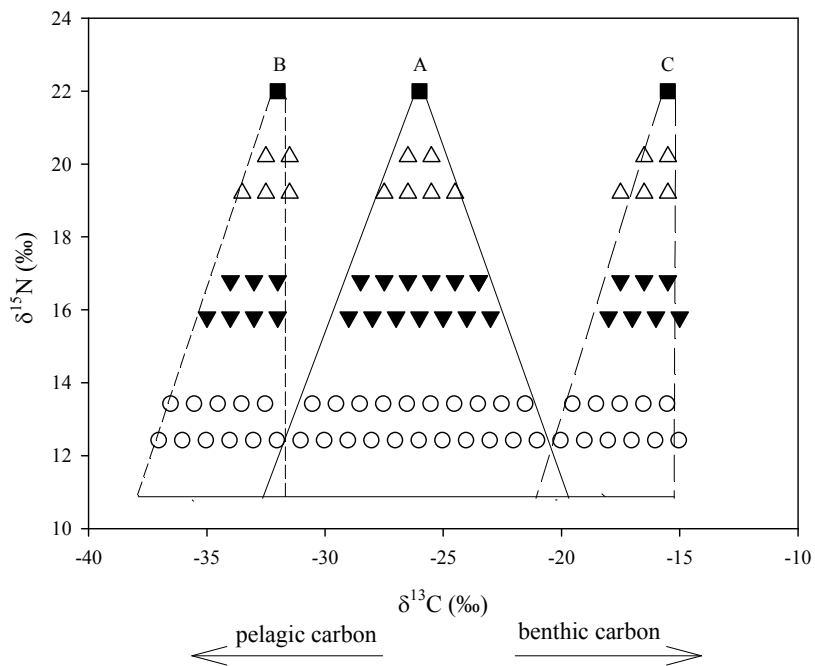
Figure 4.1 Three hypothetical scenarios in a simplified shallow lake food web. Open circles represent primary consumers; filled triangles - secondary consumers; open triangles - fish; filled squares - piscivore (e.g. pike). Scenario A (solid lines) represents a typical food web where fish access both littoral and benthic basal resources, with the apex piscivore equidistant between sources. In scenario B (short dash lines), the importance of littoral/benthic production is reduced in proportion to pelagic resources and the food web is skewed accordingly. In scenario C (long dash lines), the benthic/littoral production is the dominant resource pool used and transferred up the food web resulting in a skew to heavier $\delta^{13}C$ values.

2. With respect to the mean metrics calculated from all the sampling events and related to the gradient of organotins as a potential stressor, I hypothesised that

    a) there would be a negative relationship between both total niche area (TA) and nitrogen range (NR) and the sediment organotin concentration as species are removed from the food web and food chains become shorter.

    b) in relation to (a), the nearest neighbour distance (MNND / SDNND) would increase as species are removed, reflecting a potential loss of stability within the food web.

    c) carbon range (CR) would be negatively related to sediment organotin concentration as species diversity is reduced in the benthos

    d) mean distance to centroid (CD) would also be negatively related to organotin sediment concentration in response to both (a) and (c).

3. Finally, the susceptibility of community metrics to seasonal variability could also be tested using the quarterly sampling regime outlined in Chapter 2, by calculating each metric for a given season. The most likely components of the shallow lake community exhibiting marked isotopic variability throughout an annual cycle would be those with the smallest body size, higher metabolism, undergoing ontogenetic dietary shift, or simply exhibiting dietary switch over the seasons. I hypothesised that zooplankton and chironomids were likely candidates to have the most influence on metric values.

4.2 Methods

The generic methods used for organotins and stable isotope analyses have been detailed in Chapter 2. Details specific to this part of the study are outlined below.

4.2.1 Study sites

This study was conducted at all eight sites referred to in Chapter 2, comprising the six broads with a varying history of boating access / connectivity to the river systems, and the two highly engineered riverine sites represented by the boatyards. The sites were selected on the premise that they would represent a gradient of organotin contamination.

4.2.2 Sampling frequency

To cater for temporal variation in isotope values for different components of the broads food webs, a quarterly regime reflecting the four seasons was chosen. Thus, for the Cromes, Cockshoot, Ranworth and Wroxham food webs, each species data point on the isotope bi-plots and used to calculate the isotope-derived metrics (see next section) represents the mean $\pm$ SD from a minimum of n = 12 samples (three replicates from four quarterly sampling events). Malthouse and Salhouse Broads were only sampled for three seasons i.e. minimum n = 9. Access to the boatyards was extremely limited by the owners due to boat traffic so those two sites were sampled only in late Autumn and Spring i.e. n = 6.

4.2.3   Community Metrics

Community metrics were calculated on the statistical package R (version 2.9.2) using the SIAR package freely available online

The metrics proposed by Layman et al. (2007) are described below:

1. δ^{15}N Range (NR). This is calculated as the Y-axis distance between the two species with the most ^{15}N-enriched and most ^{15}N-depleted values (e.g. maximum δ^{15}N - minimum δ^{15}N). An increase in NR is suggestive of more trophic levels within the community and hence greater trophic diversity. Conversely, a decrease in NR might suggest a reduction in trophic levels or increased omnivory.

2. δ^{13}C Range (CR). Similar to NR, CR is calculated as the X-axis distance between the two species with the most ^{13}C-enriched and most ^{13}C-depleted values (e.g. maximum δ^{13}C - minimum δ^{13}C). It might be expected that higher CR values are found in food webs where multiple basal resources with varying δ^{13}C values provide for niche diversification at the base of the food web. CR reduction might indicate removal of particular basal resources.

3. Total Area (TA). TA is essentially the convex hull area encompassed by all species in δ^{13}C- δ^{15}N bi-plot space and represents the total niche space occupied. It is therefore indicative of the total extent of trophic diversity within a food web, and is computed using the Windows traithull interface programme (see Cornwell et al., 2006)

4. Mean Distance to Centroid (CD). CD is the average Euclidean distance of each species to the δ^{13}C- δ^{15}N centroid, where the centroid is the mean δ^{13}C and δ^{15}N value for all species

in the food web. CD provides a measure of the average trophic diversity within a food web.

5. Mean Nearest Neighbour Distance (MNND). MNND is calculated as the mean of the Euclidean distances to each species' nearest neighbour within bi-plot space. As such, it provides a measure of the overall density of species packing within that space. In food webs where many species are characterised by similar feeding ecologies (or trophic niches) MNND will be low, suggesting greater trophic redundancy than one in which species are on average more divergent with respect to their trophic niches.

6. Standard Deviation of Nearest Neighbour Distance (SDNND). SDNND is a measure of the evenness of species packing within bi-plot space. This metric is less influenced by sample size than MNND. Lower SDNND values are indicative of more evenly distributed trophic niches.

To demonstrate the impact of seasonal variability in the stable isotope values of particular components of the community on whole community metric values, the metrics were calculated for three seasons from Wroxham and Salhouse Broads.

4.2.4   Data analyses and statistics

Normality and equality of variances were ascertained for all data by performing Anderson-Darling and Levene's tests, respectively. As sediment butyltin were non-normal and there

was unequal variance between site specific butyltin data, non-parametric Kruskall-Wallis tests were used in place of ANOVA. Butyltin data was log transformed for comparison with community metrics. Regression analyses were used to examine the relationship between butyltin in sediments and community metrics.

4.3   Results

4.3.1   Organotin concentrations in surficial sediments

Organotin analyses from control sites showed no quantifiable butyltin species. In contrast TBT was quantifiable at the six remaining sites (Fig. 4.2) and ranged from 87 to 4625 ng g^{-1} (mean 1150 ± 230 ng g^{-1}), and was the dominant species at all sites where organotins were detected. DBT was present in quantifiable concentrations at all connected sites, although concentrations were lower relative to TBT, ranging from 165 to 850 ng g^{-1} (mean 286 ± 46 ng g^{-1}). MBT was quantifiable from boatyard sediments only and ranged from 267 to 514 ng g^{-1} (mean 268 ± 37 ng g^{-1}). Thus, there was a gradient of increasing sediment contamination in the order of Wroxham < Salhouse < Ranworth < Malthouse < Loyne's Boatyard < Horning Ferry Marina (Fig. 4.2).

Total butyltin ($\sum$MBT+DBT+TBT) concentrations from boatyard sites were up to an order of magnitude greater than those from three of the four connected broads; those from Malthouse Broad yielded concentrations of DBT (266 ± 11 ng g^{-1}) and TBT (1368 ± 68 ng g^{-1}) which was similar to those observed in boatyard sediments. Thus, there were significantly greater concentrations of total butyltins sediment samples from Malthouse Broad and the two

boatyard sites (Kruskall-Wallis χ^2 =14.8, DF=5, p< 0.05) compared to those from Wroxham, Salhouse and Ranworth.
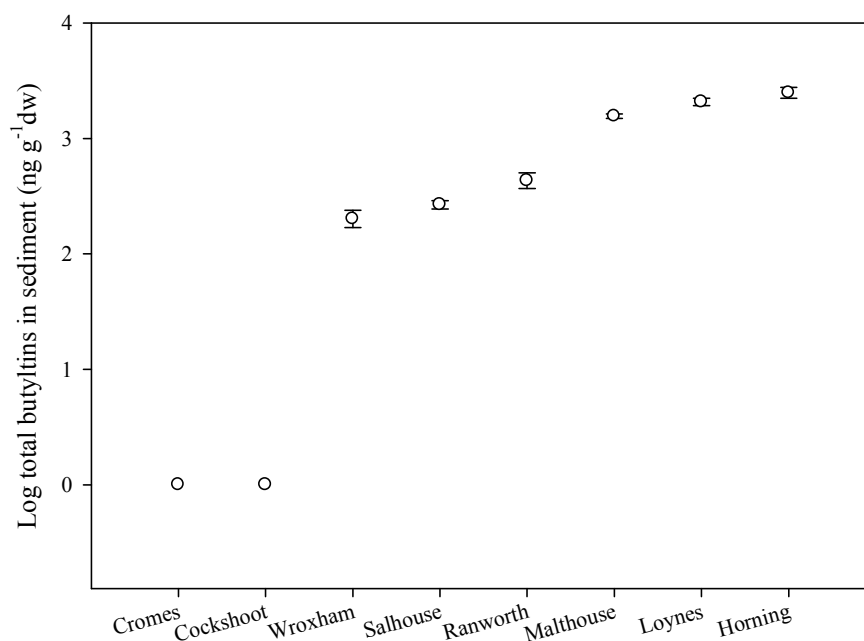
Figure 4.2 Mean total butyltin ($\sum$ MBT + DBT + TBT) concentrations (ng g^{-1} dry weight) in surficial sediments from sampled sites. Error bars represent ± SE. Mean values derived from pooled data from 2006/2008 (n=6), except Malthouse and Salhouse Broads - data from 2008 only (n=3)

4.3.2   Stable isotope bi-plots

A full species list with data for each seasonal sampling is given in Appendix 1. The number of species representing primary and secondary consumers and fish for each food web are given in Table 4.1, along with the isotope values representing the food web centroid.

Site	1° Cons sp.	2° Cons sp.	Fish sp.	δ^{13}C (‰)	δ^{15}N (‰)
Cromes	15	12	7	-33.2 ± 0.3	17.4 ± 0.4
Cockshoot	13	12	7	-31.2 ± 0.4	13.3 ± 0.4
Wroxham	12	6	6	-32.6 ± 1.3	12.6 ± 0.8
Salhouse	11	6	5	-32.9 ± 1.4	13.1 ± 0.7
Ranworth	12	9	7	-31.6 ± 0.4	15.3 ± 0.4
Malthouse	12	9	7	-32.4 ± 0.4	15.0 ± 0.4
Loyne's	10	0	0	-34.0 ± 0.6	13.2 ± 0.2
Horning	4	0	0	-33.3 ± 0.9	15.4 ± 1.2

Table 4.1 Summary of species forming invertebrate primary consumers (1° Cons sp.), secondary invertebrate consumers (2° Cons sp.) and fish in each food web. The mean δ^{13}C and δ^{15}N values (± SD) are presented as the co-ordinates of the food web centroid.

Non-contaminated broads

Isotopic characterisation of food webs for the control sites of Cromes and Cockshoot Broads are presented in Figure 4.3a & b. In general the food web components of Cromes were ^{13}C-depleted by ~2 ‰ relative to Cockshoot, and primary consumers from Cromes were ^{15}N-enriched by 3.8 ‰ compared to Cockshoot, likely reflecting a higher isotopic baseline. Thus, despite similar species composition, the δ^{15}N values for Cromes were on average enriched by 4.1 ‰ compared to Cockshoot (Table 4.1). Within the macroinvertebrate components, temporal variability of isotopic values (particularly δ^{13}C) was often substantial. For example,
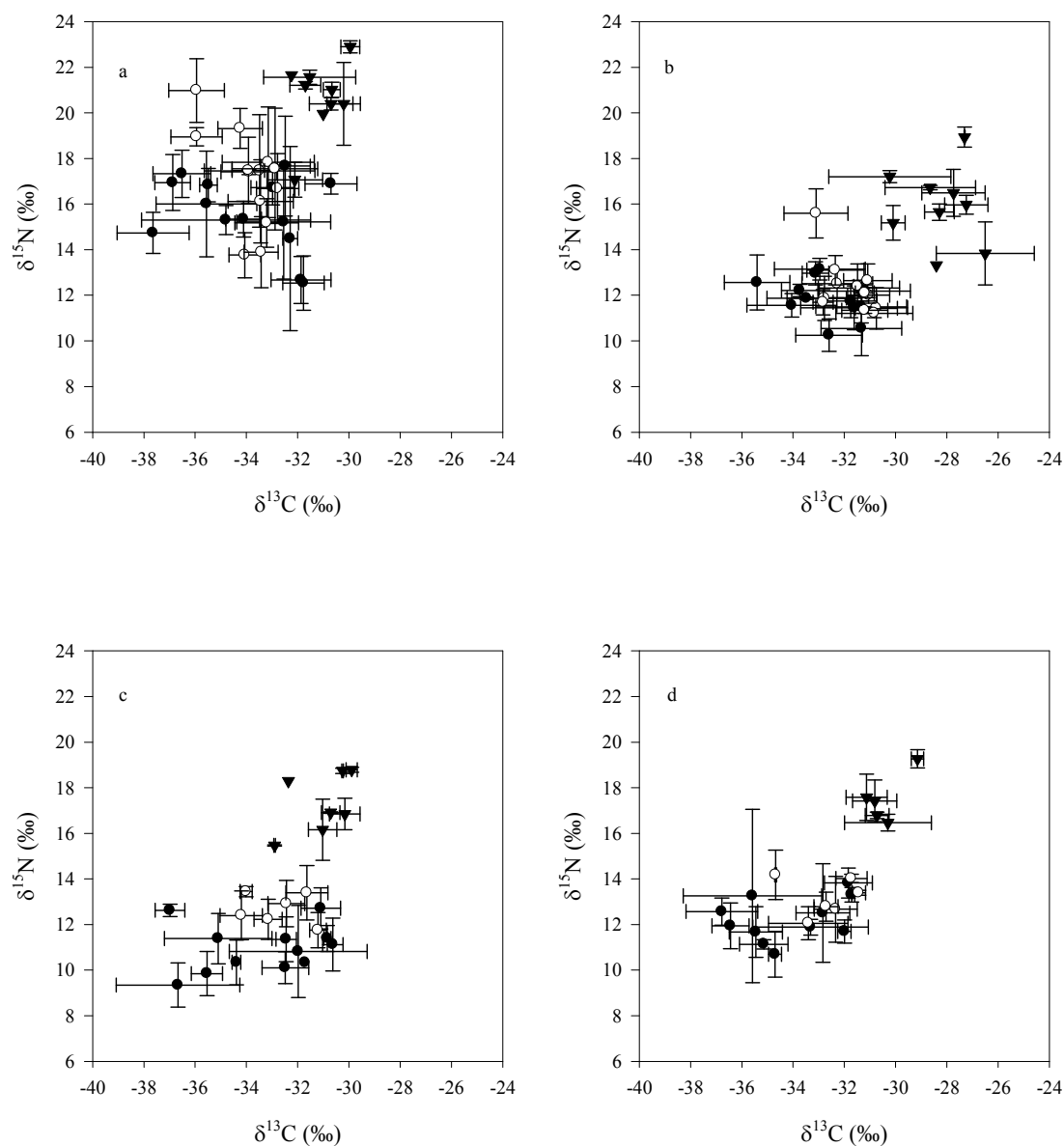
Figure 4.3 a-d. Stable isotope bi-plots derived from quarterly sampling (mean ±SD) of food web components collected from eight sites in the Norfolk Broads. Sites are ranked along a scale of tin loading: a - Cromes Broad; and b - Cockshoot Broad (both TBT undetected); c - Wroxham, (lowest quantifiable TBT); d - Salhouse (intermediate TBT). Symbols: triangles - fish; open circles - invertebrate secondary consumer; and closed circles - invertebrate primary consumers. Continued overleaf.
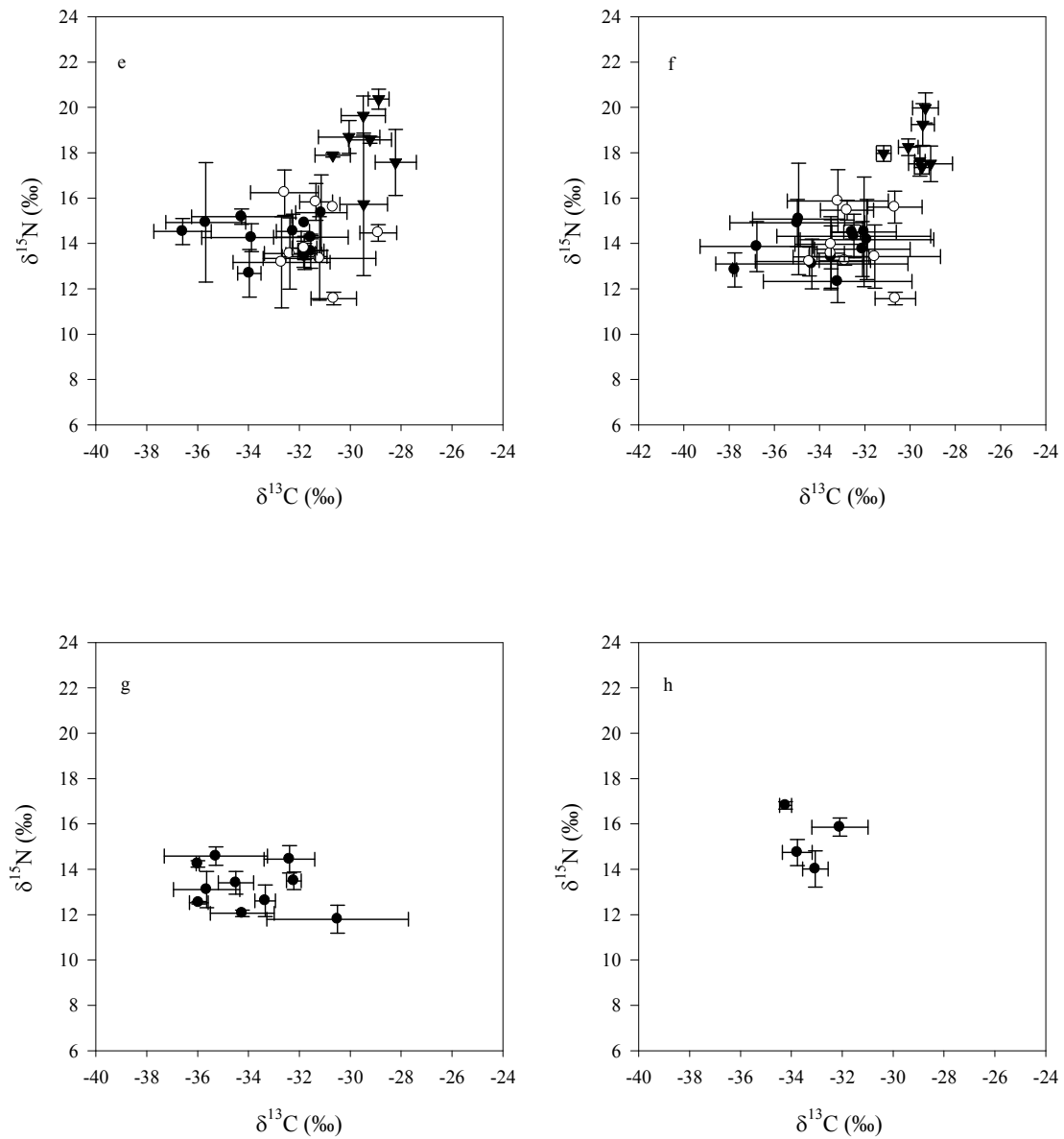
Figure 4.3 e-h. Stable isotope bi-plots derived from quarterly sampling (mean ±SD) of food web components collected from eight sites in the Norfolk Broads. Sites are ranked along a scale of TBT loading: e - Ranworth Broad (intermediate TBT); f- Malthouse Broad (high TBT); g – Loyne's Boatyard (high TBT); h - Horning Ferry Marina (highest TBT). Symbols: triangles - fish; open circles - invertebrate secondary consumer; and closed circles - invertebrate primary consumers

δ^{13}C values of *Chironomus plumosus* spanned 7.4 ‰ at Cromes and 4.4 ‰ at Cockshoot among the seasons. The same pattern was evident among the majority of macroinvertebrate consumers at both sites (see the SDs in Fig 4.3a & b).

The mean δ^{13}C of primary consumers at control sites provided some evidence of consumer resource partitioning between littoral-benthic and pelagic energy pathways. For example, at Cromes, the unionid mussel *Anodonta cygnea* (-35.5‰) and bulk zooplankton (-35.6‰) were ^{13}C-depleted compared to typical littoral consumers such as *Limnaea stagnalis* (-30.6‰) and *Asellus aquaticus* (-31.9‰). The same species exhibited similar differences within Cockshoot where δ^{13}C of pelagic consumers *A. cygnea* (-33.1‰) and bulk zooplankton (-35.4‰) were depleted relative to *A. aquaticus* (-31.3‰) and *L. stagnalis* (-31.6‰).

At Cromes, only two predatory macroinvertebrate taxa were δ^{15}N-enriched compared to all herbivorous counterparts; the dragonfly larva *Aeshna isoceles* (19.3‰) and the zooplanktivorous phantom midge of the genus *Chaoborus* (21‰; Fig. 4.3a). The majority of other predatory macroinvertebrates were frequently isotopically indistinct from herbivores suggestive of considerable omnivory (trophic plasticity) within the invertebrate sub-web.

In Cockshoot, *Chaoborus* spp. was almost classically ^{15}N-enriched (e.g. 3.0‰) relative to its putative zooplankton prey (15.6‰ and 12.6‰ respectively). Excluding *Chaoborus*, the damselfly larva *Erythromma najas* and the predatory caddis *Holocentropus dubius* (both 13.1‰) were the most ^{15}N-enriched of all predatory macroinvertebrates within the

Cockshoot food web being enriched by 2.6‰ compared to *A. aquaticus* (10.5‰). As at Cromes, the remaining invertebrate food web appeared complex, with dense species packing (low NND) and similar $\delta^{15}N$ values shared between predators and primary consumers.

Both control sites displayed similar fish assemblages (Table 4.1), comprising roach (*Rutilus rutilus*), rudd (*Scardinius erythrothalamus*), tench (*Tinca tinca*), perch (*Perca fluviatalis*), eel (*Anguilla anguilla*) and pike (*Esox lucius*); with the addition of juvenile bream (*Abramis brama*) at Cromes, and ruffe (*Gymnocephalus cernuus*) at Cockshoot.

At Cromes, all fish were positioned above the invertebrate food web except for juvenile perch. This size class of perch (68-76 mm) exhibited mean $\delta^{13}C$ and $\delta^{15}N$ values of -32.1‰ and 17.1‰, respectively, placing them in a similar position in the food web as intermediate invertebrate predators such as damsel fly larvae (*E. najas*), leeches (*Erpobdella* sp) and notonectids (*Notonecta viridis* and *Ilyocoris cinicoides*). Some evidence of isotopic overlap was evident between roach and rudd both in terms of $\delta^{13}C$ (-30.6‰ and -30.1‰), and $\delta^{15}N$ (both 20.3‰), as they were isotopically indistinct. Similar mean isotopic values were also recorded between juvenile pike ($\delta^{13}C$: -31.5‰, $\delta^{15}N$: 21.5‰) and large perch ($\delta^{13}C$: -31.6‰, $\delta^{15}N$: 21.2‰). Both these results could be perceived as evidence for a degree of inter-specific niche overlap.

Large pike (535 to 740 mm) occupied the position of top predator with the most ^{13}C-enriched (-29.9‰) and highest $\delta^{15}N$ (22.9‰), and were almost classically enriched in both carbon and

nitrogen relative to roach and rudd (Fig 4.3a). Comparison of the ^{13}C-enriched top predator (pike) and ^{13}C-depleted zooplanktivorous *Chaoborus* could be seen as evidence for the existence of benthic-littoral and pelagic energy pathways, respectively, within the Cromes Broad food web. However, it is not possible to rule out the incidence of pike feeding on invertebrate predators.

Elevated δ^{15}N of fish species positioned them above macroinvertebrate consumers within the Cockshoot food web (Fig 4.3b). Excluding a single eel, juvenile perch were the most ^{13}C-depleted fish consumer (-30.1‰), and in addition relatively low δ^{15}N (15.1‰) placed them in a similar position to conspecifics within Cromes Broad (Fig 4.3a&b). Of all fish species, rudd exhibited the most ^{13}C-enriched (-26.5‰) and ^{15}N-depleted (13.8‰) values. Roach were ^{15}N-enriched (15.9‰) by 2.3‰ relative to rudd, and further segregated in terms of ^{13}C (-27.2‰ and 26.5‰, respectively), suggested little evidence of niche overlap within these two species.

Low contamination broads

Compared to the control sites, the number of species forming the invertebrate food web was lower in both Wroxham and Salhouse food webs (Table 4.1). Accordingly, species packing was less dense (Table 4.2) and there was clearer vertical delineation between primary and secondary consumer invertebrates, particularly within Wroxham (Fig 4.3c&d). The food web centroid δ^{13}C and δ^{15}N values of these broads were also similar (Table 4.1). Leeches (*Erpobdella* spp.) and libellulid nymphs (*Sympetrum* spp.) were the most ^{15}N-enriched

invertebrate predators in the Wroxham food web (14‰). Leeches were also one of the most ^{15}N-enriched invertebrate predators in the Salhouse food web, although the vertical position of libellulid nymphs (13.4‰) in Salhouse was substituted by damselfly larvae (*Erythromma najas*: 14.2‰). Within both food webs the carbon signatures of these consumers were consistent with their assumed energy sources, with leeches more benthic/littoral (Wroxham: -31.6‰; Salhouse; -31.2‰) and dragonfly and damselfly larvae were more pelagic (Wroxham: -34‰; Salhouse: - 34.7‰). There were various other similarities between primary consumers within these two food webs. Mayfly larvae (*Baetis* spp.) shared the same position in their respective food webs (δ^{13}C: -36.9‰; δ^{15}N: 12.6 ‰). Similarly swan mussel isotopes were remarkably similar; at both sites δ^{15}N measured 11.7‰ while mussels from Salhouse were only marginally ^{13}C-enriched compared to Wroxham (0.5‰).

On average, bulk zooplankton were similar in terms of δ^{13}C (Wroxham: -35.1‰; Salhouse: -35.6‰) although δ^{15}N was higher in Salhouse (13.3‰) compared to Wroxham (11.4 ‰). However, zooplankton exhibited considerable isotopic variability over an annual cycle varying by 5.4 ‰ and 7.0‰ for δ^{13}C and δ^{15}N, respectively.

Chironomids (*C. plumosus*) from Wroxham also exhibited considerable annual variation in δ^{13}C. The most enriched values were observed in summer (-34.6‰) but a value of -40.1‰ was recorded when sampled in spring, the lowest δ^{13}C value recorded throughout the entire study, and could be seen as evidence for utilisation of methane oxidising bacteria (cf. Grey et al 2004). This was in marked contrast to chironomids from Salhouse that varied <1‰ in

δ^{13}C. In terms of δ^{15}N, variation was approximately the same between broads (Wroxham: 2.3‰; Salhouse: 2.6‰).

As with respective invertebrate food webs, the fish assemblages of Wroxham and Salhouse were similar in both number (Table 4.1) and species formed by roach, bream, eel, perch and pike. The only notable difference was the presence of tench in Wroxham but not Salhouse. Pike exhibited the highest δ^{15}N in both broads, however in Wroxham the position of perch was virtually indistinguishable from pike (Fig 4.3c) suggesting Wroxham perch were piscivorous and little niche differentiation between these two species. This was in contrast to Salhouse, where pike (δ^{13}C: -29.1‰, and δ^{15}N: 19.3‰) were clearly higher than perch and almost classically isotopically enriched compared to roach (δ^{13}C: -30.3‰ and δ^{15}N: 16.5‰). In Wroxham, juvenile pike (0+) were positioned centrally above the invertebrate sub web. Aside from a single eel, tench and bream were the most ^{13}C-depleted fish in Wroxham (-31.0‰ and -30.7‰, respectively), although bream had marginally higher δ^{15}N (0.7‰). Little inference can be made about the isotopic niche of eel in Wroxham due to the small sample size (n=1). However, within Salhouse eel (δ^{13}C: -30.8 ‰ and δ^{15}N: 17.4‰ were positioned similar to perch (δ^{13}C: -31.1‰ and δ^{15}N: 17.6‰) suggesting some potential niche overlap between these two species. In general fish appeared to be supported primarily by the benthic-littoral energy pathway as indicated by the skew toward more enriched values on the carbon axis.

Medium-high contamination broads

Relative to Wroxham and Salhouse, the number of invertebrate species comprising the invertebrate sub web increased slightly and as a result species packing was denser in the Ranworth and Malthouse food webs (Table 4.1 & 4.2). As with the other broads, food web averaged isotopic values (i.e. centroids) were similar although Ranworth consumers were ^{13}C-enriched relative to Malthouse by ~1‰ (Table 4.1). There were a number of structural similarities between these two food webs. For example, the most ^{15}N-enriched invertebrate predator in both broads was the damselfly larvae *E. najas* (Ranworth: 16.2‰ and Malthouse: 15.9‰), which combined with their respective carbon values (Ranworth: -32.6‰ and Malthouse: -33.2 ‰) placed them centrally above the remainder of the invertebrate sub- web (Fig 4.3e&f). This was a similar position to 0+ pike in Wroxham (Fig 4.3c) and is suggestive of utilisation of both pelagic and benthic/littoral energy pathways. Other isotopically distinct invertebrate predators were leeches (*Erpobdella* sp) and acarid mites. The former were similar in terms of their δ^{15}N between sites (Ranworth: 15.6‰ and Malthouse: 15.5‰). Both these predators exhibited lower δ^{13}C than damsel fly larvae; and acarid mites shared similar isotopic niches in both broads (Ranworth, δ^{13}C: -30.6 ‰, δ^{15}N: 15.6‰; Malthouse, δ^{13}C: -29.9 ‰, δ^{15}N: 14.1‰).

Chironomids (*C. plumosus*) exhibited considerable seasonal variability in terms of δ^{13}C in both broads (4.8‰ in Ranworth and 8‰ in Malthouse) and similar to Wroxham, this probably indicates some degree of seasonal feeding mode plasticity. In contrast, variability of chironomid δ^{15}N was reduced and varied <1‰ across all seasons in Ranworth and ≤2‰ from Malthouse (Fig 4.3e&f). Similar to *Anodonta* spp. in Wroxham and Salhouse, these consumers shared virtually identical positions in Ranworth (δ^{13}C: -32.2 ‰, δ^{15}N: 14.5‰) and

Malthouse (δ^{13}C: -32.6‰, δ^{15}N: 14.5‰) and varied <1.5‰ in both δ^{13}C and δ^{15}N (Fig 4.3e&f).

Ranworth and Malthouse fish species compositions were identical and formed by roach, bream (skimmer and adult analysed separately), tench, eel, and perch. Pike were not caught from either broad and thus are apparently absent from both food webs, being substituted by perch in the position of top predator (Fig 4.3e&f; Ranworth δ^{13}C: -28.8‰, δ^{15}N: 20.4‰; Malthouse δ^{13}C: -29.3‰, δ^{15}N: 19.9‰) and typically enriched compared to conspecifics from Wroxham and Salhouse Broads (Fig 4.3c&d). Indeed, perch were positioned higher than Wroxham and Salhouse pike in terms of δ^{15}N. These features could be seen as indication of perch replacing pike as top predator in these food webs. However, as primary consumers were generally δ^{15}N enriched compared to Wroxham and Salhouse, this is more likely a reflection of a higher δ^{15}N baseline. In both broads, perch were isotopically enriched relative to roach (Ranworth δ^{13}C: -29.4‰, δ^{15}N: 15.7‰; Malthouse δ^{13}C: -29.6‰, δ^{15}N: 17.6‰) and rudd (Ranworth δ^{13}C: -28.2‰, δ^{15}N: 17.6‰; Malthouse δ^{13}C: -29.0‰, δ^{15}N: 17.5‰). In fact, rudd were the most ^{13}C-enriched species while tench were the most ^{13}C-depleted fish species in both broads. Eel shared almost identical positions in both food webs (Ranworth δ^{13}C: -29.4‰, δ^{15}N: 19.6‰; Malthouse δ^{13}C: -29.4‰, δ^{15}N: 19.2‰) and were ^{15}N-depleted relative to perch by <1‰ in both food webs.

High contamination boatyards

The food webs of the two boatyard sites were species poor with secondary consumers apparently absent from Horning and fish absent from both. The food web averaged $\delta^{13}C$ varied by <1‰ between food webs although in terms of $\delta^{15}N$, consumers from Horning were elevated by ~2‰ (Table 4.1). The markedly reduced number of species in the Horning boatyard meant there were few similarities between sites. The invasive zebra mussel was present at both sites (*Dreissena polymorpha*) and was the most ^{13}C-depleted consumer (-35.1‰) at Loyne's. The most ^{13}C-depleted consumers in the Horning boatyard were gallery-building chironomids (-34.2‰) that also exhibited the highest $\delta^{15}N$ (16.8‰). Pea mussels (*Psidium* spp.) were present in both food webs and were the most ^{13}C-enriched consumer in the Horning boatyard.
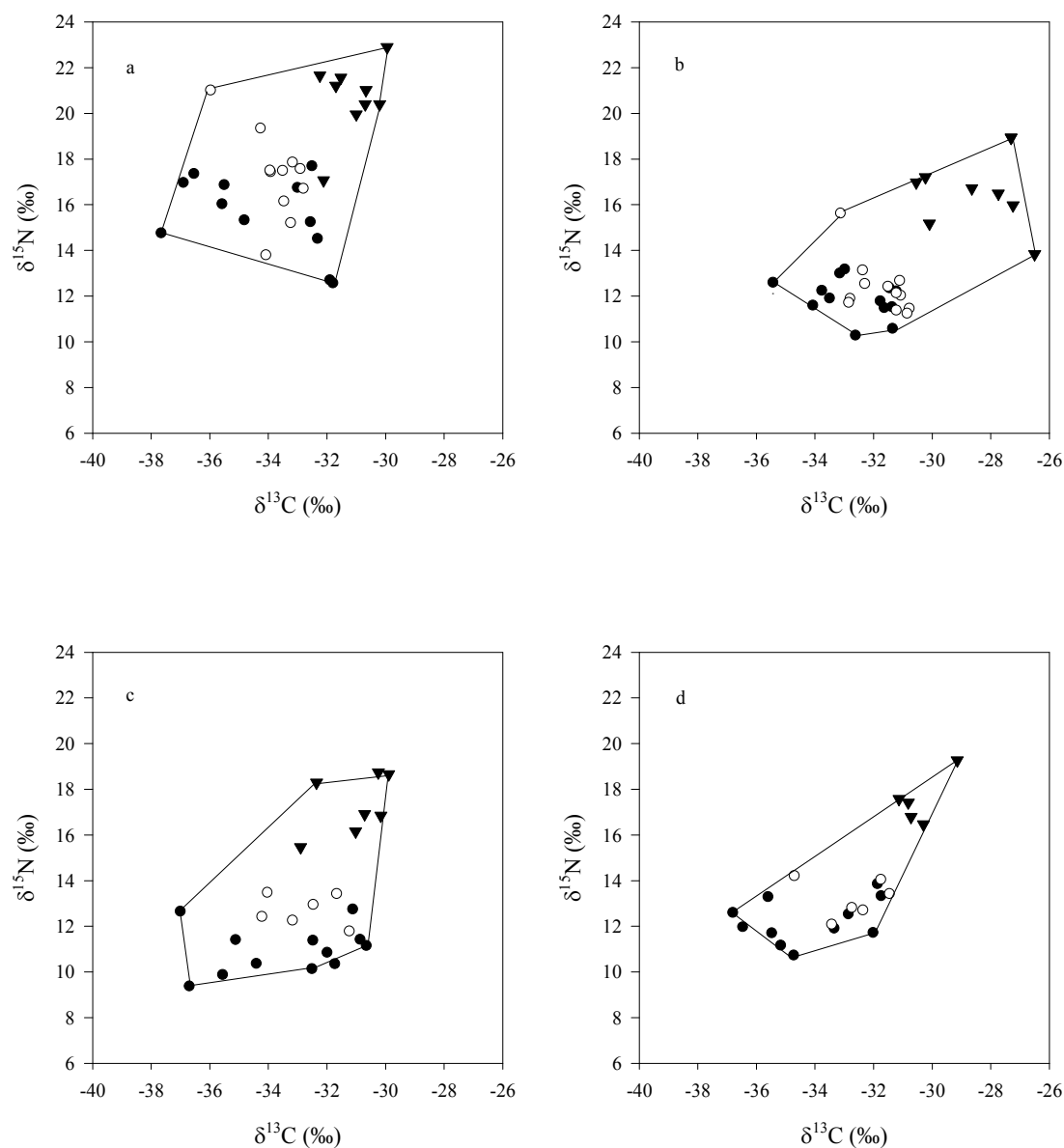
Figure 4.4 a-d Stable isotope bi-plots derived from quarterly sampling (error bars omitted for simplicity) of food web components collected from eight sites in the Norfolk Broads. Solid lines represent the convex hulls from which community metrics were calculated. Sites are ranked along a scale of TBT loading: a - Cromes Broad; and b - Cockshoot Broad (both TBT undetected); c - Wroxham, (lowest quantifiable TBT); d - Salhouse (intermediate TBT). Symbols: triangles - fish; open circles - invertebrate secondary consumer; and closed circles - invertebrate primary consumers.
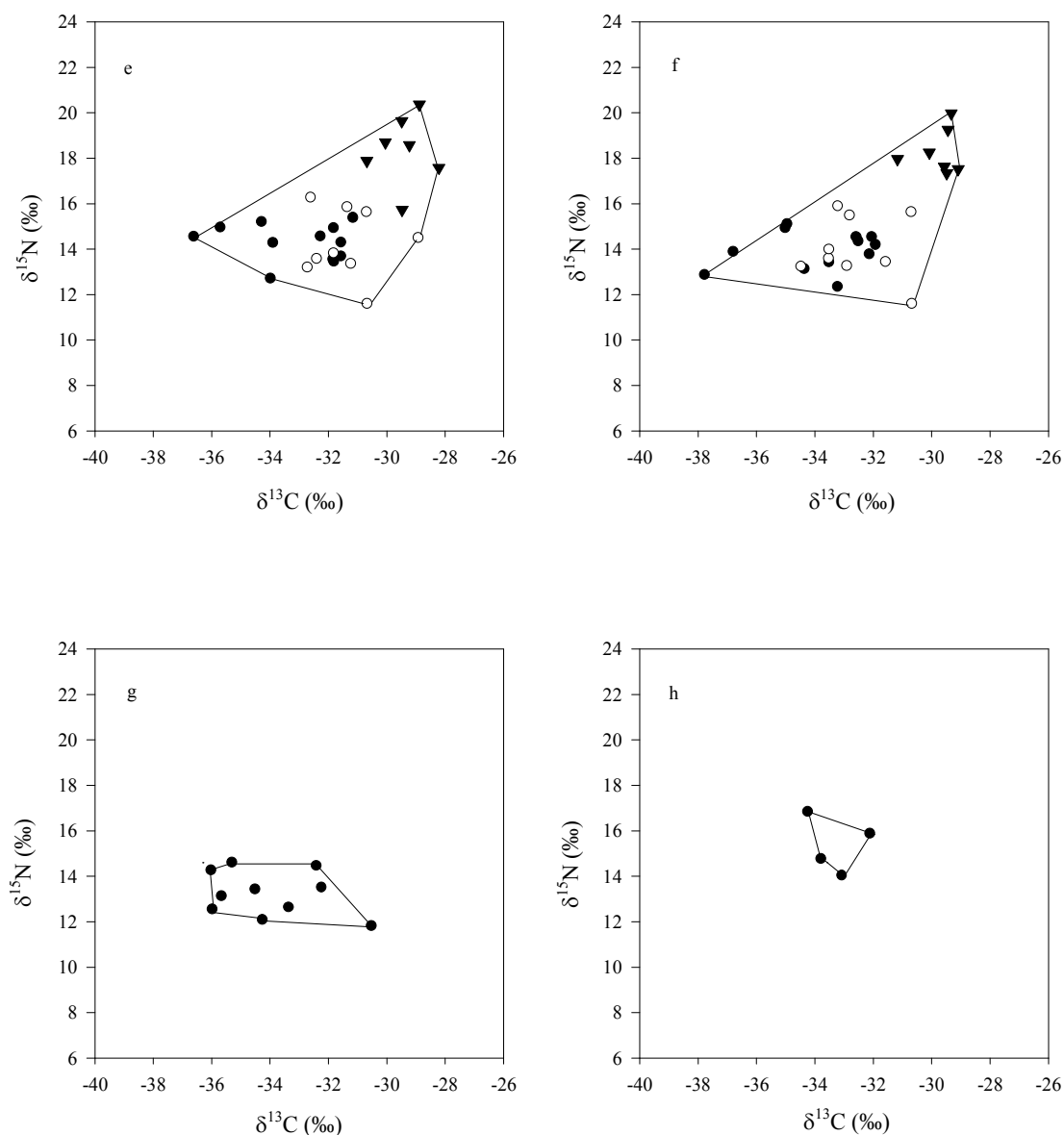
Figure 4.4 e-h Stable isotope bi-plots derived from quarterly sampling (error bars omitted for simplicity) of food web components collected from eight sites in the Norfolk Broads. Solid lines represent the convex hulls from which community metrics were calculated. Sites are ranked along a scale of TBT loading: e - Ranworth Broad (intermediate TBT); f- Malthouse Broad (high TBT); g – Loyne's Boatyard (high TBT); h - Horning Ferry Marina (highest TBT). Symbols: triangles - fish; open circles - invertebrate secondary consumer; and closed circles - invertebrate primary consumers.

4.3.3   Stable isotope metrics

The food webs were re-plotted in a simplified format omitting error bars to show the dimensions of the convex hulls used to boundary the metric TA (Fig 4.4a-h). In general, the hulls from the two control sites were square in shape, whereas the hulls from the broads containing contaminated sediments tended toward an isosceles triangle form, with comparative skewing of the top predators toward higher $\delta^{13}C$ values. The two boatyards were very simplified in terms of species richness (four species for Horning) and thus it appears prudent not to draw too much from their shape when other metrics are more revealing.

Site	TA	NR	CR	CD	MNND	SDNND
Cromes	51.04	10.3	7.7	2.82	0.74	0.56
Cockshoot	48.02	9.6	10.2	2.8	0.61	0.57
Wroxham	39.4	8.6	7.11	2.86	0.93	0.50
Salhouse	23.7	8.6	7.1	2.71	0.68	057
Ranworth	37.48	8.8	8.4	2.54	0.78	0.44
Malthouse	34.3	8.4	8.7	2.64	0.60	0.51
Loynes	10.65	2.8	5.5	1.8	1.05	0.65
Horning	3.04	2.8	2.1	1.34	1.61	0.65

Table 4.2. Community metrics from the eight sample sites, and are listed in descending order of increasing contamination profile, where Cromes and Cockshoot are controls and Horning the most contaminated Boat yard. Descriptions of the metrics are provided in section 4.2.3

Three of the community metrics revealed no pattern across the contamination gradient except for when comparing the boatyards (i.e. the most contaminated sites) with all the 'true' broads. CR and CD were typically higher in the broads (~7 to 8 and ~2.6, respectively) whereas these values were reduced in the boatyards (2.1 to 5.5 and 1.3 to 1.8, respectively; Table 4.2). Conversely, MNND was lower in the broads and higher in the boatyards (Table 4.2), as a result of increased species packing at these sites. SDNND varied <2 across all sites, suggesting reduced influence of sample size compared to MNND. There was a significant negative relationship between both NR (y = 10.69x - 1.52; $p < 0.05$) and TA (y = 52.18x - 10.09; $p < 0.05$) with sediment organotin concentration (Fig 4.5 & 4.6).
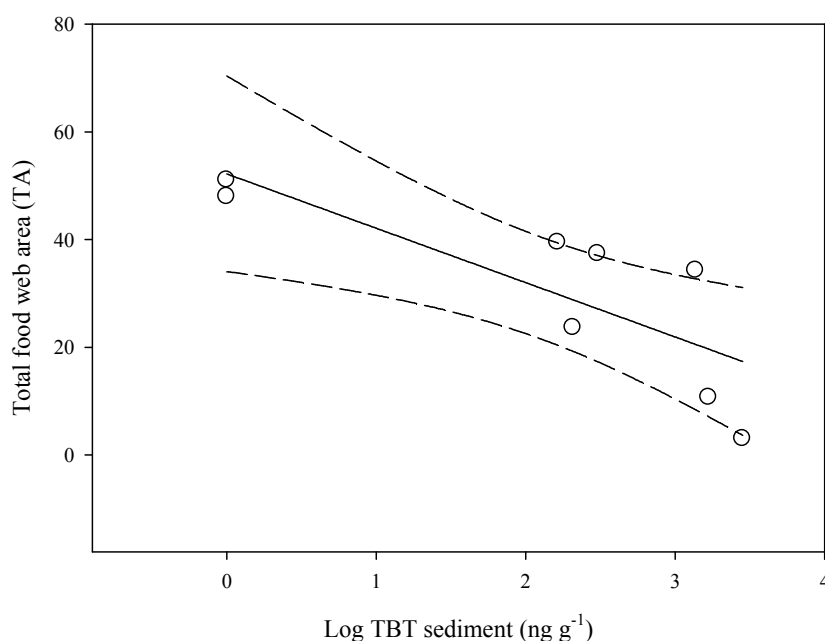
Figure 4.5 Relationship between Total food web area (TA) and mean log TBT in sediment (ng g^{-1}). The regression line (± 95% CI) was fitted using the equation y = 52.18x - 10.09; r^2 = 0.65; $p < 0.05$
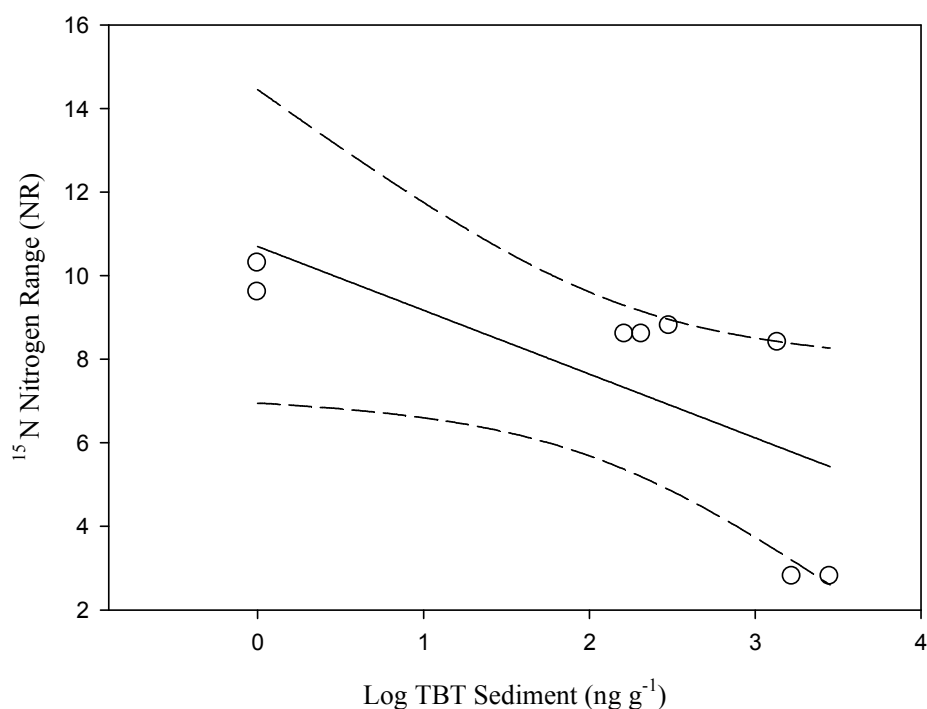
Figure 4.6 Relationship between ^{15}N nitrogen range (NR) and mean log TBT in sediment (ng g^{-1}). The regression line ($\pm$ 95% CI) was fitted using the equation y = 10.69x - 1.52; r^2 = 0.50; p < 0.05.

To examine the variation in isotope metrics over time, each metric was calculated for three sampling events for Wroxham and Salhouse broads (Table 4.3). In the Wroxham food web TA and CR were the most variable metrics. TA varied by as much as 13.34 between sampling events (Table 4.3). This variability was influenced primarily by the wide range of δ^{13}C exhibited by chironomids. In the Salhouse food web the variation was greatest in TA. CR and NR also showed some variability although in the case of CR this was not as great as in

Wroxham (Table 4.3). Much of the observed change in these metric values from Salhouse

was influenced by small bodied calanoid and copepod zooplankton (Figure 4.7).

Site	Month	TA	NR	CR	CD	MNND	SDNND
Wroxham	Jul 07	39.11	9.9	6.35	3.5	0.9	0.5
Wroxham	Oct 07	29.9	9.11	4.63	2.91	0.84	0.60
Wroxham	Mar 08	43.24	8.85	10.33	2.95	0.98	0.88
Salhouse	Jul 07	61.03	9.7	9.0	3.13	1.15	1.12
Salhouse	Oct 07	25.3	8.9	6.68	2.47	0.73	0.58
Salhouse	Mar 08	24.8	8.12	7.6	2.6	0.69	0.46

Table 4.3. Calculated metrics for three sampling events from Wroxham and Salhouse Broads.
A description of each metric is provided in section 4.2.3.
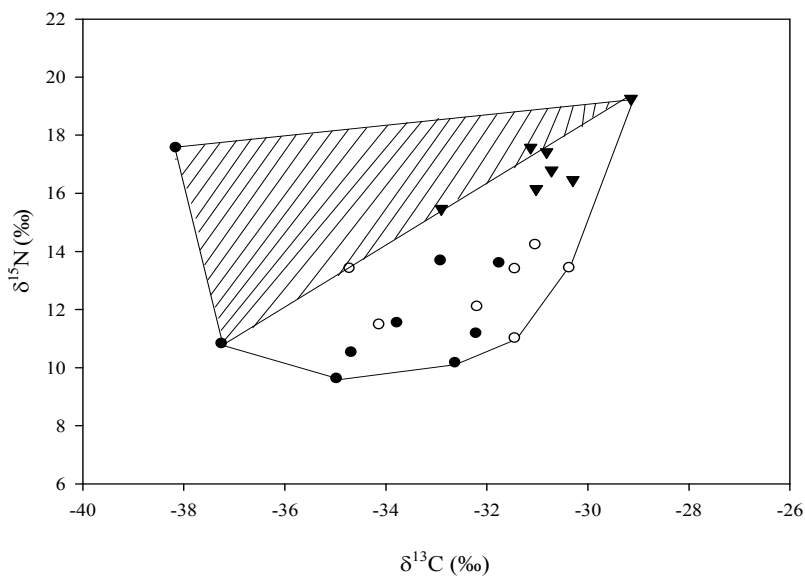
Figure 4.7 Salhouse food web constructed for July. The hatched lines indicate the influence of an outlying primary consumer (in this case small bodied zooplankton) on the calculation of the TA metric. TA was more than doubled (61.03) by the influence of this outlier relative to other seasons (25.3 and 24.8).

4.4. Discussion

The sites selected were of sufficiently different mean sediment organotin concentrations to provide an adequate gradient of potential contaminant stressor (see Fig 4.1) against which to isotopically characterise the food webs (both conventionally as standard $\delta^{13}C$ / $\delta^{15}N$ bi-plots and also using the recently developed community metrics of Layman et al., 2007). It is clear from Table 4.1 that the food web components available to be sampled in sufficient quantity for stable isotope analyses differed considerably between broads with a general decline in

species richness from uncontaminated broads to highly contaminated boatyards. It must be noted that Cromes and Cockshoot both retain considerable macrophyte growth which has been lost from the other sites and thus have a structurally complex habitat with considerable refugia for macroinvertebrates. In terms of species composition across all lakes I recorded the common occurrence of shredder species such as *Asellus aquaticus*, and *Gammarus pulex* scrapers such as *Baetis* spp., filter/deposit feeders such as *Chironomus plumosus* and *Anodonta cygnea*; grazers such as *Bithynia tentaculata, Radix bathica, Psidium* spp. and predators such as *E. najas, Holocentropus dubius, Sympetrum* spp, and *N. viridis*. Generally control sites were more species rich with the addition of species such as *Lymnaea stagnalis, Chaoborous* spp., *Ilyocoris cinicoides, Aeshna* spp. for example. Thus, I appear to have effectively sampled the food webs of my chosen sites as these species compositions are typical of shallow Norfolk lakes; this was true of fish as well as invertebrates (Mason, 1977; Jones & Waldron., 2003; Rawcliffe et al., 2010). Isotopic food webs do not capture the same degree of resolution as connectance food webs (see Rawcliffe et al., 2010) especially at the basal resource level where phytoplankton / benthic diatoms may be described to the species level. However, this degree of resolution is not necessarily essential in studies such as mine when I am looking simply for routing from either the littoral / benthic or the pelagic (Post, 2002). In addition, in those lakes where macrophytes were absent (Wroxham, Salhouse, Ranworth and Malthouse) it was necessary to sample exhaustively to obtain the species listed as habitat complexity was reduced. I have thus characterised the food webs of the sites as fully as logistically possible. There are however some caveats that should be considered, notably, the absence of fish from boatyards and pike from the Ranworth and Malthouse food webs means that metrics such as NR, CR, and TA could be affected as the absence of these species may have artificially truncated respective food webs in both the vertical and

horizontal plains (Vander Zanden & Vadeboncoeur, 2002) Pike do exist in these broads as a particularly large individual was caught during sampling in Malthouse but had to be returned in order to comply with the conditions of Environment Agency licensing. Some circumstantial evidence exists to suggest numbers of pike have declined since the saline incursions during November 2006 (A. Hinds, Environment Agency pers. comm.). Certainly the potential for biomagnification of organotins seems slight in these broads (see Chapter 3) but pike as apex predators do still contain a measurable body burden. If such factors combine to reduce their abundance then their importance as top predator could be reduced in broads food webs. The higher position of perch in both Ranworth and Malthouse food webs relative to other contaminated broads could be seen as substitution of perch as top predators although this could be an artefact of a higher δ^{15}N baseline. It should be acknowledged also that fish are highly mobile and are known to be present in the boatyards during particular periods (e.g. cyprinids overwintering in marinas) but are unlikely to feed at this time, and therefore can be theoretically excluded as being a part of that specific site food web. The relevant literature concerning fish in the broads deals primarily with non-connected broads and so does not detail migration and overwintering in boatyards (Perrow et al., 1997; 1999). However, there is some isotopic evidence to suggest that feeding activity is reduced in overwintering fish, (Perga & Gerdeaux, 2005).

With reference to other shallow lakes, some similarities can be drawn. For example, the vertical structure of the food webs was not clearly delineated by δ^{15}N; although apparent in all lakes this was particularly true within the more structurally complex lakes such as Cromes and Cockshoot (Figure 4.2 a&b). In these lakes the apparent 'disorder' of primary consumer

and predatory invertebrates suggests the occurrence of omnivory in the invertebrate sub-web. Similar feeding plasticity has been recorded in other Norfolk lakes, e.g. Jones and Waldron (2003) noted such patterns of isotopic overlap in both invertebrates and fish.

With regard to my specific hypotheses, the architecture of the food webs from uncontaminated sites (Cromes & Cockshoot) was as expected i.e. relatively square in shape suggesting food chains stemming upwards from all the basal resources available. However, based upon the results from Chapter 3 and Hoare (2007) suggesting that the benthic sediments rather than the pelagic (e.g. open water) would be the source of the contaminants to the wider food web, the upper tiers of the food web were still skewed toward the benthic / littoral (i.e. more ^{13}C-enriched; scenario C in Fig 4.1) and not toward reliance upon pelagic resources (scenario B in Fig 4.1) in the contaminated broads (Fig 4.3c-f). Indeed, they have a very similar species composition and isotopic food web architecture to that found in the Plußsee, Germany (Harrod & Grey, 2006), which is a kettle lake but the hypolimnion is so anoxic during the summer that it effectively functions as a shallow hypertrophic lake. There may be several explanations for this apparent maintenance of the food web by benthic resources, which are not mutually exclusive.

It appears from my findings outlined in Chapter 3 that the organotin concentrations currently to be found in the primary consumers such as chironomids and swan mussels from the intermediate contaminated broads (Wroxham, Salhouse, Ranworth and Malthouse) are not sufficiently high to be causing complete eradication of benthic infauna. Indeed, it appears that chironomids are capable of metabolising TBT to some extent (Looser et al, 2000; Chapter 3).

That said, the benthic infauna is considerably depauperate compared to the non-contaminated sites, a fact confounded by the lack of macrophytes to provide structural complexity. The majority of the macroinvertebrate consumers from the contaminated broads (excluding chironomids and mussels) were collected from the littoral edges rather than the benthic sediments. A number of these (such as *A. aquaticus, G. pulex,* and *Agrypnia* spp.) are classically considered as collector- gatherers of terrestrial detritus and thus would not be expected to contain an organotin burden associated with diet. The littoral edges may be providing a relatively rich hunting ground for fish to forage in. Furthermore, in contrast to larger deeper lakes, the littoral and pelagic habitats in shallow lakes are less clearly defined and this makes isotopic distinction between the two less likely. (Jones & Waldron, 2003, Vander Zanden & Vadeboncoeur, 2002) Indeed using gut content analysis others have shown that fish readily feed on both zooplankton and benthic/littoral invertebrates suggesting that in shallow lakes energy may not be channelled through discrete pelagic or benthic littoral pathways (Jones & Waldron, 2003. It certainly appears from the isotope data that fish such as rudd may be heavily subsidised by allochthonous dietary items as rudd were generally too ^{13}C-enriched to 'fit' within the isotopic confines of the aquatic food web. The potentially wide trophic niche of this species has been demonstrated elsewhere. Rawcliffe et al (2010) recorded terrestrial insects in the guts of rudd and Jones & Waldron (2003) recorded 29 different invertebrate species in a single gut of the species.

Although the benthic infauna may be comparatively depauperate when assessed alongside the uncontaminated broads, it must also be considered that the zooplankton may not be an ideal alternative food source. Zooplankton is abundant in these broads (Perrow et al, 1994; 1999), but individuals are typically small in body size and although a good source of food for YOY and juveniles (Vander Zanden et al, 2006, Perrow et al., 1997), it is simply not efficient for

larger fish to target such small prey items.   Indeed fish such as perch rapidly undergo dietary ontogeny from a zooplankton diet to the inclusion of more benthic prey as their gape size increases and tench and bream are more adapted to benthic feeding (Perrow et al., 1994).

So, it appears that my initial hypotheses regarding food web architecture derived from classical isotope bi-plots in the contaminated sites were incorrect. My next hypotheses were based upon the community metrics derived from stable isotope data developed by Layman et al. (2007). Only two of the metrics calculated (NR and TA) were significantly related to the sediment organotin concentration, while the remainder only were markedly different for the two highly contaminated boatyard sites when compared to the 'natural' broads (contaminated or otherwise).

My initial hypothesis that NR and TA would contract in response to the increasing contamination gradient was supported by significant relationships between both metrics and total sediment butyltin loading (Figs. 4.5 and 4.6). However, as NR affects TA in the vertical plane these two metrics are not necessarily mutually exclusive. Vertical compression of food webs along the contamination gradient could indicate increasing omnivory and/or loss of species in response to the shortening of food chains. In the case of the former, no reduction in the number of species need occur. However, for food chain length to shorten then species must by definition be lost from the system. Whilst omnivory may not necessarily weaken food web stability (for example if it increases trophic redundancy shorter food chains are likely to destabilise food webs by weakening their capacity to dampen the effects of perturbations (Rawcliffe et al., 2010). If one assumes a food web $\delta^{15}N$ trophic fraction factor of 2.5. (Post 2002b), then boatyard food webs are compressed by 7.3‰ relative to Cromes;

this is equal to approximately three trophic levels. Discounting fish, which may be transient visitors to boatyards; this is still equivalent to ~ two trophic levels. In other words, the reduced NR (and thus TA) appears to represent a significant reduction in the trophic height of these food webs that is not just an artefact of the absence of fish. Of course it is not possible to isolate TBT as the cause of this as there are likely to be other stressors at work. However, the potential for TBT to partially maintain these stressed food webs in conjunction with other stressors such as disturbance regime, and lack of habitat heterogeneity cannot be ruled out.

Although there was no significant relationship between MNND and the organotin gradient this metric increased markedly in the two boatyard food webs (Table 4.2). The broader spacing within the bi-plot 'niche- space' is suggestive of a lack of trophic redundancy and by proxy, functional diversity. Similar patterns have been observed in fragmented tidal creeks which led to a collapse in niche width of the top fish predator (Layman et al, 2007b). The lack of species sharing similar trophic niches is likely to weaken the stability of these food webs and increase vulnerability further simplification by future perturbations (Rawcliffe et al., 2010).

I hypothesised that there would be a significant reduction in CR in response to the increasing organotin gradient, as trophic diversity was reduced at the base of the food web. Although no significant change was apparent there was (as with MNND), a marked drop in CR from broads to boatyards. A reduction in basal diversity could be expected at these sites due to homogenous habitat structure and the lack of a true littoral margin. Disturbance is also increased relative to broads at these sites due to year round boat traffic. As CD is a measure of the Euclidean distance of each food web member to the centriod (the the food web mean

$\delta^{13}C$ and $\delta^{15}N$) and there was no significant contraction of CR there was no significant relationship between CD and the organotin gradient. However, as with MNND, a reduction was again apparent in the boatyards food webs, suggesting that on average trophic diversity was lower (Layman et al., 2007).

In a critique of the community metrics approach developed by Layman et al. (2007), Hoeinghaus and Zueg (2007) identified temporal variability in the isotopic composition of components of the community (related to metabolism and isotopic turnover) as one factor capable of inducing considerable bias if not accounted for by a sampling protocol of sufficient resolution. It is clear from the literature that small-bodied organisms such as those comprising the zooplankton which a) feed upon a highly isotopically variable diet (e.g. Jones et al., 1999; Grey et al., 2001), and b) have rapid growth and tissue turnover (Lampert & Sommer, 1987 and refs. therein) will exhibit rapid isotopic turnover (e.g. Grey, 2000; Grey et al., 2001) and indeed may vary considerably in their stable isotope values over an annual cycle (~20‰, Harrod & Grey, 2006). However, the majority of such isotopic variability is caused between summer and winter from switching diets (e.g. Grey et al., 2001; Harrod & Grey, 2006) and so by sampling across seasons, I should have encompassed the majority of isotopic change likely to occur. To test the assertions of Hoeinghaus and Zueg (2007) and examine how influential a bias of restricted seasonal sampling might be, I calculated all the metrics for Wroxham and Salhouse (Table 4.3).

There was clearly an influence of season on the calculated metrics, particularly TA and CR, and as hypothesised it appeared to be the primary consumers such as chironomids and

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zooplankton that exerted the most influence on temporal metric variability. For example in the Wroxham food web, the high variability of chironomid (*C. plumosus*) δ^{13}C values caused expansion of the convex hull along the carbon axis and resulted in considerable change in the calculated total niche space of the food web (TA). This was manifest in increasingly depleted δ^{13}C values as the seasons progressed possibly as a result of feeding mode plasticity (e.g. methane utilisation; Grey et al. 2004; Jones et al, 2008). As isotopic tissue turnover time is typically longer in fish they are less isotopically variable over temporal scales than macroinvertebrates (Jardine et al 2006). Thus, the most ^{13}C-enriched fish species served as a relatively stable 'anchor' and expansion and contraction of CR occurred depending on the position of chironomids relative to fish. Changes in NR are likely to occur in the same way, in that the trophic position of top piscivores is unlikely to change significantly; this is particularly true of species such as pike. Thus, it is likely to be variability in primary consumers at the base of the food web that results in significant change in NR (and TA). One example of this was provided in the Salhouse food web where chironomid (*C. plumosus*) δ^{15}N increased by ~ 3‰ over the three sampling events, despite <1‰ variability in terms of δ^{13}C over the same temporal period (see Salhouse results). Zooplankton exerted a similar influence on TA via increases in both NR and CR (Table 4.3), likely as a result of selective feeding on a variable resource. For example, feeding on phytoplankton fractions such as the cyanobacteria with highly variable δ^{13}C or δ^{15}N (shown to vary seasonally up to 8 ‰ and 4‰, respectively, Vuorio et al. 2006) could result in such changes. The influence of zooplankton on the metrics calculated for July was interesting as it resulted in a large area of the convex hull that is effectively 'dead' niche space. It was also considerably influential on other metrics and in particular MNND and SDNND. This illustrates well the problems that could potentially arise from sampling programmes that fail to account for seasonal

variability; in this case a gross over-representation of the total trophic diversity of the food web (see Figure 4.7).

Chapter 5

Cross ecosystem boundary transfer of organotin contaminants mediated by emergent imagos

5.1 Paper submitted to *Oikos*

The following chapter is presented in the format required for submission to the journal *Oikos*. At the time of thesis submission, the paper is still under review.

To briefly recap on my aim for this final part of my thesis, I have already established that chironomid larvae tend to dominate the benthic sediments of the Norfolk Broads and that those chironomids from broads with sediments containing measurable concentrations of organotins also carry an organotin burden (Chapter 3). Despite some evidence that chironomids may be able to metabolise organotins (Chapter 3), the detectable quantities I have measured in larval biomass has the potential to be transferred from the aquatic to the terrestrial environment as the chironomids undergo emergence to the adult winged imago life stage.

Therefore, using a combination of stable isotope analyses and organotin analyses I will examine the potential for chironomids to act as vectors of the contaminants across the aquatic-terrestrial interface via predation by riparian spiders. Hypothesis: *There will be a positive correlation between the OTC burden found in spiders collected from the immediate riparian and the degree to which they are subsidised by aquatically derived prey*

No such thing as a free meal: organotin transfer across the aquatic-terrestrial interface

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Running head: Contaminant transfer from aquatic to terrestrial consumers

Summary

The detrimental effects of the organotin tributyltin (TBT) have been well documented globally, from the level of the individual to the community. However, work has focused on marine systems overlooking the fact that TBT was also applied widely in freshwaters. We used the Norfolk Broads, a series of shallow lakes in the UK, as a model system to examine the wider ecological ramifications of organotin contaminants, in particular the potential for export across the aquatic- terrestrial interface mediated by emergent chironomids to terrestrial predators such as spiders.

A combination of spider and chironomid stable isotopes (principally $\delta^{13}C$) and isotope mixing models were used to assess the contribution of chironomid carbon to spider biomass from around four lakes; two with a contaminant history, and two without. Mixing model outputs indicated considerable chironomid contribution to spider biomass at all four sites (34-88%). Organotin analyses revealed consistent butyltin (dibutyltin; ~7.6 ng g^{-1} DBT) contamination in chironomids from the most contaminated site (assessed by sediment concentration). Further, organotin analyses of spiders revealed similar concentrations of DBT to their chironomid prey. There was a significant, positive relationship between the contribution of chironomid to spider biomass and total butyltin burden in spiders.

To determine contaminant biomagnification, we used $\delta^{15}N$ values of sediments, chironomids, and spiders against respective DBT concentrations. Spider and chironomid butyltin burdens were statistically indistinct, and thus, there was a significant negative relationship between increasing trophic position and DBT contamination suggesting no biomagnification occurred in this short chain. These results are evidence that despite ~ 25 years elapsing since the last

known TBT use, butyltin contamination stemming from sediments are still mediated across ecosystem boundaries to terrestrial predators via emergent chironomid imagos.

Introduction

For over a century, aquatic ecologists have recognised the importance of terrestrially derived allochthonous subsidies and much research has focussed on the unidirectional flow of material such as senescent terrestrial plant matter inputs to both lotic and lentic ecosystems (Jansson et al, 2007). A significant proportion of productivity in the recipient ecosystem may be supported via detritus-driven food chains. For example, Grey et al (2001) demonstrated that crustacean zooplankton derived up to 40% body carbon from terrestrially derived particulate organic matter (POM) in the pelagic food web of Loch Ness. However, with a more holistic view of aquatic–terrestrial linkages recently, ecologists have identified the reciprocal transfer of aquatic subsidies to terrestrial systems, and begun to examine the complex interactions which they often support. An aquatic subsidy may impact the functioning of terrestrial food webs across multiple trophic levels; Pacific salmon form a direct subsidy for terrestrial predators (Ben David, 1997; Gende et al, 2002) but they also subsidise terrestrial primary producers and consumers via more indirect pathways such as nutrient recycling resulting from carcass decomposition (Cederholme et al, 1999; Helfield et al, 2001).

Aquatic subsidies to terrestrial ecosystems can also alter the dynamics of trophic interactions, often with 'cascading' effects. Subsidies that drive resource based 'bottom up' processes can result in increased production of higher consumers subsequently increasing the strength of 'top down' control exerted on intermediate trophic levels. Thus, primary production of the recipient terrestrial system may be increased. Indeed, Henschel et al (2001) were able to demonstrate that herbivory on nettles, (principally by leaf hoppers, Cicadellidae) was reduced in riparian microhabitats compared to more landward areas; a spider population subsidised by

emergent aquatic chironomids was able to exert increased top down control on leafhoppers, thereby depressing herbivory to a greater extent than spiders living further from the stream edge.

Emergent aquatic invertebrates represent perhaps the most conspicuous of freshwater subsidies as imagos are often highly abundant and flight period is tightly synchronised. In a summary of 20 studies from Europe, Canada, the USA and Africa, Jackson & Fisher (1986) reported an average emergence of 10,000-20,000 individuals m^{-2} yr^{-1}. Imagos can constitute a significant proportion of benthic secondary production. For example, from a desert stream in Arizona, Jackson & Fisher (1986) measured annual benthic production as 120 g m^{-2} yr^{-1}; ~ 20% of this total emerged from the stream as adult imagos. Productive shallow lakes such as Lake Esrom (Denmark) and Lake Myvatn (Iceland) contain considerable benthic secondary production (respectively, 35.1 g C m^{-2} yr^{-1}, Jonasson et al. 1990; 670 Kj m^{-2} yr^{-1}, Lindegaard & Jonasson, 1979), and it is the chironomids that often form a significant proportion of both production and emergence, typically 50-80%. The synchronicity of such hatches potentially represents a 'pulse' of aquatic-derived production to adjacent terrestrial habitats.

Post emergence, imago biomass may be preyed upon by a broad range of terrestrial consumers including adult odonates (Sukhacheva, 1996), spiders (Collier et al, 2002; Kato et al, 2003), mammals (Power & Rainey, 2000), and birds (Ormerod & Tyler, 1991, Murakami & Nakano, 2002). Until relatively recently, the magnitude of the impact of the subsidy on receiving food webs was poorly understood. However, an increasing body of literature biased toward stream ecosystems (but see Gratton et al, 2008) documents the influence of imagos on various aspects of consumer ecology revealed primarily by the application of stable isotope tracer techniques (e.g. Collier et al, 2002; Kato et al, 2003; Paetzold et al, 2005).

Advances in the field have been aided considerably using measures of the natural abundance (and to a lesser extent artificial enrichment) of stable carbon isotopes (^{13}C and ^{12}C; the ratio of the two reported as $\delta^{13}C$). Aquatic and terrestrially derived carbon sources typically exhibit markedly different $\delta^{13}C$ values due to differences in the source CO_2 pools of terrestrial and freshwater ecosystems, and subsequent fractionation by primary producers (Peterson & Fry, 1987). Thus, if material is transferred across ecosystem boundaries, then the distinguishable $\delta^{13}C$ should allow that material to be traced with confidence (e.g. Grey et al, 2001). Mathematical mixing models allow for the quantitative assessment of the contribution of two or more carbon sources to a given consumer (e.g. Phillips & Gregg, 2001).

The larval instars of many chironomids inhabit profundal sediments throughout much of their life history. Such sediments can act as a sink for anthropogenic contaminants (Burton, 1991) and thus we might predict that larvae have increased exposure and potential to accumulate persistent contaminants (Menzie, 1980; Runck, 2007). Following emergence, imagos may vector accumulated contaminants across ecosystem boundaries, where subsequent predation results in the transmission of the contaminant burden to the terrestrial food web. Studies of cross-ecosystem, trophic transfer of contaminants are relatively rare (but see Echols, 2004 and Walters et al, 2008; 2009), but obviously essential if we are to correctly identify contaminant fate and before we contemplate their management.

The organotin tributyltin (TBT) has been described as the most toxic substance ever deliberately introduced to the aquatic environment (Goldberg, 1986). The widespread use of TBT as an antifoulant applied to boat hulls in the late 1970s – early 1980s resulted in a number of well documented, examples of immune system toxicity and endocrine disruptions in non-target organisms. TBT was responsible for the virtual collapse of oyster (*Crassostrea*

gigas) production in western France as a result of shell thickening and reduced spatfall (Claisse & Alzieu, 1993); and in southwest England, TBT induced masculinisation (imposex) of female dog whelk (*Nucella lapillus*) resulting in serious population decline and local extinctions (Bryan et al, 1986); imposex induction as a consequence of exposure to TBT was later described in at least 72 gastropod species globally (Oehlman, 1991). Deleterious effects were also documented in vertebrates, such as fish (O'Halloran et al, 1998) and mammals (Omura et al, 2001). In light of this research, restrictions were imposed on the use of TBT antifoulants (1987 in the UK), banning their application on craft <25m in length.

Although the effects of TBT-based antifoulants on marine ecosystems are well documented, there have been relatively few studies of the effects of TBT in freshwaters (see Kannan 1997; Tessier et al, 2007). There has been a tendency to detail its occurrence or estimate trophic transfer between aquatic consumers only (Stab et al. 1996; Traas et al. 1996). Such research is undoubtedly valuable, yet thus far has overlooked the potential for TBT to be transferred across the aquatic – terrestrial ecosystem boundary, where its potential impacts on recipient terrestrial system are unknown.

In light of this, we aimed to trace the transfer of organotins from an aquatic ecosystem source into terrestrial consumers mediated via emerging insects in the Norfolk Broads, an interconnected shallow lake system in eastern England. TBT was used on pleasure craft on the Broads from 1960 until the ban in 1987. Consequently, a considerable sediment-bound legacy of TBT has been reported (Sayer et al, 2006). The productive nature of the broads supports high densities of chironomids (largely *Chironomus plumosus*), and a further characteristic of these shallow ecosystems is the dense fringe of *Phragmites* spp. and alder carr (*Alnus glutinosa*) that forms the littoral margins, providing ideal habitat for web-building

spiders such as *Tetragnatha* spp. known to specialise on aquatic prey (Yoshida, 2002). Given that *C. plumosus* is the most abundant component of the zoobenthos in the majority of the broads, we hypothesised that spiders would readily prey upon their imagos throughout the emergence period. We further hypothesised that those chironomids emerging from sites contaminated with organotins would carry with them an organotin burden, which would subsequently be reflected in spiders via trophic transfer. Thus dependence on larval biomass could present a paradox to the spider, whereby an important nutrient resource subsidy also represents a source of aquatic-derived contaminant. We tested these hypotheses at four sites in the Norfolk Broads: two control sites with no boating access and thus no history of organotin contamination; and two sites which have previously yielded high sediment-bound concentrations of TBT. We used stable isotope analysis to estimate larval contribution to spider biomass coupled with measures of organotins.

Methods

Sampling locations

The main physical and chemical parameters of the four study sites are summarised in Table 1. Cromes Broad is situated on the River Ant and connected by a fragmented network of small dykes. The lake has been non-navigable throughout its history. Cockshoot Broad is isolated from the River Bure by a damned dyke and has been non-navigable for >20 years. Malthouse and Ranworth broads are situated adjacent to one another and are connected via a gated dyke which currently renders Ranworth Broad non-navigable; both broads are connected to the tidal River Bure via Ranworth Cut. Ranworth Broad is a designated nature reserve with Site of Special Scientific Interest status.

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Chironomids and spiders

Chironomus plumosus were collected (March, June, October 2007, March 2008 at all sites, and June 2008 at Malthouse broad only) from profundal sediments using an Ekman grab to determine seasonal variability in isotope values. Spiders (*Tetragnatha* spp.) were collected at roughly two week intervals from April to early August, then monthly until early October 2008, from littoral habitats and the riparian zone and were sampled no further than 2m landward from lake margins. Spiders were either detected visually and removed using forceps, or vegetation was shaken vigorously over beating trays. Following capture, spiders and chironomids were immediately placed into hexane rinsed glassware to eliminate contamination.

Stable isotope analyses

Chironomid larvae were placed in tap water and were allowed up to 24 hrs for clearance of gut content (Feuchtmayr and Grey, 2003). Three replicate samples of five pooled individuals from four sampling occasions (n=12) were used to calculate annual means. Tetragnathids are suctorial, which precludes the necessity for gut clearance, and so spiders were frozen immediately on return from the field. Spider and chironomid tissues were dried at 65°C for a minimum of 48hrs before being homogenised using an agate pestle and mortar. Samples were weighed (0.6 ± 0.05 mg) into tin capsules and were then analysed for stable carbon and nitrogen isotope composition using an elemental analyser coupled to a continuous flow

isotope ratio mass spectrometer (Thermo-Finnigan, Delta Matt Plus) in house at Queen Mary, University of London. Results are given using the standard δ notation:

$$\delta = [(X_{sample} / X_{reference}) - 1] \times 1000$$

expressed in units of per mille (‰) where $X = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Reference materials were international standards of ammonium for nitrogen, and sucrose for carbon. A secondary internal standard of a known relation to international standards was run every ten samples to provide a measure of instrumental precision ($\pm$ 0.15‰ for $\delta^{13}C$ and $\delta^{15}N$).

Data analyses and isotope mixing models

A dual-source, single isotope mixing model was used to estimate the percentage contribution of chironomid carbon to spider biomass (Phillips & Gregg, 2001). In accordance with Doi et al (2007), we assumed no significant change in $\delta^{13}C$ between fourth instar *C. plumosus* larvae and adults. A mean terrestrial insect $\delta^{13}C$ value (4 different families; $n = 14$ derived from all sites) was used as the opposite end-member to chironomid carbon, with spiders assumed to be a mixture of the two. We assumed no fractionation between dietary and spider $\delta^{13}C$ as little dietary enrichment has been demonstrated previously in fluid feeding consumers (McCutchan et al, 2003). If spider carbon exceeded the limits of the model in a particular direction, then the contribution was capped at 100%. Normality and equality of variances were ascertained for all data by performing Anderson-Darling and Levene's tests, respectively. As terrestrial insect $\delta^{13}C$ data were non-normal and there was unequal variance between isotopic signatures of terrestrial and chironomid $\delta^{13}C$ non-parametric Kruskall – Wallis tests were used in place of ANOVA. Regression analyses were used to examine the

relationship between chironomid contribution to spider biomass and total butyltin burden in spiders and $\delta^{15}N$ and butyltin concentrations in sediments, chironomids and spiders. In spider samples where butyltins were not detected, 50% of the LOD for the relevant sample was assumed for statistical purposes (Murai et al, 2008). An unpaired Students t – test was used to test for differences between spider and chironomid dibutyltin (DBT) concentrations. All statistical analysis was conducted using Minitab 15 (Minitab inc. 2007), with a significance level of ≤ 0.05.

Organotin analyses

Analyses of the organotin compounds tributyltin (TBT) and triphenyltin (TPhT), and the di-, and mono-substituted daughter compounds (DBT, MBT, DPhT, and MPhT, respectively), were carried out at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) laboratory facility at Lowestoft, Suffolk. Briefly, samples were homogenised then, in accordance with methods developed by Waldock et al (1989), organotin compounds were extracted from the sample matrix (either tissues or sediments) by addition of sodium borohydride and methanol, converted to hydrides and partitioned into hexane. Derivatives were then analysed by gas chromatography with flame photometric detection (GC-FPD). Quality control in each sample batch included an analytical blank; a certified reference material (CRM 477) spiked with target compounds; and a Response Factor sample analysed prior to every three environmental samples. Each sample contained a tripropyltin (TPT) internal standard. All quantification was calculated through comparison of the known concentration of the standard. No contamination was detected in any analytical blank. Limits of detection for the method were approximately 2ng g^{-1} for butyltin species and 14ng g^{-1} for

phenyltins. Relative Standard Deviations (RSDs) of the Response Factor sample heights were < 15 % for butyltins for all sample runs. Organotin concentrations are expressed in terms of the cation (TBT$^+$, DBT$^+$ and MBT$^+$) as ng g^{-1} wet weight (ww). One bulked sample of chironomids was analysed from a total of four sampling events (each site: n=4). Single bulked samples of spiders were analysed from a total of eight sampling events at Malthouse (n=8) and Cromes (n=8). Reduced sampling events at Ranworth and Cockshoot meant that n=5 at both these sites. Due to the large amount of biological material required for organotin analysis (~3g wet weight) replication was not possible.

Results

Variability in chironomid δ^{13}C

The variability in *C. plumosus* tissue δ^{13}C did not exceed 10‰ at either Ranworth or Malthouse on any sampling occasion (Fig 2). Annual mean (±SD) chironomid δ^{13}C was -34.1 ± 3.64‰ and -34.46 ± 3.75‰ from Malthouse and Ranworth Broads, respectively. A similar range of values were evident at Cromes and Cockshoot although larval tissue δ^{13}C variability was reduced by approximately 5‰ relative to conspecifics at contaminated sites (-33.76 ± 2.29‰ and -30.97 ± 1.83‰, respectively). Terrestrial insect δ^{13}C values (-26.3 ± 0.7‰) did not vary more than 3‰ and were isotopically distinct from chironomids (P<0.05).

Contribution of chironomid biomass to spiders

Spider δ^{13}C values spanned 6‰ among sites, tending to increase throughout the study (Table 2). At all sites, the relative contribution of the aquatic subsidy to spider biomass derived from

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chironomid imagos was of considerable importance and was greater from April through to early July, typically between 80 to 100% (Fig 3). Thereafter, the importance of the subsidy generally decreased. This decrease was least evident at Cromes where the proportion of aquatic-derived carbon remained >70% throughout the sampling period. Spiders from around Cockshoot reverted to terrestrial carbon rather rapidly during July as the proportion of aquatic-derived carbon dropped from >90% to <40%. Spiders from Ranworth initially appeared atypical in that the contribution was lower but the temporal pattern was similar in form, and it is likely that the peak contributions may have been missed as sampling commenced later at this site in comparison to Malthouse and Cromes (Fig 3).

Organotin analyses

No quantifiable concentrations of phenyltins were found in any sample, and no butyltins were quantified in chironomid larvae from Cromes or Cockshoot. Chironomids from Ranworth exhibited quantifiable concentrations of butyltins in one sample only (3ng g^{-1} DBT). All chironomid samples from Malthouse broad yielded quantifiable concentrations of DBT; the mean (±SE) being 7.8 ± 0.6ng g^{-1}.

Butyltins were not detected in spiders from the control sites, Cromes and Cockshoot. Spiders from around Ranworth yielded a quantifiable concentration of MBT (15 ng g^{-1} wet weight) only in late August. In contrast, spiders from around Malthouse contained quantifiable concentrations of both MBT and DBT. Thus, total butyltin burden in spiders from Malthouse in April was 14ng g^{-1} (MBT= 8ng g^{-1}, DBT = 6ng g^{-1}), 23 ng g^{-1}in May (MBT= 16ng g^{-1}, DBT = 7ng g^{-1}), and 19 ng g^{-1} (MBT= 11ng g^{-1}, DBT = 8ng g^{-1}) in June. Spiders collected later yielded diminishing concentrations of MBT only. There was a significant positive

relationship between aquatic subsidy measured as chironomid-derived carbon contribution to spider biomass and the total butyltin burden of spiders ($P < 0.05$; Fig 4).

Discussion

To trace the incorporation of aquatic-derived carbon mediated via chironomid imagos into terrestrial spider biomass using stable isotope analyses and therefore test our first hypothesis, aquatic prey and terrestrial prey needed to be isotopically distinct. The terrestrial prey $\delta^{13}C$ values (-26.3 ± 0.7‰) were remarkably invariable (Fig 2) reflecting consumption of C3 plant material, typically around -28 to -26‰ (Peterson & Fry, 1987). Chironomid larval $\delta^{13}C$ revealed some temporal variability at both contaminated and control sites (~10‰ and ~5‰, respectively). This is fairly typical of chironomids over an annual cycle, even in relatively shallow lakes that do not undergo seasonal stratification (Grey et al 2004b) and is certainly not indicative of the incorporation of substantial methane-derived carbon into larval biomass which can induce seasonal shifts in larval $\delta^{13}C$ of up to 40‰ in *C. plumosus* (Grey et al 2004a). More importantly however, mean larval chironomid values were consistently ^{13}C-depleted relative to terrestrial insects throughout the study period by at least 5‰ allowing us to trace the transfer of chironomid carbon into the terrestrial food web and model the contribution of the aquatic subsidy to spiders with confidence.

Our first hypothesis that chironomid carbon would contribute to spider biomass following emergence was supported well by mixing model outputs; spiders obtained between 34-88% of their biomass from imagos across the four study sites. Patterns of chironomid contribution to spider biomass were similar from June to September at all sites, indicating a temporal decrease in the importance of this subsidy to spiders. Hence, it seems likely that earlier in the

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season spiders from around Ranworth and Cockshoot would have been subsidised by chironomids to a similar degree as conspecifics from Malthouse and Cromes.

Our data are remarkably similar to several other studies that span a range of geographical locations and habitats. In New Zealand, Collier et al (2002) reported that an average of 58% of spider carbon was obtained from in-stream sources. Similarly, spiders from the riparian zone of a desert stream in Arizona (USA) were found to be subsidised up to 100% by aquatic insects (Sanzone et al. 2003). Fewer studies have examined these relationships between lentic systems and adjacent terrestrial habitats; however those that do report similar patterns. For example, Walters et al (2009) estimated emergent invertebrate contribution to spider biomass up to 92% in Lake Hartwell (South Carolina, USA), and Gratton et al (2008) reported increased spider utilization of aquatic-derived carbon in subarctic Icelandic lakes.

The high spider dependence on chironomid biomass (as inferred from mixing models) early in the season could be seen as evidence for the temporal peak in aquatic invertebrate emergence. At Cromes this peak occurred ~14 days earlier than at Malthouse. Temperature is known to be a key environmental variable triggering chironomid emergence (Pinder et al 1991). Given that Cromes is shallower and smaller, a plausible explanation for this difference in respective peaks of emergence could be that the critical 'trigger' temperature was reached earlier.

The increases in chironomid contribution to spider biomass during late summer at Malthouse, Ranworth, and Cockshoot can be seen as evidence for a smaller, secondary, peak in chironomid emergence which is not surprising as many species are bi-voltine. Indeed, Mason (1977) inferred a similar pattern from the relative abundance of different *C.plumosus* instars at nearby Alderfen Broad. This secondary pulse of chironomid contribution to spider biomass

was absent at Cromes. Sampling from this site consistently revealed not only high densities of homogenously distributed *C. plumosus* but numerous other chironomid larvae associated with the dense macrophyte stands (largely *Ceratophyllum demersum*) that characterise the pelagic habitat. Macrophytes are known to support high abundance and diversity of chironomids via associated epiphyton biomass (Balci & Kenndey 2003), and provision of refugia from key predators such as invertivorous fish (Hershey, 1985). Such factors may increase the abundance and diversity of aquatic-derived prey, accounting for the consistently higher dependence of spiders on aquatic biomass at this site. In contrast, macrophytes were absent from Malthouse; thus, sampling of profundal sediments yielded high densities of *C. plumosus* larvae only and we assume that this species were the dominant aquatic contributor to spider biomass at this site.

We hypothesised that chironomids emerging from organotin contaminated sediments would carry a contaminant load across the aquatic-terrestrial interface where it would be reflected in spiders via trophic transfer. Generally, results from Malthouse supported this hypothesis as there was a significant positive relationship between chironomid biomass contribution to spiders and spider butyltin burden. The lack of butyltins in spiders from Ranworth is unsurprising, given that butyltins were detected only once in *C. plumosus* and at a concentration close to the limit of detection. Furthermore, spiders were not sampled during the time at which they were likely to be most heavily subsidised by emergent biomass.

There are a number of subtle features in our data that offer insight into the nature of butyltin contamination, the factors which control it, and the subsequent exposure implications for biota. For example, DBT concentrations in sediments and chironomids were separated by an order of magnitude. Butyltin compounds have a log organic carbon partition coefficient (log

K_{oc}) of ~4.2-5.0 (Meador et al, 2002); values indicative of a high affinity for organic carbon. In addition, sediments from the Norfolk Broads are extremely rich in organic matter, comprising up to 40% total organic carbon (TOC) (J. Laws unpublished data). Thus, butyltin compounds are bound strongly to organic carbon in sediments, which probably reduces bioavailability to benthic primary consumers such as chironomids. Indeed, various authors have demonstrated a negative relationship between organism butyltin concentration and sediment TOC (e.g. chironomids - Looser et al, 1998; marine polycheates - Meador et al, 1997). Despite high concentrations of TBT in Malthouse sediments ($1368 \pm 6.3 ng\ g^{-1}$), chironomids contained no quantifiable concentrations of the parent compound. However, the presence of DBT and MBT in biota is often associated with the step-wise biological degradation of TBT. Experimental evidence has demonstrated that members of the genus *Chironomus* (e.g. *C. riparius*) have an efficient metabolic capacity for TBT degradation (Looser et al, 1998; Looser et al, 2000). Thus, the low concentrations of butyltins in chironomids observed here could be a combination of abiotic factors such as sediment TOC, and a metabolic capacity for de-butylation of TBT. These two processes could be important, effectively reducing the amount of butyltins transferred to terrestrial habitats, thereby limiting concentrations available for uptake by predators via trophic transfer.

Some groups of organic contaminants have a tendency to biomagnify from their source through subsequent trophic levels. For example, Kidd et al (2001) and Power et al (2002) have demonstrated clear patterns of contaminant biomagnification with increasing trophic position (as measured by $\delta^{15}N$) of DDT and mercury in aquatic food webs. However, organochlorines such as DDT are both highly persistent and have a high affinity for lipids; in contrast, butyltins are moderately lipophilic and relatively unstable (Sun et al, 1999). Coupled

with differences in species-specific butyltin metabolism, this means they are less predictable in terms of biomagnification potential (see Takahashi *et al*, 1999).

Using $\delta^{15}N$ values of sediments, chironomids, and spiders against respective DBT concentrations we were able to test whether DBT concentrations increased in response to trophic level (as measured by $\delta^{15}N$; Fig 5.) Despite ^{15}N-enrichment of spiders relative to chironomids, concentrations of DBT were statistically indistinct (unpaired *t*-test, $t = 0.88$ *P*, <0.05). Sediments exhibited the lowest $\delta^{15}N$ but highest DBT concentrations (Fig 5). Regression analysis revealed a significant negative relationship (Fig 5; *P* <0.05) between $\delta^{15}N$ and DBT concentrations, suggesting that biomagnification did not occur in this short food chain.

Conclusions

Various studies across a range of geographical habitats have demonstrated the importance of chironomids as a subsidy to terrestrial spiders. However, the majority detail this aquatic-terrestrial linkage in lotic systems (but see Walter et al, 2009). Our results extend this pattern to a shallow eutrophic lake system in Eastern England. In our study, dependence on chironomids emerging from contaminated sediments resulted in a butyltin burden in spiders. We could not find any data on the lethal and sub-lethal effects of butyltins on terrestrial invertebrates but there is a wealth of experimental data for a wide range of aquatic organisms (see Eisler, 1989). Concentrations at which lethal and sub lethal effects are recorded are typically higher than those we observed in chironomids and spiders from the Norfolk Broads; indeed, the 96h LC50 (50% mortality in 96h) for *C. plumosus* is reported as ~ 50 ng g^{-1} (USA

EPA). Combined with an apparent lack of biomagnification potential between sediment, chironomid and spiders (which is not unusual for butyltins e.g. Hu et al, 2006; Murai et al, 2008), then terrestrial biota may not be exposed to hazardous concentrations of butyltins. However, it may be prudent to consider life-stage because many invertivorous birds such as swallows (Hirundinidae) prey upon chironomid imagos and spiders and target their breeding to coincide with mass emergence of insects to better provision for their young. Chicks and juveniles are more vulnerable to contaminant exposure due to their small body size and high ingestion rate (Walters et al 2009). This has been previously documented in tree swallows (*Tachycineta bicolour*), where polychlorinated biphenyl (PCB) concentrations were up to three fold higher in young than adults, principally as a result of extensive feeding on emerging diptera (Echols et al 2004).

Twenty five years have elapsed since TBT based antifoulants were last (legally) applied to craft navigating the Norfolk Broads. Despite this, a considerable sediment bound legacy of the contaminant still exists, and is quantifiable not only in aquatic biota, but also in terrestrial predators such as spiders. In addition this research highlights the potentially long lasting implications of the application of persistent, bioaccumulative and toxic organic compounds in the aquatic environment.

References - removed

Tables

Site	SA (ha)	Z_{mean} (m)	Mean TP (ug l^{-1})	Mean NO$_3$-N (mg l^{-1})	Secchi Depth (m)	Mean TBT (ng g^{-1} ± SE)	Mean DBT (ng g^{-1} ± SE)	Plant richness
Cromes Broad	2.3	0.58	59	0.2	0.58	ND	ND	5
Cockshoot Broad	5.1	1	48	0.01	0.92	ND	ND	4
Malthouse Broad	10	1.2	*	*	*	1368 ± 6.3	266.3± 10.9	2
Ranworth Broad	29.2	1.3	72	0.7	0.8	234 ± 3.4	78.3± 3.8	3

Table 1. Limnological characteristics of the four study sites in the Norfolk Broads: surface area (SA) and mean annual values (n = 22 each site) for total phosphorous (TP), total nitrate, Secchi depth and plant richness. * indicates data not available. TBT data are mean values derived from n=3 from the current study; ND indicates TBT not detected. Physiochemical data for Wroxham, Ranworth and Cockshoot Broads provided by

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the Environment Agency, and from Sayer et al, 2009 for Cromes Broad. Plant species richness refers to number of species of submerged and floating-leaved macrophytes found (Cromes, and Cockshoot data from Sayer et al 2009, remainder from current study).

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Site	Mid-Apr	Late-Apr	May	June	Jul	Aug	Sep	Oct
Cromes Broad	-33.6 ± 0.8	-33.3 ± 0.7	-33.1 ± 0.8	-32.9 ± 0.2	-32.4 ± 0.5	-31.8 ± 1.1	-31.5 ± 1.0	-31.2 ± 1.3
Cockshoot Broad	*	*	*	-31.2 ± 0.7	-30.7 ± 0.4	-27.9 ± 1.6	-28.7 ± 1.6	-28.1 ± 0.6
Malthouse Broad	-32.8 ± 1.0	-33.7 ± 1.0	-32.7 ± 0.7	-32.6 ± 0.7	-31.4 ± 0.2	-29.9 ± 0.3	-30.7 ± 0.4	-30.7 ± 0.6
Ranworth Broad	*	*	*	-31.1 ± 0.4	-29.1 ± 0.5	-28.8 ± 0.3	-28.8 ± 0.3	-29.7 ± 0.2

Table 2. Mean (±SD; $n = 5$) of Tetragnathid spider δ^{13}C (‰) from the four sample sites throughout the study period. These data were used in mixing models to estimate chironomid contribution to spider biomass.

Cromes   Isolated Broads
Ranworth   Connected Broads
Malthouse   Navigable Broads

N O R F O L K

R. Ant

R Bure

Cromes Broad

R. Thurne

N

Cockshoot Broad

Wroxham Broad

Ranworth Broad
Malthouse Broad

0   km   5

North
Sea

London
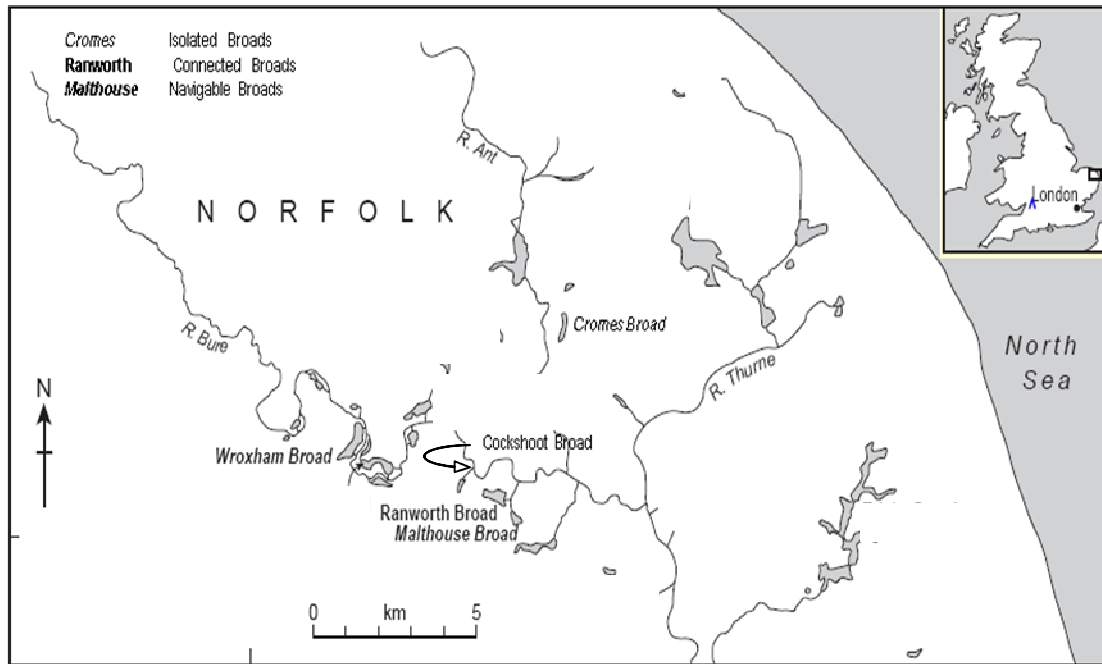
Figure 1. Map of the Norfolk Broads, showing main broads and the four study sites. Cockshoot Broad (River Bure) and Cromes Broad (River Ant) were control sites. Ranworth and Malthouse Broads (adjacent on River Bure) were contaminated study sites.
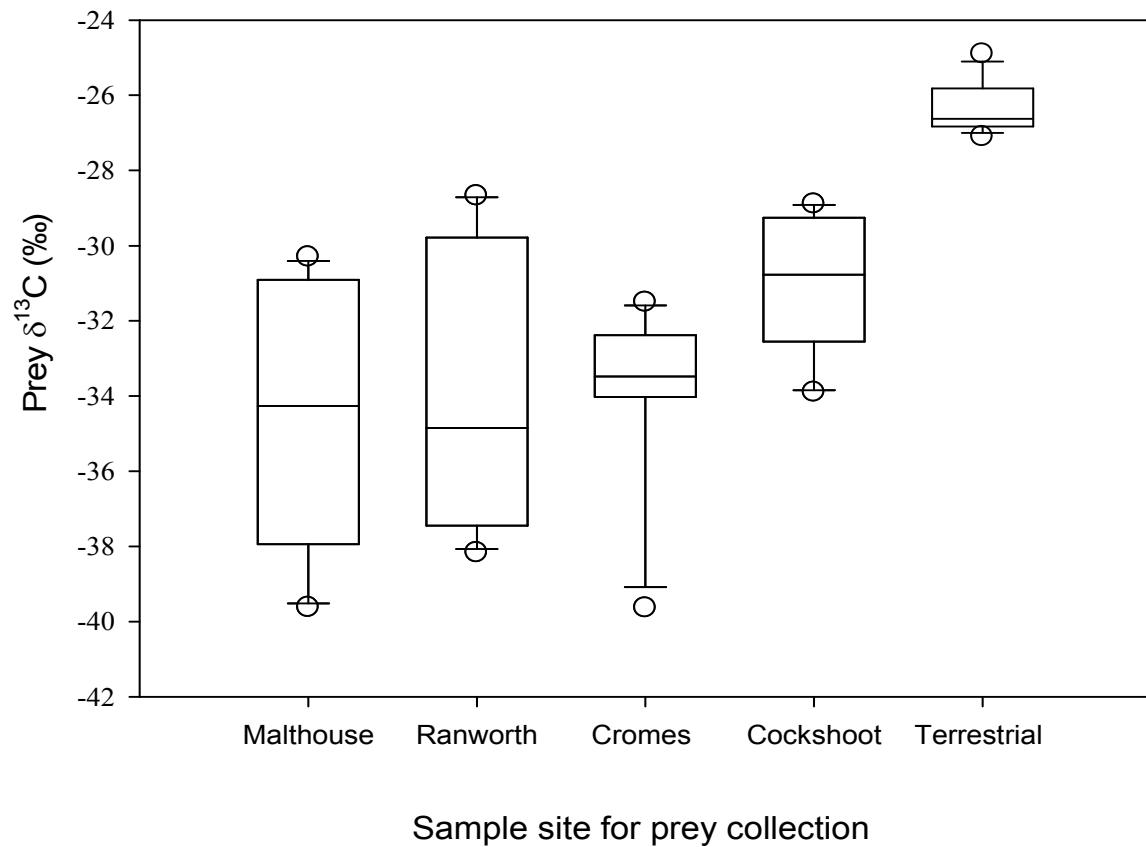
Figure 2. Box and whisker plots for larval *C. plumosus* δ^{13}C values collected during 2007-2008 from four Norfolk broads. Boxes represent inter-quartile range, the solid line represents the median, the whiskers minimum and maximum values, and outliers are included as open circles. Terrestrial insects from the riparian zone are shown for comparison.
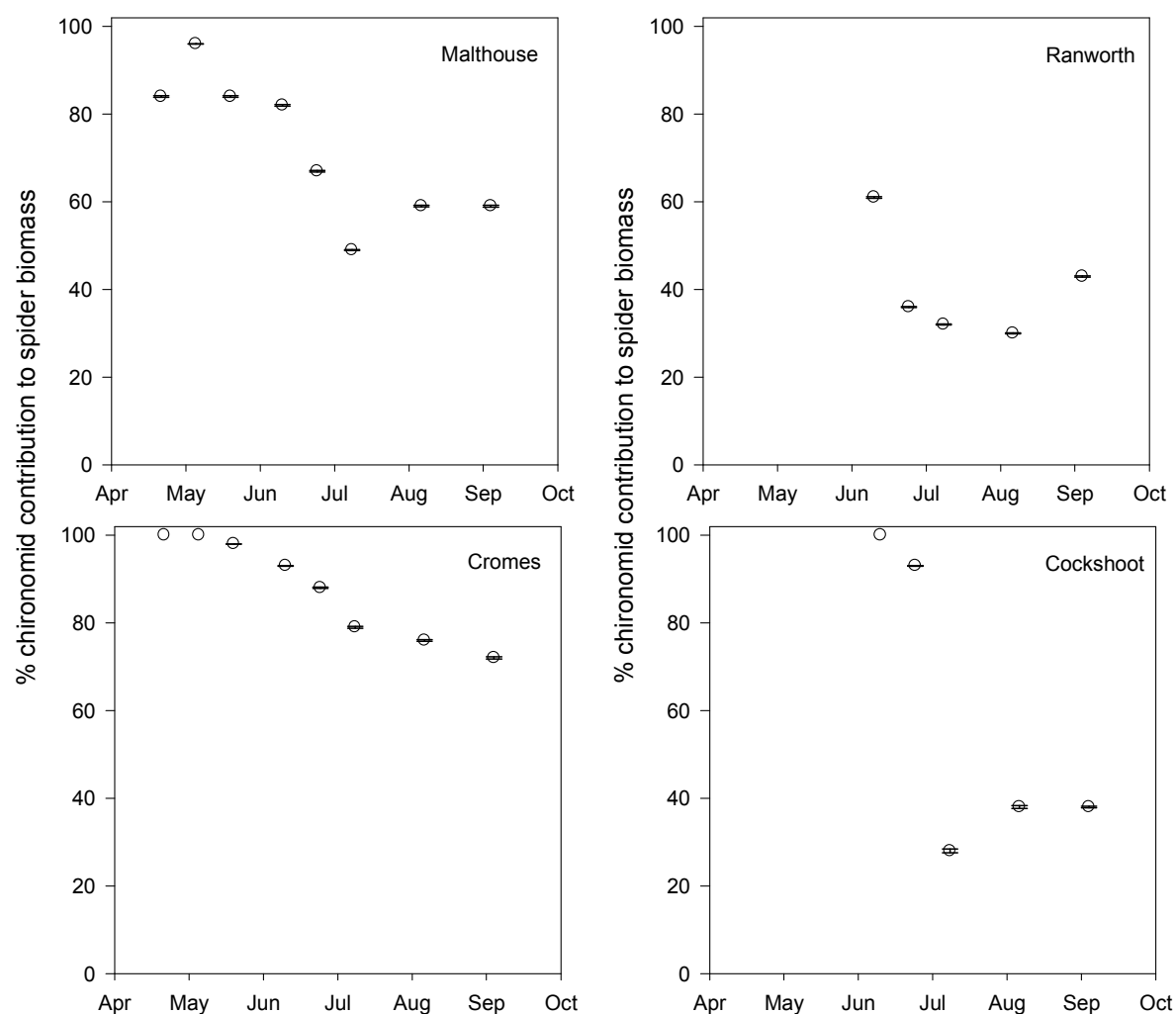
Figure 3. Mean percentage (± 95% CI) chironomid contribution to *Tetragnatha* spp. biomass during 2008 modelled from $\delta^{13}C$ values. Malthouse and Ranworth broads (top panels) are butyltin contaminated sites. Cromes and Cockshoot broads (bottom panels) are control sites.
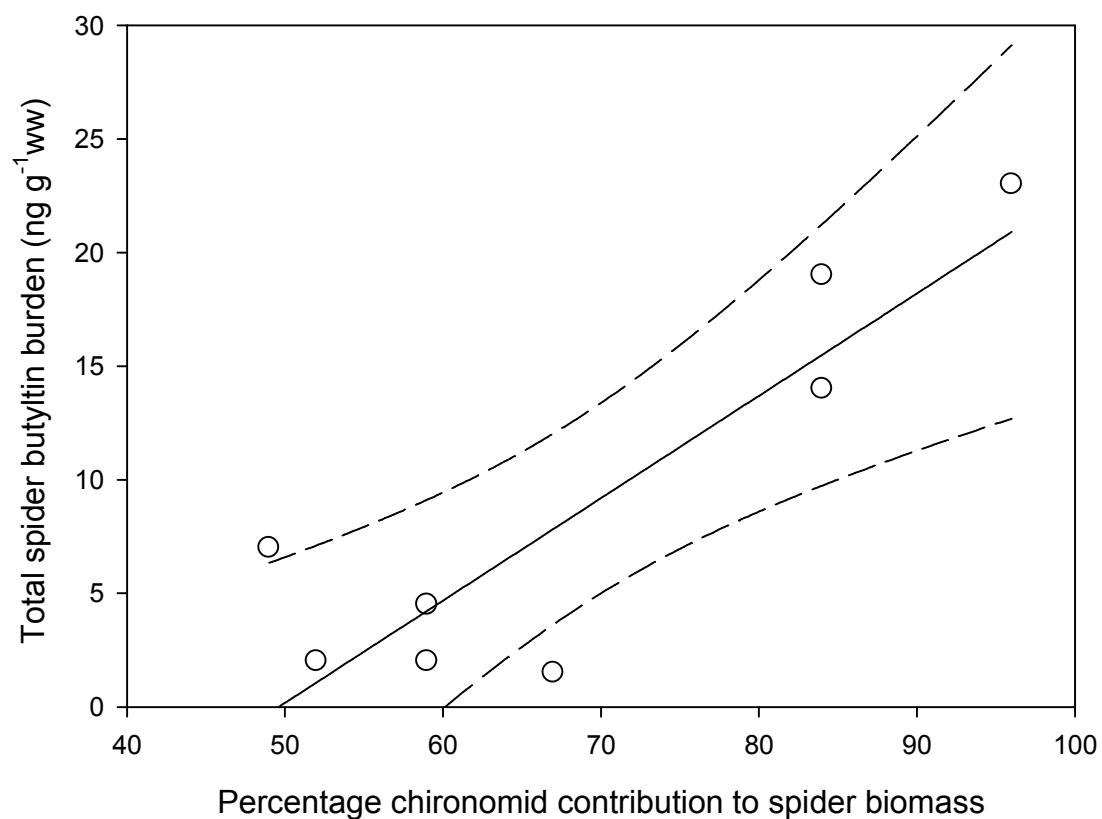
Figure 4. Relationship between total butyltin burden (MBT + DBT) in spiders and the percentage of chironomid contribution to spider biomass from Malthouse Broad. The regression line ($\pm$ 95% CI) was fitted by the equation y = 0.430x -22.49 (R^2 = 0.77; P < 0.05).
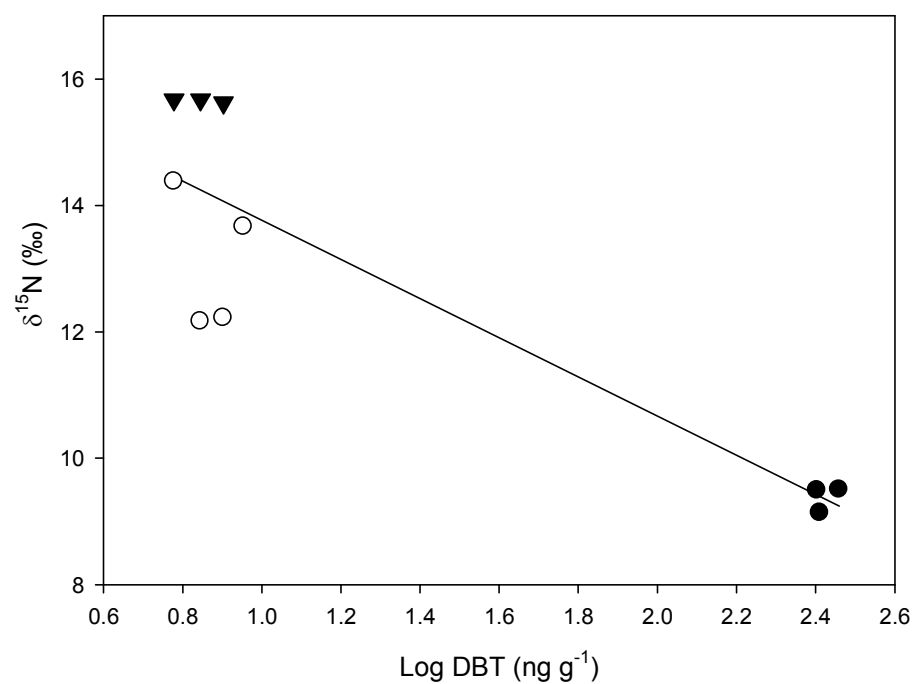
Figure 5. Relationship between $\delta^{15}N$ and DBT in sediments (filled circles), chironomids, (open circles) and spiders (filled triangles) from Malthouse Broad. The regression line was fitted by the equation $y = 3.1x + 16.86$ ($R^2 = 0.78$; $P < 0.05$).

Chapter 6

Synthesis

In this thesis, I have confirmed the continued presence of the organotin contamination legacy in the broads sediments previously unveiled by Hoare (2007) and further, that there has been no significant change in the ratio of TBT:DBT (i.e. negligible metabolic breakdown) over the four years between studies. Such slow degradation implies that organotin contamination will persist in these shallow lake ecosystems for some considerable time into the future. However, it appears that the toxicity of this legacy is mitigated to a certain extent by the chemistry of the ecosystem; by sorption of TBT to organic rich sediments and dissolved organic carbon in interstitial pore waters. These processes are important in the context of the wider food web as they effectively limit the amount of biologically available TBT that can be taken up by consumers from what could otherwise be considered a substantial reservoir.

Despite this, TBT does still enter the food web as all consumers targeted and analysed for butyltin burdens from the most highly contaminated sites in this study exhibited quantifiable concentrations of organotins. Furthermore, dredging in Malthouse Broad undertaken by the Broads Authority, and similar private operations in Loynes Boatyard and Horning Ferry Marina appear to have had little impact on sediment TBT concentrations; implying that the TBT concentrations I found may result from the redistribution of existing contaminated anoxic sediments. Whether the mechanical disturbance promotes desorption from sediments to the water column is unknown. Given the increased bioavailability of TBT when it is present in the water column, there is an amplified potential for deleterious effects on biota, warranting further investigation in order that suitable mitigation techniques may be implemented if deemed necessary.

As would be expected, consumers that either inhabit sediments or use benthic resources appear most at risk to TBT exposure. Yet, it seems that some of the primary consumers also play a role in limiting the provision of TBT to higher consumers. I have effectively demonstrated that *Chironomus plumosus* exhibits an efficient metabolic capacity for TBT (*sensu* Looser et al, 1998, 2000). This was manifest in consistently quantifiable concentrations of only the breakdown product (DBT) in chironomids from the most contaminated site, Malthouse Broad. Although the longer lived primary consumer *Anodonta* spp. contained much higher concentrations of TBT, these are still not at concentrations that would induce deleterious effects (US EPA). Furthermore, I consider *Anodonta* to effectively represent a 'trophic dead end' within aquatic food webs as the large body size of adults means they are not viable prey for gape-limited predators such as fish, although exceptionally large cyprinids such as tench and carp may represent an exception to this rule. There is circumstantial evidence to suggest that some terrestrial consumers may be subsidised by these mussels and an avenue for future investigation might be whether this is a potential route for the cross-ecosystem transfer of TBT.

Despite low concentrations in chironomids and the unavailability of larger mussels to fish, specialist benthivores such as adult bream accumulated high hepatic concentrations of TBT. The likely routes are probably a combination of feeding on contaminated prey and ingestion of sediment whilst foraging. Another potential explanation is the passing of ingested sediment over the gill filaments that could result in uptake direct from sediment to blood. However, it was impossible to quantify the contribution of these uptake routes to bream organotin burden. Stomach content analysis could prove useful to this end in future work.

Pike also exhibited very high hepatic concentrations of organotins but when considered as an average total body burden, I found no evidence of biomagnification. These results suggest that in ecosystems contaminated largely by TBT as opposed to other organotins such as TPhT, biomagnification is unlikely to occur. Furthermore, in the absence of biomagnifications, there is little concern for TBT entering human diet from fish, and besides freshwater fish are a not a popular food source in the U.K. However, mammalian predators such as otter frequently target the liver of fish so may be exposed to considerable TBT if feeding on contaminated prey.

Despite demonstrating strong relationships between food web architecture and increasing sediment TBT concentrations, I acknowledge that it is not possible to quantify the role (if any) that TBT played in the compression and simplification of food webs along this gradient because of the correlational nature of the study. However, the changes in isotopic architecture mirror the palaeolimnological evidence so far presented by Sayer et al. (2006) for the same sites. I have shown also that the Layman et al. (2007) community metrics derived from stable isotopes could prove useful in other contaminant studies under experimental mesocosm scenarios. Future field based research should attempt to measure other key environmental stressors in an attempt to better understand the manner in which TBT could affect current food web structure and function. However, in complex systems such as the Norfolk Broads where multiple stressors may be difficult to isolate or control for, an experimental approach would prove more insightful. For example, mesocosm experiments involving representative food webs and site-specific sediments could control for other potential stressors on food web architecture such as disturbance, eutrophication, macrophyte biomass and habitat complexity

in order to quantify the effect (if any) of the sediment-based TBT reservoir on current food web structure and function.

The role of chironomids in the transfer of organotins was two-fold in the environs of the Norfolk Broads. I was able to demonstrate using stable isotopes as a tracer, that the seasonal flux of chironomid imagos across the aquatic-terrestrial interface was utilised by spider predators that were subsidised substantially by the aquatic-derived carbon source and resulted in a butyltin burden in spiders. The concentration of organotins was low, as would be expected given the concentrations evident in the aquatic larval life stage of chironomids. For this reason the potential for terrestrial consumers to be exposed to harmful concentrations of butyltins may be limited. However, it is suggested that life stage should be considered as invertivores prey upon chironomid imagos and target their breeding to coincide with mass emergence of insects to better provision for their young. Chicks and juveniles are more vulnerable to contaminant exposure due to their small body size and high ingestion rate. Future research should assess the potential risks of hazardous exposure to these life stages. To my knowledge, the results detailed in chapter five represent the first documented account of organotin transfer across the aquatic- terrestrial interface. Chironomids have an almost global distribution in freshwater habitats, and so are likely to inhabit the majority of such habitats that are contaminated with organotins. Thus redistribution of organotins from aquatic to terrestrial ecosystems by chironomid (or indeed other) imagos may be widespread. Concentrations in terrestrial predators were low in the Norfolk Broads, probably as a result of reduced bioavailability, lack of recent inputs, and species-specific chironomid TBT metabolism. However, it should be considered that this may not be the case in developing countries where TBT use may be ongoing as a result of weak monitoring programmes and poorly enforced environmental legislation. Further research is therefore required to establish

both the extent of the occurrence of this phenomenon and its potential ecotoxicological impact on recipient terrestrial consumers.

The results from this study can be viewed largely as a positive outcome with reference to the story of TBT in the Norfolk Broads. Despite a large and potentially ecologically harmful TBT reservoir, a combination of the chemical characteristics of TBT and physiochemical conditions in the Norfolk Broads ecosystem appear to have mitigated the bioavailability of TBT thereby limiting ecotoxicological impacts on both aquatic and terrestrial food webs. However, despite nearly a quarter of a century since their last known (legal) use in the Norfolk Broads ecosystem, organotins are still quantifiable throughout the food web from basal resources, through primary producers, to piscivorous fish, and also into the terrestrial ecosystem. It therefore seems likely that similar TBT contamination of aquatic and terrestrial consumers will occur in other systems where TBT has been used, even if its use is now restricted. Despite this, few studies document TBT occurrence stemming from a truly historic legacy, and even fewer have attempted to trace TBT contamination from its source to the wider food web. To this end results from this thesis should be directly transferable to other similarly contaminated freshwater systems and should provide insight in to the behaviour, fate, and trophodynamics of TBT, and may aid aquatic ecosystem management strategy. Beyond the historic context, more urgent research should be directed at other freshwater systems where TBT use may be ongoing. A global ban on TBT usage was implemented in 2003 by the International Maritime Organisation (van Wezel and van Vlaardingen 2004) yet TBT use is likely still widespread in the freshwater habitats of non-signatory, developing nations (Hoare 2007). In such cases, persistent organotin inputs into the water column may result in freely bioavailable concentrations of TBT that are unrestricted by sediment

chemistry where the potential for deleterious effects at all organisational levels from that of the individual through to the ecosystem may be greatly increased.

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Cromes Broad	March 07 δ¹³C (‰)	δ¹⁵N (‰)	June 07 δ¹³C (‰)	δ¹⁵N (‰)	Sept 07 δ¹³C (‰)	δ¹⁵N (‰)	March 08 δ¹³C (‰)	δ¹⁵N (‰)
1° Consumers								
C.plumosus	-39.6±0.2	14.5±0.4	-32.3±0.6	15.1±0.8	-33.9±0.2	15.9±0.3	-33.3±0.3	15.8±0.3
Chironomidae	-36.2±0.7	15.9±1.1	-37.8±0.5	18.3±0.9	-35.1±0.6	17.2±2.4	-36.9±0.4	17.9±0.4
G. pulex	-32.1±0.4	15.1±0.3	-32.3±0.5	19.2±0.6	-31.7±0.1	16.6±0.6	-33.9±0.3	19.7±0.7
A. aquaticus	-31.0±0.5	12.2±0.4	-31.0±0.6	13.7±0.7	-31.9±0.1	11.5±1.0	-33.5±0.3	13.3±0.3
Baetis spp.	-32.3±0.8	11.1±0.6	-32.7±0.7	11.6±0.5	-32.0±1.5	15.6±0.5	-32.2±0.3	19.7±0.2
Bulk zoop.	-33.7±0.6	16.5±0.1	-35.3±0.6	13.5±1.1	*	*	-37.6±1.0	18.0±1.0
Cladoceran zoop.	-36.0	13.7	-39.4	16.0	-37.1	14.9	-37.9	14.2
Agrypnia spp.	-30.4±1.3	12.3±3.0	-33.5±0.7	16.4±0.8	-33.1±0.7	15.4±0.5	-33.7±0.2	16.9±0.2
Psidium spp.	-37.2±0.5	15.5±0.5	-36.4	16.9	-36.2±1.0	17.5±2.0	-37.7±0.1	17.9±0.1
Anodonta spp.	-35.6±0.2	15.9±0.6	-35.0±0.2	16.5±0.2	-35.6±0.2	17.4±0.2	-35.8±0.2	17.4±0.3
B. tentaculata	-33.9±0.6	15.3±0.4	-34.9±0.3	15.6±0.3	-33.3±0.4	14.3±0.4	-34.5±0.3	16.2±0.4
L. stagnalis	*	*	-31.1±1.3	16.6±1.1	-29.6±0.3	16.7±1.2	-31.5±0.2	17.4±0.3
R. Bathica	-32.4±0.4	16.4±0.3	-32.2±0.8	16.3±0.3	-33.9±0.5	16.3±0.2	-33.5±0.3	17.9±0.9
2° Consumers								
Sympetrum spp.	-31.7±0.9	13.6±1.2	-34.4±1.9	16.4±1.0	-33.2±0.4	17.9±1.0	-34.3±0.02	16.5±0.3
Aeshna spp.	*	*	-34.5±0.6	18.5±0.8	-33.2±0.9	19.1±2.4	-34.9±1.0	20.2±1.3
E.najas	-30.6±0.6	14.5±0.3	-33.7±0.7	17.9±0.5	-33.5±0.7	18.7±0.7	-34.7±0.1	20.1±0.1
R. linearis	-32.7±1.4	14.6±3.0	-34.1±0.5	18.8±0.2	-34.8±0.4	18.7±0.6	*	*
N. viridis	-31.6±0.8	14.7±1.2	-34.4±1.4	16.3±1.3	-32.7±1.7	18.2±1.0	-33.3±0.3	17.4±0.2
I. cinicoides	*	*	-33.2±1.3	15.9±1.3	-33.9±0.1	18.8±0.4	-34.5±0.6	17.6±1.3
S. fuliginosa	-34.3±0.3	15.7±0.8	-31.4±0.2	16.3±0.9	-33.3±0.8	16.2±0.9	-33.7±0.6	15.0±0.8
Chaobrous spp.	*	*	-35.9±0.1	19.3±0.1	-34.8±1.0	21.9±1.6	-37.0±0.7	21.6±0.1
Sigara spp.	-33.8±0.6	13.1±0.8	34.6±0.5	15.1±0.4	-34.6±0.7	13.7±1.5	-33.4±0.5	13.3±0.3
Erpobdella spp.	-31.0±0.7	14.6±0.5	-32.02±0.3	16.1±0.7	-34.5±0.1	20.5±0.1	-33.9±1.2	18.8±2.9
Acaridae	-35.2	18.5	-36.4	19.4	-36.2	18.6	-35.8	19.1
Hydrophilidae	-32.7	12.8	-33.8±0.6	16.1±1.5	-34.0±0.8	13.5±0.9	-33.1±0.4	13.2±1.0
Fish Consumers								
R. rutilus	*	*	-29.7±1.3	20.2±0.2	*	*	*	*
S. erythropthalamus	*	*	-31.0	19.1±0.7	-30.6±0.6	21.7±0.3	*	*
A. brama (j)	*	*	-30.6±0.3	19.9	*	*	*	*
T .tinca	*	*	-32.2	21.0±0.3	*	*	*	*
A. anguilla	*	*	-32.1±1.0	21.6	*	*	*	*
P. fluviatilis (j)	*	*	-32.1±0.4	17.1±0.7	*	*	*	*
P. fluviatilis	*	*	-32.1±0.5	21.1±0.3	-31.2±0.7	21.3±0.3	*	*
E. lucius (j)	*	*	-31.5±1.7	21.0±0.3	-30.3±0.3	21.3±0.4	*	*
E. lucius	*	*	-29.9±0.3	22.8±0.2	*	*	*	*

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Appendix 1a (previous page): mean (± SD) seasonal isotopic data for each species from Cromes Broad;* indicates organism not encountered during sampling. Invertebrate values without ±SD are bulked samples comprising a minimum of 5 individuals, excepting Cladoceran zooplankton where each sample comprised a minimum of 50 individuals. Fish consumer isotope values without ±SD represent a single individual; (j) next to species name indicates juvenile.

Cockshoot Broad	March 07 δ¹³C (‰)	δ¹⁵N (‰)	June 07 δ¹³C (‰)	δ¹⁵N (‰)	Sept 07 δ¹³C (‰)	δ¹⁵N (‰)	March 08 δ¹³C (‰)	δ¹⁵N (‰)
1° Consumers								
C. plumosus	-32.3	9.6	-29.2±0.1	13.4±0.1	-30.1±1.1	14.1±0.1	-33.0±0.9	11.5±0.8
Chironomidae	*	*	-34.4±0.9	12.1±0.2	-33.7±0.4	11.9±0.5	-33.0±1.1	12.4±0.9
G. pulex	-32.1±0.4	12.3±0.7	-29.7±0.9	12.7±0.8	-30.5±0.9	12.0±0.8	-33.3±0.8	12.1±0.5
A. aquaticus	-30.0±0.6	11.1±0.4	-33.0±0.5	11.7±0.5	-30.0±0.4	8.9±0.7	-32.3±0.2	10.4±0.3
Baetis spp.	-31.9±0.3	11.4±0.4	-35.3±0.5	12.1±0.7	-34.1±0.9	12.3±0.2	-32.7±0.4	11.8±0.3
Bulk zoop.	-36.4±0.4	12.3±0.6	-33.5±0.7	12.2±0.9	-35.9±0.1	14.0±0.1	-35.7±0.2	12.7±0.1
Agrypnia spp.	-31.4±1.0	12.4±0.5	-31.2±1.2	11.9±0.5	-31±0.8	11.9±1.5	-33.2±1.1	11.6±0.8
Psidium spp.	-33.2±0.3	11.0±0.2	-32.9±0.2	11.9±0.3	-33.3±0.7	11.7±0.3	-36.6±0.4	11.5±0.1
Anodonta spp.	-33.2±0.4	12.2±0.6	-32.5±0.1	12.6±0.6	-32.9±0.1	13.3±0.4	-33.8±0.6	13.3±0.3
B. tentaculata	-33.4±2.7	9.2±0.2	-32.1±1.3	10.8±0.5	-30.9±1.7	10.7±0.5	-33.8±1.0	10.2±1.0
L. stagnalis	-29.0±0.7	10.4±0.4	-33.6	12.3	-32.7±0.5	12.1±0.5	-31.1	11.2
R. bathica	*	*	-29.9±1.1	11.0±0.4	-30.7±0.6	11.1±0.1	-33.3±1.0	12.3±0.7
2° Consumers								
Sypetrum spp.	-31.5±1.1	10.3±0.1	-29.5±0.9	12.3±0.3	-30.8±0.4	11.9±0.3	-31.0±0.6	11.1±0.6
Aeshna spp.	*	*	-33.2±0.5	13.8±0.1	-30.8±0.5	12.5±0.9	-33.5±0.6	11.1±0.7
E. najas	-31.5±0.4	12.4±0.8	-31.8±0.5	13.0±0.1	-32.1±0.02	12.9±0.1	-33.9±0.4	13.9±0.3
R. linearis	*	*	*	*	-31.4±1.1	11.6±3.0	-31.5±1.1	13.1±0.4
N. viridis	-31.2±1.2	11.6±0.4	-29.9±0.2	12.1±0.2	-31.2±0.3	11.8±0.5	-31.8±0.2	12.4±0.4
I. cinicoides	-29.7±0.6	11.8±0.5	-30.9±0.5	12.1±0.1	-31.6±0.4	13.3±0.1	-31.8±0.4	13.1±0.3
S. fuliginosa	-31.8±0.4	11.3±0.1	-30.1±0.8	11.1±0.3	-29.0±0.1	10.9±0.1	-32.3±0.3	11.4±1.0
Chaobrous spp.	-32.0±0.2	15.1±0.3	-34.4±0.1	14.2±0.3	-31.9±0.2	16.1±0.3	-33.9±0.8	16.6±0.2
G. caspius	-30.3±1.0	8.7±1.5	*	*	-31.8	11.4	-30.5±0.9	11.2±0.6
H. dubius	*	*	-32.2±0.8	16.6±0.5	-31.7±0.9	13.2±1.1	-35.0±0.6	13.5±0.6
Sigara spp.	-32.2±0.7	11.7±0.3	-34.2±1.5	13.2±0.9	-33.7±0.1	11.5±0.4	-31.1±0.4	11.2±0.7
Erpobdella spp.	-30.6±0.5	12.1±1.0	-31.3±1.7	12.5±0.8	-31.4	11.8	-31.5±0.9	12.0±1.0
Hydrophilidae	-31.5±1.2	10.8±0.6	-33.4±0.8	12.1±0.6	-32.1	11.4	-32.6	12.1
Fish Consumers								
R. rutilus	*	*	-27.0±0.8	15.9±0.4	*	*	*	*
S. erythropthalamus	*	*	-26.5±1.9	13.8±1.3	*	*	*	*
G. cernuus	-28.6±1.7	16.7±0.1	*	*	*	*	*	*
T .tinca	*	*	-28.2±0.5	15.6±0.4	*	*	*	*
A. anguilla	*	*	*	*	-30.2±2.3	17.1±0.2	*	*
P. fluviatilis (j)	-30.0±0.4	15.1±0.7	*	*	*	*	*	*
P. fluviatilis	*	*	-27.7±1.2	16.4±1.0	*	*	*	*
E. lucius	*	*	-27.3±0.1	18.9±0.4	*	*	*	*

Appendix 1b (previous page): mean (± SD) seasonal isotopic data for each species from Cockshoot Broad; * indicates organism not encountered during sampling. Invertebrate isotope values without ± SD are bulked samples comprising a minimum of 5 individuals. Fish consumer isotope values without ±SD represent a single individual; (j) next to species name indicates juvenile.

Wroxham Broad	March 07 δ^{13}C (‰)	δ^{15}N (‰)	June 07 δ^{13}C (‰)	δ^{15}N (‰)	Sept 07 δ^{13}C (‰)	δ^{15}N (‰)	March 08 δ^{13}C (‰)	δ^{15}N (‰)
1° Consumers								
C.plumosus	-36.3±0.4	10.5±0.2	-34.6±0.2	8.8±0.2	-35.6±0.6	8.3±0.3	-40.1±1.9	9.8±0.5
Chironomidae	*	*	-36.2±0.3	9.2±0.1	-35.2±0.2	9.4±0.3	-35.2±0.2	10.9±0.4
G. pulex	-30.0±0.2	12.5±0.3	-31.3±1.2	11.8±1.0	-31.8±0.3	12.6±0.2	-31.1±0.6	13.9±0.2
A. aquaticus	-30.7±0.1	11.2±0.3	-30.2±1.0	9.7±1.2	-30.4±0.6	11.0±0.1	-31.1±0.9	12.6±0.2
Baetis spp.	-37.5±0.1	12.6±0.1	-36.8±1.2	12.7±0.4	-37.2±0.7	12.2±0.3	-36.1±0.8	12.9±0.2
Bulk zoop.	-37.5±0.3	10.2±0.7	-32.8±0.6	12.2±0.2	-34.1±0.9	12.4±0.3	-36.0±0.4	10.8±0.5
Agrypnia spp.	-28.9±0.4	8.5±3.0	*	*	-34.05±0.6	11.9±0.6	-32.9±0.6	12.1±0.9
Psidium spp.	*	*	-34.5	9.7	-34.4±0.1	10.3±0.4	-34.1±0.1	12.3±1.2
Anodonta spp.	-32.8±0.3	11.2±0.3	-32.6±0.3	10.1±0.3	-32.5±0.9	11.8±0.6	-31.9±0.6	12.4±1.2
B. tentaculata	-31.3	9.5	-32.3±0.3	11.1±0.5	-33.1±0.4	10.2±0.3	-33.3±0.4	9.6±0.4
L. stagnalis	*	*	-30.9±0.4	11.7±0.3	-30.7±1.2	10.9±0.9	*	*
R. bathica	-31.1±0.6	10.6±0.4	-29.7±0.9	9.8±1.0	-32.1±0.3	9.6±0.7	-31.4±0.9	11.3±0.4
2° Consumers								
Sympetrum spp.	-32.9±0.6	12.4±0.4	-34.6±0.5	14.3±0.4	-34.9±0.5	13.7±0.3	33.6±0.2	13.2±0.7
E.najas	*	*	-34.8±0.1	11.5±0.5	-34.5±0.3	12.1±0.3	-33.3±1.5	13.6±0.3
N. viridis	*	*	-31.8±0.4	13.8±0.1	-33.2±0.5	11.8±0.3	-32.3±0.7	13.1±0.4
S. fuliginosa	*	*	-31.6±0.1	10.9±0.2	-31.1±0.6	12.3±0.6	-30.9±1.8	12.0±1.2
Sigara spp.	*	*	-33.8±0.2	11.3±0.2	-32.8±1.0	13.0±1.8	-32.9±0.1	12.4±0.1
Erpobdella spp.	*	*	-31.4±0.9	14.7±0.7	-32.5±0.5	12.9±0.6	-30.9±1.7	12.5±1.9
Fish Consumers								
R .rutilus	-30.5±1.0	17.3±1.5	-29.7±2.6	16.3±0.4	*	*	*	*
A. brama	*	*	-30.9±1.0	16.9±0.6	-30.4±0.7	16.8±0.7	*	*
T .tinca	*	*	-31.0±0.5	16.5±1.3	*	*	*	*
A. anguilla	*	*	*	*	-32.3	18.2	*	*
P. fluviatilis	*	*	-30.2±0.1	18.7±0.1	*	*	*	*
E. lucius (0+)	*	*	-32.9±0.1	15.4±0.1	*	*	*	*
E. lucius	*	*	-29.8±0.2	18.6±0.1	*	*	*	*

Appendix 1c: mean (± SD) seasonal isotopic data for each species from Wroxham Broad; * indicates organism not encountered during sampling. Invertebrate isotope values without ± SD are bulked samples comprising a minimum of 5 individuals. Fish consumer isotope values without ±SD represent a single individual.

Salhouse Broad	June 07 δ¹³C (‰)	δ¹⁵N (‰)	Sept 07 δ¹³C (‰)	δ¹⁵N (‰)	March 08 δ¹³C (‰)	δ¹⁵N (‰)
1° Consumers						
C. plumosus	-34.9±1.3	9.6±0.8	-34.4±0.6	10.3±0.8	-34.7±0.2	12.2±0.2
Chironomidae	-37.2	10.8	-35.8±2.0	12.3±1.0	-36.2±2.3	12.8±0.1
G. pulex	-32.9±1.2	13.7±0.8	-31.4±0.3	14.5±0.6	-31.2±0.9	13.2±0.8
A .aquaticus	-31.7±0.5	13.6±0.8	-31.8±0.6	13.4±0.7	-31.6±0.2	13.0±0.7
Baetis spp.	-37.2±1.2	12.1±0.4	-35.2±0.6	12.5±0.4	-38.0±1.1	13.2±0.8
Bulk zoop.	-38.1±0.6	17.6±0.9	-32.8±0.04	10.6±0.2	-35.8±0.3	11.6±0.1
Cladoceran zoop.						
Agrypnia spp.	-34.1±0.4	10.9±1.0	-35.8±0.7	11.1±0.8	-35.4±0.9	11.3±0.2
Psidium spp.	-34.7±0.3	10.5±0.5	-35.1±0.7	11.8±0.2	-36.6±0.5	12.7±0.2
Anodonta spp.	-32.2±0.5	11.2±2.3	-31.7±0.3	12.2±0.3	-32.1±0.2	11.7±0.1
B. tentaculata	-33.8±1.0	11.5±0.7	-30.9±0.8	11.9±0.2	-35.3±0.5	12.2±0.5
R. bathica	-32.6	10.2	-31.9±0.5	14.4±1.0	-33.9±0.6	12.9±0.5
2° Consumers						
Sympetrum spp.	-31.2±0.3	13.0±0.1	-31.3±0.4	13.3±0.3	-31.7±0.6	13.9±0.9
E.najas	-34.7±0.3	13.4±0.4	*	*	-34.6±0.3	14.9±0.1
N. viridis	-31.4±0.4	11.0±0.3	-33.1±0.4	13.6±0.1	-32.5±1.2	13.4±2.0
S. fuliginosa	-32.2±0.5	12.1±0.6	-32.9±0.7	13.3±0.1	-33.1±0.7	12.9±0.2
Sigara spp.	-34.1±0.7	11.9±0.5	-31.6±0.2	11.8±0.8	-34.5±0.1	12.9±1.0
Erpobdella spp.	-31.0±0.4	14.2±0.4	-32.2±0.2	14.0±0.2	-32.0±0.8	13.9±0.5
Fish Consumers						
R.rutilus	-29.1±0.8	16.7±0.8	-31.4±0.4	16.2±0.3	*	*
A. brama (j)	-30.7±0.4	16.7±0.1	*	*	*	*
A. anguilla	-30.8±0.8	17.4±0.9	*	*	*	*
P. fluviatilis	-31.1±0.7	17.5±1.0	*	*	*	*
E. lucius	-29.3±0.8	18.9±0.8	-28.9	19.5	*	*

Appendix 1d: mean (± SD) seasonal isotopic data for each species from Salhouse Broad; * indicates organism not encountered during sampling. Invertebrate isotope values without ± SD are bulked samples comprising a minimum of 5 individuals. Fish consumer isotope values without ±SD represent a single individual; (j) next to species name indicates juvenile.

Ranworth Broad	March 07 δ^{13}C (‰)	δ^{15}N (‰)	June 07 δ^{13}C (‰)	δ^{15}N (‰)	Sept 07 δ^{13}C (‰)	δ^{15}N (‰)	March 08 δ^{13}C (‰)	δ^{15}N (‰)
1° Consumers								
C.plumosus	-35.1±0.4	14.7±0.3	-33.3±0.6	15.3±0.2	-36.3±0.2	15.2±0.1	-37.9±0.2	15.5±0.1
Chironomidae	-35.7±0.8	14.1±0.4	*	*	-35.6±0.3	15.2±0.2	-37.6±0.8	14.3±0.2
G. pulex	*	*	-30.2±0.4	13.6±0.6	-31.0±0.1	16.9±0.1	-32.2±0.5	15.5±0.3
A .aquaticus	-32.4±0.6	13.3±0.7	-30.4±0.6	12.7±0.3	-32.1±0.4	13.8±0.4	-32.2±0.3	13.9±0.4
Baetis spp.	-33.5±0.9	11.5±0.5	-34.6±1.5	13.9±1.7	-34.2±0.3	12.4±0.6	-33.7±0.3	13.0±0.9
Bulk zoop.	-37.4±0.5	13.8±0.4	*	*	-34.3±0.1	17.9±0.1	-35.3±0.1	13.0±0.1
Agrypnia spp.	-31.1±1.6	12.7±0.1	-32.3±1.0	14.6±0.9	-31.3±0.7	13.9±0.9	-31.7±0.5	13.2±0.8
Psidium spp.	*	*	-32.2±0.4	13.9±0.2	-33.4±0.1	14.9±0.4	-36.0±0.3	13.9±0.8
Anodonta spp.	-32.1±0.6	13.6±0.4	-32.1±0.2	14.2±0.7	-31.6±0.5	15.2±0.9	-33.1±0.4	15.1±0.3
B. tentaculata	-31.5±0.3	13.3±0.2	-31.4±0.01	12.9±0.2	-31.5±0.5	13.6±0.2	-32.9±0.5	14.3±0.5
L. stagnalis	*	*	-30.3±0.4	14.2±0.4	-33.0±0.3	14.3±0.6	-31.2±0.4	14.4±0.3
R. bathica	-30.4	14.5	-32.0±0.6	15.0±0.4	-33.3±0.9	`15.4±0.3	-31.5±0.2	14.7±0.4
2° Consumers								
Sympetrum spp.	-29.3±0.3	11.5±0.7	-30.8±0.8	13.2±0.2	*	*	-33.6±0.2	15.2±0.3
Aeshna spp.	*	*	-31.3±0.2	15.2±0.2	-33.9±0.4	11.4±0.4	*	*
E. najas	*	*	-31.1±0.3	15.1±0.4	-32.9±0.2	16.5±0.1	-33.7±0.3	17.1±0.3
R. linearis	-28.4±0.6	14.1±0.4	-29.7±0.7	14.9±0.4	-28.6±0.6	14.2±0.4	*	*
N. viridis	*	*	-31.3±1.4	12.5±0.9	-33.2±0.3	12.8±0.3	-32.6±0.2	15.4±0.3
S. fuliginosa	-31.2±0.3	13.2±0.6	-32.1	14.3	-32.3±0.5	13.8±0.5	-31.6±0.3	13.5±0.4
Sigara spp.	*	*	-30.8±0.3	13.5±0.7	-32.7±0.2	14.9±0.1	-34.6±0.3	11.0±0.3
Erpobdella spp.	-30.6±0.3	15.0±0.1	*	*	-31.7±0.2	15.9±0.7	-31.8±0.3	16.6±0.2
Acaridae	-29.1	15.1	-32.0	16.8	-31.4	15.3	-30.3	15.2
Fish Consumers								
R.rutilus	*	*	-29.4±0.9	15.7±3.1	*	*	*	*
S. erythropthalamus	*	*	-28.2±0.8	17.5±1.4	*	*	*	*
A. brama (j)	*	*	-30.0±1.2	18.6±0.7	*	*	*	*
A. brama	*	*	-29.2±0.8	18.5±0.1	*	*	*	*
T .tinca	*	*	-30.6±0.6	17.8±0.1	*	*	*	*
A. anguilla	*	*	-29.4±0.8	19.6±0.8	*	*	*	*
P. fluviatilis	*	*	-28.8±0.4	20.3±0.4	*	*	*	*

Appendix 1e: mean (± SD) seasonal isotopic data for each species from Ranworth Broad; * indicates organism not encountered during sampling. Invertebrate isotope values without ± SD are bulked samples comprising a minimum of 5 individuals. Fish consumer isotope values without ±SD represent a single individual; (j) next to species name indicates juvenile.

Malthouse Broad	June 07 δ¹³C (‰)	δ¹⁵N (‰)	Sept 07 δ¹³C (‰)	δ¹⁵N (‰)	March 08 δ¹³C (‰)	δ¹⁵N (‰)	July 08 δ¹³C (‰)	δ¹⁵N (‰)
1° Consumers								
C.plumosus	-31.5±0.6	12.2±0.03	-36.2±0.2	14.4±0.1	-39.4±0.2	13.7±0.1	-30.6±0.1	12.2±0.1
Chironomidae	-34.2	12.9	-36.9±0.2	15.1±0.1	-39.2±0.5	13.6±0.4		
G. pulex	-30.9±0.6	12.0±0.7	-31.5±0.3	16.9±0.2	-33.6±0.8	14.6±0.4		
A. aquaticus	-30.6±0.8	11.4±0.04	-32.1±0.2	13.3±0.2	-36.9±0.3	12.2±0.5		
Baetis spp.	-37.5±0.4	11.9±0.6	-38.3±0.5	13.0±0.7	-37.6±0.1	13.5±0.8		
Bulk zoop.	-33.6±0.6	16.3±0.3	-33.9±0.5	16.7±0.4	-37.3±0.2	12.3±0.1		
Agrypnia spp.	-28.6±1.2	12.3±0.7	-33.9±1.0	15.6±0.1	-33.2±0.6	14.4±0.9		
Psidium spp.	-32.1	12.9	-32.9±0.3	14.9±0.4	-35.3±0.4	12.3±0.3		
Anodonta spp.	-32.1±0.3	14.1±0.3	-32.5±0.3	15.0±0.7	-32.9±1.0	14.5±0.6		
B. tentaculata	-30.8±0.4	12.9±0.4	-30.9±0.6	15.1±1.0	-34.5±0.1	13.1±0.1		
R. bathica	-29.4±0.5	13.3±0.7	-31.9±0.3	14.9±0.7	-35.9±0.2	14.7±0.2		
2° Consumers								
Sympetrum spp.	-31.6±0.9	13.3±1.5	-33.1±0.4	13.3±1.0	-33.9±0.5	13.0±2.0		
Aeshna spp.	-30.9±0.6	15.8±0.5	-32.5±0.5	15.9±0.2	*	*		
E.najas	-31.0±0.2	14.3±0.1	-33.1±0.2	16.2±0.1	-35.5±0.2	17.1±0.3		
R. linearis	-33.2±0.8	12.3±1.8	-34.2±0.6	12.9±1.0	-33.1±0.3	15.3±1.6		
N. viridis	-32.1±1.3	14.6±0.2	-33.1±0.2	14.6±0.2	-35.3±0.4	12.7±1.3		
S. fuliginosa	-28.4±0.9	11.8±1.1	-32.3±0.2	14.2±0.4	-34.0±1.5	14.2±0.5		
Sigara spp.	-32.2±0.4	12.6±0.7	-32.2±0.5	13.9±0.1	-36.9±0.5	13.1±0.3		
Erpobdella spp.	-32.0±02	15.3±0.4	-34.0±0.5	16.0±0.3	-32.4±0.6	15.2±1.0		
Acaridae	-29.5	13.3	-31.2	14.4	-29.0	14.6		
Hydrophilidae	-32.2±0.6	11.1±0.8	-33.7±0.4	11.5±0.5	-32.5±0.3	11.9±0.6		
Fish Consumers								
R. rutilus	-29.4±0.9	17.1±0.3	-29.5±0.2	17.6±0.6	*	*		
S. erythropthalamus	-29.0±0.9	17.5±0.7	*	*	*	*		
A. brama (j)	-29.4±0.3	17.3±0.2	*	*	*	*		
A. brama	-30.0±0.4	18.2±0.3	*	*	*	*		
T. tinca	-31.1±0.3	17.9±0.3	*	*	*	*		
A. anguilla	-29.3±0.5	19.9±0.6	*	*	*	*		
P. fluviatilis	-29.3.1±0.5	19.9±0.6	*	*	*	*		

Appendix 1e: mean (± SD) seasonal isotopic data for each species from Malthouse Broad; * indicates organism not encountered during sampling. Invertebrate isotope values without ± SD are bulked samples comprising a minimum of 5 individuals. Fish consumer isotope values without ±SD represent a single individual; (j) next to species name indicates juvenile. Sampling in July 2008 conducted for *C.plumosus* only.

Loynes Boatyard	Sept 07 δ^{13}C (‰)	δ^{15}N (‰)	March 08 δ^{13}C (‰)	δ^{15}N (‰)
1° Consumers				
G. pulex	-36.6	14.2	-33.8	14.8
A. aquaticus	-32.2±0.2	13.4±0.3	*	*
C. curvispinum	*	*	-35.9±0.3	12.5±0.1
Baetis spp.	-34.7±0.4	13.7±0.9	-36.5±0.7	12.5±1.0
Psidium spp.	-33.6	12.1	-33.0	13.1
Anodonta spp.	*	*	-34.4±0.7	13.4±0.5
B. tentaculata	-33.4	12.1	-35.0	12.0
D. polymorpha	*	*	-35.9±0.3	12.5±0.1
R. bathica	-32.4±0.9	12.1±0.3	-28.6±1.2	11.3±0.8
2° Consumers				
H. dubius	-33.0±0.3	14.8±0.7	-31.6±0.9	14.0±0.2

Appendix 1g (above): mean (± SD) seasonal isotopic data for each species from Loynes Boatyard; * Indicates organism not encountered during sampling. Invertebrate isotope values without ±SD are bulked samples comprising a minimum of 5 individuals.

Horning Ferry	March 07 δ^{13}C (‰)	δ^{15}N (‰)	Sept 07 δ^{13}C (‰)	δ^{15}N (‰)
1° Consumers				
Chironomidae	*	*	-34.2±0.2	16.8±0.2
Psidium spp.	-31.2	15.5	-32.8	16.1
Anodonta spp.	-33.4±0.5	14.6±0.3	-32.6±0.5	13.4±1.0
D. polymorpha	-33.3±0.2	14.3±0.4	-34.1±0.7	15.1±0.3

Appendix 1h (above): mean (± SD) seasonal isotopic data for each species from Horning Ferry Marina; * Indicates organism not encountered during sampling. Invertebrate isotope values without ±SD are bulked samples comprising a minimum of 5 individuals.

Sample/site	TBT treatment conc. ($\mu g\ L^{-1}$)	Log K_{oc} value
W1	40	1.8
W2	40	1.8
W3	40	1.8
W4	80	1.8
W5	80	1.8
W6	80	1.6
W7	120	1.7
W8	120	1.8
W9	120	1.8
S1	40	1.6
S2	40	1.8
S3	40	1.6
S4	80	1.7
S5	80	1.7
S6	80	1.5
S7	120	1.5
S8	120	1.6
S9	120	1.5

Appendix 2a: experimentally derived log K_{oc} values for Wroxham and Salhouse Broad sediments; W: Wroxham Broad samples; S: Salhouse Broad samples.

Sample/site	TBT treatment conc. ($\mu g\ L^{-1}$)	Log K_{oc} value
M1	40	1.7
M2	40	1.8
M3	40	2.0
M4	80	1.5
M5	80	1.4
M6	80	1.3
M7	120	1.5
M8	120	1.4
M9	120	1.3
R1	40	1.3
R2	40	1.5
R3	40	1.4
R4	80	1.5
R5	80	1.4
R6	80	1.4
R7	120	1.4
R8	120	1.4
R9	120	1.3

Appendix 2b: experimentally derived log K_{oc} values for Malthouse and Ranworth Broad sediments; M: Malthouse Broad samples, R: Ranworth Broad samples.

Sample/site	TBT treatment conc. ($\mu g\ L^{-1}$)	Log K_{oc} value
C1	40	0.7
C2	40	0.6
C3	40	0.6
C4	80	0.7
C5	80	0.5
C6	80	0.6
C7	120	0.6
C8	120	1.0
C9	120	0.6
M1	40	1.6
M2	40	1.7
M3	*	*
M4	80	1.3
M5	80	1.1
M6	80	0.5
M7	120	0.7
M8	120	1.2
M9	120	1.5

Appendix 2c: experimentally derived log K_{oc} values for Malthouse and Cockshoot Broad sediments;

C: Cockshoot Broad samples, M: Malthouse Broad samples; * indicates analytical failure.